Regulating Interparticle Interactions to Control the Spatio-Temporal Assembly of Gold Nanoparticles

A thesis submitted for the partial fulfillment of

Doctor of Philosophy

by

Anish Rao

Department of Chemistry

Indian Institute of Science Education and Research (IISER)

Pune, India - 411008.

March 2020

Certificate

This is to certify that the work incorporated in the thesis entitled **"Regulating Interparticle Interactions to Control the Spatio-Temporal Assembly of Gold Nanoparticles"** towards the partial fulfilment of the Integrated PhD dual degree programme at the Indian Institute of Science Education and Research (IISER), Pune represents original research carried out by *Anish Rao* under my supervision. The work presented here or any part of it has not been included in any other thesis submitted previously for the award of any degree or diploma from any other university or institution.

Date:

Place: Pune

Dr. Pramod P. Pillai

Thesis Supervisor

Declaration

I declare that this written submission represents my ideas in my own words and where others ideas have been included, I have adequately cited and referenced the original sources. I also declare that I have adhered to all principles of academic honesty and integrity and have not misrepresented or fabricated or falsified any idea/data/fact/source in my submission. I understand that violation of the above will be cause for disciplinary action by the Institute and can also evoke penal action from the sources which have thus not been properly cited or from whom proper permission has not been taken when needed.

Date: **Anish Rao**

Place: Pune Registration I.D. : 20132009

Acknowledgements

The time that I have spent at IISER Pune was way more fun experience than anyone could reasonably expect. I am deeply grateful to IISER, and everyone who was a part of my life during my time here, as directly or indirectly, you all have allowed me to grow personally, academically and also literally (*AngryFace*).

This thesis would not have been possible without the love, support, guidance, and friendship that I received from many many people who I met along the way. It is not possible for me to adequately express the gratitude I feel for everyone who have helped me along the way, nor is it possible for me to list everyone who deserves that gratitude, but that won't stop me from trying ☺.

- My advisor - **Dr. Pramod P. Pillai**; You've taught me more than just how to do experiments, how to write papers, and present the work. The time that I have spent in the lab has taught me how to think, and inculcated in me a love for science and research. The mentor/mentee relationship is may be how we began, but now, and for all times, you are and will be a cherished friend. Special thanks for all the patience that you have showed me over the years. I doubt that I would have found a better lab at IISER to work at. Thank you!

- My RAC members - Along the way, I have had wonderful discussions with **Dr. B. L. V. Prasad**, and **Dr. Angshuman Nag**. I hope that I will get a chance to stay in touch with you for years to come.

- My lab mates, the NanoAlchemy lab, and no that logo is not a caterpillar (*AngryFace*).

– **Soumendu** and **Gayathri**; all of us joined the lab at same time, *(technically, I came 1 month before you guys ☺, but who is counting...).* Now, we are leaving together as well. Your smartness, sincerity, and hard work has been a subject of my envy as well as irritation. Soumendu - I will suggest that people who I know and like, do not end up in your group (if you decide to start a lab, or your startup). The guy who drives awfully, abuses the traffic light, is 3 years younger to sir, asks Shana to divide and multiply by the same number, makes the most amount of noise when hungry, but eats the least, has right priorities (he was about to throw NPs away, so as to get a clean vial), guy who loves to visit bangalore, has not broken anything ever in life, is the most soft-spoken and well tempered person I ever met. Gayathri - The reason for Soumendu's fall... literal fall.. she is a chair puller. Everyone who is around her, has been hereby warned. I hope that you will have a job for me in your entrepreneurial endeavour. Also, remember the mendeleev incident? *the time when you randomly pressed enter, and something happened?*

– To the first **Sumit** (Sumit Bhosale)- I wanted you to know that you are popular among the new group members. You have been the star of so many of my stories that I like telling to the younger members of the group. SPOILER! In many of them, you ended up dying because of Soumendu's anger. I don't know who will tell these stories to the generations to come...

– The second **Sumit** (Sumit Roy) - I hope you know that a part of the reason why you ended up in the group is because you shared the names of two awesome men - SUMIT bhosale, and soumendu ROY. Particularly memorable are the things you do; the amount of biryani you eat when you are not hungry, the way you remove silica out of the column, your expressions while dancing. To **Indra** - I have no idea how you manage to get a bike, iPad, gaming laptop, and 75k cycle in such a short span of time with the fellowship we get. Curious. Also, the noise that you make while laughing... that too at random things... *kerala*,*seat-belt*, *sumit*, *AC*, *car* Remember?

  - **Pradyut** (Mr. PMRF)- One of the most confident, sincere, charming, hard-working person and knowledgeable person that I have come across. I don't know you manage to do it all at once man. Kudos! and Good luck to you! and *BadhaiHo* **Vanshika** and **Kashyup**; Vanshika - Another PMRF from the lab. Your sincerity and street smartness is beyond compare! You really are going to be a rockstar! **Kashyup** - I really like your leadership skills man. I know many other people love it too...

- Another fun part of being in the group was the constant influx of project students, the presence of whom, made the lab a bit younger, and a bit more fun place to come to (every morning between 9:00-10:00 ☺). Especially **Mahima**, **Preethi**, and **Ajesh**, whose have carved a special place in my memory of the initial times of our lab.

- I also got the opportunity to help mentor 4 amazing masters thesis students (*Ajesh, Govind, Sarah,* and *Shana*). **Govind** - for coming up with the worst of the worst (that can ever exist) PJs. Your help in the successful, so many unsuccessful projects is deeply valued. The gift wrap **Shana** - for being so talented and sincere. While working with you, just to keep up, I had to work wayyy harder. (*TrustFall*) Thank you! for all the malayalam movies, songs, pazham puri, kothu parathas, taking my awesome photos (*TrustFallAgain*). Both of you especially have shown how much fun this mentoring experience can be, and I hope you guys like the experience as well. Because of your awesomeness, you guys have raised unfair and unreasonable expectations in me from people :D. I hope I can keep my unreasonableness in check in future. Thanks to you once again.

- My sincere gratitude to **Dr. Anirban Hazra**, **Dr. Harinath Chakrapani**, **Dr. Partha Hazra**, and **Dr. Sujit K. Ghosh** for giving me an opportunity to rotate in their labs. Interactions with you, as well as your group members were key in instilling in me a love for science, and research.

- My batchmates - (Amar, Dhriti, Divya, Akhila, Harpreet, Neel, Ron, Swati, Aditi,

Bharat, Mehak, Shivani, Sandip, Adarsh, Anshul, Charu, Deepak, Gudibanda Ananth Raghavendra Shiv Rajkumar K. (you have another K in your name!) Kashyap, and Jay). The best Int. Ph.D. Batch ever - 2013 batch; our seniors know it and our juniors do it too. We managed to be one of the very few batches where there is no bengali. Please don't count Neel as a bengali... You guys have been one of the most energetic, understanding, and fun-loving bunch of people. This batch is super-powerful; sugar spice and everything nice... Thank you for being around and making the life at IISER so much fun.

- Special thanks to the other members of The Four Indian Wisemen; Sandip, Ron and Neeladri, or The Controlled Aggregation + the Manager.

  - **Sandip**- For all the insightful discussions, being so wise, being so dramatic, and now being so fat. Thank you for taking so much enthusiasm in all the random things that we used to do. Like making a movie for celebrating Ron's b'day; a movie that has the potential of re-defining entire world of cinema - as we know it. I have had so many discussions, and arguments with you on maybe everything that one can think of; at the end of which, at least I have emerged a bit more wiser. I really like your taste in gifts as well. What better gift to give a stranger than 'One Hundred Years of Solitude'. I solemnly swear that I will finish this book soon. Lastly, thanks for all the hashtags that you initiated to get me do things that for some reason or the other, I would not have done; like tagging on to the Goa trip, and Divya's shaadi. I guess you would have finished your Ph.D. 2 years earlier, had you not been involved in such things. :D But thank you for doing all the awesome things that you did.

  - **Neeladri** - for doing things... We have tried to document your life in a book. We are waiting for you to do some more important things, like solving COVID-19 (after solving Nipah), so that we can work on another boook; **Neeladri Sen Doing Important Things**. And just for the record, not

just Pune, you are also a reason why I am getting bald *AngryFace*. A very short biographical introduction for Sen (which can be used to introduce him when he wins Nobel prize; maybe in peace!). ***Neeladri*** *- a kind hearted and gentle soul who is against all kinds of violence and killing. I remember once, he saw some bugs infect his hostel room, and did nothing - as he did not want to kill them. Later, he outsourced the work to pest control people to peacefully get the bugs out of his room - an awesome way of resolving conflict. Another incident comes to mind, when, Sandip sent his car to Kerala, and the people in charge made some real mess. The whole hostel floor is an alibi to the kindness with which our Neel requested the concerned authorities to fix their mistake. And SPOILER: They did fix it. We all can learn a lot from this gentle soul, who has never hit anyone, never bit anyone, never pulled anything, never kicked anything, and never killed anything (FISH: he does not like). I just added the last part so that you remember to not cook fish for the banquet dinner. There is no relation between killing and fish okay. I am glad that the world could recognize the potential of this great man, and made the obvious choice of giving him the Nobel prize.* (*P.S. - This was written under duress from him, similar to the duress the Nobel committee was in while making their final decision.*)

– **Ron**- If someone pulls a gun to my head, and asks me to call someone, I am never calling you... Despite being always on the phone, I don't understand how you manage to not pick any of our calls... A field ecologist, whose science matters, who has been the constant source of great questions regarding plant ecology, but never of answers. I believe you have taken quotes regarding 'having unanswered questions' a teeny-tiny bit too seriously. One of the great things that I remember of the time that was spent with you, is the freedom, and the lack of judgement to any question/ opinion that we thought about. A selfless person who joined the batch with the image of being the most sick person (literally), and when Neel stole this unique quality of yours, you

never made a fuss. A person who somehow, always managed to steal people's thunder. When some was celebrating her/ his b'day - you got sick and got attention, when someone gave pre-synppsis - you got sick and got attention. **For all the three combined: I deeply value the time that I had the opportunity to share with each one of you. It has definitely made me a wiser, and thanks to you Neel - balder person. But to be fair, I think I would not have it any other way. On a serious note, I think if I can choose, I would hvave it exactly the same way, with a bit more hair on my head. I am sure I have not said it enough during my time at IISER, but thank you guys for being an essential and continuous source of happiness and wisdom in my life at IISER.**

- Along the way, I've had helpful conversations, and musings about science, philosophy, politics, faith, teaching, etc. with Sandip, Ron, and Deepak. Also, thanks to Sandip, Ron and Neeladri for being involved in several projects like Ronjuring, Neeladri Sen Doing Things, Balubali, and some more stuff. Sukrut, Mukul, Deepak, Sandip and Ron - for being with me either in Taekwondo, Cross-Fit, Swimming classes. Other than sandip, I am glad that no-one lost any weight. Sandip managed to lose 3kgs in a month by swimming like a whale... He is so irresponsible... he keeps on loosing his stuff.

- My parents, and my sister; It is an age old question whether nature or nurture governs a person's behaviour. Whatever the answer to this question may be, the outcome is that my family's visible as well as invisible support has pushed me a step closer to science.

- **Neha, Shikha, Abhishek,** and **Sheetal**. Many of you have been an invisible, but a constant source of help, and support. Especially **Neha** and **Shikha** - both of your support during a much needed time is deeply valued. Thank you!

- To all those randomly said 'Yes-es' that made me sign-up for, or volunteer for

things that I would not have done. Like being the joint secretary of the Chemphillic club, being in the social media team for India March for Science, CLM lecture, and some other things as well...

- I thank all the Chemistry Department's HODs (Head of Department) Dr. H. N. Gopi, and Dr. M. Jayakannan for their support to our lab.

- To IISER Faculties, Chemistry Directors; for providing all the necessary facilities to efficiently carry out the research.

- To IISER Pune's administrative staff; for their constant invisible support and help. Special thanks to Mayuresh Ji, Tushar, and Sayalee; Having you guys around is like having some order in our chaotic Ph.D. life.

- A big thank you to all the funding agencies for their financial support, and CSIR, as well as IISER Pune for my fellowship.

Aap Chronology Samajhiye!

- First I came to IISER, as someone with **NO** interest in research.

- Then I found some awesome teachers especially Anirban Hazra, Harinath Chakrapani, Sudipta Basu; for introducing me to the joy of finding things out during your lectures/ discussions.

- I did more rotations than I was supposed to ☺ - to find a Ph.D. lab.

- Then I stumbled on Dr. Pramod's website (a newly joined faculty at IISER Pune). Had discussions with him, saw him presenting his past work and show future projects (in a departmental talk). Got fascinated by it, and took a leap of faith to join his lab for Ph.D.

- Now, after 6-and-a-half years, I am a little older, a little balder, a little wiser, and I daresay a scientistt!

I humbly acknowledge all the help, support, guidance and love that I have been so generously given to me.

- Anish

Thesis Synopsis

Self-Assembly is nature's preferred *'zero-waste'* means of creating animate matter. It typically involves the realization of functional materials from individual building blocks, without the need for any external or human intervention. Researchers are focused on understanding the principles underlying the self-assembly process, so as to form purposeful and useful structures, despite the lack of human intervention. In light of this, the aim of my thesis is to study the effects of finely tuned interparticle interactions in governing the outcomes of both spatial, as well as temporal self-assembly processes. We demonstrate that a control over interparticle interactions can be successfully translated to create systems with fascinating degrees of complexity. This thesis contains a summary of our efforts that show how finely tuned interparticle interactions can (a) improve existing nanoparticle (NP) properties, (b) show the emergence of inherently absent properties, and (c) mimic complex *'life-like'* behaviour. Here, the property of our choice was aggregation mediated identification of heavy metal ions, while the behaviour of our choice was the formation of transiently stable self-assembled structures.

With these specific goals in mind, in **Chapter 2**, we designed heterogeneously charged gold nanoparticles ([+/-] AuNPs), where, strengths of different interparticle interactions could be regulated by simply changing the ratio of oppositely charged ligands on the NP surface. We used these NP systems, to balance different attractive and repulsive interparticle interactions and reveal an unprecedented phenomenon of controlled aggregation. These NP systems could *reversibly "arrest"* toxic ions like lead (Pb^{2+}) and cadmium (Cd^{2+}) through the formation of controlled aggregates,

making them a recyclable trapping and scavenging system. A key advantage of the present system is the simplicity with which the mixed-Self-Assembled Monolayer (m-SAM) on the NPs could be tuned to trap and scavenge different triggers of interest (like Pb^{2+}, Cd^{2+}, H^{+}, and citrate). More importantly, we showed that the regulation of interparticle interactions could impart a new function of selectivity towards trapping of toxic ions over biologically relevant ones. These initial signs of selectivity encouraged us to design an identification protocol capable of identifying a specific M^{2+} ion.

With this challenge in mind, in **Chapter 3**, we worked towards introducing the notion of selectivity towards strongly binding divalent metal ions (M^{2+}), to inherently nonselective carboxylate functionalized gold nanoparticles ([-] AuNPs). Here, we chose the abilities of M^{2+} ions to break the interactions between the oppositely charged AuNPs (in a nanoionic precipitate) as the means of identification, rather than the conventional idea of forming an interaction. We observed that out of all the M^{2+} ions tested, only Pb^{2+} could break the electrostatic interactions in the nanoionic precipitates, and release [+] AuNPs to the solution (turn-on response). Interestingly, both [+] and [-] AuNPs, despite being *"blind"* in terms of selectivity toward M^{2+} ions, gave rise to an assembled state that showed remarkable selectivity towards Pb^{2+} ions. Furthermore, by tuning the strengths of interparticle interactions, the sensitivity as well as selectivity of our identification protocol could be improved to ~ 4 μM. Note that traditionally, similar tasks of selective identification are undertaken with the help of analyte-specific ligands, where the property of selectivity is simply *'added on'* to the NPs. This work, therefore, showed a conceptually different strategy of identification, where, the self-assembled state shows the emergence of selectivity.

In **Chapters 2**, and **3**, we demonstrate that to create nanosystems with desirable or improved properties, one need not have to come up with novel materials. A careful understanding, and control over different interparticle interactions can help in not only improving the known properties of NPs (**Chapter 2**), but can

impart inherently absent properties to NPs (**Chapter 3**). These finding motivated us to use our control over interactions to install *life-like* properties to a NP system. More specifically, we thought of creating systems that come into existence only for a limited amount of time (transient self-assembly). In order to mimic the formation of such systems, in **Chapter 4**, we demonstrate a fundamental discovery of creating self-assembled structures that show transient switching/ shuttling between completely precipitated and redispersed stages of nanoparticles (NPs) – *a first of its kind in plasmonic NPs*. The chemical trigger driven transient self-assembly was accomplished by using the temporal control over electrostatic attractions between positively charged gold nanoparticles ([+] AuNP) and negatively charged EDTA (chemical trigger). Consequently, some of the desirable feats in the field of transient self-assembly were realized such as easy removal of waste, formation of a transiently stable precipitate state and negligible dampness in redispersion. We also reveal the so far unknown ability of atmospheric components to transform a mundane mixture of chemicals into a dynamically active one – *a task usually accomplished with a network of chemical reactions*.

In summary, my thesis demonstrates the effectiveness of establishing a control over interactions between the building blocks as a potent way of creating intelligent passive as well as active states.

Summary

This thesis is divided into four chapters, where,

- In **Chapter 1**, we introduce the terminologies, and make a case for using finely tuned interparticle interactions as our design principle.

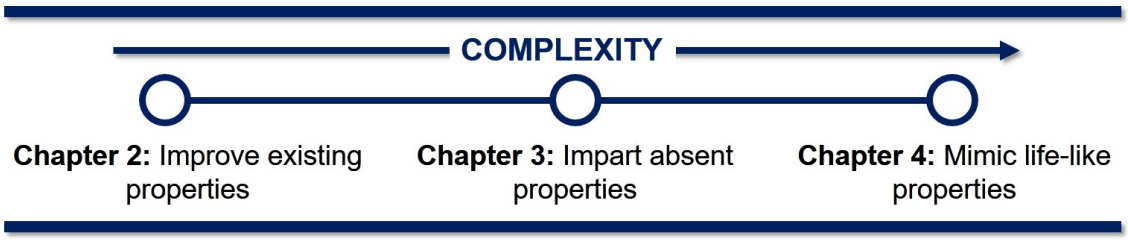
- In **Chapter 2**, we show how finely tuned interparticle interactions can improve existing NP properties. It is known in the literature that most of the NP based detection protocols are dependent on precipitation/ aggregation of NPs, and are oftentimes irreversible and nonselective in nature. In this study, we could balance

attractions and repulsions to reveal the formation of controlled aggregates.

- In **Chapter 3**, we design an identification protocol to carry out selective identification, without the use of any analyte-specific ligand. Here, we utilized the abilities of different M^{2+} ions to break an interaction, as opposed to formation of an interaction as the identification protocol.

- In **Chapter 4**, we utilize our control over interparticle interactions to choreograph the formation of a transiently stable self-assembled state. Nature routinely employs such states to form create life-like systems.

In my thesis, you would see the principle of finely-tuned interparticle interaction being employed to create systems of increasing complexity.



Contents

Chapter 1

Design Principles to Complex Matter

1.1 Introduction

Interest in the area of nanoscience is slowly transitioning from the design and synthesis of nanoparticles (NPs) with exotic structures and properties, to their assembly into larger systems.[1] This transition is fuelled by the realization that a collective assembly of interacting NPs is responsible for applications in the fields of diagnostics, drug delivery, electronic devices, etc.[2–5] For instance, carbon atoms, when arranged in two different ways can be either used to make a great conductor (*graphene*), or an insulator (*diamond*). This is a classic example where, two completely contrasting properties can be realized from the same building blocks. This, in turn, demonstrates the importance of relative arrangements of building blocks in deciding the functions associated with an assembled state. Because of this, it has become increasingly important to find ways of controlling and realizing the arrangement of different building blocks in desired and purposeful ways. Most popular and widely used strategies are - a) assisted/ guided assembly (commonly seen in assembly lines), and b) self-assembly (commonly seen in natural systems), see Figure 1.1.

Self-Assembly is the science of things that form on their own. This method

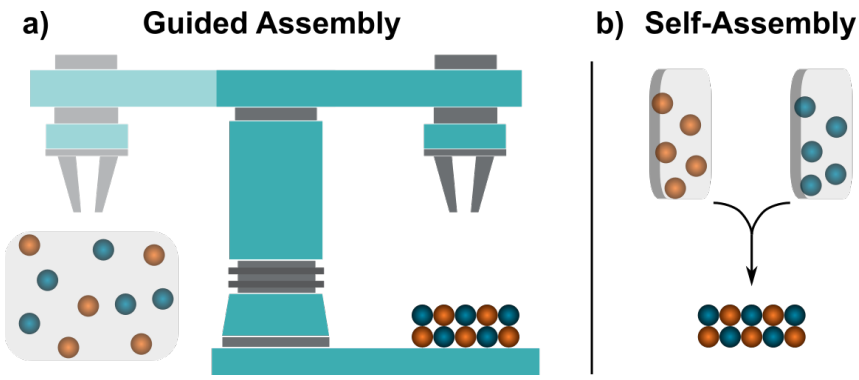


Figure 1.1: Schematics illustrating the formation of an assembled state through two different methods. (a) Assisted/ guided assembly where an external agency (like a robot) is needed to form the assembled state, and (b) self-assembly where the interacting components (here, two types of spherical particles) assemble into the crystalline state on their own. *Schematics adapted from Freepik.*

of making things involve no external agency, as opposed to the guided formation commonly utilized in assembly lines, and therefore have a magic-like promise of assembling useful entities. In particular, the power of self-assembly lies in synthesizing things that are too small or too many in number - a task which is impossibly difficult to be handled robotically. For instance, if the task of forming a 100 μm NaCl crystal is assigned to a very fast working robot (that can assemble $\sim$1 million atoms/second), it will take $\sim$1800 years to make one such crystal - a devastatingly long time to get a grain of salt. In real life, evaporation driven self-assembly/ crystallization protocols are set in place, to form salt within a matter of hours to days. In contrast to the way humans work, biological systems typically utilize both guided as well as self-assembly protocols to create useful entities. For instance, living systems utilize guided assembly protocols to link amino acids into a polypeptide chain, a task championed by the ribosome. Once the linear polypeptide chains are formed, using the principles of self-assembly this linear polymeric chain folds into the correct functional state in a matter of milliseconds. In a typical self-assembly process, the organized structures are formed by vigorously shaking the individual components .[6,7] This picture is deceptively simple and conceals the need of appropriately *'programmed'* building blocks.[7] One needs to master the control over different chemical and physical interactions so as to *code* the positional information onto the building

blocks - a crucial task to realize the next generation of complex matter.

There are numerous ways of coding the positional information of individual building blocks in the self-assembled structure. Such a code acts as a selective glue, which becomes sticky only when the building blocks are in their assigned/ desired places. This kind of information can be coded onto the building blocks either by using appropriate molecular glue, such as the idea of complementary shapes *(similar to a jigsaw puzzle)*, chance *(entropy)*, external fields, appropriate molecular coatings, etc. Each one of these ways of coding are outlined in Section 1.2. An appropriate understanding of these gluing techniques is like learning the fundamentals of music theory or understanding the rules of a chess game. The act of playing a good game of chess or composing a musical masterpiece, comprises a different skill of how complex one can go without breaking the rules. A detailed understanding of these design principles can not only help in creating complex structures, but also help in understanding the origins of life. Although a well-accepted definition of complexity is not around, but one can clearly see that some structures are more complex than others. For instance, cells transfer a cargo from one place to another by constructing highways made up of actin filaments. These highways are distinctly different, and are more complex than man-made highways. A key distinction between these two is that the cellular highways are inherently dynamic, and spontaneously dissolve away upon completion of the work. Biological systems, therefore, present an interesting contradiction to the conventional human wisdom, and thus present challenges to design similar complex systems. This Chapter lays out the rules and design principles to create different classes of self-assembly. These design principles will then be used in the subsequent Chapters to create different functional states from a small set of nanoparticle (NP) building blocks.

1.2 Fantastic Structures and How to Make Them

It has been shown by Phillips and co-workers that molecular machines that form the basis of life, operate in regimes where the energy-versus-length curves for a wide

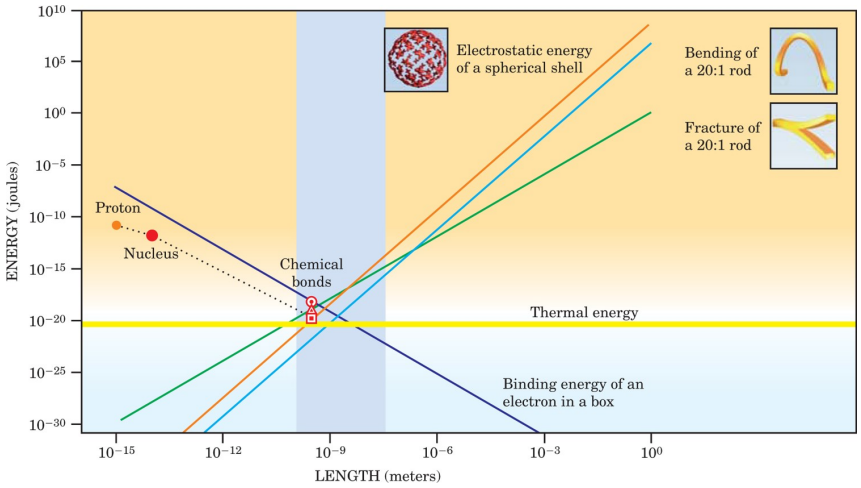


Figure 1.2: Graph illustrating how different deterministic energies like chemical, mechanical, electrostatic interactions as well as non-deterministic thermal energies associated with an object vary as a function of its size. Note the confluence of different deterministic as well as non-deterministic energies as the characteristic object size approaches nanometre regime (shaded in blue) (Reproduced in part with permission from [8] Copyright 2006 AIP Publishing).

variety of phenomena converge. Figure 1.2 shows that at the nanometer length-scale (shaded in blue), different *deterministic* energies like electrostatic, mechanical and chemical are of comparable strengths to the *non-deterministic* thermal energies. This regime, therefore, allows nature to design complex life-like entities through an interplay of different deterministic and non-deterministic energies.[8,9] For instance, consider restriction enzymes (proteins that recognize and cut specific nucleotide sequences), which finds the target sequence by searching through millions or billions of base pairs in a genome, at rates inconsistent with simple 1D or 3D diffusion of the enzyme along the DNA. It has been shown that entropic forces, resulting in folding of DNA, and hopping of enzyme from one strand to another is used to speed up the search process.[8,10] In order for one to design systems capable of similar functions, it is useful to understand how to control different interparticle interactions. The kinds of interactions that can be tuned are given in the following sections.

1.2.1 Molecular Interactions

Ligands are an indispensable component of NP architecture, as they not only impart stability to them, but dictate their interactions with the environment as well.[13]

Because of the ubiquitousness of ligands, researchers have started utilizing the interactions originating from the NP surface to create different ordered self-assemblies.

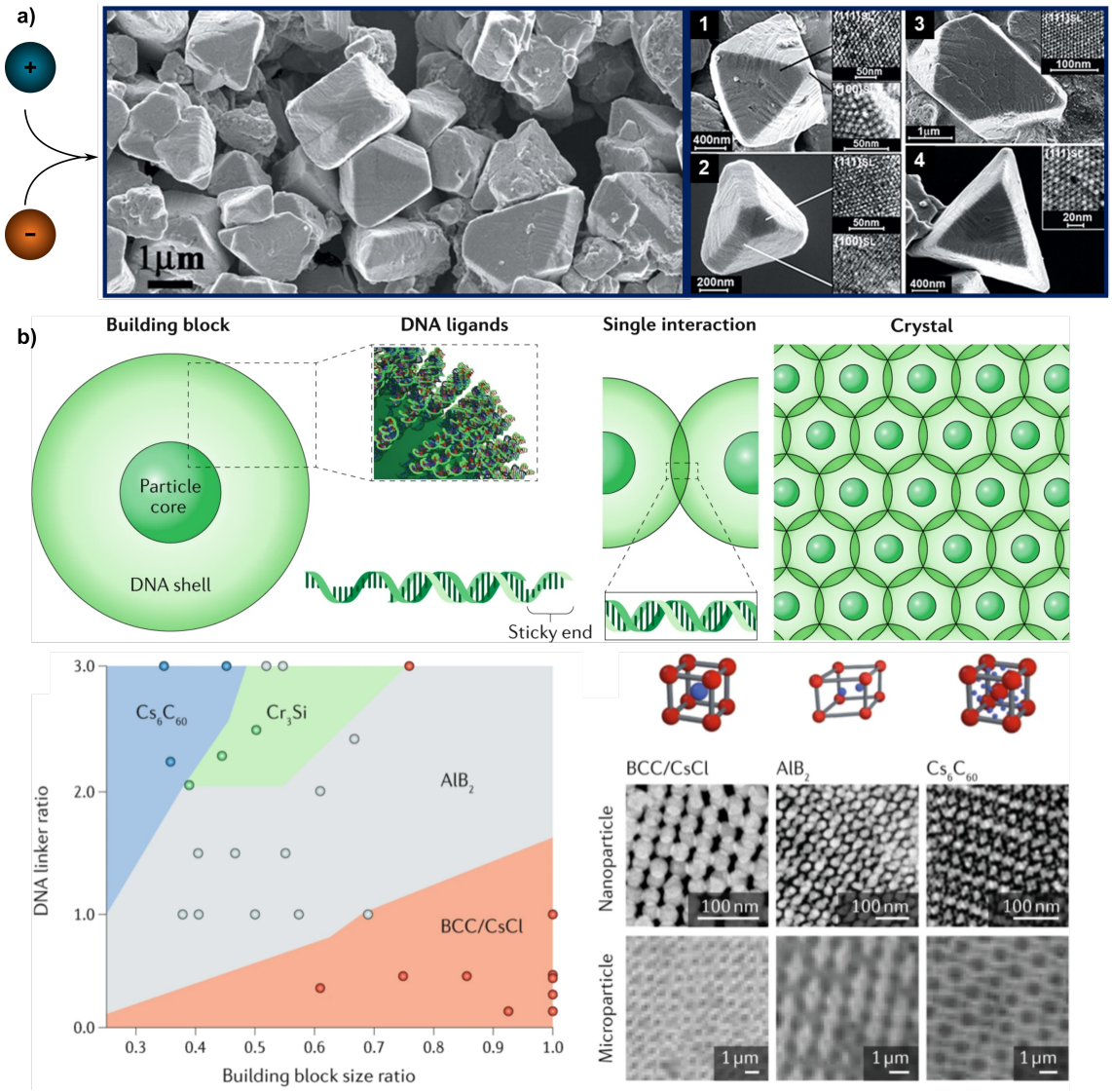


Figure 1.3: a) Schematics showing the use of oppositely charged like-size metallic NPs to form a non-closed packed crystal. A large area SEM image showing the formation of binary crystals obtained from [+] Ag and [-] Au NP precipitates. Different morphologies of the NP crystals (1) octahedron, (2) cut tetrahedron, (3) octahedron with two triangular faces cut, and (4) truncated tetrahedron are also shown (Reproduced in part with permission from [11] Copyright 2006 American Association for the Advancement of Science). (b) Schematics showing the formation of a crystal from building blocks comprising a DNA shell. The phase diagram shows the phase behaviour of crystal obtained from two-component systems containing complimentary DNA strands. Here, the ratio of hydrodynamic diameter of the building block and the number of DNA linkers were used to control/ direct the lattice symmetry. Electron and fluorescence microscopy images showing the generality of the idea for both micro and nanoparticle crystals (Reproduced in part with permission from [12] Copyright 2019 Springer Nature).

In this direction several studies have been undertaken to understand the design rules to form self-assemblies at different length-scales, popular among them is the crystallization of NPs.[6,13,14] Decades of research in the field of crystallization of micrometre or nanometre sized particles has shown that a dispersion of particles, when evaporated, usually results in the formation of a close-packed assemblies.[15,16] It is therefore an attractive feat to realize crystals with a wide variety of structures, porosity, and symmetries for various optoelectronic, photonic, and memory storage applications.[5] In a seminal work, Grzybowski and co-workers showed the formation of a non-close packed diamond like crystal lattice using oppositely charged, nearly equal sized metal NPs (Figure 1.3a).[11,17] Here, the authors used a mixture of oppositely charged Au and Ag NPs, which precipitate out from the solution at the electroneutrality point, resulting in the formation of nanoionic precipitates.[11,18] These precipitates were then converted into micrometre-sized and regularly faceted crystals, by first re-dispersing the precipitates in DMSO-water mixture, and slowly evaporating the water. Interestingly, the obtained crystals were diamond-like with coordination number (CN) of 4, as opposed to the commonly observed ones with coordination numbers of 6 or 8. The formation of these *open crystals* were rationalized based on slight charge asymmetry, as well as considerably high thickness of the screening layer in comparison to the size of NPs (κ^{-1}).[11,17] In another class of AuNP crystal engineering, thiolated DNA is used as a ligand to create organized structures of arbitrary shape and complexity.[12,19–21] The use of DNA as ligand is very attractive as it can be used to design and control the thermodynamics as well as kinetics of crystallization.[12] Here, the bases present at the end of the DNA sequence are unpaired and therefore act as sticky end. These sticky ends can be used to install sequence specific interactions between the NP building blocks, and drive the formation of an ordered crystal (Figure 1.3b). In a landmark study, Mirkin and co-workers developed a model based on a set of rules, that maximizes DNA hybridization events, and could predict the thermodynamically most favoured crystal (complementary contact model, CCM).[21] They constructed a phase diagram of DNA-driven crystallizaiton, where the ratio of

hydrodynamic diameter of the building block and the number of DNA linkers could direct the lattice symmetry, irrespective of the sizes of the building blocks (Figure 1.3b).

1.2.2 Field Dependent Forces

The use of external fields like light, magnetic field, and electric field are attractive strategies for the manipulation of a wide variety of NPs.[22] Of particular interest are light and magnetic field, that can be instantaneously delivered, as well as removed from the system. In such cases, these external fields act as a template for the organization of NPs in various complex structures. Here, Klajn and co-workers evaporated a monodisperse dispersion of superparamagnetic magnetite nanocubes in hexane at the liquid-air interface in the presence of magnetic field (Figure 1.4a-c).[23] Under the influence of the applied field, the magnetic dipoles partially aligned with the field, resulting in 1-Dimensional (1D) helical NP chains, with ∼90 % yield. These observations were rationalized on the basis of a clever interplay between magnetic dipole-dipole interactions, van der Waals interactions, entropic forces and Zeeman coupling.[23] Here, under the influence of a magnetic field, chiral nanocube clusters formed spontaneously, and in order to maximize the packing neighboring helices adopted the same handedness, thereby revealing a novel mechanism for the symmetry breaking and chiral amplification (Figure 1.4a-c). Similar to magnetic field, light can also be used as an external stimulus to direct the formation of appropriately functionalized NPs. In this direction, Grzybowski and co-workers used light controlled dipole-dipole and covalent interactions for the assembly of colloidal crystals and supraspheres (Figure 1.4d).[24] They used AuNPs decorated with a mixture of dodecylamine (DDA) and a photo-isomerizable azobenzene dithiol ligand, which reversibly self-assembled under the action of UV light, because of dipole-dipole and crosslinking interactions. Here, the degree of reversibility depended on the strength of dipole-dipole, as well as covalent interactions. For instance, at low dithiol surface coverage, reversible (*metastable*) crystalline assemblies were formed, whereas

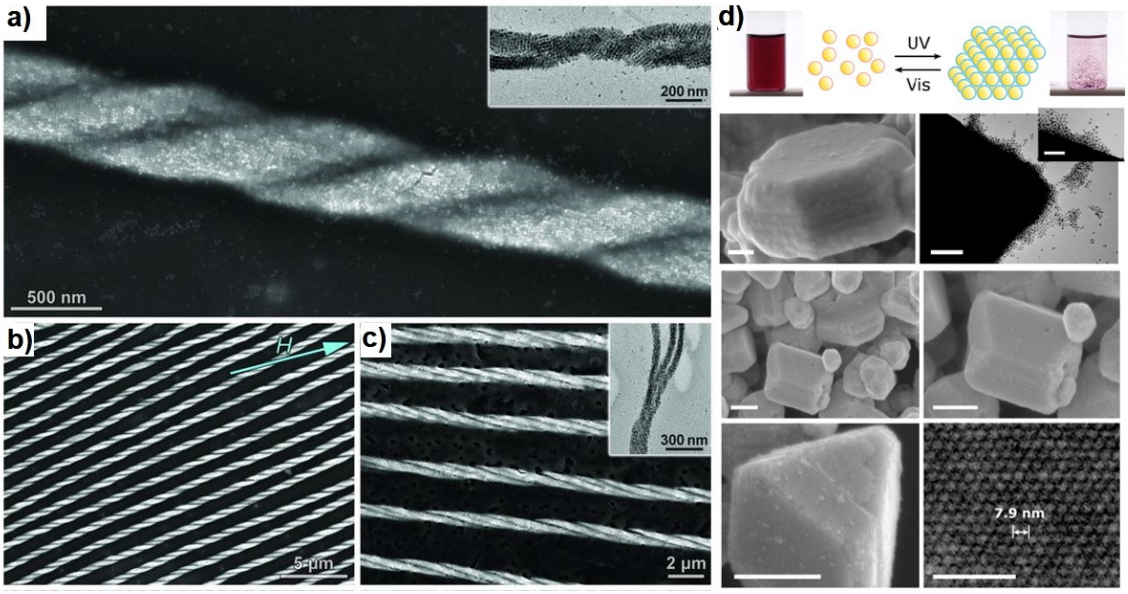


Figure 1.4: Self-assembly of helical 1D nanowires from cubic iron oxide NPs. SEM images showing a well-defined double helix structure in (a) an individual, and (b) large array of single-stranded 1D nanowires. (c) SEM and TEM images showing an array of triple helices (Reproduced in part with permission from [23] Copyright 2014 American Association for the Advancement of Science). (d), (e) Optical photographs, SEM, and TEM images showing the light induced reversible self-assembly of AuNP crystals. (d) Scale bars = 100 nm in main images, 50 nm in inset. (e) Scale bars = 200 nm, and 50 nm in the magnified lower right image (Reproduced in part with permission from [24] Copyright 2007 National Academy of Sciences).

permanent cross-links resulted in the formation of irreversible supraspheres at high dithiol surface coverages.[24]

1.2.3 Shape-Dependent and Entropic Interactions

As opposed to the other methods of controlling NP organization, where the interactions between the NPs are coded with the help of suitable ligands, assembling interactions can also emerge from the NP core. This alternative recognition mechanism uses shape-complementarity of particles for creating self-assembled structures. Here, the key interaction between the particles is depletion interaction, where it becomes entropically more favourable for the smaller *solute* particles to stay in the bulk solution as compared to staying in between two colloids.[27] This results in a net osmotic pressure acting to push the particles together. In a seminal work, Pine and co-workers demonstrated these geometrical interactions by using colloidal spheres

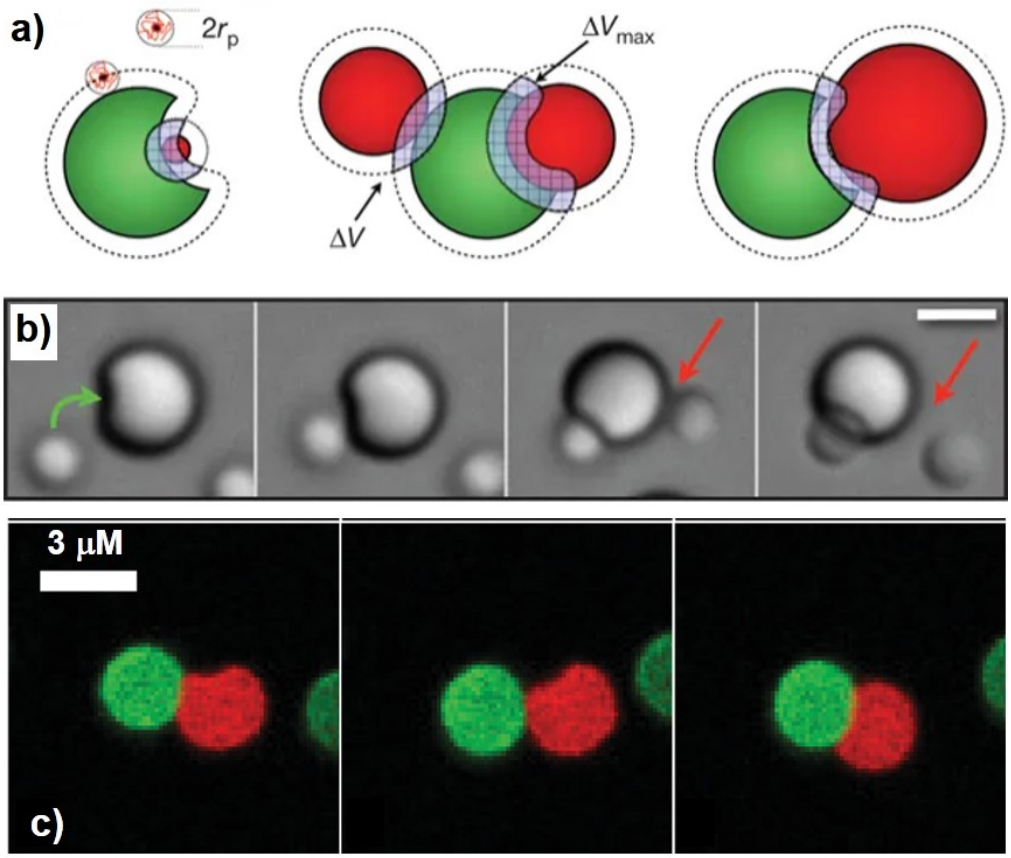


Figure 1.5: (a) Schematics showing the depletion attraction between lock (shown in green), and key (shown in red) colloids, which is proportional to the excluded volume (shown in blue). (b) Snapshots showing the size-specific depletion-driven self-assembly of lock and key colloids. Scale bar is 2 μm (Reproduced in part with permission from [25] Copyright 2010 Springer Nature). (c) Confocal microscopy images showing the transition from non-specific (middle frame) to specific binding between the lock and key colloids. Scale bar is 3 μm (Reproduced in part with permission from [26] Copyright 2015 AIP Publishing).

as keys (shown in red, Figure 1.5a) and a buckled colloid as a lock (green sphere in Figure 1.5a).[25,28] Here, the specific binding occurred only if the size of colloidal key matched closely with the size of the cavity in the colloidal lock (Figure 1.5b). These systems could spontaneously bind to each other via the depletion interaction, resulting in shape complimentary lock-and-key binding. The authors then laid out geometrical rules for creating directional, selective as well as reversible interactions for engineering smart machinery.[25] Later, investigations were carried out to understand the kinetics of bond formation between such lock and key colloids. It was found that the bond formation between the colloids proceeded at comparable rates

in both specific as well as non-specific interactions.[26] Here, the colloids associate directly from the free particles during specific binding, while in the indirect pathway a key particle nonspecifically binds to the lock surface, which after surface diffusion results in a specific bond (Figure 1.5c).

In another landmark study, Glotzer and co-workers demonstrated the formation of ordered self-assembled structures under the influence of chance (*entropy*).[29–31] Here, the authors performed a systematic and exhaustive modelling of the assembly

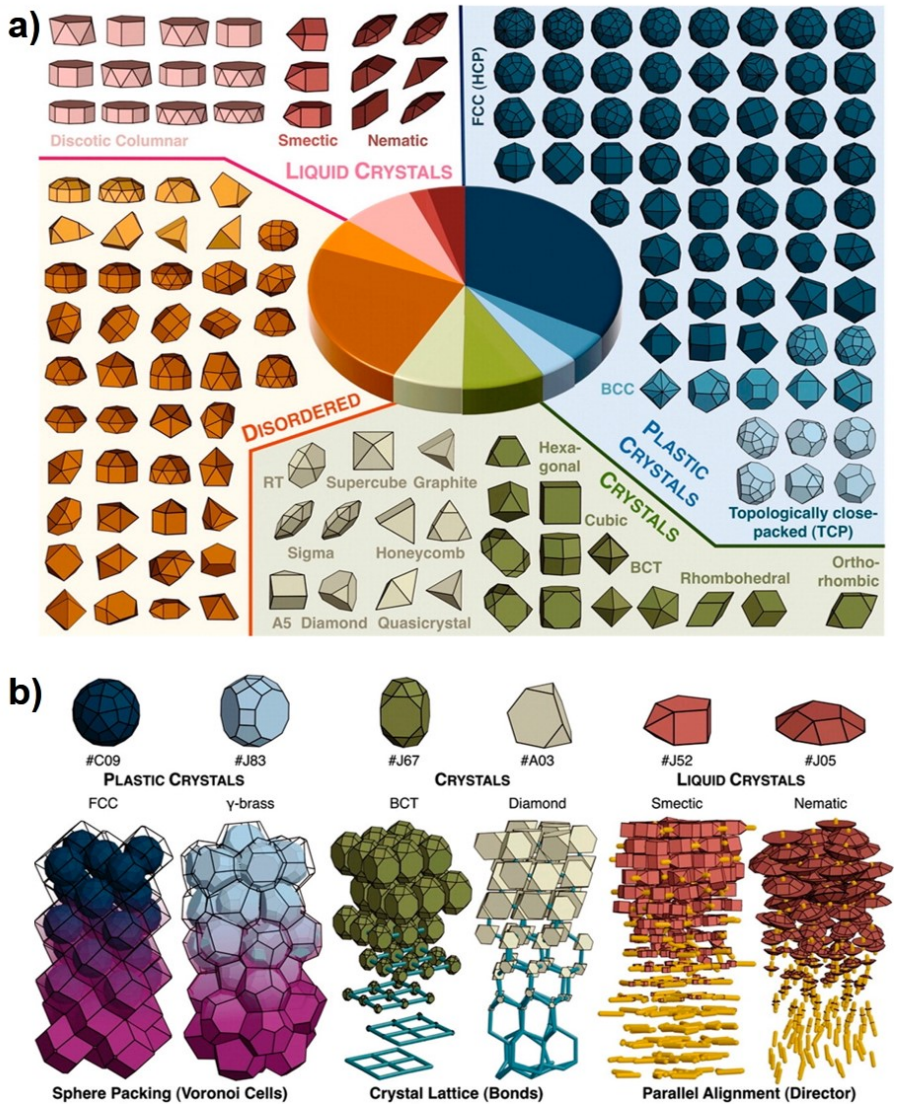


Figure 1.6: (a) Systematic classification of different polyhedral particles into four different assembled organizations. (b) Illustrations of six systems of polyhedra that form either plastic crystals, crystals, or liquid crystals (Reproduced in part with permission from [29] Copyright 2012 American Association for the Advancement of Science).

of polyhedral hard particles upon evaporation (Figure 1.6).[29] Note that for hard particles, all the possible conformations are similar due to lack of enthalpic contribution, and therefore are governed only by the maximization of orientational and positional entropy. This entropy maximization rule, for a polyhedral particle, favours organization along the facets, resulting in the formation of an *'entropic bond'*. They observed that the systems assembled in either of the four categories; i.e. crystals, plastic crystals, liquid crystals, or completely disordered structures (Figure 1.6). A design rule was then established depending on the high correlation between the *'coordination number'* of the polyhedron in the fluid phase, and the *'isoperimetric quotient'* (IQ) which measures the deviation of particle's shape from a sphere. According to this rule, (i) highly faceted particles with high coordination number formed plastic crystals, (ii) low faceted particles having low coordination numbers formed liquid crystals, and (iii) polyhedra with intermediate coordination numbers formed crystals. This study, therefore provided a comprehensive framework allowing one to predict the assembly of particles by simply determining its shape parameter and coordination number in the fluid phase.

1.3 What kinds of Complex Structures?

Having understood the rules that can be used to govern and dictate the outcomes of a self-assembly process, we lay down the design principles for creating complex self-assembled structures. Here, the complexity that is associated with a self-assembled state can be attributed to its position in the Gibbs free energy landscape (Figure 1.7).[32–36] Although there is no universal definition for complexity that is associated with a system, but it is agreeable that, cells are fairly more complex than crystals, despite both being the products of self-assembly.[6,37] Several distinctions can be drawn between the two, for instance, a crystal - once formed does not change with time and is plastic in nature, while living cells keep on evolving with time. Furthermore, most crystals do not need a constant supply of energy to stay in the organized state. On the other hand, cells do need a continuous influx of energy

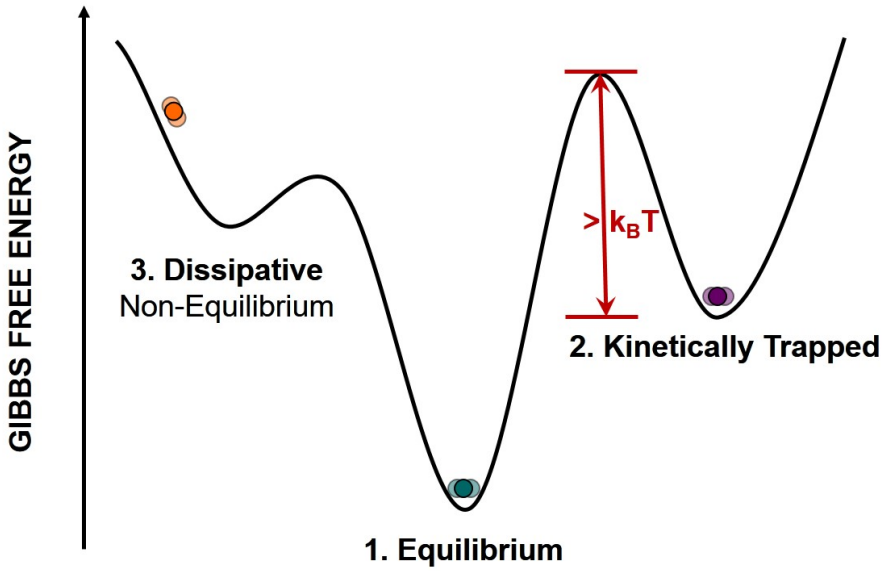


Figure 1.7: Schematic illustration of Gibb's free energy diagram showing different thermodynamic states associated with the outcomes of a self-assembly process.

to stay alive and function as suitable building block for life. Although distinctions between different kinds of self-assembled states is still a matter of inquiry, but some broad distinctions can be made between each of these processes. For instance, the re-configurability of a self-assembled state is dictated by the depth of the interaction potential, where, states that occupy a global minima cannot be reconfigured, while the ones in a local minima can be modified under the action of a suitable stimulus. Broadly speaking, one can classify the self-assembled states in the following three categories:-[34,36]

1. **Thermodynamic Equilibrium State -** Here, the self-assembled state occupies a global minima, and can require some energy to form (say in the form of stirring). Such structures, once formed, does not need any energy to sustain themselves. Most of the research in the field of artificial self-assembly has been focused on this kind of self-assembly.

2. **Kinetically Trapped State -** Here, the self-assembled state occupies a local minima, instead of a global minima, and if the energy barrier (marked in red) is higher than the thermal energy ($k_b T$), it results in the formation of long lived kinetically trapped states. These states, under the influence of a suitable trigger,

can be modified or re-configured, and can install the much needed property of reversibility and adaptation to a system.

3. **Dissipative State -** Here, the self-assembled state comes into existence only under a constant supply of energy and/or matter. A key feature of these systems is the necessity of a constant energy supply to sustain the self-assembled state. In this class of self-assembly the interactions that hold the building blocks together come into existence only if the system continuously dissipates energy.

1.3.1 Examples of Thermodynamic Self-Assembly

Nature frequently utilizes equilibrium self-assembly mechanisms to reproducibly create a wide variety of functional materials like lipid bilayers, DNA, proteins, virus, enzymes, etc. Furthermore, most of the observed self-assembly processes like formation of micelles, folding of proteins, crystallization, etc. are thermodynamically favoured processes, where the self-assembled state lies at a global minima.[6] In order to effectively design such equilibrium self-assemblies, the interactions between the building blocks needs to drive the self-assembled state to a thermodynamic minima so as to reproducibly form the desirable structure. In this direction, Stupp and co-

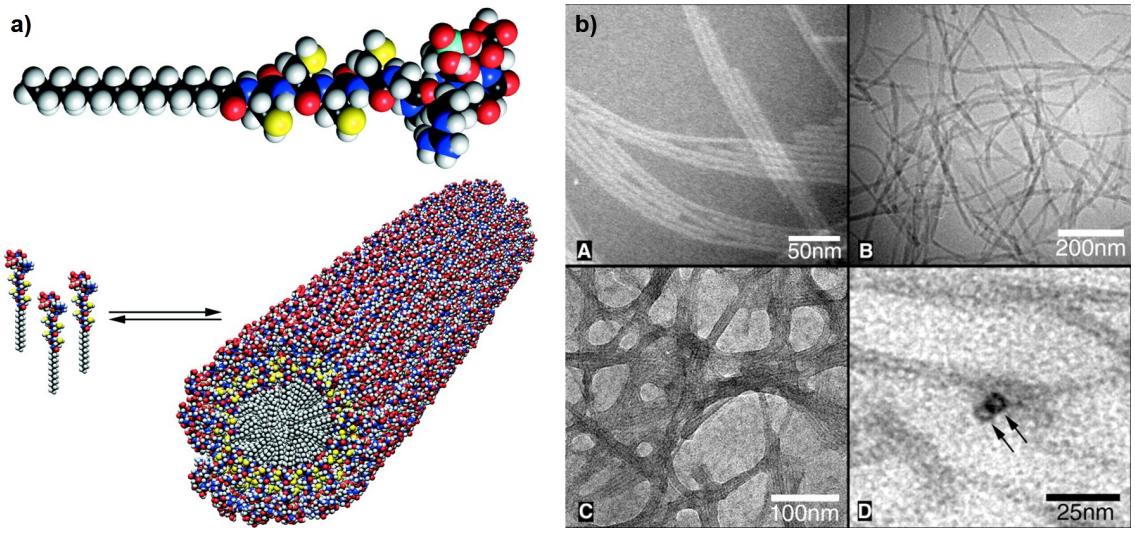


Figure 1.8: (a) Schematic model of the PA and its self-assembled cylindrical micelle. (b) TEM images of the self-assembled nanofibers (Reproduced in part with permission from [38] Copyright 2001 American Association for the Advancement of Science).

workers, in a pioneering example, constructed nanostructured fibrous scaffolds from organic self-assembling building blocks that closely mimics bone (Figure 1.8).[38–40] Here, they used pH induced self-assembly of peptide-amphiphiles (PAs), where hydrophobic and hydrophilic moieties are attached to a peptide moiety (Figure 1.8). This allowed a reversible enhancement of the structural integrity of the nanofibers through re-configurable cross-linking interactions. After cross-linking, the fibers could direct the mineralization of hydroxyapatite and form a composite material having alignments similar to that observed between collagen fibrils and hydroxyapatite in bones. These polymers were found to be active for in vivo applications and were shown to promote several biological events like regeneration of axons in injured spinal cord, regeneration of cartilage and bone, and growth of blood vessels.[39–41]

In another class of thermodynamic self-assembly, a solution of NPs was evaporated over a solid or liquid phase so as to form two dimensional (2D) crystals. In order to have maximal domain of such 2D crystals, interactions between the NPs and the support needs to be finely tuned. In this direction, Heinrich and co-workers prepared long-range hexagonal monolayers of dodecanethiol (DDT) capped AuNPs by evaporating a toluene solution of the NP (Figure 1.9a).[42] In this approach, the early-stage evaporation traps the AuNPs at the liquid-air interface, from which

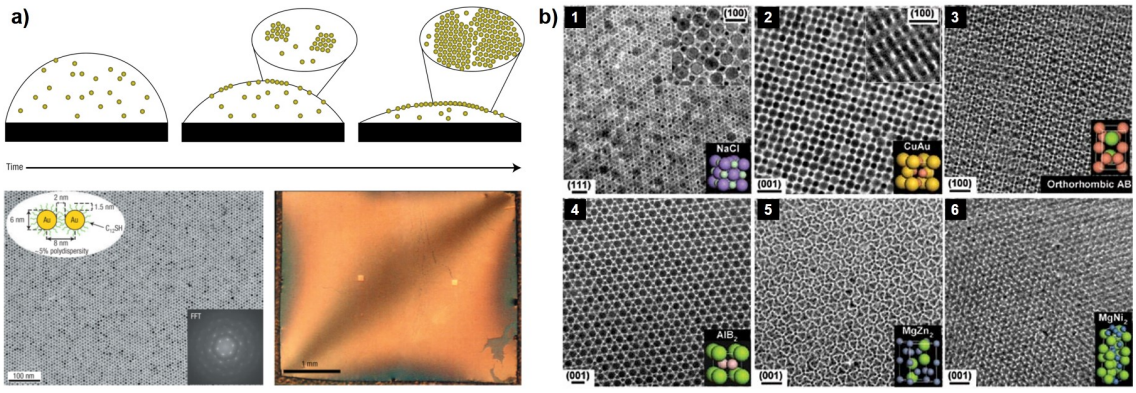


Figure 1.9: (a) Schematic diagram showing the self-assembly of DDT capped AuNPs to form large domains of 2D superlattice at the liquid-air interface. TEM and optical photographs of a typical monolayer produced (Reproduced in part with permission from [42] Copyright 2006 Springer Nature). (b) TEM images of binary NP superlattices, self-assembled from different nanoparticles, and their modelled unit cells (Reproduced in part with permission from [43] Copyright 2006 Springer Nature).

nanoparticle islands nucleate and grow to form μm sized AuNP films (Figure 1.9a). This new drop-drying regime was a robust, simple, and scalable way of creating high quality and long range 2D crystals. Similar experiments have been performed with NPs of various sizes to create a wealth of 2D close packed crystal lattices (Figure 1.9b).[16,43] In an alternate approach, Klajn and co-workers designed a strategy

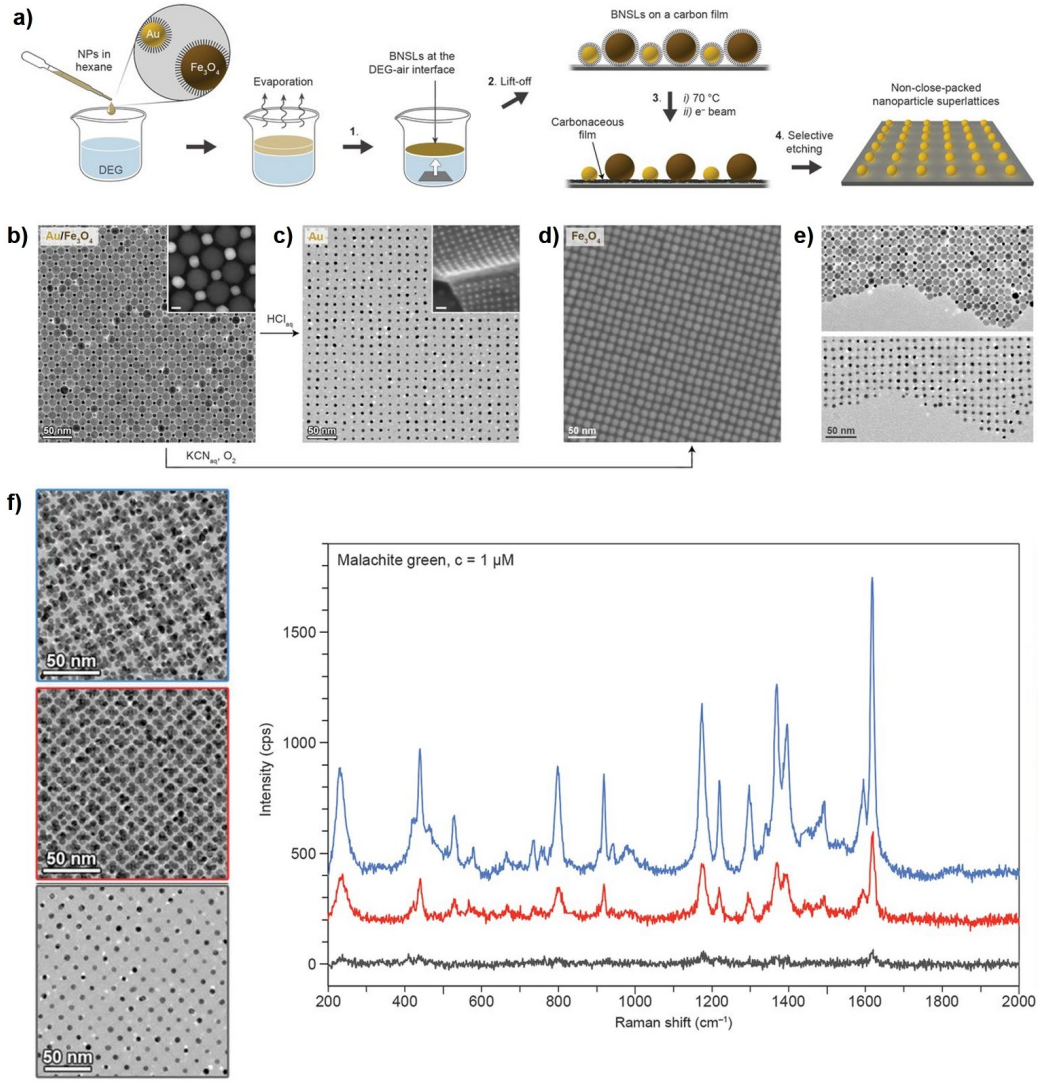


Figure 1.10: (a) Schematic diagram showing the method of formation of a non-closed packed superlattice. (b) TEM image showing the formation of an AB-type superlattice between Au and Fe_3O_4 NPs. Scale bar of inset = 5 nm. (c) TEM image of a non-closed packed AuNP array formed after selective etching of Fe_3O_4. (d) SEM image of a non-closed packed Fe_3O_4 NP array formed after selective etching of AuNPs. (E) TEM image of a close packed AB-type superlattice before (top) and after (bottom) etching of Fe_3O_4 NPs. (f) SERS spectrum of malachite green, recorded on three different NP arrays (Reproduced in part with permission from [44] Copyright 2017 American Association for the Advancement of Science).

to create non-close packed self-assemblies.[44] Here, the authors used a mixture of monodisperse Au and iron oxide (Fe_3O_4) NPs, which were assembled at the diethylene glycol-air interface, to intially form a close-packed superlattice (Figure 1.10a, b). These superlattices were then transferred to a suitable substrate like carbon coated TEM grids (Figure 1.10a). The authors then selectively etched either AuNPs, or Fe_3O_4 NPs resulting in non-close-packed arrays with vacancies stabilized by the underlying carbon substrate (Figure 1.10c, d, e). Such non-closed packed assemblies are useful for a wide range of optical, mechanical as well as catalytic properties. For instance, the authors observed that some films were better suited for Surface Enhanced Raman Scattering (SERS) based sensing as they showed superior signal enhancements in the SERS intensity (Figure 1.10f, g). This is because of higher density of electromagnetic hotspots in superlattices with the vacancies of specific shape and size (Figure 1.10f, g). In another class of self-assembly, destabilization of the NPs in the presence of a suitable trigger, can be used to form a self-assembled state. Such destabilization induced assemblies are promising candidates, especially in the field of sensors.[2] Such sensors, result in the formation of a thermodynaically stable precipitate, and hence can not be re-used again.[2] A key challenge therefore, is to control the interactions between the interacting components so as to create reversible and re-usable assemblies.

1.3.2 Example of Kinetically Trapped Self-Assembly

A key characteristic of kinetically trapped self-assembled states is their reversible nature. In order to create such assemblies, the interparticle interactions should guide the assembled state to a local minima, as opposed to a global minima. This will enable the reversal of the self-assembly process under the influence of a suitable trigger. With this principle in mind, several systems have been designed that show reversible transformations in response to a wide variety of signals like solvents, metal ions, temperature, light, magnetic field, biomolecules, etc.[22,45] In a pioneering study, Liz-Marzán and co-workers have employed solvent as a trigger to assemble a

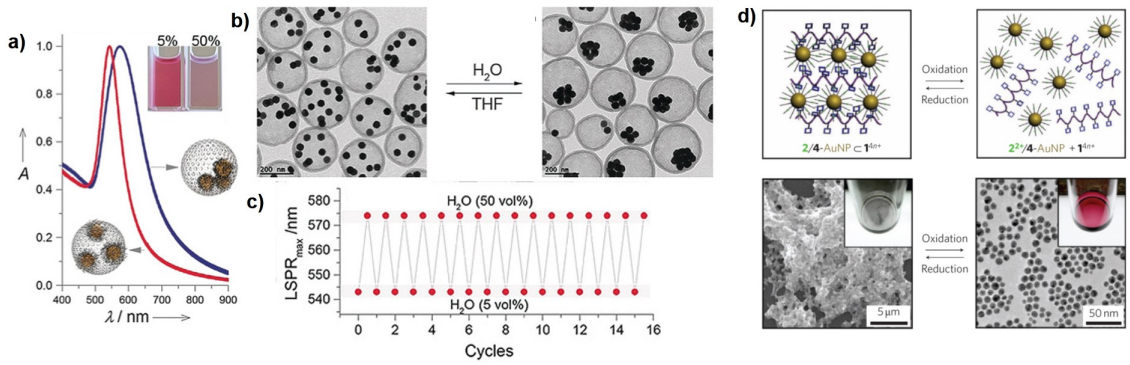


Figure 1.11: (a) UV-Vis. absorption spectrum of the nanocapsules containing hydrophobic AuNPs under low (shown in red), and high (shown in blue) water content. (b) TEM images showing the reversible clusturing of AuNPs. (c) Variation of LSPR maximum for ∼15 cycles (figures are reproduced from an open access article [47]). (d) Schematic diagram showing the reversible binding of TTF functionalized AuNP with polymer containing pendant $CBPQT^{4+}$ units. SEM image (bottom left) showing the AuNP-polymer complex. TEM image of redispersed AuNPs. Insets show optical photographs of the samples (Reproduced in part with permission from [48] Copyright 2009 Springer Nature).

dispersion of polystyrene (PS) stabilized hydrophobic AuNPs.[46,47] Here, they used water to trigger the assembly of PS-AuNPs dispersed in tetrahydrofuran (THF). A key challenge in the use of solvent as a trigger is the inevitable dilution of the solution, which affects the extent as well as kinetics of the self-assembly process, resulting in irreproducible aggregation kinetics. In order to resolve this challenge, the authors used a permeable silica nanocapsule to confine the hydrophobic AuNPs, so as to limit the number of particles participating in the aggregation process.[47] Here, water being permeable to the silica nanocapsule could efficiently trigger the aggregation of PS-AuNPs, resulting in a bathochromic shift in the absorbance spectrum (Figure 1.11a, b). Upon confining these hydrophobic AuNPs, the number of particles participating in the aggregation process could be controlled and preserved, resulting in highly reproducible plasmon band shifts (Figure 1.11c) despite changes in the solvent compositions.

In another class of stimuli responsive self-assembly, NP systems were constructed that could show reversible transformations upon the addition of a redox stimuli. In a classic study, Stoddart and co-workers used redox controlled host-guest complex formation between the electron-rich tetrathiafulvalene (TTF), and a polymer con-

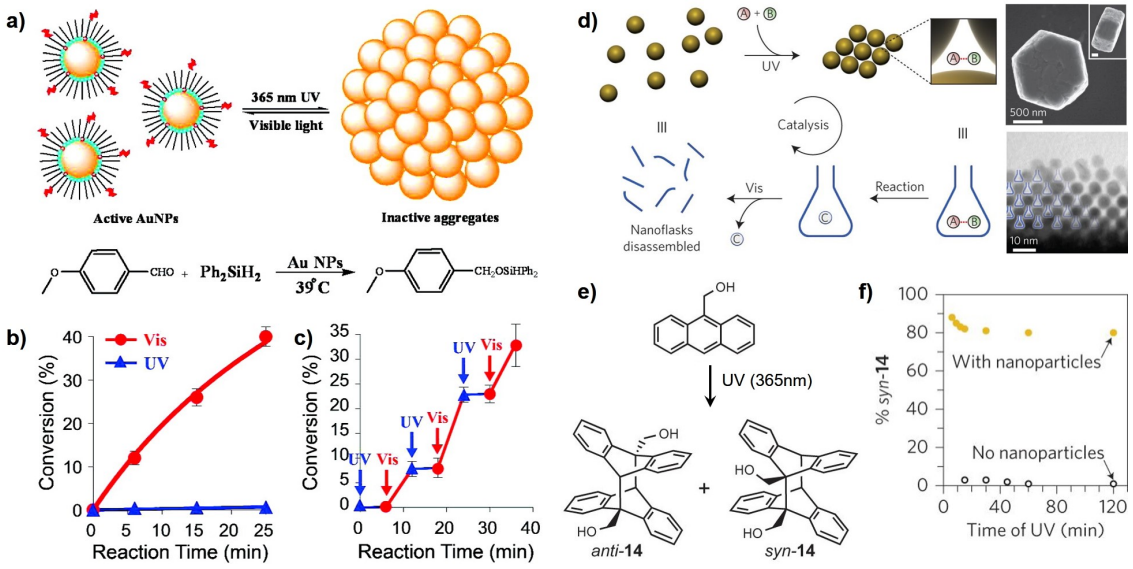


Figure 1.12: (a) Schematic diagram showing reversible photo-switchable AuNP catalysts, where dispersed AuNPs are catalytically active, while the aggregated AuNPs are catalytically inactive for the hydrosilylation reaction. (b) AuNP-catalyzed hydrosilylation of 4-methoxybenzaldehyde with respect to time in visible light (showing in red), and under UV irradiation (shown in blue). (c) Conversion graph showing the photo-switchable catalysis for 3 cycles (Reproduced in part with permission from [49] Copyright 2010 American Chemical Society). (d) Schematics showing the reversible formation of nanoflasks (scale bar in the inset = 200 nm). (e) and (f) UV-induced dimerization of anthracene in the presence and absence of AuNP, showing the stereoselectivity of the reaction to form preferentially *syn*-isomer (Reproduced in part with permission from [50] Copyright 2015 Springer Nature).

taining electron-deficient macrocycle cyclobis(paraquat-p-phenylene) ($CBPQT^{4+}$).[48] Here, AuNPs were functionalized with the electron rich TTF that can form a host-guest inclusion complex with the macrocyle resulting in the aggregation of AuNPs (Figuer 1.11d). In the presence of a mild oxidising agent, TTF could be easily oxidised to TTF^{2+}, resulting in the loss of AuNP's affinity towards the macrocycle, and a release of AuNPs to the solution. In the presence of a reducing agent (ascorbic acid), the complex could be reformed, thereby driving the complexation between AuNPs and the polymer. (Figure 1.11d). After carefully tuning the interactions between the polymer and a range of metallic NPs, the authors demonstrated the use of polymer as a redox controlled *selective sponge* for the capture and release of different metallic NPs (Figure 1.11d).

The reversible nature of such kinetically trapped self-assemblies is key in the design and fabrication of systems with re-usable chemical functionalities. For in-

stance, one can create re-usable sensors for a wide range of chemical, and biological stimuli. Alternatively, one can design catalysts, whose activity can be controlled on demand, thereby imparting the much needed ability of switchability to the system. In a fantastic example, Grzyowski and co-workers used light as a trigger to assemble AuNPs functionalized with azobenzene thiols in a reversible fashion.[49] The photo-switchable aggregation of AuNPs was later extended to control their catalytic activity towards a hydrosilylation reaction (photo-switchable catalysis, Figure 1.12a). In their study, AuNP catalysts decorated with a mixed self-assembled monolayer (m-SAM) of azobenzene terminated thiols and alkyl amine (DDA), which readily dispersed in a toluene solution containing the p-anisaldehyde and diphenylsilane (reactant molecules for the hydrosilylation reaction). When dispersed, AuNPs could readily catalyze the formation of 4-methoxybenzyloxy-diphenylsilane (product) (see red curve in Figure 1.12b). Upon irradiating the mixutre with UV light, AuNPs aggregated from the solution, resulting in switching-off of the reaction (see blue curve in Figure 1.12b). Furthermore, this photo-modulation of the catalytic activity could be efficiently performed for at least three cycles (see Figure 1.12c). In another seminal study, Klajn and co-workers demonstrated another attractive application of azobenzene-functionalized NPs by utilizing the voids between the NPs in the aggregates as *'nanoreactors'* for accelerating various reactions (Figure 1.12d).[50] These voids, formed between azobenzene functionalized AuNPs contain a high number of polar cis-azobenzene moieties, and therefore, can be used to trap a variety of polar guest molecules during the assembly process. The authors, using this strategy, could trap a range of polar reactant molecules inside these cavities, and demonstrated accelerated reaction rates (Figure 1.12d). This observed acceleration of reactions is possibly because of increased *effective* molarity of the reactant molecules, as well as their pre-organization inside the cavities. Upon completion of the reaction, the nanoflasks could be conveniently disassembled using visible light, thereby releasing the products into the solution (Figure 1.12d). Most fascinatingly, the reactions underwent altered stereoselectivities, when performed in dynamic nanoflasks. For

instance, 9-(hydroxymethyl)anthracene dissolved in toluene can be dimerized in the presence of UV light to afford the thermoduynamically favoured *anti* isomer in the solution, whereas, the same reaction when conducted in the nanoflasks results in >80 % syn isomer (Figure 1.12e, f), suggesting substrate preorganization in the nanoflasks. Furthermore, these nanoflasks could be re-used again, thereby demonstrating the potential of using similar kinetically trapped assemblies for different catalytic applications.

1.3.3 Example of Dissipative Self-Assembly

Dissipative self-assembled systems differ noticeably from all the other classes of self-assembly that have been discussed so far, as they come into existence only under a constant influx of matter and/or energy. Because of this constant need of energy and/or matter to sustain the assembled state, these systems are classified as non-equilibrium or out-of-equilibrium systems. Typical examples of such systems include microtubules, which, in biological systems can be used to *'grasp'* chromosomes by acting as robotic arms during the cell division, or act as a highway for the transport of molecular cargo within the cell.[52] Fascinatingly, these structures are transient in nature, and conjure-up only when their function is required by the cell.[10] These systems have captivated the interest, as well as imagination of several

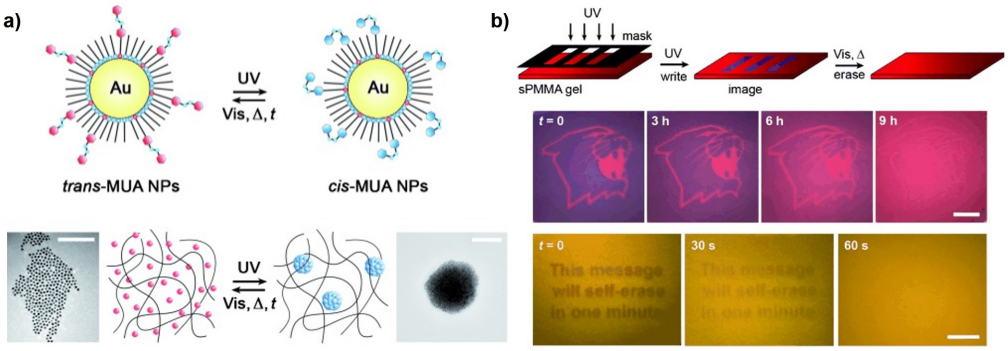


Figure 1.13: (a) Schematics showing the use of light to control the dissipative self assembly of AuNPs. Photo-isomerization of azobenzene thiol with UV light on AuNPs resulting in the onset of aggregation. (b) Self-erasing images created by exposing Au, and AgNP films with UV light through a photomask, scale bar = 1 cm (Reproduced in part with permission from [51] Copyright 2009 John Wiley and Sons).

researchers. A key challenge in the design and realization of such systems is the ability to vary the interparticle interactions in a time-dependent fashion. In this direction, Grzybowski and co-workers used light as a trigger to vary the interparticle interactions and create a dissipative self-assembled state.[51] Here, they used AuNP or AgNP functionalized with a mixed self-assembled monolayers (m-SAM) of dodecylamine (DDA) and a photoswitchable thiol containing azobenzene headgroup (Figure 1.13a). These NPs, because of the low dipole moment of trans-azobenzene (μ = 0 D), readily dispersed in hydrophobic solvents like toluene or hexane. Upon exposure to UV light, however, the thermodynamically more stable trans-azobenzene isomerizes to its metastable cis-configuration, which has higher dipole moment (μ = 4.4 D).[24] As a result, AuNPs lose their colloidal stability and starts aggregating, resulting in a distinct colour change (Figure 1.13a). Interestingly, these aggregates were *dissipative* in nature and persisted only under the continuous irradiation of UV light. Once the irradiation was discontinued, the aggregates gradually disassembled, resulting in a spontaneous colour change from blue to red. This strategy was then used to create high contrast, re-writable *'self-erasable'* patterns by exposing a film of AuNPs through a photomask (Figure 1.13b). This inspired researchers to routinely employ groups like azobenzene, and spiropyran to skillfully create a range of dissipative self-assembled systems.[53–55] Such light powered systems, are very attractive because of *'zero-waste'* production and almost instantaneous deactivation, but differ markedly from the natural systems which use chemical fuels to drive the dissipative self-assembly process.[52,56]

In a pioneering example, van Esch and co-workers developed a strategy to create a chemically fuelled dissipative self-assembled system by using triggered esterification, and a spontaneous de-esterificaiton reaction.[57,59] In their study, they established a control over reaction rates and amounts of chemical fuel, to not only choreograph the formation of a transient gel, but also govern properties like lifetime, stiffness, and self-regeneration capability of the gel state. Here, they used a carboxylate containing molecular gelator (chemical structures given in Figure 1.14a) which is

Figure 1.14: Dynamic self-assembly using covalent modification of the precursor. a) Chemical structures of the building blocks. Reaction cycle showing the use of triggered esterification, autonomous de-esterification of the building blocks to show the formation of transient fibres/ gels. (b) Cryo-TEM images of the formed gel (scale bar = 100 nm), and optical photographs showing a typical reaction cycles of transient self-assembly (Reproduced in part with permission from [57] Copyright 2015 American Association for the Advancement of Science). (c) Schematic diagram showing ATP-templated dissipative self-assembly of vesicular nanostructures (Reproduced in part with permission from [58] Copyright 2016 Springer Nature).

readily soluble in water at basic pH, and shows no tendency for assembly because of electrostatic repulsions. Upon addition of the chemical fuel (an esterifying agent - dimethyl sulfate (DMS)), the system loses its charge and gets activated for the self-assembly process. These activated molecules assembled into 1D nanofibers, stabilized with intermolecular H-bonds as well as hydrophobic interactions, ultimately resulting in the formation of self-healing gels at room temperature (Figure 1.14b). These esterified fibres/ gels, reacted with the base present in the system to yield the initial carboxylate gelator, resulting in the autonomous disassembly of the gels (Figure 1.14b). The lifetime, as well as the mechanical properties of the gels could be conveniently tuned by varying the amount of fuel added to the system. Later, this idea of using two competing and opposite reactions was utilized to create a wide

variety of dissipative self-assembled systems.[60–63] In another class of dissipative self-assembly, Prins and co-workers used adenosine triphosphate (ATP) as a chemical fuel for the transient stabilization of vesicles through non-covalent interactions, as opposed to covalent activation.[58] Here, they used a *'cationic'* surfactant molecule having 1,4,7-triazacyclononane·Zn^{2+} (TACN·Zn^{2+}) head group, whose propensity to self-assemble increased in the presence of ATP, resulting in the formation of vesicles. The presence of an enzyme potato apyrase (capable of hydrolysing ATP) in the system led to the in situ depletion of ATP. This results in the loss of stabilizing interactions and, consequently the formed vesicles undergoes autonomous dissociation. These vesicles could sustain a chemical reaction, where the lifetime of the vesicles dictated the extent of the reaction. The lifetime of the aggregates could be conveniently tuned by controlling the rate of ATP hydrolysis, which was controlled by tuning the amount of enzyme in the system.

In all these examples, the addition of a trigger results in the spontaneous assembly of systems into higher order structures, that are inherently unstable in the reaction conditions. These structures therefore disassemble autonomously once the trigger is removed. This strategy results in the construction of systems with tuneable lifetimes and properties, which can have far reaching impacts. For instance the design and realization polymers with tuneable lifetime, that can spontaneously degrade after the completion of their pre-designed lifetime is attractive to combat the widespread problem of plastic pollution. Furthermore, systems with self-healing, and self—replication capabilities can be used to design products that can self-repair if damaged. The possibilities and promises of these class of materials are endless. Nature has created life by controlling similar self-assembly processes, and we have to wait and see what kind of complex structures mankind will create!

1.4 Conclusion and Outline

The examples highlighted in this Chapter outline the principles involved in the design and synthesis of different self-assembled systems (see Figure 1.15). A key

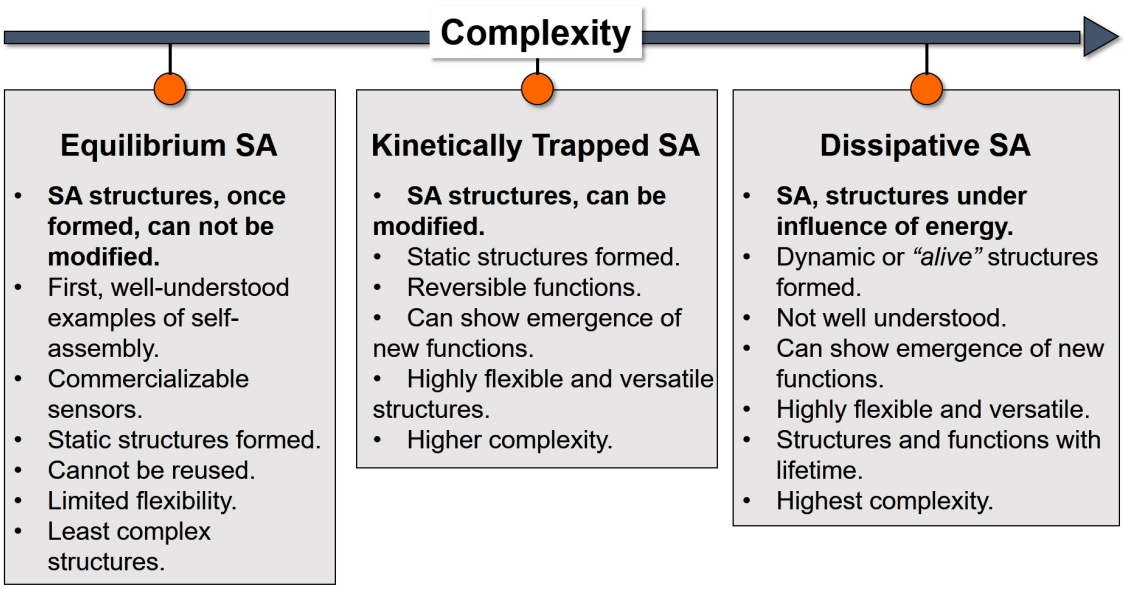


Figure 1.15: Schematics summarizing key characteristics of different self-assembly processes, and the evolution of complexity.

feature in the design and synthesis of self-assembled systems is the ability to program interactions between the building blocks. Several approaches have been shown in Section 1.2 that can be used to design suitable interaction-potential governing the process of self-assembly. In order for nanoscience and self-assembly to be the next technological revolution, design principles governing the formation of complex and useful structures need to be laid out. Some understandings regarding complexity associated with a self-assembled structure is mentioned in Section 1.3. This chapter, therefore projects fine-tuning of interparticle interactions as an versatile tool to create self-assembled states of varying complexity and usefulness.

In light of this, the underlying principle in the present thesis is to investigate the effects of finely tuned interactions between NP building blocks in governing the outcomes of self-assembly in both spatial, as well as temporal domains. More specifically, we studied the effects of finely tuned interparticle interactions as a versatile tool capable of (a) improving existing NP properties, (b) demonstrating the emergence of inherently absent properties, and (c) mimicking of complex *'life-like'* behaviour. The effects of interparticle interaction in governing these spatio-temporal properties are discussed in **Chapter 2** to **4**.

References

(1) Grzybowski, B. A.; Huck, W. T. S. *Nat. Nanotechnol.* **2016**, *11*, 585–592.

(2) Saha, K.; Agasti, S. S.; Kim, C.; Li, X.; Rotello, V. M. *Chem. Rev.* **2012**, *112*, 2739–2779.

(3) Kim, B.; Han, G.; Toley, B. J.; Kim, C.-k.; Rotello, V. M.; Forbes, N. S. *Nat. Nanotechnol.* **2010**, *5*, 465–472.

(4) Doane, T. L.; Burda, C. *Chem. Soc. Rev.* **2012**, *41*, 2885–2911.

(5) Talapin, D. V.; Lee, J.-S.; Kovalenko, M. V.; Shevchenko, E. V. *Chem. Rev.* **2010**, *110*, 389–458.

(6) Whitesides, G. M.; Grzybowski, B. *Science* **2002**, *295*, 2418–2421.

(7) Grzybowski, B. A.; Fitzner, K.; Paczesny, J.; Granick, S. *Chem. Soc. Rev.* **2017**, *46*, 5647–5678.

(8) Phillips, R.; Quake, S. R. *Phys. Today* **2006**, *59*, 38–43.

(9) Mann, S. *Angew. Chem. Int. Ed.* **2008**, *47*, 5306–5320.

(10) Karsenti, E. *Nat. Rev. Mol. Cell Biol.* **2008**, *9*, 255–262.

(11) Kalsin, A. M.; Fialkowski, M.; Paszewski, M.; Smoukov, S. K.; Bishop, K. J. M.; Grzybowski, B. A. *Science* **2006**, *312*, 420–424.

(12) Laramy, C. R.; O'Brien, M. N.; Mirkin, C. A. *Nat. Rev. Mater.* **2019**, *4*, 201–224.

(13) Boles, M. A.; Engel, M.; Talapin, D. V. *Chem. Rev.* **2016**, *116*, 11220–11289.

(14) Min, Y.; Akbulut, M.; Kristiansen, K.; Golan, Y.; Israelachvili, J. *Nat. Mater.* **2008**, *7*, 527–538.

(15) Velev, O. D. *Science* **2006**, *312*, 376–377.

(16) Dong, A.; Chen, J.; Vora, P. M.; Kikkawa, J. M.; Murray, C. B. *Nature* **2010**, *466*, 474–477.

(17) Bishop, K. J. M.; Chevalier, N. R.; Grzybowski, B. A. *J. Phys. Chem. Lett.* **2013**, *4*, 1507–1511.

(18) Kalsin, A. M.; Kowalczyk, B.; Smoukov, S. K.; Klajn, R.; Grzybowski, B. A. *J. Am. Chem. Soc.* **2006**, *128*, 15046–15047.

(19) Rogers, W. B.; Shih, W. M.; Manoharan, V. N. *Nat. Rev. Mater.* **2016**, *1*, 1–14.

(20) Kim, Y.; Macfarlane, R. J.; Jones, M. R.; Mirkin, C. A. *Science* **2016**, *351*, 579–582.

(21) Macfarlane, R. J.; Lee, B.; Jones, M. R.; Harris, N.; Schatz, G. C.; Mirkin, C. A. *Science* **2011**, *334*, 204–208.

(22) Grzelczak, M.; Liz-Marzán, L. M.; Klajn, R. *Chem. Soc. Rev.* **2019**, *48*, 1342–1361.

(23) Singh, G.; Chan, H.; Baskin, A.; Gelman, E.; Repnin, N.; Král, P.; Klajn, R. *Science* **2014**, *345*, 1149–1153.

(24) Klajn, R.; Bishop, K. J. M.; Grzybowski, B. A. *Proc. Natl. Acad. Sci.* **2007**, *104*, 10305–10309.

(25) Sacanna, S.; Irvine, W. T. M.; Chaikin, P. M.; Pine, D. J. *Nature* **2010**, *464*, 575–578.

(26) Colón-Meléndez, L.; Beltran-Villegas, D. J.; van Anders, G.; Liu, J.; Spellings, M.; Sacanna, S.; Pine, D. J.; Glotzer, S. C.; Larson, R. G.; Solomon, M. J. *J. Phys. Chem.* **2015**, *142*, 174909.

(27) Bishop, K. J. M.; Wilmer, C. E.; Soh, S.; Grzybowski, B. A. *Small* **2009**, *5*, 1600–1630.

(28) Sacanna, S.; Korpics, M.; Rodriguez, K.; Colón-Meléndez, L.; Kim, S.-H.; Pine, D. J.; Yi, G.-R. *Nat. Commun,* **2013**, *4*, 1–6.

(29) Damasceno, P. F.; Engel, M.; Glotzer, S. C. *Science* **2012**, *337*, 453–457.

(30) Glotzer, S. C.; Solomon, M. J. *Nat. Mater,* **2007**, *6*, 557–562.

(31) Haji-Akbari, A.; Engel, M.; Keys, A. S.; Zheng, X.; Petschek, R. G.; Palffy-Muhoray, P.; Glotzer, S. C. *Nature* **2009**, *462*, 773–777.

(32) Ashkenasy, G.; Hermans, T. M.; Otto, S.; Taylor, A. F. *Chem. Soc. Rev.* **2017**, *46*, 2543–2554.

(33) Lehn, J.-M. *PNAS* **2002**, *99*, 4763–4768.

(34) Sorrenti, A.; Leira-Iglesias, J.; Markvoort, A. J.; de Greef, T. F. A.; Hermans, T. M. *Chem. Soc. Rev.* **2017**, *46*, 5476–5490.

(35) Lehn, J.-M. *Angew. Chem. Int. Ed.* **2018**, 2836–2850.

(36) Rossum, S. A. P. v.; Tena-Solsona, M.; Esch, J. H. v.; Eelkema, R.; Boekhoven, J. *Chem. Soc. Rev.* **2017**, *46*, 5519–5535.

(37) Grzybowski, B. A.; Wilmer, C. E.; Kim, J.; Browne, K. P.; Bishop, K. J. M. *Soft Matter* **2009**, *5*, 1110–1128.

(38) Hartgerink, J. D.; Beniash, E.; Stupp, S. I. *Science* **2001**, *294*, 1684–1688.

(39) Hendricks, M. P.; Sato, K.; Palmer, L. C.; Stupp, S. I. *Acc. Chem. Res.* **2017**, *50*, 2440–2448.

(40) Cui, H.; Webber, M. J.; Stupp, S. I. *Peptide Science* **2010**, *94*, 1–18.

(41) Aida, T.; Meijer, E. W.; Stupp, S. I. *Science* **2012**, *335*, 813–817.

(42) Bigioni, T. P.; Lin, X.-M.; Nguyen, T. T.; Corwin, E. I.; Witten, T. A.; Jaeger, H. M. *Nat. Mater,* **2006**, *5*, 265–270.

(43) Shevchenko, E. V.; Talapin, D. V.; Kotov, N. A.; O'Brien, S.; Murray, C. B. *Nature* **2006**, *439*, 55–59.

(44) Udayabhaskararao, T.; Altantzis, T.; Houben, L.; Coronado-Puchau, M.; Langer, J.; Popovitz-Biro, R.; Liz-Marzán, L. M.; Vuković, L.; Král, P.; Bals, S.; Klajn, R. *Science* **2017**, *358*, 514–518.

(45) Grzelczak, M.; Vermant, J.; Furst, E. M.; Liz-Marzán, L. M. *ACS Nano* **2010**, *4*, 3591–3605.

(46) Sánchez-Iglesias, A.; Grzelczak, M.; Altantzis, T.; Goris, B.; Pérez-Juste, J.; Bals, S.; Van Tendeloo, G.; Donaldson, S. H.; Chmelka, B. F.; Israelachvili, J. N.; Liz-Marzán, L. M. *ACS Nano* **2012**, *6*, 11059–11065.

(47) Sánchez-Iglesias, A.; Claes, N.; Solís, D. M.; Taboada, J. M.; Bals, S.; Liz-Marzán, L. M.; Grzelczak, M. *Angew. Chem.* **2018**, *130*, 3237–3240.

(48) Klajn, R.; Olson, M. A.; Wesson, P. J.; Fang, L.; Coskun, A.; Trabolsi, A.; Soh, S.; Stoddart, J. F.; Grzybowski, B. A. *Nat. Chem.* **2009**, *1*, 733–738.

(49) Wei, Y.; Han, S.; Kim, J.; Soh, S.; Grzybowski, B. A. *J. Am. Chem. Soc.* **2010**, *132*, 11018–11020.

(50) Zhao, H.; Sen, S.; Udayabhaskararao, T.; Sawczyk, M.; Kucanda, K.; Manna, D.; Kundu, P. K.; Lee, J.-W.; Kral, P.; Klajn, R. *Nat. Nanotechnol.* **2016**, *11*, 82–88.

(51) Klajn, R.; Wesson, P. J.; Bishop, K. J. M.; Grzybowski, B. A. *Angew. Chem. Int. Ed.* **2009**, *48*, 7035–7039.

(52) Hess, H.; L. Ross, J. *Chem. Soc. Rev.* **2017**, *46*, 5570–5587.

(53) Bian, T.; Chu, Z.; Klajn, R. *Adv. Mater.* **2019**, 1905866.

(54) Klajn, R. *Chem. Soc. Rev.* **2013**, *43*, 148–184.

(55) Kundu, P. K.; Samanta, D.; Leizrowice, R.; Margulis, B.; Zhao, H.; Börner, M.; Udayabhaskararao, T.; Manna, D.; Klajn, R. *Nat. Chem.* **2015**, *7*, 646–652.

(56) Ragazzon, G.; Prins, L. J. *Nat. Nanotechnol.* **2018**, *13*, 882–889.

(57) Boekhoven, J.; Hendriksen, W. E.; Koper, G. J. M.; Eelkema, R.; Esch, J. H. v. *Science* **2015**, *349*, 1075–1079.

(58) Maiti, S.; Fortunati, I.; Ferrante, C.; Scrimin, P.; Prins, L. J. *Nat. Chem.* **2016**, *8*, 725–731.

(59) Boekhoven, J.; Brizard, A. M.; Kowlgi, K. N. K.; Koper, G. J. M.; Eelkema, R.; van Esch, J. H. *Angew. Chem. Int. Ed.* **2010**, *49*, 4825–4828.

(60) Della Sala, F.; Neri, S.; Maiti, S.; Chen, J. L.-Y.; Prins, L. J. *Curr. Opin. Biotech.* **2017**, *46*, 27–33.

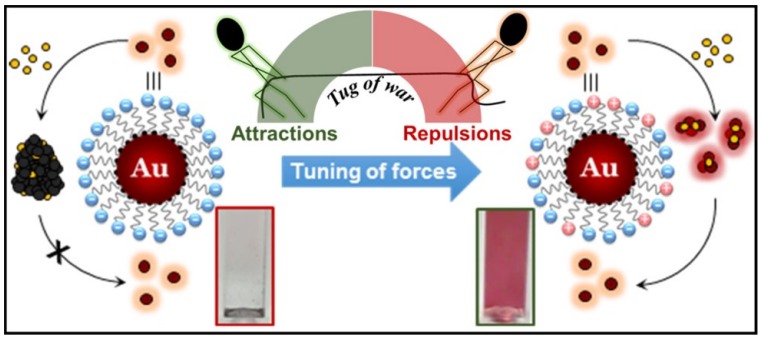
(61) Tena-Solsona, M.; Wanzke, C.; Riess, B.; Bausch, A. R.; Boekhoven, J. *Nat. Commun.* **2018**, *9*, 1–8.

(62) Kariyawasam, L. S.; Hartley, C. S. *J. Am. Chem. Soc.* **2017**, *139*, 11949–11955.

(63) Grötsch, R. K.; Angı, A.; Mideksa, Y. G.; Wanzke, C.; Tena-Solsona, M.; Feige, M. J.; Rieger, B.; Boekhoven, J. *Angew. Chem. Int. Ed.* **2018**, *57*, 14608–14612.

Chapter 2

Regulation of Interparticle Forces Reveals Controlled Aggregation in Charged Nanoparticles



Making the Unstable, Stable

This chapter has been adapted from the following paper:- **Rao, A**, Roy, S., Unnikrishnan, M., Bhosale, S. S., Devatha, G., and Pillai, P. P.*, Regulation of Interparticle Forces Reveals Controlled Aggregation in Charged Nanoparticles. *Chem. Mater.* **2016**, *28*, 2348 − 2355.

2.1 Abstract

The ability to control interparticle forces can not only improve existing nanoparticle (NP) functionalities, but can pave the way for newer properties as well. A proof of concept in this direction is presented here, wherein the regulation of interparticle forces reveals the phenomenon of controlled aggregation, which is successfully translated into trapping and scavenging of toxic ions. A perfect balance between the attractive and repulsive forces is achieved by tuning the [+] and [−] ligands on the surface of heterogeneously charged metal NPs. The NP-ion aggregates are stable for ∼2 days, with a visible color change ($\Delta\lambda_{max.}$ = 12- 15 nm), which makes them available for scavenging from the site of action. The incorporation of *'potent'* forces like repulsions, rather than a mere dilution of attractive forces, is necessary to ensure the formation of controlled aggregates. The net surface charge of NPs is conveniently modified to trap different ions/ molecules irrespective of their charge and binding strengths. More importantly, the regulation of interparticle forces imparts a new function of selectivity toward trapping of toxic ions in a carboxylate functionalized NP system. Thus, the present work introduces a conceptually unprecedented approach to impart long-term stability (∼2 days) to NP- ion aggregates by controlling the interparticle forces.

2.2 Introduction

The possibility of *'coding'* functions onto the surfaces of nanoparticles (NPs) has led to numerous exciting applications in the fields of sensing,[1–11] drug delivery,[12–17] plasmonics,[18–20] light harvesting,[21] and so on. Common methodologies based on H-bonding,[22,23] electrostatics,[24,25] and host-guest[26–28] interactions have been successfully employed to *'style'* NP functions in an anticipated manner.[29–43] In principle, the fine tuning of NP surface chemistry can render a precise control over the magnitudes of different interparticle forces,[44–49] thereby improving or even dictating NP functionalities. Such a precise control over forces has led to an outburst

of exciting applications, both at the macroscale (like *'elimination of friction'* in maglev fans, trains, etc.), and at molecular level[50,51] (like development of molecular motors, propellers, shuttles, etc.). At the nanoscale, however, such a careful tweaking of interparticle forces remains a challenging task and thus an emerging area of research. With an objective to judiciously alter the magnitudes of attractive and repulsive forces, thereby revealing the controllable interparticle interactions, we operated with electrostatic, H-bonding, and bridging interactions in charged NPs. As a proof of concept, we wanted to understand how modifications made in the interparticle forces '*add*' to already known functions of NPs. For this, trapping phenomena of charged NPs was selected as the model study. In a seminal work, Hupp and co-workers designed a simple colorimetric technique for the identification of *'spectroscopically silent'* heavy metal ions. Here, carboxylate functionalized AuNPs ([-] AuNPs) showed a nonselective color change from red to blue in the presence of Pb^{2+}, Cd^{2+}, and Hg^{2+} ions (Figure 2.1).[1] Later, Mirkin and co-workers used DNA-functionalized AuNPs, and thymidine-Hg^{2+}-thymidine chemistry to carry out selective, as well as sensitive identification of Hg^{2+}, over other ions (Figure 2.2).[11] A careful survey of literature shows that most of the metal NP based identification/ trapping of toxic ions is achieved through the precipitation of NPs.[1–7,27] However, their scavenging ability *(the ease with which the NPs can be separated out from the system)* is questionable as the precipitates of NP- ion aggregates can interfere with

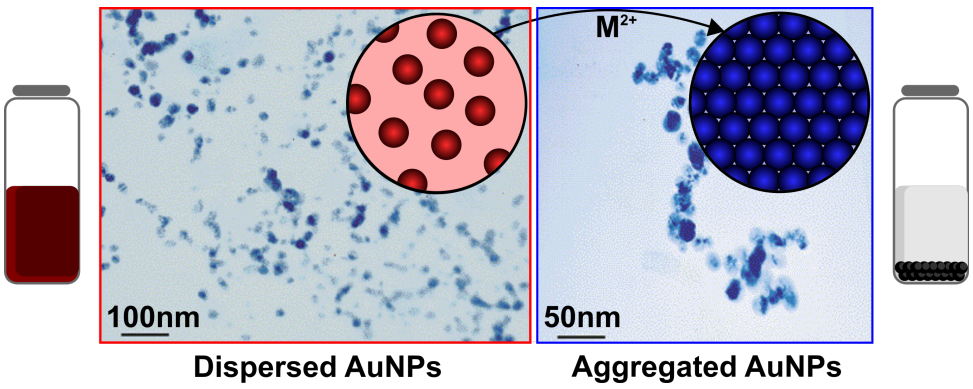


Figure 2.1: Schematic description of red-to-blue colour change and ultimately precipitation of AuNPs in the presence of an aggregating trigger. TEM images of [-] AuNPs, and [-] AuNP - Pb^{2+} precipitates (Reproduced in part with permission from [1] Copyright 2001 American Chemical Society).

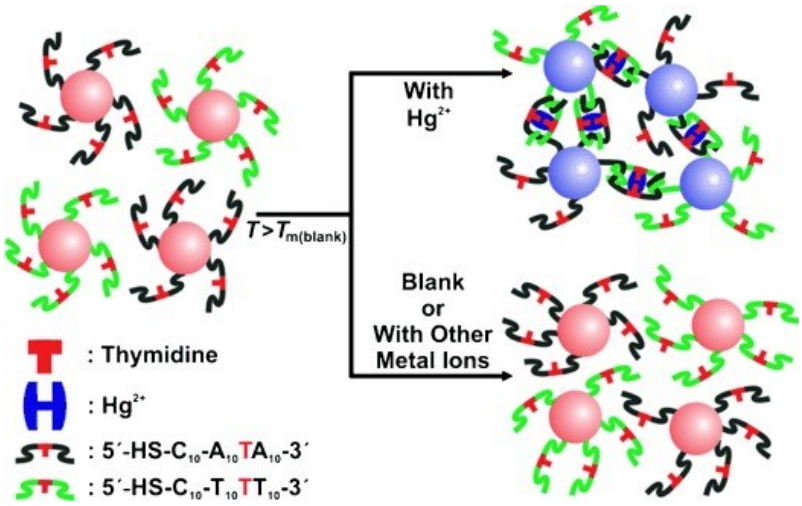


Figure 2.2: Colorimetric detection of Hg^{2+} using DNA functionalized AuNPs exploiting thymidine-Hg^{2+}-thymidine coordination chemistry (Reproduced in part with permission from [11] Copyright 2007 John Wiley and Sons).

the system under study. For instance, precipitates clogging the water filters during water decontamination process. Thus, an ideal trapping and scavenging system should be able to *'arrest'* the toxic ions without any compromise on the colloidal stability of aggregates. For realization of such a system, one needs to picture the observed NP behavior in terms of a *'tug of war'* between different interparticle forces. On addition of a trigger, the attractions should result in the formation of aggregates followed by either a decrease in attractions or an increase in repulsions. Such an interesting interplay between attractions and repulsions can lead to the *'arrest'* of aggregation triggers without precipitation (Figure 2.3).

We envisaged the use of heterogeneously charged NPs, where a simple change in the ratio of oppositely charged ligands regulates the strengths of different interparticle forces. The heterogeneously charged gold nanoparticles (AuNPs) functionalized with mixtures of (11-mercaptoundecanoic acid, MUA [-]) and (N,N,N-trimethyl(11-mercaptoundecyl)ammonium chloride, TMA [+]) were able to '*arrest*' and '*release*' the aggregation trigger on call (Figure 2.3b). The present NP design '*arrests*' toxic ions like lead (Pb^{2+}) and cadmium (Cd^{2+}) through the formation of controlled aggregates, which on disassembly enabled the release of ions, making them a recyclable trapping and scavenging system. One of the main advantages here is the simplicity

with which the mixed self-assembled monolayer (m-SAM) on NP can be tuned to trap and scavenge different triggers of interest (like Pb^{2+}, Cd^{2+}, H^+, etc.). Our studies also show that '*potent*' forces like repulsions, rather than a mere dilution of attractive forces are crucial to ensure the formation of controlled aggregates, especially with stronger triggers. Also, a reversal in NP net surface charge translates into the trapping of negatively charged molecules like trisodium citrate. More importantly, the regulation of interparticle forces imparts a new function of selectivity toward trapping of toxic ions in carboxylate functionalized NP system.

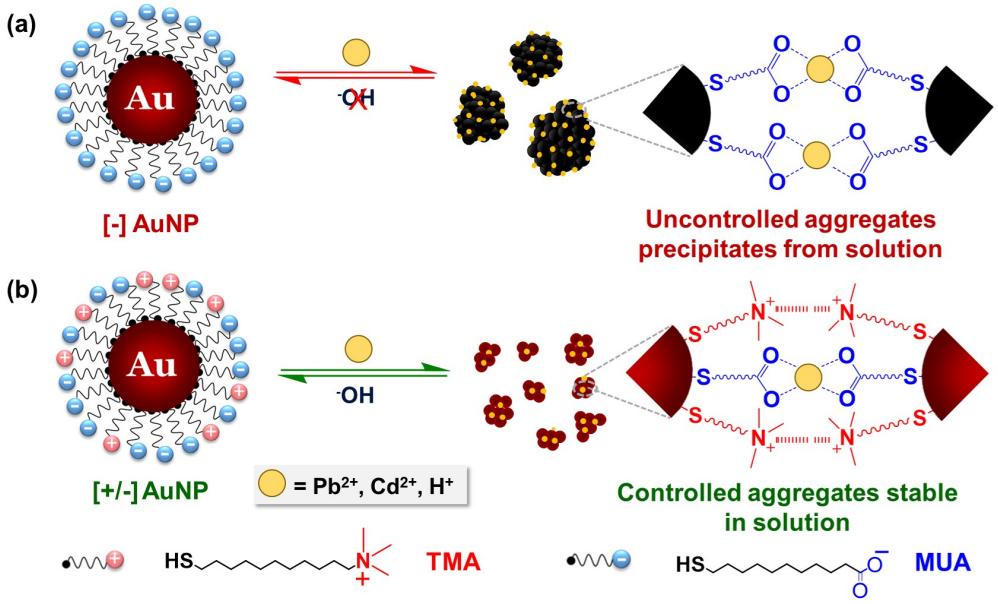


Figure 2.3: Concept of regulating interparticle forces to achieve controlled aggregation in charged NPs. The interactions of (a) [-] and (b) [+/-] AuNPs with triggering ions results in the formation of unstable and stable AuNP-ion aggregates, respectively. The colloidal stability of [+/-] AuNPs is retained in the aggregates due to the electrostatic repulsions experienced from the like charged head groups on adjacent NPs.

2.3 Experimental Section

2.3.1 Synthesis of Charged AuNPs

The primary aim of the present work is to show the effects of finely tuned interparticle interactions, *primarily electrostatics*, on the self-assembly properties of AuNPs.

For this, we chose positively and negatively charged ω-functionalized thiols having around ∼11 carbon chains. It has been shown in the literature that ligands with smaller chain lengths oftentimes result in unstable AuNP dispersions, because of strong van der Waals attractions at small interparticle separations.[52] The use of longer chain lengths helps in overcoming van der Waals interactions, thereby imparting ***long-term stability*** to AuNP dispersions.[46,52] Furthermore, the place exchange protocols and kinetics have been well studied for TMA and MUA ligands.[53] Also, similar ligands have been routinely utilized by several researchers to prove different hypothesis in a wide range of processes like self-assembly, sensing, targeting and drug-delivery, etc.[25,54,55] Based on all these considerations, we chose TMA and MUA as ligands of our choice. Heterogeneously charged AuNPs were prepared using a place exchange reaction method. First, dodecylamine (DDA)-coated AuNPs were synthesized using a modified literature procedure.[56,57] We used tetrachloroaurate trihydrate ($HAuCl_4{\cdot}3H_2O$) instead of $AuCl_3$ as the gold salt and a mixture of hydrazine monohydrate ($N_2H_4{\cdot}H_2O$) and tetrabutylammonium borohydride (TBAB) as the reducing agent instead of anhydrous hydrazine (N_2H_4). Briefly, a toluene solution (7 mL) of $HAuCl_4{\cdot}3H_2O$ (24 mg) containing 222 mg of dodecylamine (DDA) and 277 mg of (Di-n-dodecyl)dimeth-ylammonium bromide (DDAB) was prepared. The solution was sonicated for ∼5 min for completely solubilizing the Au(III) salt. This was followed by a rapid injection of another toluene solution (containing 58 mg of TBAB and 111 mg of DDAB in 3 mL toluene) to ensure the reduction of Au(III) salts. The resulting DDA-AuNP solution (seed solution) was aged for ∼24 h. These NP seeds were then grown to form ∼6.0 nm sized DDA-AuNPs. Here, a growth solution containing 1 g of DDAB, 2.6 g of DDA, 224 mg of $HAuCl_4{\cdot}3H_2O$, and 10 mL of seed was prepared in 60 mL of toluene. Next, the growth solution was reduced by dropwise addition (in ∼30 min) of 22 mL of toluene containing 300 μL of $N_2H_4{\cdot}H_2O$ and 3.5 mg of TBAB. The slow stirring was continued overnight to yield monodisperse 6.0 ± 0.7 nm sized DDA-AuNPs. All the steps were carried out at room temperature. Next, we employed a place exchange protocol to synthe-

size charged AuNPs (Figure 2.4a). For this, DDA-AuNPs (20 mL) was quenched with 50 mL of methanol to yield a black precipitate and the supernatant containing excess of ligand was decanted. The precipitate was then redissolved in 20 mL of toluene, to which, a mixture of TMA ([+]) and MUA ([-]) (dissolved in 10 mL of dichloromethane, DCM) was added. The solution was kept undisturbed for ∼15 h to equilibrate. Next, the supernatant solution was decanted, and the precipitates were extensively washed with DCM (3 × 50 mL) followed by acetone (1 × 50 mL). The precipitate was then dried, redispersed in water, and 20 μL of TMAOH was added to deprotonate all the carboxylic acid groups in the [-] ligands. A ∼40 fold molar excess of ligands was added during the place exchange to confirm the complete removal of DDA. Similarly, various ratios of [+] and [-] were used during the place exchange reaction to synthesize heterogeneously charged $[+/-]_n$ AuNPs ($n_{soln} = \frac{[-]}{[+]} = 9, 4, 3\ldots$ etc.). The prepared AuNPs showed a characteristic absorption peak in the visible region at ∼520 nm (Figure 2.4b), because of surface plasmon resonance (SPR). This strong absorbance imparted a brilliant wine-red colour to

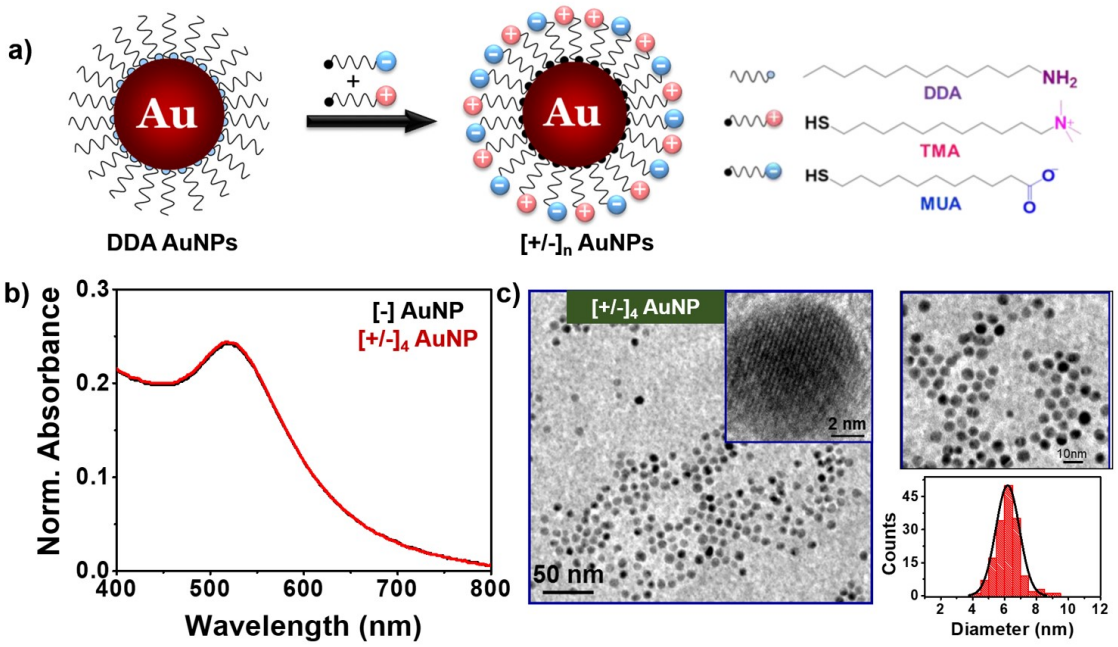


Figure 2.4: Synthesis and characterization of AuNPs. (a) Schematic representation of place exchange reaction. Here, $[+/-]_n$ AuNPs are prepared by replacing DDA ligand with a mixture of [+] and [-] charged ligands. (b) UV-Vis. absorption spectra of [-]AuNP and $[+/-]_4$ AuNP. (c) TEM images and size distribution of 6 ± 0.7 nm $[+/-]_4$ AuNPs. The size distribution was estimated from ∼ 350 NPs.

the NP solution. The average size of the nanoparticles thus synthesized were 6.0 ± 0.7 nm, as estimated from TEM analysis (Figure 2.4c).

2.3.2 Characterization of AuNPs

In order to verify the presence of both surface ligands on the same AuNP surface (formation of $[+/-]_n$ AuNPs), rather than the presence of a mixture of [+], and [-] AuNPs, we performed a series of control experiments (Figure 2.5a). Firstly, we added ∼10μL of HCl, and NaOH to test the stability of the prepared AuNPs in acidic and basic media respectively. The rationale being that if the solution contained a mixture of charged AuNPs, under acidic conditions, [-] AuNPs will lose their stability and ultimately precipitate out from the solution. This will result in a decrease in absorption intensity under acidic conditions, while both homogeneous, and heterogeneously charged AuNPs will retain their stability under basic conditions. As can be seen from Figure 2.5a, there is negligible change in the absorption intensity of AuNPs under both acidic and basic conditions, indicating the presence of both [+] and [-] ligands on the same NPs (formation of heterogeneously charged $[+/-]_n$ AuNPs). Furthermore, a gradual variation in the zeta potential of AuNPs from completely -ve to completely +ve values, with varying fraction of [+] ligands during the place exchange prove our control over the functionalization of AuNP

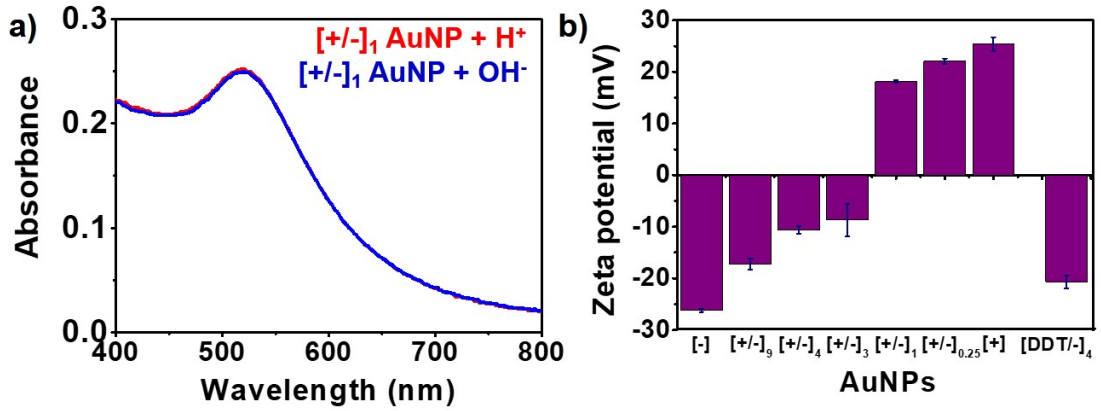


Figure 2.5: Characterization of AuNPs. (a) Negligible changes in the UV-Vis. absorption of [+/-] AuNPs in the presence of acid and base. (b) Plot showing the variation of ζ potential with m-SAM composition at pH ∼ 11. The error bars correspond to standard deviations based on three different sets of experiments.

surface (Figure 2.5b).

The exact composition of the [+] and [-] ligands on the surface of [+/-] AuNPs was determined by ^{1}H NMR experiment. In a typical experiment, before performing NMR investigation, gold cores of the NPs were etched with molecular I_2, using the reported literature procedure.[58] Excess of I_2 was removed by washing with methanol and drying at 65^0C. The thiol mixtures were then dried under vacuum for ∼15 h to remove the traces of water and methanol. The purified thiol mixtures were dissolved in deuterated DMSO and ^{1}H NMR spectrum was taken on a 400 MHz Bruker apparatus. A typical spectrum of $\frac{[-]}{[+]}$ thiol solution ($\alpha_{soln.}$ = n = 4) in d^6-DMSO is shown in Figure 2.6. The composition of the mixture was estimated as follows:-

$$\begin{aligned} \frac{[-]^{surf}}{[+]^{surf}} &= \frac{\text{Mixed Peak} - [-]^{surf}}{[+]^{surf}} \\ &= \frac{[+]^{surf} - [-]^{surf}}{[+]^{surf}} \end{aligned} \tag{2.1}$$

The mixed peak corresponds to methylene protons next to thiol (-CH_2-S-) from both [+] and [-] ligands (at $\delta \sim 2.7$ ppm). In order to calculate the α_{surf}, all the peaks were integrated with respect to the reference peak at $\delta \sim 3.26$ ppm (corresponding to (-CH_2—$N^+(CH_3)_3$). The integration values corresponding to the mixed peak ([+] + [-]) at $\delta \sim 2.69$ ppm) and [+] peak at $\delta \sim 3.03$ ppm (recalculated for single methyl proton in —$N^+(CH_3)_3$) were substituted in equation 2.1 to get α_{surf} to be 3.0. The α_{surf} value obtained here is in accordance with the literature values.[53,57]

2.3.3 Trapping and Binding Experiments

Trapping experiments were performed with different AuNP ratios (differing in the magnitudes of surface charges) in the presence of varying concentrations of aggregation triggers. In a typical trapping experiment, aqueous solution of [-] AuNP (3 mL) was prepared such that the optical density was ∼0.25 (∼5 nM in terms of AuNPs),

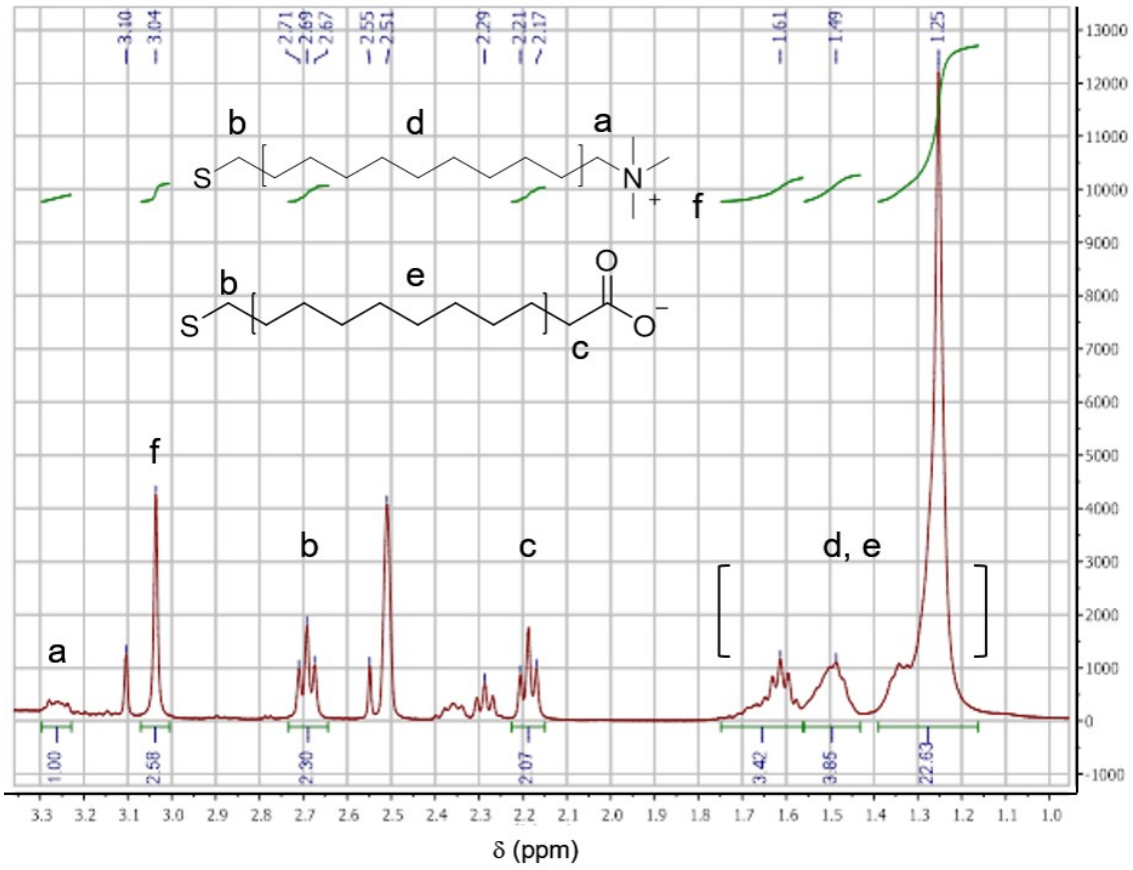


Figure 2.6: ^{1}H NMR spectrum of $\frac{[-]}{[+]}$ thiol solution ($\alpha_{soln.}$ = n = 4) in d^6-DMSO after etching the Au cores.

followed by the addition of 1 mM of Pb^{2+}. The progress of trapping was monitored by UV-Vis. absorption, Dynamic Light Scattering (DLS), Zeta potential and microscopy (Transmission Electron Microscopy (TEM), Atomic Force Microscopy (AFM) and Scanning Electron Microscopy (SEM)) studies. Spectral changes were followed by monitoring the UV-Vis. absorption spectrum for at least 2 days. DLS measurements were performed for ∼3 h so as to gain insight on the variation of hydrodynamic diameter of AuNPs with time. Microscopy experiments were carried out by drop casting ∼100 μL of AuNP-Pb^{2+} aggregate solution (after 3 h of incubation in Pb^{2+}) on the respective substrates. The drop was subsequently removed with a tissue paper after ∼10 min so as to minimize the 'drying effect'.

2.3.4 Reversibility Studies

The reversibility experiments were performed on $[+/-]_9$ AuNP because the spectral responses were faster, and $[+/-]_9$ AuNP-Pb^{2+} aggregates were stable for ∼7 h. Sodium hydroxide (NaOH) and hydrochloric acid (HCl) were used as the disassembly and reassembly triggers, respectively. The addition of 1 mM Pb^{2+} to $[+/-]_9$ AuNP resulted in the formation of AuNP-Pb^{2+} aggregates. For the disassembly of the aggregates, 10 μL aliquots of 1 M NaOH were added to the solution. Here, NaOH reacted with Pb^{2+} to form insoluble lead hydroxide ($Pb(OH)_2$), which is further converted into soluble plumbate (PbO_2^{2-}).[59] The added NaOH, thus removes all the Pb^{2+} from the NP-ion aggregates as is evident from the complete reversal of plasmon band to its initial position. This was followed by the addition of 10 μL aliquots of 1 M HCl, which caused the release of Pb^{2+} from plumbate (PbO_2^{2-}) to the solution, resulting in the reassembly of aggregates. The addition of acid first neutralized the excess base present in the system and then dissociated the PbO_2^{2-} to $Pb(OH)_2$ and ultimately releasing the Pb^{2+}. The free Pb^{2+} then chelates with the carboxyl group resulting in the reassembly of NPs. The retrapping of Pb^{2+} was confirmed by performing control experiments with AuNPs and HCl in the absence of Pb^{2+}.

2.4 Results and Discussion

The present work focuses on regulating the interparticle forces in NPs and investigates how it '*adds*' to the existing NP functionality. For this, we selected NP's ability to trap toxic ions as the model study, which has been used in numerous sensing applications.[1–10] Frequently, NP-based sensing methods utilizes carboxylic acid groups as a targeting moiety,[1–4,22,27] and hence, we chose carboxylic acid as the headgroup in our NP design. AuNPs functionalized with MUA ligands, [-] AuNPs, having a core diameter of 6.0 ± 0.7 nm, were synthesized using a modified literature procedure[56,57] (see Figures 2.4, 2.5), and systematic Pb^{2+} binding studies were car-

ried out. Homogeneously charged [-] AuNPs (∼5 nM in terms of NPs) precipitated in the presence of 1 mM Pb^{2+} with a visible color change from wine red to blue and finally to black, over a period of 3-4 h (see Figures 2.3a, 2.7, 2.8a, c, d, 3.17). Such a system serves as good trapping agent but fails to cater to the need of an ideal scavenger, owing to the instability of NP-ion aggregates. In order to achieve the stability, attractive bridging interactions were diluted by incorporating hydrophobic ligands (dodecanethiol, DDT) into the SAM of [-] AuNPs using place exchange reaction (see Section2.3.1 in the Experimental Section). $[DDT/-]_4$ AuNPs (where α_{soln} = ligand feed radio during place exchange = $\frac{[-]_{sol}}{[DDT]_{sol}} = 4$) also precipitated in the presence of 1 mM Pb^{2+} similar to [-] AuNPs (Figure 2.7). It has been shown that dilution of m-SAM with ethylene glycol ligands (inert ligand) can improve the stability of the AuNP-Eu^{3+}-ion aggregates.[26] However, our study indicates that incorporation of more '*potent*' forces like repulsions rather than a mere dilution of attractive forces is required to accomplish controlled aggregation phenomenon. Accordingly, we introduced positive charges into the SAM of [-] AuNPs and a series of heterogeneously charged [+/-] AuNPs were prepared by adopting modified literature procedure.[57] The composition of [+] and [-] in m-SAM was systematically varied to generate heterogeneously charged NPs with different magnitudes of surface charge ($[+/-]_n$ where n = α_{soln} = ligand feed ratio during place exchange = $\frac{[-]}{[+]}$ = 9, 4, 3 etc.). All the AuNP systems were well characterized using UV-Vis. absorption, TEM, and zeta potential studies (Figure 2.4, 2.5). The addition of 1 mM Pb^{2+} to 5 nM of $[+/-]_9$ AuNPs resulted in an immediate bathochromic shift in the plasmon band followed by precipitation (Figure 2.7). Interestingly, the kinetics of precipitation in $[+/-]_9$ AuNPs was slower than that of [-] and $[DDT/-]_4$ AuNPs (Figure 2.7a). Hence, incorporation of a small percentage of positive charges in the SAM proved to be vital in slowing down the NP precipitation. Consequently, systematic Pb^{2+} induced aggregation studies were carried out with [+/-] AuNPs system to impart long-term stability to NP-ion aggregates (Figure 2.7).

The screening of charges on the NP surface by the addition of Pb^{2+} resulted in

scenarios where either attractive/repulsive interactions were dominating or were of comparable strengths. For instance, the addition of Pb^{2+} to $[+/-]_9$ AuNPs resulted in NP precipitation indicating the dominance of attractive forces. Motivated by the slower kinetics of precipitation for $[+/-]_9$ AuNPs in Pb^{2+}, the [+] charges in the m-SAM were increased to see its effect on interparticle forces. No appreciable shift in the plasmon band was observed for $[+/-]_4$ AuNPs in 1 mM Pb^{2+}, which indicated a complete dominance by repulsive interactions (Figure 2.7). The magnitude of interparticle attractive forces was then gradually raised, to balance the repulsive forces, by increasing the concentration of Pb^{2+} in solution (Figure 2.7b). It was found that $[+/-]_4$ AuNPs in 35 mM of Pb^{2+} exhibited a shift of 12-15 nm without undergoing precipitation, and remained stable for ~2 days (Figures 2.7b, 2.8b, c). A further increase in the concentration of Pb^{2+} resulted in a greater spectral response but compromised on the stability of the aggregates (Figure 2.7b). For instance, 100 mM addition of Pb^{2+} resulted in a bathochromic shift of ~20 nm and the $[+/-]_4$ AuNPs precipitated over a period of 7-8 h. This by itself was interesting as the

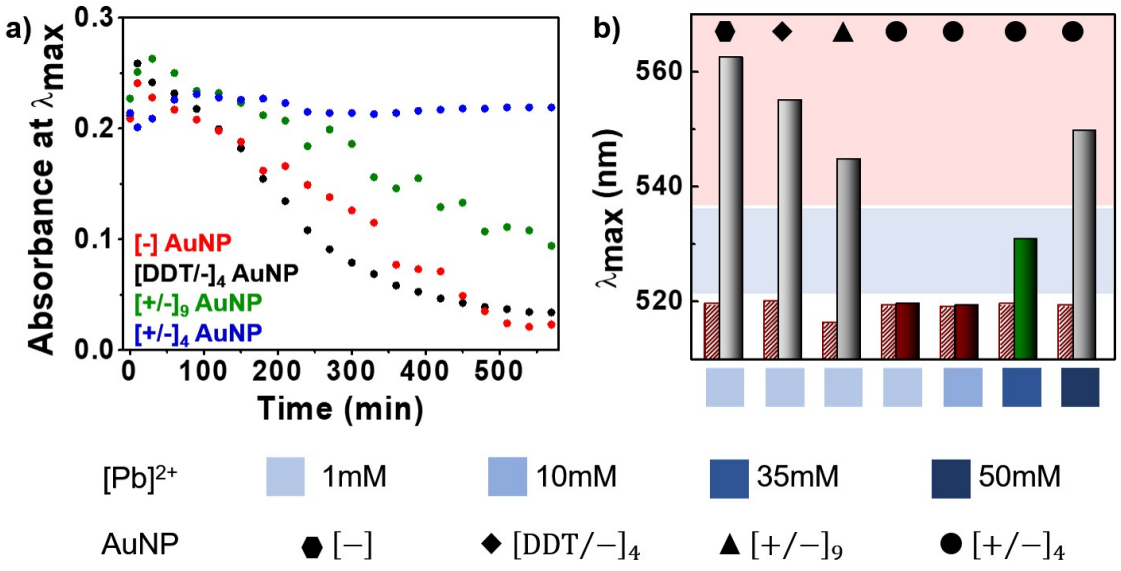


Figure 2.7: Optimization of controlled aggregation phenomenon in charged AuNPs. (a) Representative UV-vis measurements showing the variation of absorbance at $\lambda_{max.}$ with time for [-], $[DDT/-]_4$, $[+/-]_9$ and $[+/-]_4$ AuNPs upon the addition of 1 mM Pb^{2+}. (b) A plot summarizing the variation of $\lambda_{max.}$ in various charged AuNP systems as a function of Pb^{2+} concentration. Based on the initial screening studies, $[+/-]_4$ AuNPs in 35 mM Pb^{2+} was selected as the working system for studying controlled aggregation phenomenon.

NP aggregates were stable for an appreciable period, providing the time to scavenge them out of the system. As the focus was on the long-term stability (∼1 day) of NP-ion aggregates, we chose $[+/-]_4$ AuNPs in 35 mM Pb^{2+} as the working system.

The interaction of [-] and $[+/-]_4$ AuNPs with 1 mM and 35 mM Pb^{2+} resulted in an instantaneous increase in λ_{max} (∼40 nm and ∼12 nm, respectively; Figure 2.8). The observed bathochromic shift was due to the formation and increase in size of NP aggregates driven by the chelation between carboxylate groups on adjacent NPs and Pb^{2+}. The decrease in interparticle distance enables '*talking*' between the plasmons of adjacent NPs, which resulted in a red-shift in the absorption maxima.[29–31] A

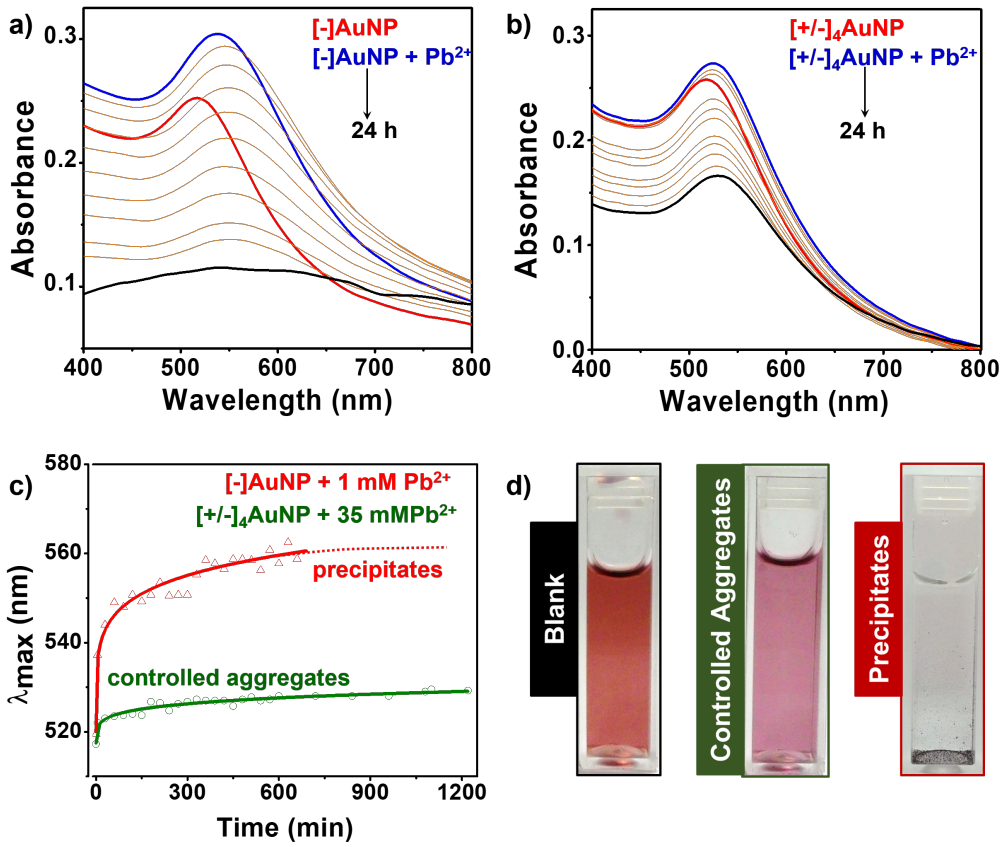


Figure 2.8: Spectral responses of homogeneously and heterogeneously charged AuNPs in the presence of Pb^{2+}. Changes in absorption spectra of (a) [-] and (b) $[+/-]_4$ AuNPs in the presence of 1 mM and 35 mM Pb^{2+}, respectively. (c) Variation of λ_{max} with time for [-] and $[+/-]_4$ AuNPs in 1 mM and 35 mM Pb^{2+}, respectively. (d) Photographs of solutions of $[+/-]_4$ AuNPs in the absence (left) and presence (middle) of 35 mM Pb^{2+}. A visible color change is observed upon the addition of 35 mM Pb^{2+} to $[+/-]_4$ AuNPs solution. The image on the right is the photograph of [-] AuNPs in the presence of 1 mM Pb^{2+}, showing complete precipitation (see the black precipitate at the bottom of the vial). All the photographs were taken after 1 day.

λ_{max} shift of ~40 nm was observed for [-] AuNPs with a collateral decrease in the absorption intensity. However, $[+/-]_4$ AuNPs showed an initial increase in both the absorbance and λ_{max} value (typical of aggregation),[53] followed by a decrease in absorbance. This decrease in absorbance ceased after ~90 min, confirming the arrest of aggregation process. Other than differences in the λ_{max} shifts, these two NP systems also differed in the stabilities of the aggregates formed. As depicted in Figure 2.8a, b, c, the [-] AuNPs sedimented within 4 h whereas $[+/-]_4$ AuNPs exhibited stability for ~2 days. The time scan and optical photographs of NP aggregates emphasizes the divergent optical behavior of [-] and $[+/-]_4$ AuNPs in the presence of Pb^{2+} (Figure 2.8c, d). The spectral responses thus obtained were well-complemented by Dynamic Light Scattering (DLS) studies (Figure 2.9). The hydrodynamic diameter of [-] AuNP-Pb^{2+} aggregates increased uncontrollably with time (up to ~1.2 μm, Figure 2.9a), and ultimately, they sedimented from solution. On the contrary, a controlled increase in hydrodynamic diameter was observed for $[+/-]_4$ AuNPs upon addition of Pb^{2+} and remained constant ~50 nm (Figure 2.9b). The repulsions arising from the quaternary ammonium groups on $[+/-]_4$ AuNPs

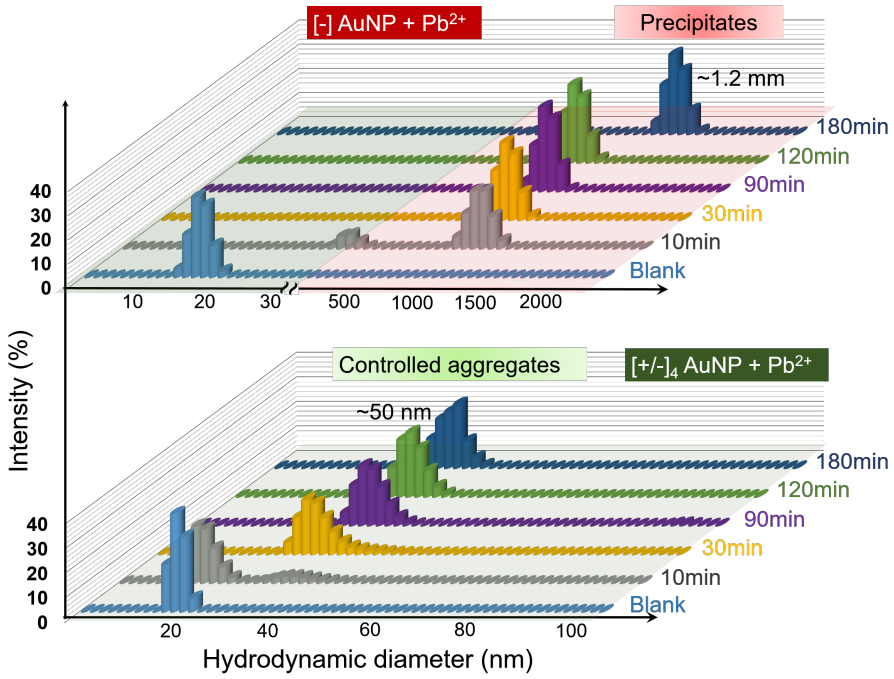


Figure 2.9: Comparative DLS plot corresponding to (a) [-] and (b) $[+/-]_4$ AuNPs in the presence of 1 mM and 35 mM Pb^{2+}, respectively.

eventually controlled the increment in the size and imparted stability to the NP-Pb^{2+} aggregates.

The size and morphology of the Pb^{2+}-trapped aggregates formed in [-] and $[+/-]_4$ AuNPs were investigated using microscopy studies. Transmission Electron Microscope (TEM) and Atomic Force Microscope (AFM) studies established the formation of large uncontrolled aggregates (several microns in size) when 1 mM Pb^{2+} was added to [-] AuNPs (Figure 2.10a). On the contrary, $[+/-]_4$ AuNPs formed many small controlled aggregates in the range of 30-50 nm (Figure 2.10a-e) upon addition of 35 mM Pb^{2+}. The 2D and 3D AFM height images clearly demarcate the boundaries between the individual NPs constituting the aggregates (bright lines and ripples in

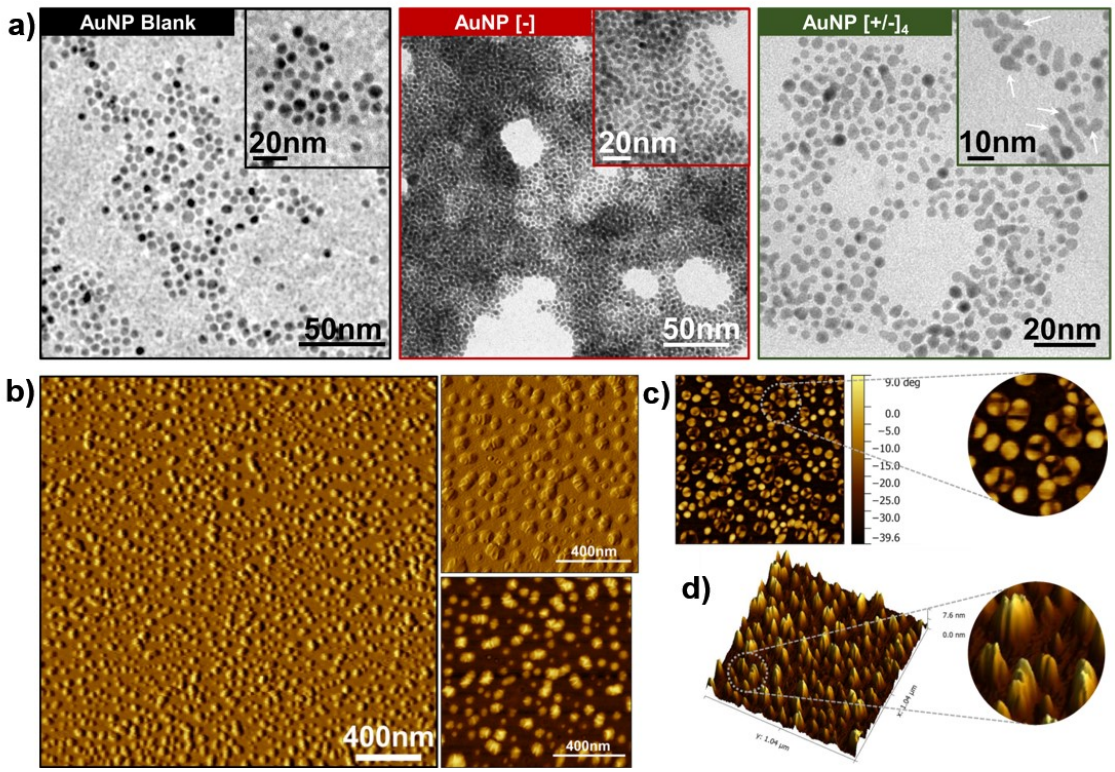


Figure 2.10: Microscopic characterization of NP-Pb^{2+} aggregate formation. (a) TEM images of 6.0 ± 0.7 nm sized AuNPs in the absence and presence of Pb^{2+}. [-] AuNPs formed large networks of uncontrolled aggregates in the presence of 1 mM Pb^{2+} (middle image). Interestingly, smaller aggregates comprising fewer NPs (mostly <5) were observed for $[+/-]_4$ AuNPs in the presence of 35 mM of Pb^{2+} (right image). A few of these smaller aggregates are highlighted with white arrows in the inset. (b-e) AFM images of $[+/-]_4$ AuNPs at different magnifications in the presence of 35 mM Pb^{2+}. (b) Height, (c) phase, and (d) contrast images of $[+/-]_4$ AuNPs aggregates showing the boundaries between the individual NPs constituting the aggregates (bright and dark lines, respectively).

Figure 2.10c, e respectively) - an observation which is seen as dark boundaries in the phase contrast image (Figure 2.10d). The image analysis done on >1000 NPs shows the preference among the $[+/-]_4$ AuNPs to participate in the formation of '*n-particle*' aggregates (where n < 10), instead of huge aggregates or remaining as individual NPs (Figure 2.10f). Spectroscopic and microscopic data thus confirmed the formation of stable and controlled aggregates between heterogeneously charged NP and Pb^{2+}.

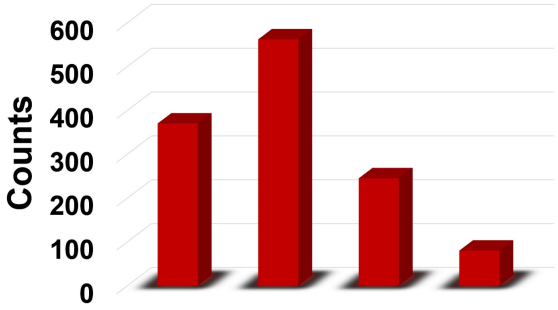


Figure 2.11: Image analysis done on >1000 NPs confirm that most of the $[+/-]_4$ AuNPs prefer to participate in the formation of aggregates constituting <10 NPs.

2.4.1 Role of Interparticle Forces

The major forces responsible for controlling the interparticle interactions in [+/-] AuNPs are (i) electrostatic repulsions among carboxylate [-] or quaternary ammonium [+] groups, (ii) electrostatic attractions between quaternary ammonium and carboxylate groups, (iii) H-bonding attraction between carboxylic acid groups (a few of the carboxylate groups are protonated at the pH under study, ~7), and (iv) Pb^{2+} mediated bridging interactions. The phenomenon of stable aggregation can be understood by observing how the strengths of these interparticle forces evolves with time. The exact composition of the [+] and [-] ligands on the surface of $[+/-]_4$ AuNPs was determined by ^{1}H NMR experiments[60] and found to be $\alpha_{surf} = \frac{[-]_{surf}}{[+]_{surf}}$ = 3 (see Figure 2.5c, and Section 2.3.2). The α_{surf} obtained is lower than the ligand feed ratio used during the place exchange reaction, which is in accordance with previous reports.[23,57] Due to the relatively smaller number of [+] in the m-SAM of $[+/-]_4$ AuNPs, the electrostatic attractions between [+] and [-] on adjacent NPs are

not strong enough to overcome the stronger electrostatic repulsions between two [-] ligands. $[+/-]_4$ AuNPs exhibited a zeta potenial of -8.0 ± 1.2 mV in the absence of Pb^{2+} (Figure 2.12c top). The aggregation of NPs in the presence of Pb^{2+} can occur either due to bridging interactions[1–7,26,27,61] or desoprtion of thiolates from the NP surface.[62] Control experiments, where $[+/-]_4$ AuNPs showed no aggregation with Pb^{2+} (at acidic pH values) overruled the desoprtion of thiolates from the NP surface (Figures 2.12a, 3.18). With the addition of Pb^{2+} into [+/-] AuNP system, bridging interaction comes into picture and the initial electrostatic repulsions between [-] ligands transformed to attractions— a phenomenon usually referred to as '*charge inversion*'. As the interparticle distance reduced, the electrostatic repulsion arising from the [+] started to dominate and the kinetics of assembly process slowed down. This is clearly evident from the reversal of zeta potential from -8.0 ± 1.2 mV to +14 ± 1.5 mV upon binding of Pb^{2+} (Figure 2.12b, c). The presence of [+]

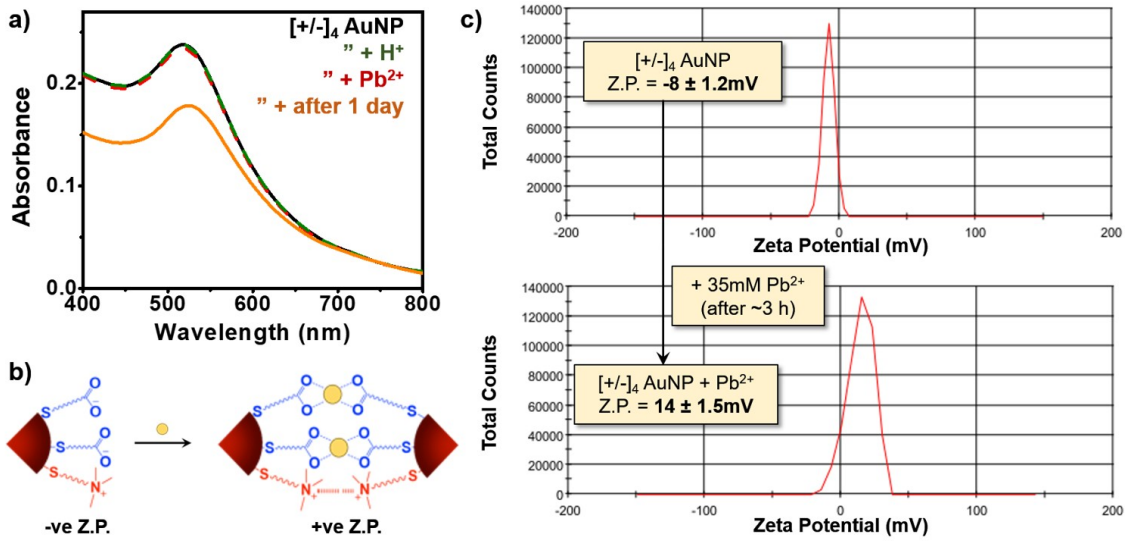


Figure 2.12: Control experiments for understanding mechanism of aggregation. (a) Interaction of Pb^{2+} with $[+/-]_4$ AuNP at acidic pH. The insignificant change ($\Delta\lambda_{max.} \sim 2$ nm) witnessed in the absorption spectrum after the addition of 35mM of Pb^{2+} to an acidic solution of $[+/-]_4$ AuNPs. This validates the necessity of carboxylate group at the NP surface for interaction with Pb^{2+}. (b) Schematics showing the effect of addition of Pb^{2+} on Zeta Potential of $[+/-]_4$ AuNPs. (c) The ζ plots for $[+/-]_4$ AuNP in the absence (top) and presence (bottom) of 35 mM Pb^{2+}. The negative ζ value indicates the presence of excess negatively charged ligands on the surface of untreated $[+/-]_4$ AuNPs. Interestingly, there is a reversal in ζ value (from -8 ± 1.2 mV to 14 ± 15 mV) upon addition of Pb^{2+} which confirms that the stability in $[+/-]_4$ AuNP-Pb^{2+} is imparted by positively charged [+] ligands in the m-SAM.

in the m-SAM, not only diluted the attractive forces by decreasing the amount of [-] but also introduced repulsive interactions. Thus, an elegant interplay of surface chemistry by careful adjustment in the number of [+] and [-] ligands resulted in the formation of stable aggregates in heterogeneously charged AuNPs.

2.4.2 Reversibility

Our next objective was to check the reversibility of controlled aggregation in [+/-] AuNP system. As chelation of Pb^{2+} is responsible for the onset of interparticle aggregation, their removal should then in principle result in the disassembly of NP-ion aggregates. The use of ethylenediaminetetraacetic acid (EDTA) as the disassembling trigger resulted in a partial release of Pb^{2+}, which is in accordance with previous studies. Alternatively, NaOH (1M) and HCl (1M) were used to disassemble and reassemble the NP-ion aggregates, respectively (Figure 2.13, 2.3, 3.19). NaOH reacts with Pb^{2+} to form insoluble lead hydroxide ($Pb(OH)_2$), which is further converted into soluble plumbate (PbO_2^{2-})[59] (see Section 2.3.4 for detailed discussion on formation and disappearance of turbidity as a function of NaOH addition). The added NaOH, thus removed all the Pb^{2+} from the NP-ion aggregates as is evident from the complete reversal of plasmon band to its initial position (Figure 2.13a, blue curve in 2.13c). The reassembling of NP-ion aggregates was achieved by releasing the Pb^{2+} from PbO_2^{2-} using 1 M HCl. The addition of acid at first neutralizes the excess ^-OH present, then dissociates the PbO_2^{2-} to $Pb(OH)_2$ and ultimately releases the Pb^{2+} (Figure 2.13b, red curve in 2.13c). The free Pb^{2+} then chelates with the carboxyl group resulting in the reassembly of NPs. The reversibility of the assembly disassembly was carried out for at least three cycles and is reflected in the shuttling of λ_{max} between 518 and 528 nm corresponding to the unaggregated and aggregated states, respectively (Figure 2.13b). We performed similar reversibility experiments with [-] AuNPs in 1 mM Pb^{2+} and failed to observe complete reversal in λ_{max}, even after addition of excess NaOH (see Figure 2.19). This indicated that Pb^{2+} are perhaps trapped too tightly between the [-] AuNPs, and addition of ^-OH failed to release

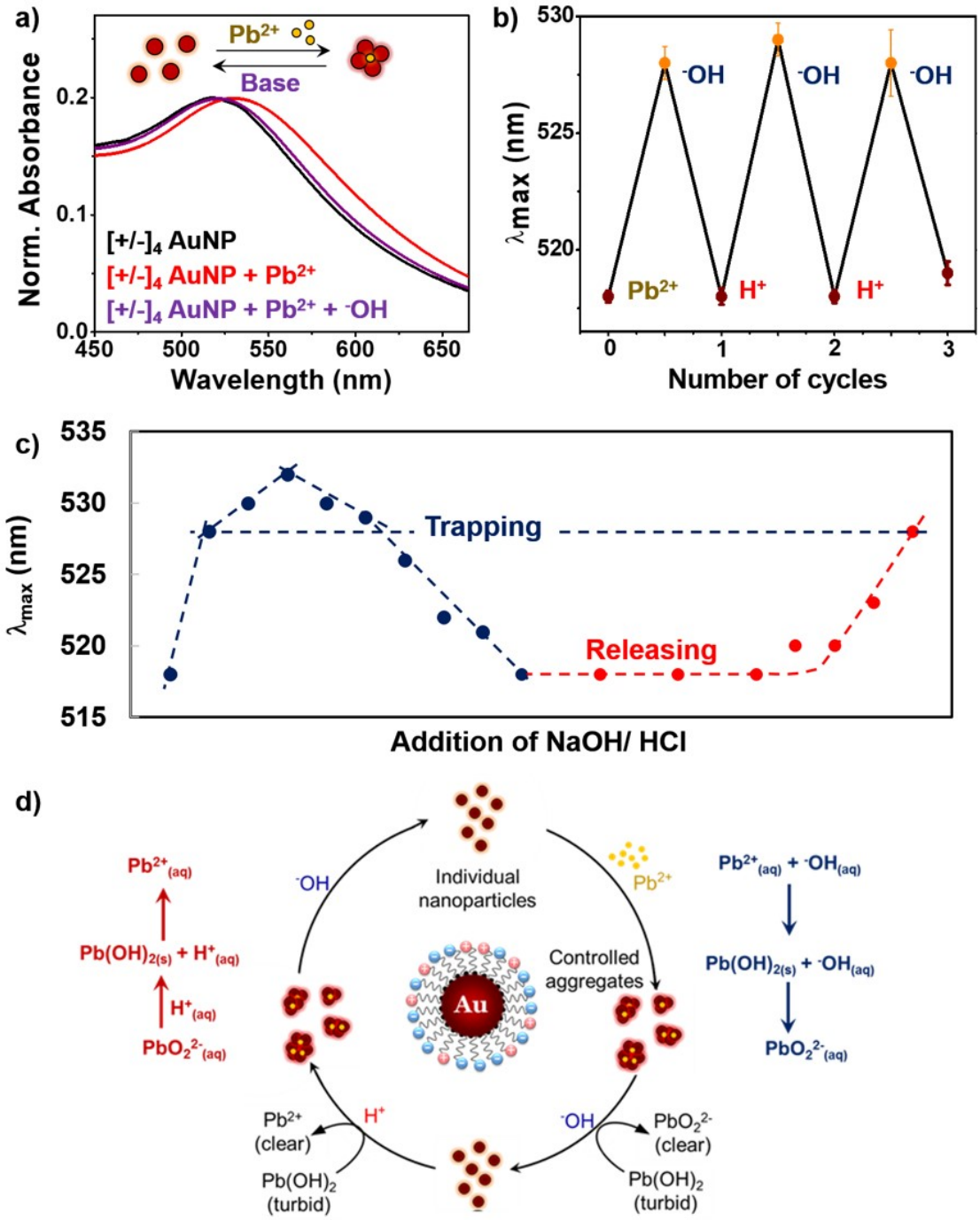


Figure 2.13: Reversible trapping of Pb^{2+} by heterogeneously charged NPs. (a) Normalized absorption spectra of $[+/-]_9$ AuNPs showing the shuttling of $\lambda_{max.}$ from monodisperse (black) to aggregated (red) and back to monodisperse (blue) NPs upon sequential addition of Pb^{2+} and ^{-}OH. (b) Three complete cycles of assembly-disassembly process monitored with UV-vis spectroscopy. The error bars correspond to standard deviations based on three different sets of experiments. (c) A plot showing the variation of $\lambda_{max.}$ upon the addition of ^{-}OH (shown in blue) and H^{+} (shown in red) during a single assembly-disassembly cycle. Each point in the plot corresponds to 10 μL addition of 1 M ^{-}OH or H^{+}. (d) Schematic representation of the changes occurring during a single assembly-disassembly cycle (Section 2.3.4).

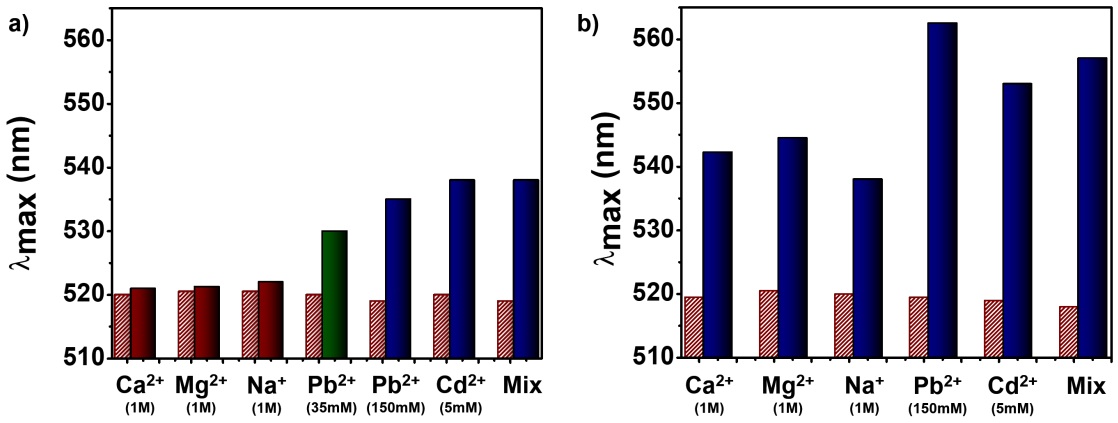


Figure 2.14: Selectivity of $[+/-]_4$ and [-] AuNPs toward the trapping of toxic ions. Variation in $\lambda_{max.}$ of (a) $[+/-]_4$ and (b) [-] AuNPs upon addition of different ions. In contrary to [-] AuNPs, $[+/-]_4$ AuNPs exhibited a preference in binding toward Pb^{2+} and Cd^{2+} in the presence of interfering cations. Thus, the inclusion of [+] ligands in the m-SAM imparts a new function of selectivity toward trapping of toxic ions in the carboxylate functionalized NP system.

them from the chelation. Here again, the presence of [+] charges in the m-SAM is an added advantage as the electrostatic repulsions in heterogeneously charged NPs decrease the COO^--Pb^{2+} attractions, thereby facilitating the reversible scavenging of ions.

2.4.3 Flexible Trapping and Selectivity

The formation of stable NP-ion aggregates is not only limited with Pb^{2+} but was also observed for 0.1 mM Cd^{2+} as well (Figure 2.15a). Further, $[+/-]_4$ AuNPs demonstrated a preference in binding toward Pb^{2+} and Cd^{2+} in the presence of commonly found cations like Ca^{2+}, Mg^{2+}, Na^+, and so on, emphasizing their suitability under physiological conditions (Figure 2.14a). However, [-] AuNPs failed to exhibit selectivity toward Pb^{2+} and Cd^{2+} in the presence of interfering cations (Figure 2.14b). The preference of $[+/-]_4$ Au NPs in trapping of Pb^{2+} and Cd^{2+} can be attributed to the stronger binding strengths of heavy metal ions compared to other divalent and monovalent ions.[63] Thus, the selectivity of charged AuNPs toward toxic ions is improved by the incoprporation of [+] ligands in the m-SAM. We also performed controlled aggregation studies in the presence of relatively weaker aggregation trig-

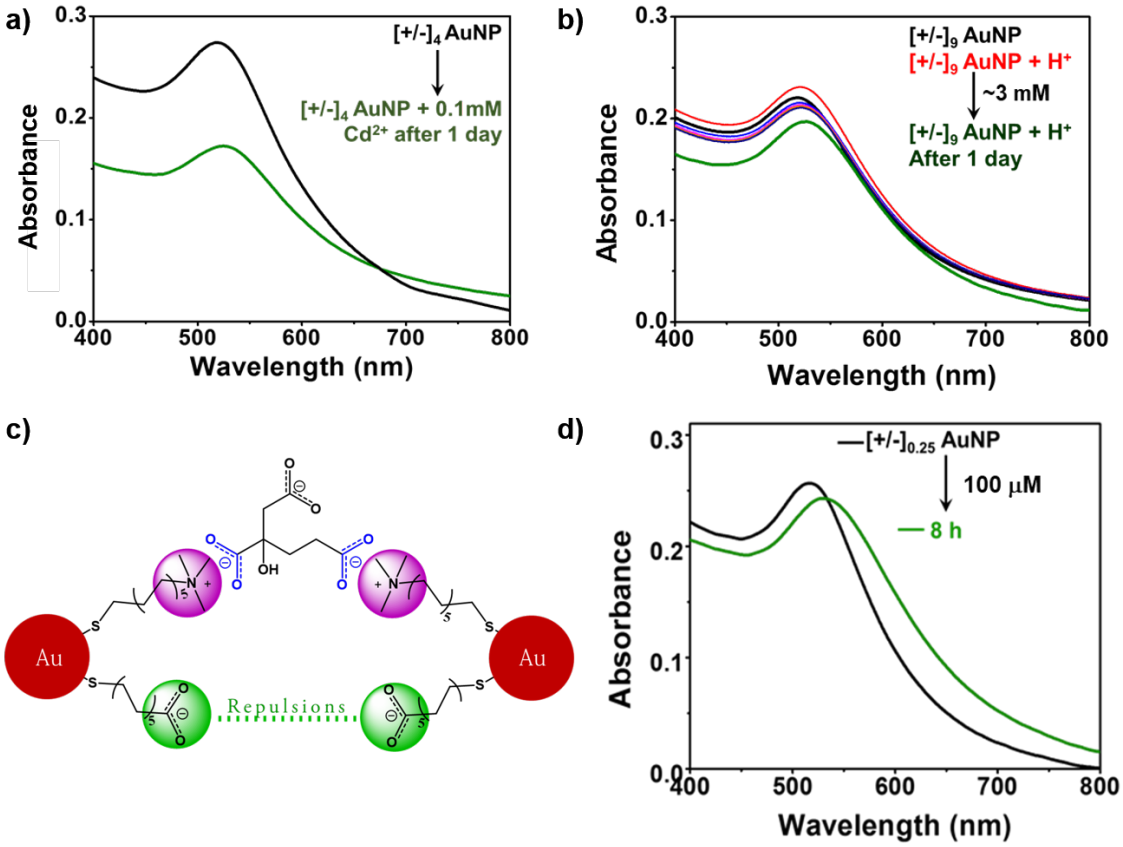


Figure 2.15: Flexibility of trapping different analytes of interest. (a) Controlled aggregation in $[+/-]_4$ AuNP with Cd^{2+}. UV-Vis. spectral changes of $[+/-]_4$ AuNPs in presence of 0.1 mM Cd^{2+} . A bathochromic shift of $\sim$ 7 nm was observed without any compromise on the colloidal stability. (b) UV-Vis. spectral changes of $[+/-]_9$ AuNPs in presence of 3.3 mM H^+. A bathochromic shift of $\sim$ 8 nm was observed without any compromise on the colloidal stability. (c) A schematic representation of citrate mediated bridging in $[+/-]_{0.25}$ AuNPs. (d) UV-Vis. spectral changes of $[+/-]_{0.25}$ AuNPs and [+] AuNPs in presence of 0.1 mM citrate ions at pH $\sim$ 11. A bathochromic shift of $\sim$ 8 nm was observed in the case of $[+/-]_{0.25}$ AuNPs without any compromise on the colloidal stability.

gers, like H^+. Interestingly, the controlled aggregation phenomenon in the presence of H^+ was observed only after reducing the magnitude of repulsive forces in [+/-] AuNPs system. For this, the protonation studies were carried out with $[+/-]_9$ AuNPs having lower percentage of [+] ligands (Figure 2.15b). The superiority of [+/-] AuNPs over other NP systems lies in the ease with which the necessary surface chemistry can be '*fitted in*' to perform analyte-specific binding, with either stronger or weaker triggers. To demonstrate the generality of our work further, we studied the interaction of $[+/-]_{0.25}$ AuNPs (the net surface charge is positive at the pH under study) with negatively charged citrate ions. Remarkably, citrate ions too behaved

in a similar way as positively charged ions, and the $[+/-]_{0.25}$ AuNP-citrate aggregates exhibited improved stability compared to [+] AuNP-citrate aggregates (Figure 2.15c,d). Here, the citrate ions chelate with the positively charged quaternary ammonium groups initializing the aggregation process. The [-] ligands on adjacent NPs provided the necessary repulsions required to achieve the perfect balance between the interparticle forces, thus stabilizing the aggregates.

2.5 Conclusion

The regulation of interparticle forces in heterogeneously charged NPs resulted in the phenomenon of controlled aggregation, which was successfully translated into reversible trapping and scavenging of toxic ions. The ratio of [+] and [-] ligands in the m-SAM was systematically tuned to achieve the formation of stable NP aggregation, in contrast to the conventionally observed phenomena of precipitation, upon the addition of charged analytes. One of the charges on the NP surface accounted for the trapping of ions of interest, whereas the other was responsible for imparting the much required repulsive forces for stabilizing the aggregates. With this strategy in mind, we were able to show the reversible trapping of Pb^{2+} ions, where the NP-ion aggregates were stable for $\sim$2 days, making them available for scavenging from the site of action. More importantly, the regulation of interparticle forces imparted a new function of selectivity toward trapping of toxic ions in a carboxylate functionalized NP systems. A rational outgrowth of these initial studies on controlled aggregation phenomenon can lead to a NP-based system capable of carrying out *in-vivo* scavenging of biologically toxic analyte molecules. The impact of controlling the interparticle forces at the nanoscale can be envisioned beyond the trapping and scavenging phenomena such as in self-assembly, optoelectronics, biotargeting, and catalysis.

2.6 Future Directions

In the present chapter, we demonstrate that by fine-tuning interparticle interactions, one can improve the existing properties of nanomaterials. Here, by working with heterogeneously charged AuNPs, and concentrations of Pb^{2+} (an aggregating trigger), we could balance attractions and repulsions and reveal the formation of controlled aggregates. This allowed us to overcome the inherent limitations of an aggregation process; i.e. instability of the aggregates, lack of reversibility, and non-selectivity. One of the immediate limitations of the present system that needs to be improved is the sensitivity of [+/-] AuNP system towards an analyte. Furthermore, we note that the present NP system could not distinguish between Pb^{2+} and Cd^{2+}. Traditionally, such levels of selectivity are imparted to a system by functionalizing the NPs with an analyte specific agent. Our next challenge was to find ways of introducing selectivity to a NP system without the use of an analyte-specific ligand.

References

(1) Kim, Y.; Johnson, R. C.; Hupp, J. T. *Nano Lett.* **2001**, *1*, 165–167.

(2) Saha, K.; Agasti, S. S.; Kim, C.; Li, X.; Rotello, V. M. *Chem. Rev.* **2012**, *112*, 2739–2779.

(3) Jimenez de Aberasturi, D.; Montenegro, J.-M.; Ruiz de Larramendi, I.; Rojo, T.; Klar, T. A.; Alvarez-Puebla, R.; Liz-Marzan, L. M.; Parak, W. J. *Chem. Mater.* **2012**, *24*, 738–745.

(4) Huang, C.-C.; Yang, Z.; Lee, K.-H.; Chang, H.-T. *Angew. Chem., Int. Ed.* **2007**, *46*, 6824–6828.

(5) Mirkin, C. A.; Letsinger, R. L.; Mucic, R. C.; Storhoff, J. J. *Nature* **1996**, *382*, 607–609.

(6) Ipe, B. I.; Yoosaf, K.; Thomas, K. G. *J. Am. Chem. Soc.* **2006**, *128*, 1907–1913.

(7) Liu, J.; Lu, Y. *Chem. Mater.* **2004**, *16*, 3231–3238.

(8) Cho, E. S.; Kim, J.; Tejerina, B.; Hermans, T. M.; Jiang, H.; Nakanishi, H.; Yu, M.; Patashinski, A. Z.; Glotzer, S. C.; Stellacci, F.; Grzybowski, B. A. *Nat. Mater.* **2012**, *11*, 978–985.

(9) Wang, L.; Ma, W.; Xu, L.; Chen, W.; Zhu, Y.; Xu, C.; Kotov, N. A. *Mater. Sci. Eng., R* **2010**, *R70*, 265–274.

(10) Ma, W.; Kuang, H.; Xu, L.; Ding, L.; Xu, C.; Wang, L.; Kotov, N. A. *Nat. Commun.* **2013**, *4*, 3689/1–3689/8.

(11) Lee, J.-S.; Han, M. S.; Mirkin, C. A. *Angew. Chem. Int. Ed.* **2007**, *46*, 4093–4096.

(12) Sun, T.; Zhang, Y. S.; Pang, B.; Hyun, D. C.; Yang, M.; Xia, Y. *Angew. Chem., Int. Ed.* **2014**, *53*, 12320–12364.

(13) Zhang, K.; Hao, L.; Hurst, S. J.; Mirkin, C. A. *J. Am. Chem. Soc.* **2012**, *134*, 16488–16491.

(14) Kim, B.; Han, G.; Toley, B. J.; Kim, C.-k.; Rotello, V. M.; Forbes, N. S. *Nat. Nanotechnol.* **2010**, *5*, 465–472.

(15) Skirtach, A. G.; Munoz Javier, A.; Kreft, O.; Kehler, K.; Piera Alberola, A.; Moewald, H.; Parak, W. J.; Sukhorukov, G. B. *Angew. Chem., Int. Ed.* **2006**, *45*, 4612–4617.

(16) Montenegro, J.-M.; Grazu, V.; Sukhanova, A.; Agarwal, S.; de la Fuente, J. M.; Nabiev, I.; Greiner, A.; Parak, W. J. *Adv. Drug Deliv. Rev.*. **2013**, *65*, 677–688.

(17) Ling, D.; Park, W.; Park, S.-j.; Lu, Y.; Kim, K. S.; Hackett, M. J.; Kim, B. H.; Yim, H.; Jeon, Y. S.; Na, K.; Hyeon, T. *J. Am. Chem. Soc.* **2014**, *136*, 5647–5655.

(18) Barrow, S. J.; Wei, X.; Baldauf, J. S.; Funston, A. M.; Mulvaney, P. *Nat. Commun.* **2012**, *3*, 2289/1–2289/9.

(19) Sonnichsen, C. *Science* **2011**, *332*, 1389–1390.

(20) Liz-Marzan, L. M. *Langmuir* **2006**, *22*, 32–41.

(21) Thomas, K. G.; Kamat, P. V. *Acc. Chem. Res.* **2003**, *36*, 888–898.

(22) Kundu, P. K.; Samanta, D.; Leizrowice, R.; Margulis, B.; Zhao, H.; Börner, M.; Udayabhaskararao, T.; Manna, D.; Klajn, R. *Nat. Chem.* **2015**, *7*, 646–652.

(23) Pillai, P. P.; Kowalczyk, B.; Grzybowski, B. A. *Nanoscale* **2016**, *8*, 157–161.

(24) Smoukov, S. K.; Bishop, K. J. M.; Kowalczyk, B.; Kalsin, A. M.; Grzybowski, B. A. *J. Am. Chem. Soc.* **2007**, *129*, 15623–15630.

(25) Kalsin, A. M.; Fialkowski, M.; Paszewski, M.; Smoukov, S. K.; Bishop, K. J. M.; Grzybowski, B. A. *Science* **2006**, *312*, 420–424.

(26) Kennedy, Z. C.; Lisowski, C. E.; Mitaru-Berceanu, D. S.; Hutchison, J. E. *Langmuir* **2015**, *31*, 12742–12752.

(27) Yoosaf, K.; Ipe, B. I.; Suresh, C. H.; Thomas, K. G. *J. Phys. Chem. C* **2007**, *111*, 12839–12847.

(28) Liu, D.; Chen, W.; Sun, K.; Deng, K.; Zhang, W.; Wang, Z.; Jiang, X. *Angew. Chem., Int. Ed.* **2011**, *50*, 4103–4107, S4103/1–S4103/16.

(29) Jain, P. K.; Huang, X.; El-Sayed, I. H.; El-Sayed, M. A. *Acc. Chem. Res.* **2008**, *41*, 1578–1586.

(30) Kumar, J.; Wei, X.; Barrow, S.; Funston, A. M.; Thomas, K. G.; Mulvaney, P. *Phys. Chem. Chem. Phys.* **2013**, *15*, 4258–4264.

(31) Shanthil, M.; Thomas, R.; Swathi, R. S.; Thomas, K. G. *J. Phys. Chem. Lett.* **2012**, *3*, 1459–1464.

(32) Mizuhara, T.; Saha, K.; Moyano, D. F.; Kim, C. S.; Yan, B.; Kim, Y.-K.; Rotello, V. M. *Angew. Chem., Int. Ed.* **2015**, *54*, 6567–6570.

(33) Huang, R.; Carney, R. P.; Ikuma, K.; Stellacci, F.; Lau, B. L. T. *ACS Nano* **2014**, *8*, 5402–5412.

(34) Verma, A.; Uzun, O.; Hu, Y.; Hu, Y.; Han, H.-S.; Watson, N.; Chen, S.; Irvine, D. J.; Stellacci, F. *Nat. Mater.* **2008**, *7*, 588–595.

(35) Nair, L. V.; Nazeer, S. S.; Jayasree, R. S.; Ajayaghosh, A. *ACS Nano* **2015**, *9*, 5825–5832.

(36) Liu, X.; Li, H.; Jin, Q.; Ji, J. *Small* **2014**, *10*, 4230–4242.

(37) Liu, X.; Chen, Y.; Li, H.; Huang, N.; Jin, Q.; Ren, K.; Ji, J. *ACS Nano* **2013**, *7*, 6244–6257.

(38) Ling, D.; Hackett, M. J.; Hyeon, T. *Nano Today* **2014**, *9*, 457–477.

(39) Albanese, A.; Tang, P. S.; Chan, W. C. W. *Annu. Rev. Biomed. Eng.* **2012**, *14*, 1–16.

(40) Walkey, C. D.; Chan, W. C. W. *Chem. Soc. Rev.* **2012**, *41*, 2780–2799.

(41) Wang, Y.; Sentosun, K.; Li, A.; Coronado-Puchau, M.; Sanchez-Iglesias, A.; Li, S.; Su, X.; Bals, S.; Liz-Marzan, L. M. *Chem. Mater.* **2015**, *27*, 8032–8040.

(42) Lee, J.-W.; Klajn, R. *Chem. Commun.* **2015**, *51*, 2036–2039.

(43) Das, S.; Ranjan, P.; Maiti, P. S.; Singh, G.; Leitus, G.; Klajn, R. *Adv. Mater.* **2013**, *25*, 422–426.

(44) Batista, C. A. S.; Larson, R. G.; Kotov, N. A. *Science* **2015**, *350*, 1242477.

(45) Walker, D. A.; Kowalczyk, B.; Olvera de la Cruz, M.; Grzybowski, B. A. *Nanoscale* **2011**, *3*, 1316–1344.

(46) Bishop, K. J. M.; Wilmer, C. E.; Soh, S.; Grzybowski, B. A. *Small* **2009**, *5*, 1600–1630.

(47) Luo, D.; Yan, C.; Wang, T. *Small* **2015**, *11*, 5984–6008.

(48) Min, Y.; Akbulut, M.; Kristiansen, K.; Golan, Y.; Israelachvili, J. *Nat. Mater.* **2008**, *7*, 527–538.

(49) Wang, L.; Albouy, P.-A.; Pileni, M.-P. *Chem. Mater.* **2015**, *27*, 4431–4440.

(50) Bruns, C. J.; Stoddart, J. F. *Acc. Chem. Res.* **2014**, *47*, 2186–2199.

(51) Balzani, V.; Credi, A.; Raymo, F. M.; Stoddart, J. F. *Angew. Chem., Int. Ed.* **2000**, *39*, 3348–3391.

(52) Zhu, Z.-J.; Tang, R.; Yeh, Y.-C.; Miranda, O. R.; Rotello, V. M.; Vachet, R. W. *Anal. Chem.* **2012**, *84*, 4321–4326.

(53) Pillai, P. P.; Kowalczyk, B.; Pudlo, W. J.; Grzybowski, B. A. *J. Phys. Chem. C* **2016**, *120*, 4139–4144.

(54) Liu, D.-B.; Qu, W.-S.; Chen, W.-W.; Zhang, W.; Wang, Z.; Jiang, X.-Y. *Anal. Chem.* **2010**, *82*, 9606–9610.

(55) Rana, S.; Bajaj, A.; Mout, R.; Rotello, V. M. *Adv. Drug Deliv. Rev.* **2012**, *64*, 200–216.

(56) Jana, N. R.; Peng, X. *J. Am. Chem. Soc.* **2003**, *125*, 14280–14281.

(57) Pillai, P. P.; Huda, S.; Kowalczyk, B.; Grzybowski, B. A. *J. Am. Chem. Soc.* **2013**, *135*, 6392–6395.

(58) Hostetler, M. J.; Templeton, A. C.; Murray, R. W. *Langmuir* **1999**, *15*, 3782–3789.

(59) Vogel, A. I., *A text-book of macro and semimicro qualitative inorganic analysis*; London Longmans: 1954.

(60) Hostetler, M. J.; Templeton, A. C.; Murray, R. W. *Langmuir* **1999**, *15*, 3782–3789.

(61) Wang, D.; Tejerina, B.; Lagzi, I.; Kowalczyk, B.; Grzybowski, B. A. *ACS Nano* **2011**, *5*, 530–536.

(62) Liu, D.; Qu, W.; Chen, W.; Zhang, W.; Wang, Z.; Jiang, X. *Anal. Chem.* **2010**, *82*, 9606–9610.

(63) Jeffery, G. H.; Bassett, J.; Mendham, J.; Denney, R. C., *Vogels Textbook Of Quantitative Chemical Analysis*, 5th ed.; Pearson Education India: 2006.

2.7 Appendix

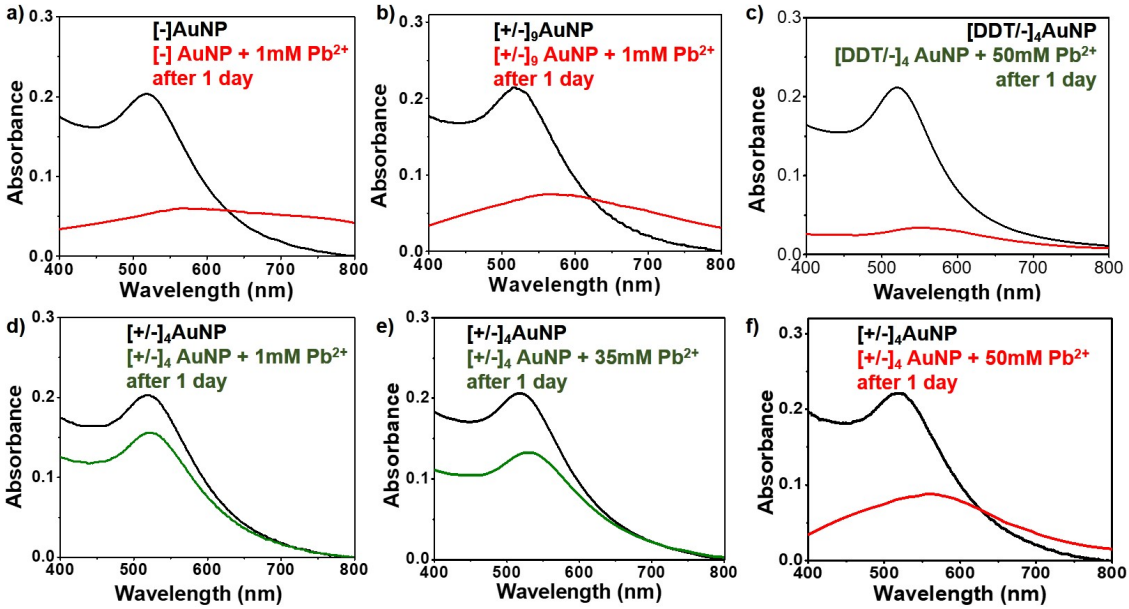


Figure 2.16: Screening of charged AuNPs for finding out the stable AuNP - Pb^{2+} aggregate system. Absorption studies of charged AuNPs in the presence of varying amount of Pb^{2+}. Details about the AuNPs and Pb^{2+} concentrations are given in the respective figures

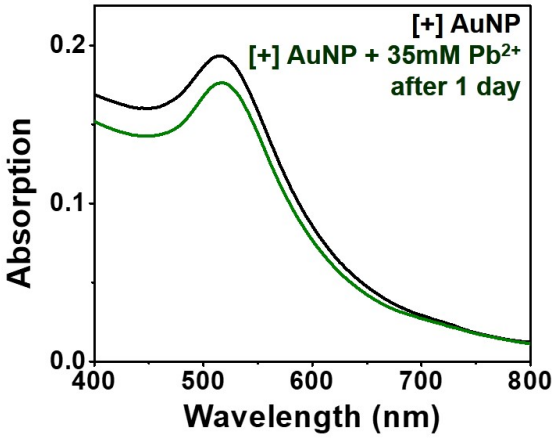


Figure 2.17: Interaction of Pb^{2+} with [+] AuNPs. The negligible change ($\Delta\lambda_{max.}$ ~1 nm) is observed in the absorption spectrum of [+] AuNPs upon addition of 35 mM Pb^{2+}.

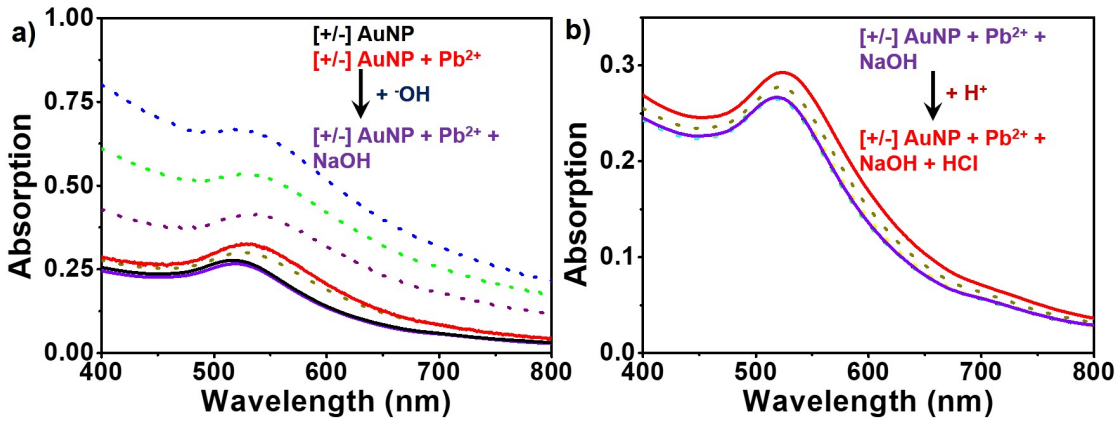


Figure 2.18: Assembly-disassembly-reassembly studies in [+/-] AuNP-Pb^{2+} system. Variation in UV-Vis. absorption spectra of [+/-] AuNPs upon (a) assembly-disassembly and (b) reassembly processes. The assembly and disassembly processes were carried out by the addition of Pb^{2+} and ^{-}OH ions, respectively. The reassembly process was carried out by the addition of HCl.

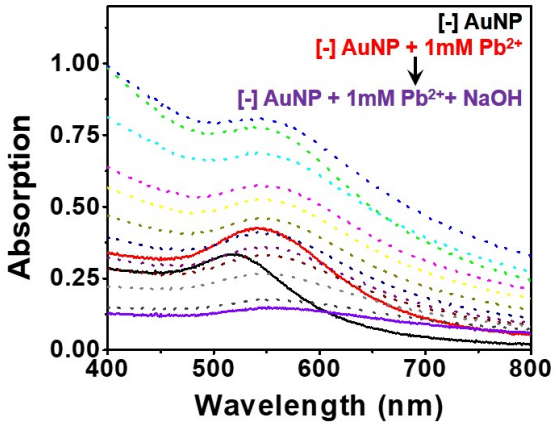
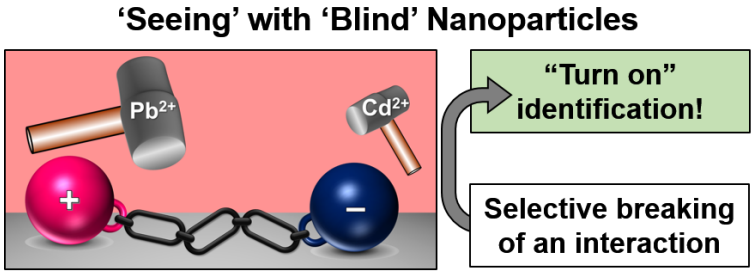


Figure 2.19: Reversibility experiment with [-] AuNP in 1mM Pb^{2+}. UV-Vis. absorption changes in [-] AuNP-Pb^{2+} upon the addition of 1 M NaOH. The decrease in the absorption intensity of [-] AuNP-Pb^{2+} (purple curve) is due to the dilution effect caused by the addition of excess NaOH (∼2 mL).

Chapter 3

Turn-On Selectivity in Inherently Nonselective Gold Nanoparticles for Pb^{2+} Detection by Preferential Breaking of Interparticle Interactions



On the Origins of Selectivity

This chapter has been adapted from the following paper:- **Rao, A.**, Kumar, G. S., Roy, S., Ajesh, T. R., Devatha, G., Pillai, P. P.*, Turn-On Selectivity in Inherently Nonselective Gold Nanoparticles for Pb^{2+} Detection by Preferential Breaking of Interparticle Interactions, *ACS Appl. Nano Mater.* **2019**, *2*, 5625 – 5633.

3.1 Abstract

Establishing a '*precise*' control over different interparticle interactions holds the promise of introducing inherently absent properties to nanosystems. In this direction, our aim is to introduce the notion of selectivity in inherently nonselective ('*blind*') carboxylate-functionalized gold-nanoparticles ([-] AuNP), towards strongly binding divalent metal ions (M^{2+}). The present system designed from such '*blind*' nanoparticles is able to discriminate between various M^{2+} ions (capable of '*seeing*'), by using differences in their abilities to break interparticle interactions. This is in stark contrast with the commonplace idea of forming an interaction between NPs and M^{2+} ions, as the means of identification, leading to the formation of precipitates. Among different ions tested, Pb^{2+} is able to break the electrostatic interactions in [+] - [-] Au nanoionic precipitates and displace [+] AuNP to solution, turning on the plasmonic wine-red color. The dominance of interaction energy for [-] AuNP - Pb^{2+} complexation over the inter-nanoparticle interactions is accountable for the selective discrimination of Pb^{2+} from other M^{2+} ions. A precise variation in strengths of different interparticle interactions helped in tuning both the selectivity and sensitivity of our identification protocol.

3.2 Introduction

Development of strategies to improve and impart newer properties to existing materials, without the aid of new components, is one of the future directions in modern nanoscience.[1,2] In this regard, strategies to control forces and interparticle interactions at the nanoscale[3–15] are emerging as an effective and widely accepted approach.[16–28] The present work demonstrates the decisive role of forces in introducing the notion of selectivity in inherently nonselective carboxylate-functionalized gold-nanoparticles ([-] AuNP) toward strongly binding divalent metal ions (M^{2+}). Overcoming nonselectivity is one of the long-standing and, thereby, well-studied challenges in the area of [-] AuNP sensors:[12,26,27,29] a case analogous to the *solvent*

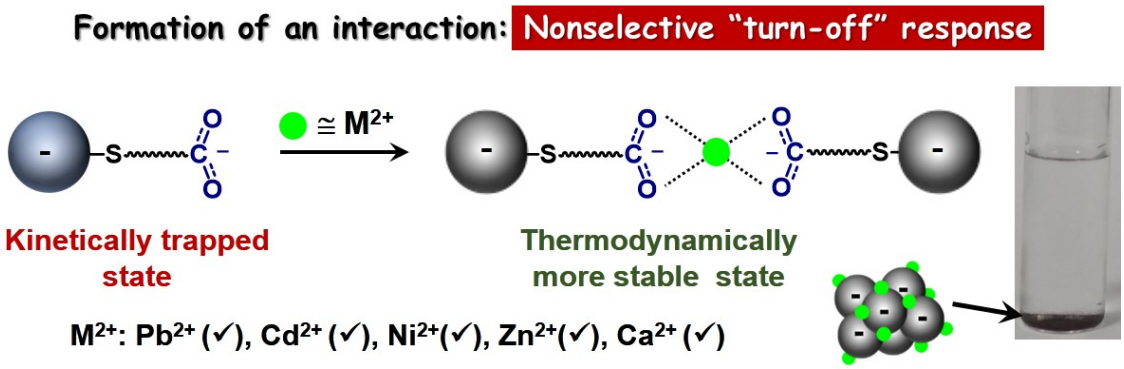


Figure 3.1: The kinetically trapped [-] AuNPs coordinate with various M^{2+} ions uniformly to reach the thermodynamically more stable precipitate state, resulting in the inherent nonselective turn-off response.

leveling effect where the solvent fails to discriminate between different strong acids or bases.[30] The leveling in [-] AuNP arises because of the strong abilities of M^{2+} ions to bridge the carboxylate groups (Figure 3.1).[12,26,27,29] The strong bridging interaction triggers the nonselective aggregation and plasmon coupling in NPs causing a rapid color change, ultimately leading to precipitation.[12,26,27,29] Essentially, a dispersed solution of NP is an example of a kinetically trapped state because of the large number of high energy surface atoms with unsatisfied valences.[33] The number of contacts between the NP increases during the process of aggregation/precipitation, which leads to a decrease in the number of high-energy surface atoms with unsatisfied valences.[33] Thus, the aggregated state of NP is thermodynamically more stable when compared to the dispersed state. Introduction of external stimuli (like M^{2+} ions) can thus uniformly trigger the thermodynamically favorable process of precipitation, imparting the nonselectivity to [-] AuNPs (Figure 3.1). Most of the available protocols overcome such nonselectivity by replacing the carboxylate groups on [-] AuNPs with analyte-specific ligands.[31,32,34–39] In a seminal work, Grzybowski and co-workers used AuNP crystals, stabilized with chemically cleavable dithiol moieties. Addition of a suitable trigger (cross-linker specific) cuts, and *'punctures'* the crystals, giving rise to a selective colour change. (see Figure 3.2a, b).[31] In another approach, Lu and co-workers used DNAzymes for the selective identification of Pb^{2+} ions. Here, out of different M^{2+} ions tested, only Pb^{2+} ions catalyzed the hydrolysis

of AuNP-DNAzyme aggregates, resulting in the disassembly of AuNPs (see Figure 3.2c).[32] On the contrary, we report here an alternate strategy to achieve the selective turn-on identification of heavy metal ions with AuNPs that are deprived of any analyte-specific ligands. Our approach is to explore the differences in the abilities of

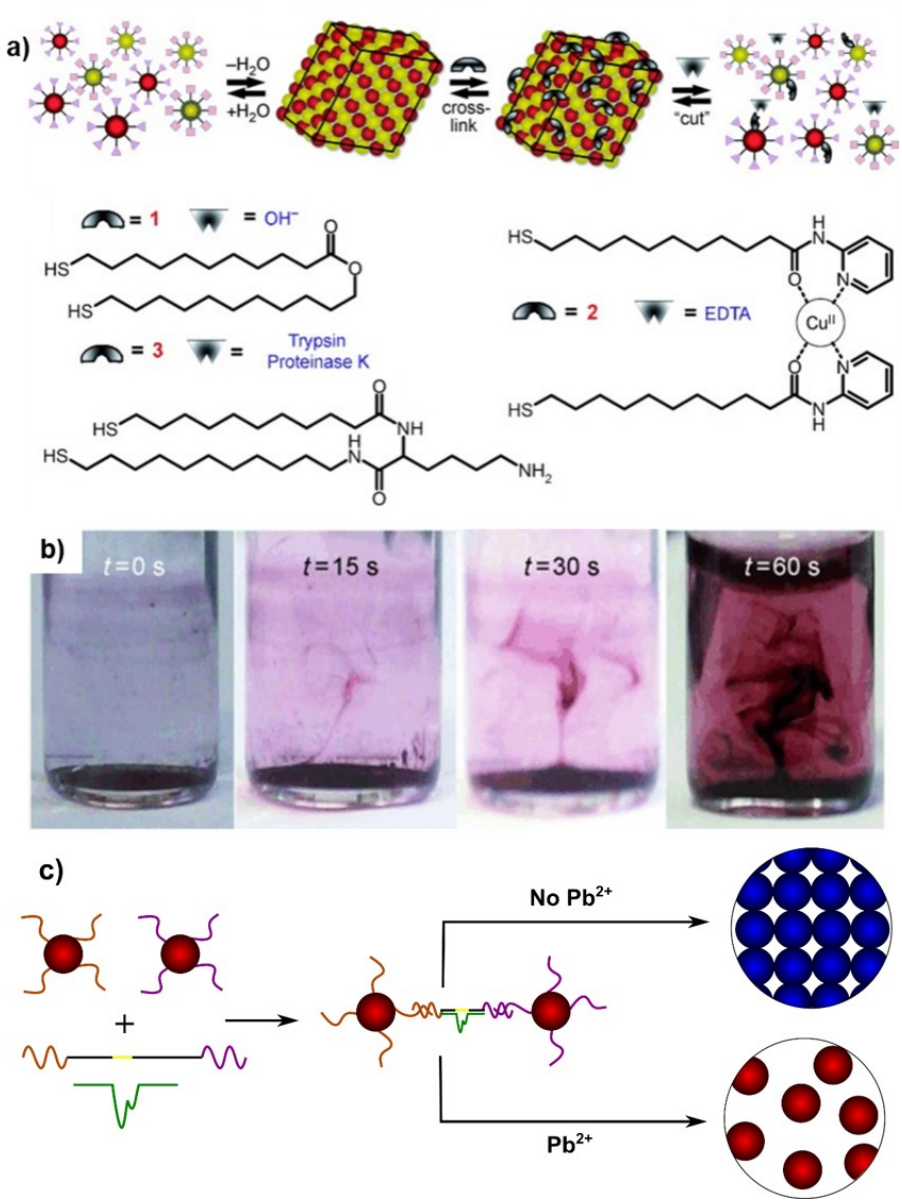


Figure 3.2: (a) Schematic diagram showing the design of crystals, cross-linked with cleavable dithiols for amplified chemical sensing. Chemical structures of the dithiol cross-linkers, and the analytes are shown in (a). (b) Optical photographs showing the dissolution/ release of NPs from the crystals upon addition of a suitable analyte (Reproduced in part with permission from [31] Copyright 2010 John Wiley and Sons). (c) Schematic illustration of a colorimetric sensor based on the disassembly of DNAzyme labelled AuNPs in the absence and presence of Pb^{2+} (Reproduced in part with permission from [32] Copyright 2003 American Chemical Society).

M^{2+} ions to interact with a thermodynamically stable[33] inter-nanoparticle precipitate containing [+] and [-] AuNPs (Figure 3.3). Here we emphasize that both [+] and [-] AuNPs, independently, were nonselective (*'blind'*) toward M^{2+} ions. Remarkably, a system composed of such nonselective nanoparticles was able to discriminate (capable of *'seeing'*) between the hard-to-distinguish pair of Pb^{2+} and Cd^{2+} ions.[12,26] The rationale is that only the strongest of strongly binding ions will be able to break the interactions in nanoionic precipitates (thermodynamically more stable state)[33] and disperse them back to the solution (kinetically trapped state).

We worked with the nanoionic precipitates having oppositely charged [+] and [-] AuNPs stitched together through electrostatic and van der Waals attractions, developed by Grzybowski and co-workers.[40] The concept of preferential breaking of interactions in [+] - [-] Au nanoionic precipitates by M^{2+} ions led to the displacement and leaking of [+] AuNPs, providing the desired turn-on selectivity (Figure 3.3). Only the Pb^{2+} ion was capable of breaking the electrostatic interactions in [+] - [-] Au nanoionic precipitates, among other M^{2+} ions including the Cd^{2+} ions. The sensitivity and selectivity of nanoionic precipitates were tuned by controlling the strength of electrostatic interactions between the NP constituents. The sensitivity of Pb^{2+} ions improved from 1 mM to 20 μM by reducing the electrostatic attractions in the nanoionic precipitates formed from heterogeneously charged $[+/-]_9$ and homogeneously charged [+] AuNPs. More importantly, 3 mM Cd^{2+} ions were also able to

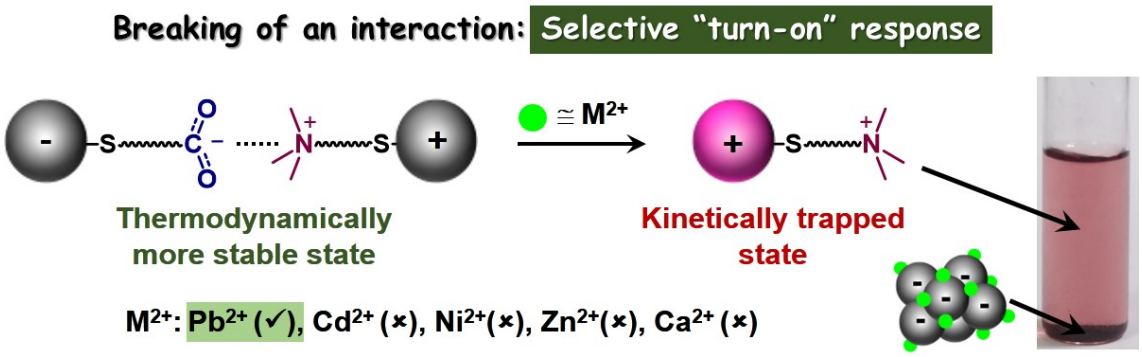


Figure 3.3: Schematics for selective turn-on response with [-] AuNPs. Our hypothesis to explore differences in the abilities of M^{2+} ions to break an interaction as the means of discrimination rather than the conventional idea of forming of an interaction.

break the electrostatic interactions between [+] - $[+/-]_9$ Au nanoionic precipitates, proving the tunability in M^{2+} ion detection (sensitivity and selectivity) as per the demand.

3.3 Experimental Section

3.3.1 Synthesis of AuNPs

AuNPs were synthesized according to a modified literature procedure.[12,41,42] We used tetrachloroaurate trihydrate ($HAuCl_4.3H_2O$) as the gold precursor and a mixture of tetrabutylammonium borohydride (TBAB) and hydrazine monohydrate ($N_2H_4.H_2O$) as the reducing agent for our AuNP synthesis. Briefly, a toluene solution (7 mL) of $HAuCl_4.3H_2O$ (23 mg, 0.058 mmol) containing 222 mg of dodecylamine (DDA) (1.2 mmol) and 277 mg (0.6 mmol) of di-n-dodecyl)dimethylammonium bromide

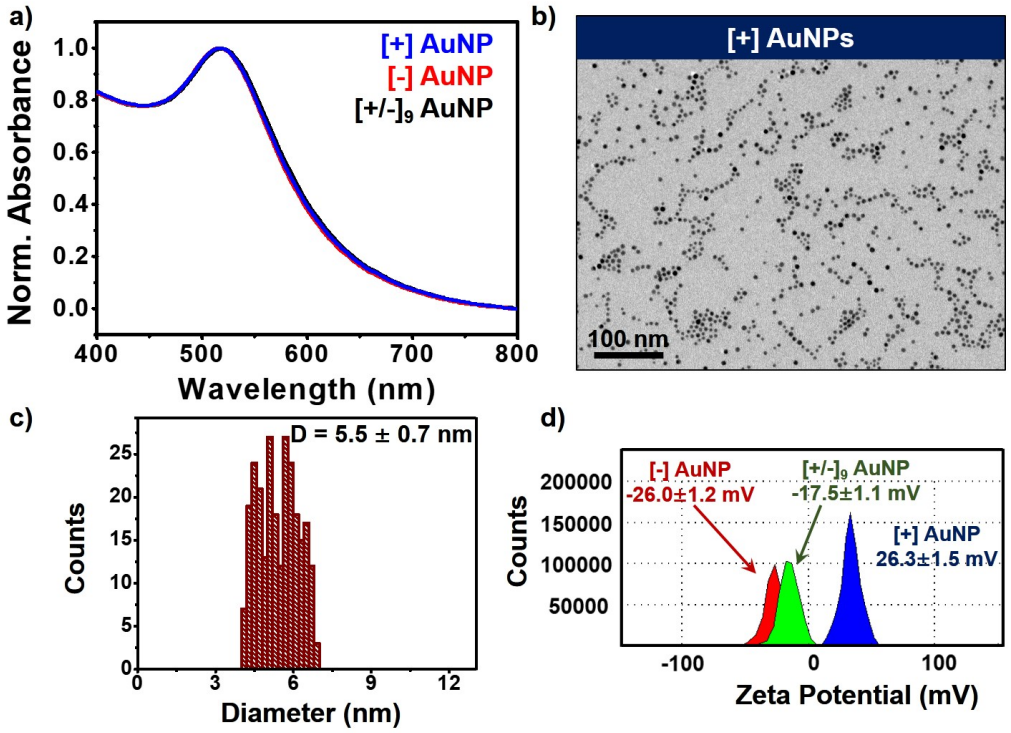


Figure 3.4: (a) UV-Visible absorption spectra of [+] (shown in green), [-] (shown in red) and $[+/-]_9$ AuNPs (shown in black). (b) Representative TEM image of [+] AuNPs with their size distribution histogram shown in (c). (d) Zeta potentials of [+], $[+/-]_9$, and [-] AuNPs were 26.3 ± 1.5 mV, -17.5 ± 1.1 mV, and -26.0 ± 1.2 mV, respectively.

(DDAB) was prepared. The solution was then sonicated for ∼5 min to completely solubilize the Au(III) salt. This was followed by a rapid injection of another 3 mL of toluene solution (containing 0.22 mmol of TBAB and 0.24 mmol of DDAB) to ensure the complete reduction of the Au(III) salt. The resulting DDA-AuNP dispersion (seeds) was aged for ∼24 h. These NP seeds were then grown to form 5.5 ± 0.7 nm sized DDA-AuNPs. For this, a growth solution containing 1 g of DDAB (2.2 mmol), 2.6 g of DDA (14 mmol), 224 mg of $HAuCl_4.3H_2O$ (0.57 mmol), and 10 mL of seed were prepared in 60 mL of toluene. Then, the growth solution was reduced by the dropwise addition (in ∼30 min) of 22 mL of toluene solution containing 300 μL of $N_2H_4.H_2O$ and 3.5 mg of TBAB. The solution was stirred overnight to yield monodisperse 5.5 ± 0.7 nm sized DDA-AuNPs (Figure 3.4a, b, c). The detailed procedure for the place exchange of DDA on AuNPs with different charged ligands is given below.

3.3.2 Place Exchange of AuNPs

DDA-AuNPs (20 mL) were first purified by quenching the reaction in methanol (50 mL), yielding a black precipitate. The DDA-AuNPs precipitate was then redissolved in 20 mL of toluene followed by the addition of 10 mL of dichloromethane solution of MUA (equal to the moles of Au(III) in the solution). The dispersion was kept undisturbed for ∼15 h to equilibrate. Next, the supernatant solution was decanted, and the precipitates were washed with dichloromethane (3 × 50 mL) followed by acetone (1 × 50 mL). The precipitate was then dried and redispersed in water, and 20 μL of TMAOH (25 wt % in water) was added to deprotonate the carboxylic acid groups in MUA. The average size of the Au nanoparticles thus synthesized was 5.5 ± 0.7 nm, as estimated from TEM analysis (∼300 NPs were counted for estimating the size distribution). A similar experimental procedure was adopted for the preparation of [+] AuNPs with TMA as the ligand. For the preparation of $[+/-]_9$ AuNPs, a mixture of [+] and [-] ligands in the molar ratio of 1:9 was fed during the place exchange reaction (Figure 3.4).

3.3.3 Synthesis of [+] - [-] Au Nanoionic Precipitates

The nanoionic precipitates composed of oppositely charged AuNPs were prepared according to the literature reports.[40,43] We prepared stock solutions of similar sized [+] and [-] AuNPs. In a typical precipitation experiment, dispersion of [+] AuNPs (~80 μM in terms of Au atoms) was titrated in a stirred vial by adding aliquots (~0.15 equiv) of [-] AuNP dispersion. After each addition, the dispersion was allowed to equilibrate for 15 min and was then transferred to a UV-Vis. cuvette for absorption measurements (see Figure 3.5). The addition continued until the AuNPs got precipitated (the precipitation occurred after the addition of ~65 μM [-] AuNPs).

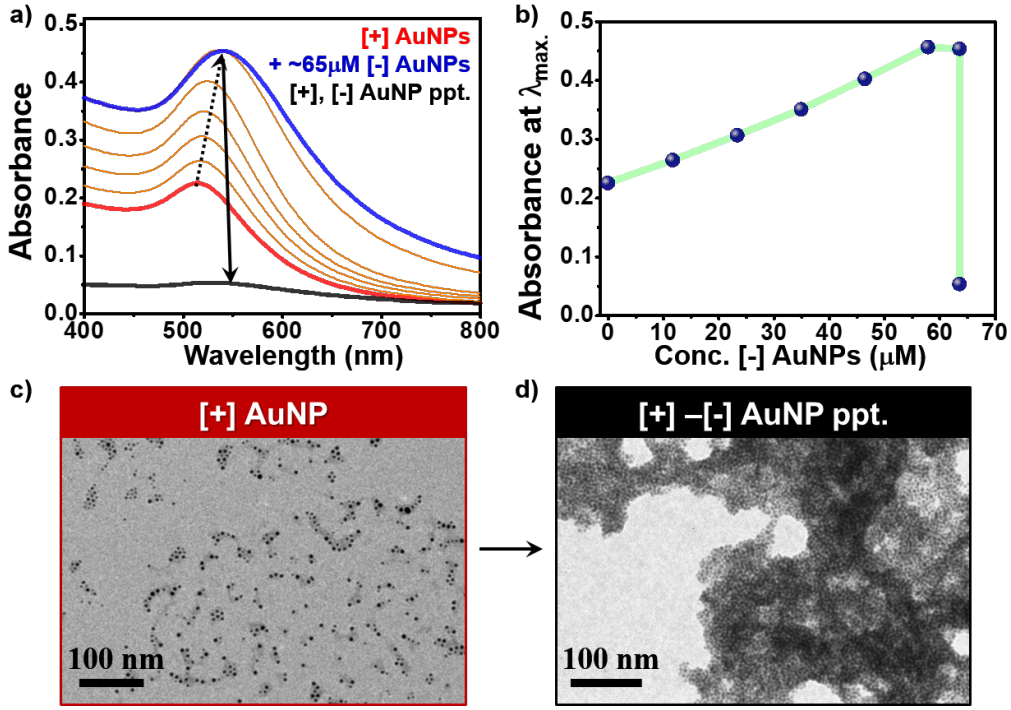


Figure 3.5: (a) Precipitation of oppositely charged AuNPs monitored by changes in the UV-Vis absorption spectrum of AuNPs. The curve shows the changes in the absorption spectrum of [+] AuNPs (shown in red), upon addition of aliquots of [-] AuNPs (shown by orange and blue curves), which leads to the formation of aggregates. This is accompanied by a gradual and continuous bathochromic shift in the λ_{max} from 515-555 nm, followed by an abrupt loss of plasmon band (shown in black). This is due to an ionic like sharp precipitation of [+] and [-] AuNPs at the charge neutrality ($\Sigma Q_{[+]} + \Sigma Q_{[-]} = 0$), confirming the formation of [+] - [-] Au nanoionic precipitates. (b) Variation in the surface plasmon band intensity of [+] AuNP at λ_{max} upon addition of different concertation of [-] AuNPs. The spectra show the sharp nature of precipitation of charged AuNPs from the solution. (c) TEM images of individual [+] AuNPs and [+] – [-] Au nanoionic precipitate.

A similar procedure was followed for the preparation of [+] - $[+/-]_9$ Au nanoionic precipitates. Here, ~80 μM $[+/-]_9$ AuNP (in terms of Au atoms) was titrated with aliquots (~0.15 equiv) of [+] AuNP dispersion. The sharp precipitation occurred after the addition of ~55 μM of [+] AuNPs. Approximately 70 % of [+] AuNPs was sufficient to form the [+] - $[+/-]_9$ Au nanoionic precipitates compared to [+] - [-] Au nanoionic precipitates, confirming the decrease in the strength of electrostatic attractions in [+] - $[+/-]_9$ Au nanoionic precipitates.

3.3.4 Response of [+] - [-] Au Nanoionic Precipitates in the Presence of Different M^{2+} Ions

The response of [+] - [-] Au nanoionic precipitates in the presence of different strong binding M^{2+} was monitored using UV-Vis. absorption studies. Here, we monitored the absorption spectrum of displaced [+] AuNPs upon addition of different amounts of M^{2+} ions. In a typical experiment, small aliquots (10-20 μL) from 50 mM stock solution of different M^{2+} were added, and the absorption spectrum of the redispersed [+] AuNPs was recorded. The limit of detection (LOD) corresponds to the point where a plasmonic wine-red color is clearly visible through the naked eye (corresponds to a change in absorbance of ~0.05). For identification studies in high ionic strength solutions, the supernatant of [+] - [-] Au nanoionic precipitates was replaced with a freshly prepared solution of 300 mM $NaNO_3$.

3.3.5 Titration Experiments for Estimating Binding Affinities

To ascertain the binding affinities of different aggregating triggers with [-] AuNPs, we performed a series of titration experiments. The minimum amount of the aggregating trigger needed to precipitate [-] AuNPs (transition point) was estimated, which is inversely related to their binding affinities. In a typical experiment to ascertain the binding affinity between [-] AuNPs and Pb^{2+}, the λ_{max} shifts of [-]

AuNPs (∼80 μM, in terms of gold atoms) were plotted against concentrations of Pb^{2+}. Briefly, we added a particular concentration (say 1 μM) of Pb^{2+} to a dispersion of [-] AuNPs and allowed it to equilibrate for ∼1 day, followed by recording the UV-Vis. spectrum. Similar experiments were performed for other concentrations of Pb^{2+} (10, 25, 50, 100, 150, 200, 300, and 500 μM). The λ_{max} values taken from each spectrum was plotted as a function of concentration of Pb^{2+}. The data were fitted with a sigmoidal fit to get the transition point (midpoint of the sigmoidal curve), which represents the minimum amount of Pb^{2+} required to precipitate [-] AuNPs. Similar experiments were performed with Cd^{2+}, Ca^{2+}, and [+] AuNPs for estimating the transition points.

3.4 Results and Discussion

3.4.1 Selective Turn-On Response towards Pb^{2+}

The AuNP systems with a core diameter of 5.5 ± 0.7 nm and varying surface chemistries were prepared by adopting a modified literature report (see Figure 3.4).[12,41,42] Nonionizable TMA ([+]) and ionizable MUA ([-]) ligands were functionalized on AuNPs to impart positive and negative surface charges, respectively (see Figure 3.4). The aggregation process of different AuNP systems in the presence of various M^{2+} ions was monitored by using time-dependent UV-Vis. absorption studies. As reported previously, [-] AuNPs complexed and ultimately precipitated out with different M^{2+} ions, confirming their inherent nonselectivity (Figure 3.6).[12,26,27] To overcome this, our approach was to use the abilities of different M^{2+} ions to break the interactions in inter-nanoparticle precipitates (*thermodynamically more stable state*)[33] and disperse them back to the solution (*kinetically trapped state*), see Figure 3.3. Accordingly, the inter-nanoparticle precipitates containing [-] AuNPs were prepared from an equimolar mixture of [+] and [-] AuNPs (Figure 3.5 and Figure 3.7a), as reported by Grzybowski and co-workers.[40] The addition of small aliquots (∼0.15 equiv) of [-] AuNP to [+] AuNP triggers the aggregation and plasmon cou-

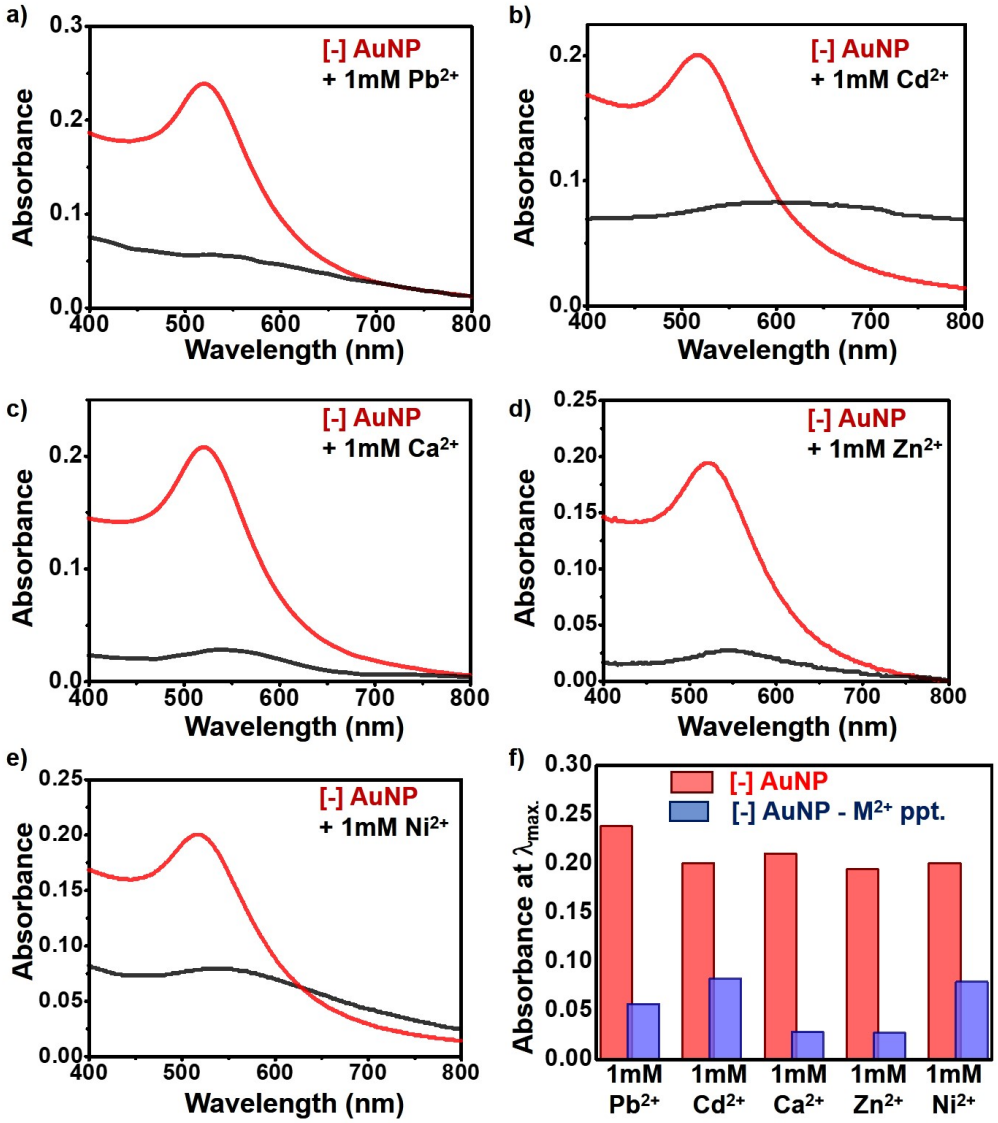


Figure 3.6: UV-Visible spectra of [-] AuNPs, before (shown in red) and after (shown in black) the addition of (a) 1 mM Pb^{2+}, (b) 1 mM Cd^{2+}, (c) 1 mM Ca^{2+}, (d) 1 mM Zn^{2+}, and (e) 1 mM Ni^{2+} . (f) Variation in the absorption intensity at $\lambda_{max.}$ of [-] AuNPs in the presence of different M^{2+} ions. The disappearance of plasmon peak after the addition of different M^{2+} (black spectrum) indicates precipitation of [-] AuNPs (turn-off response). [-] AuNPs complexed and ultimately precipitated with different M^{2+} ions uniformly, confirming their inherent nonselectivity.

pling phenomena through the strong electrostatic attraction. This is accompanied by a gradual bathochromic shift in the λ_{max} from 515 to 555 nm, followed by an abrupt loss of plasmon band (Figures 3.5). This is due to an ionic-like sharp precipitation of [+] and [-] AuNPs at the charge neutrality (i.e., when $\Sigma Q_{[+]} + \Sigma Q_{[-]} = 0$), confirming the formation of [+] - [-] Au nanoionic precipitates.[40] The nanoionic precipitation occurred, under stirring, well beyond the threshold values as reported by

Istvan and co-workers.[44]

The ability of various strongly binding M^{2+} ions to break the nanoionic precipitates was systematically studied (Figure 3.7). It was observed that the addition of Pb^{2+} ions to [+] - [-] Au nanoionic precipitates resulted in the revival of plasmon color to the solution (Figure 3.7b). For instance, a clear reappearance of wine-red color was observed after the addition of ~1 mM of Pb^{2+} ions (we define the limit

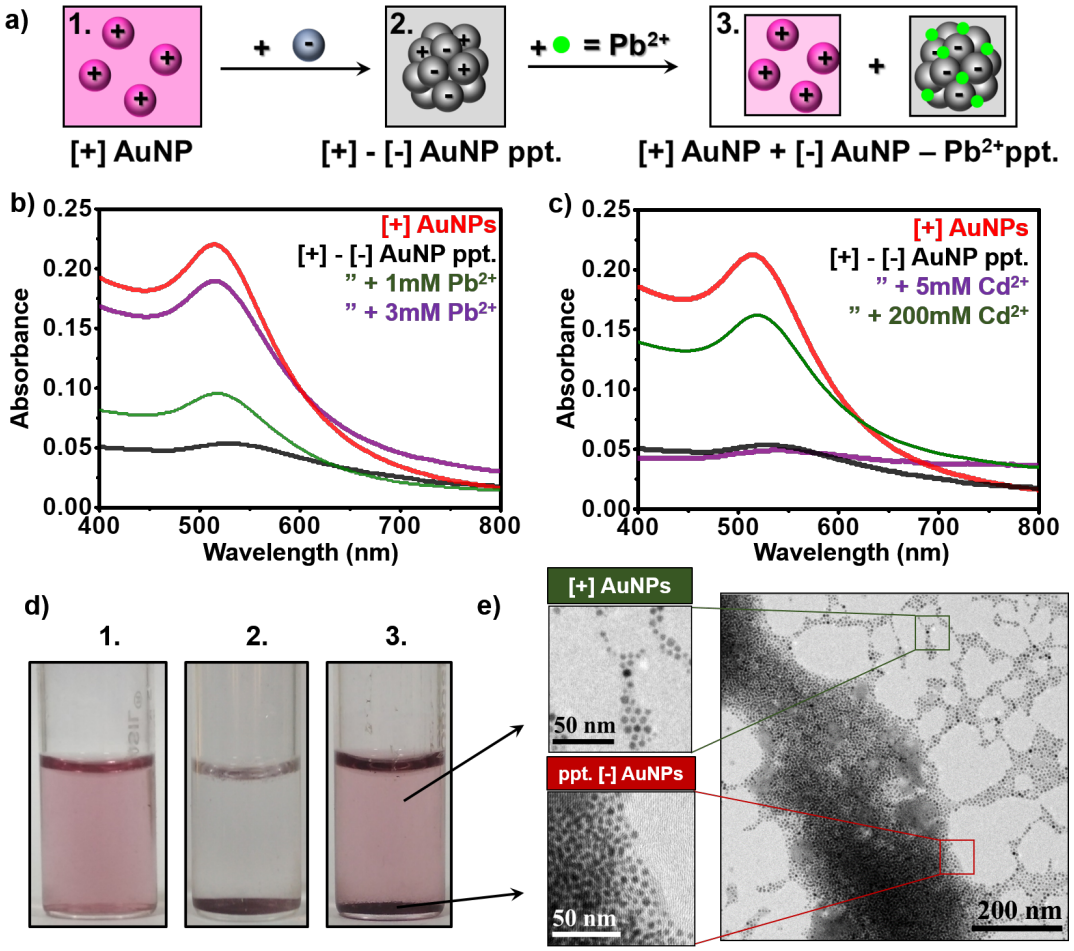


Figure 3.7: Turn-on response of [+] - [-] Au nanoionic precipitates in identifying Pb^{2+} ions. (a) Schematics for the preparation of [+] - [-] Au nanoionic precipitates and the turn-on detection of Pb^{2+} ion. Variation in the absorption of [+] - [-] Au nanoionic precipitates in the presence of (b) Pb^{2+} and (c) Cd^{2+} ions. An ~85% revival of plasmon band was observed upon the addition of 3 mM Pb^{2+} (with LOD = 1 mM, green spectrum), whereas even 3 mM Cd^{2+} ions failed to break the [+] - [-] Au nanoionic precipitates. (d) Photographs of the vials corresponding to stages 1, 2, and 3 in part (a). (e) A representative TEM image of [+] - [-] Au nanoionic precipitate after the addition of 3 mM Pb^{2+} ions. The enlarged TEM images on the left show the selected portions corresponding to dispersed [+] AuNP and [-] AuNP-Pb^{2+} precipitate.

of detection, LOD, as the point where a clear wine-red color can be visually seen- corresponding to $\Delta A \sim 0.05$, which is equivalent to 25% of initial plasmon intensity of [+] AuNPs, green spectrum in Figure 3.7b), while ∼85% of revival in the plasmon intensity was observed upon the addition of ∼3 mM of Pb^{2+} ions (purple spectrum in Figure 3.7b). The redispersed solution contained black precipitates corresponding to [-] AuNP-Pb^{2+} aggregates as well. The photographs of the solution at each stage are presented in Figure 3.7d, which clearly shows the revival of plasmon color and

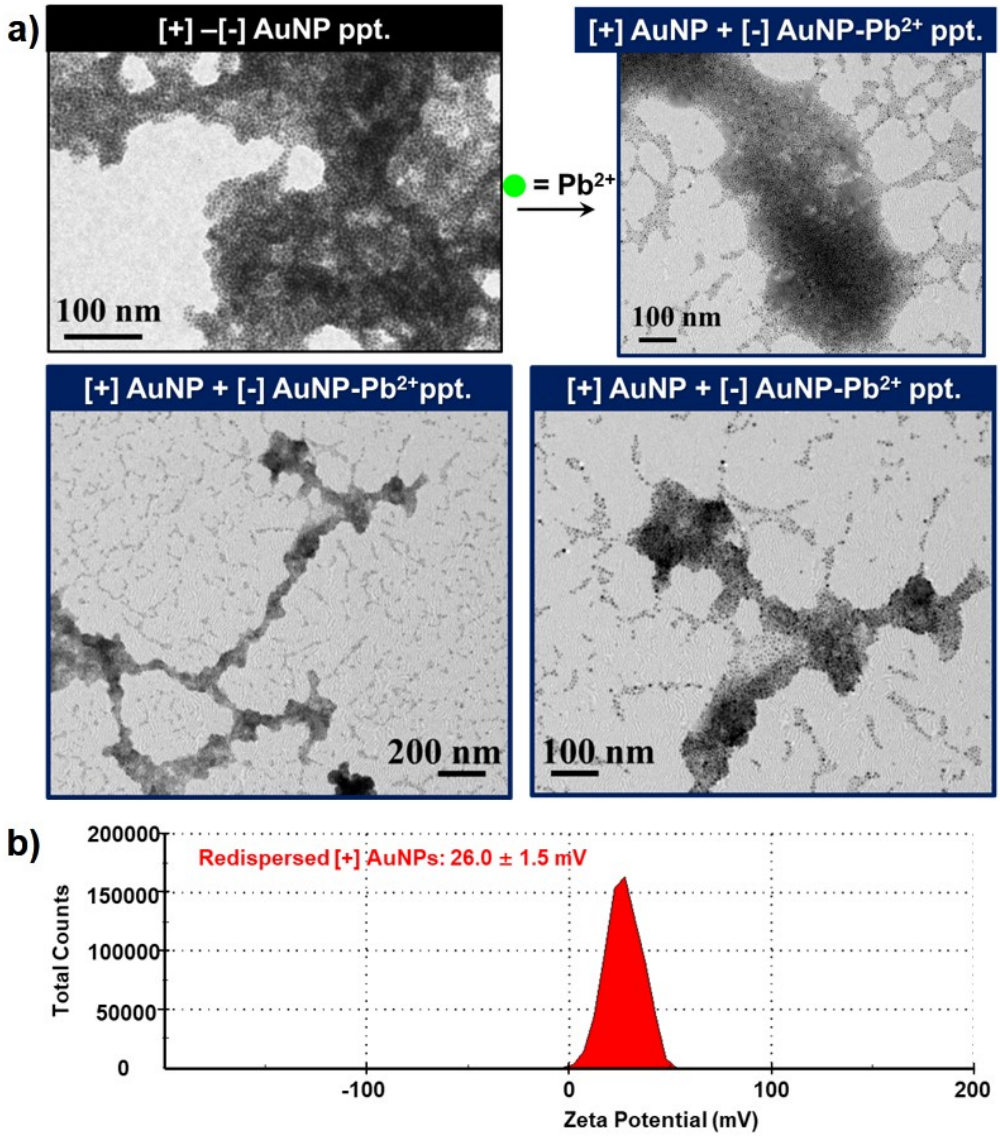


Figure 3.8: (a) TEM images of [+] – [-] Au nanoionic precipitate before and after the addition of 3 mM Pb^{2+} ions. The presence of displaced [+] AuNPs and [-] AuNP – Pb^{2+} precipitate is clearly visible.(b) Zeta Potential of redispersed AuNPs upon addition of 3 mM Pb^{2+} to [+] – [-] Au nanoionic precipitates.

the sedimented precipitates. The transmission electron microscope (TEM) images prove the leakage of [+] AuNPs from nanoionic precipitates, where both individual [+] AuNP and [-] AuNP-Pb^{2+} precipitates are clearly visible (Figures 3.7e, 3.8, 3.17). Interestingly, all the other M^{2+} ions (Cd^{2+}, Ni^{2+}, Zn^{2+} and Ca^{2+}), despite being capable of coordinating with individual [-] AuNPs, failed to revive the plasmon color under similar conditions, proving the selectivity of [+] - [-] Au nanoionic precipitates toward Pb^{2+} ions (Figures 3.7c, 3.11a, 3.18, 3.19). Even a mixture of other M^{2+} ions (interfering ions: Cd^{2+}, Ni^{2+}, Zn^{2+} and Ca^{2+}) was unable to break the interparticle interactions in [+] - [-] Au nanoionic precipitates (Figure 3.11a). On the other hand, a revival of plasmon band was observed when 3 mM Pb^{2+} ions were included in the mixture (Figure 3.11a). The other important ions like Hg^{2+} and Sn^{2+} were not included in this study due to their inappropriateness with the [+] - [-] Au nanoionic systems (see Section 3.7.2). In addition, a high concentration of Cd^{2+} ($\sim$200 mM) was able to break the electrostatic interactions in [+] - [-] Au nanoionic precipitates (green spectrum in Figure 3.7c). In comparison with Pb^{2+} ion, $\sim$200 times excess of Cd^{2+} ion was required to revive $\sim$50% of plasmon color to the solution. This confirms that the nature as well as the strengths of interaction between [-] AuNP and M^{2+} ions forms a key step in our identification protocol. A similar selectivity toward Pb^{2+} was obtained with [+] - [-] Au nanoionic precipitates that were aged for 2 weeks, with a lower revival in the plasmon intensity (see Figure 3.9a, b). Furthermore, the system retained its selectivity towards Pb^{2+} ions over Cd^{2+} even when bigger AuNPs (11.4 ± 1.2 nm) were used to prepare the [+] - [-] Au nanoionic precipitates (see Figure 3.9c-f). This confirms that preferential breaking of interactions can work well with larger AuNPs (having higher van der Waals interactions) as well , demonstrating the generality and flexibility of our identification protocol.

The identification strategy presented in our work uses the abilities of M^{2+} ions to carry out displacement of [+] AuNPs from a nanoparticle precipitate. The identification protocol, therefore, not only relies on how well M^{2+} can complex with [-]

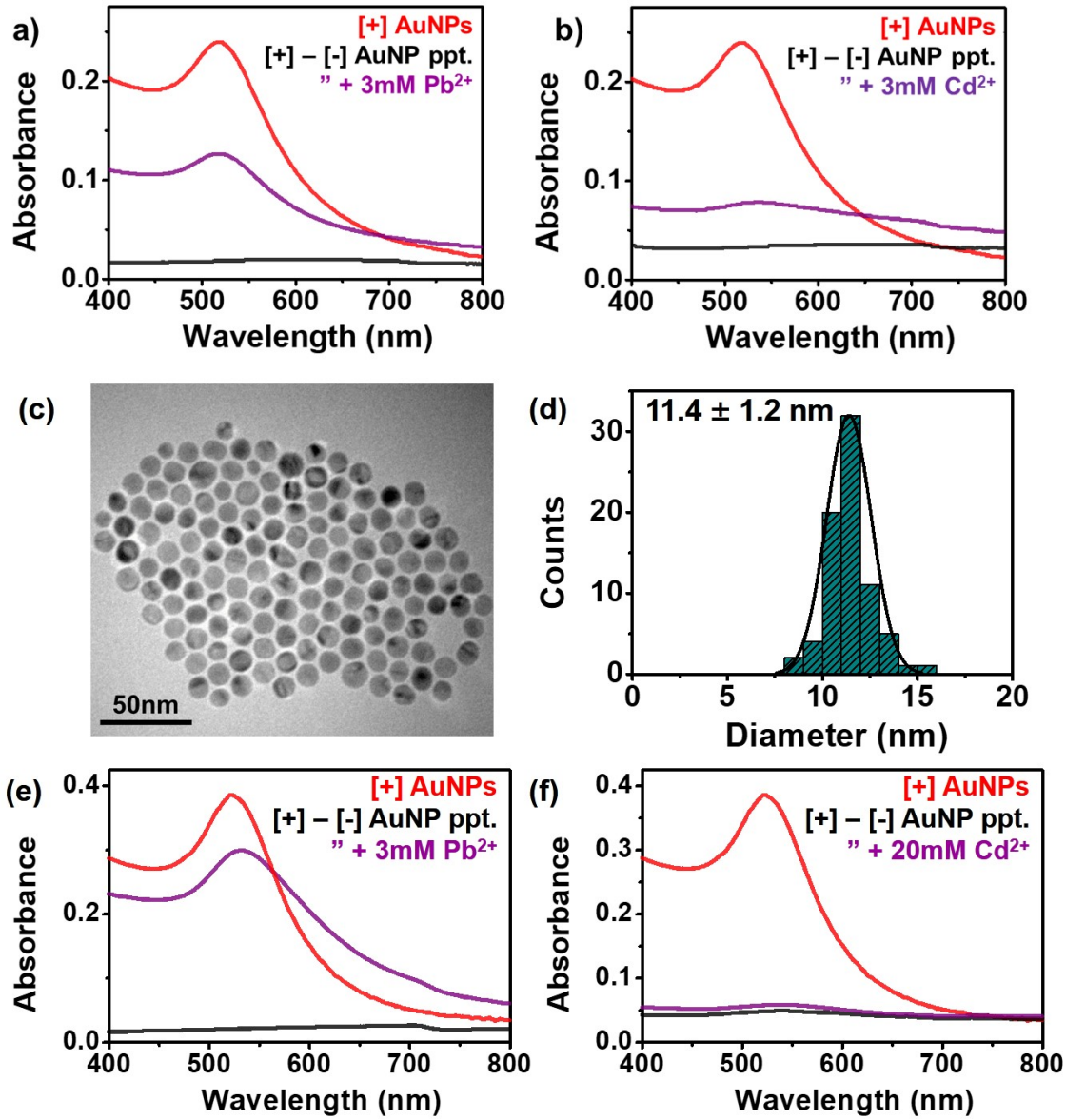


Figure 3.9: UV-Vis. absorption studies showing the revival of plasmon colour when [+] - [-] Au nanoionic precipitates (aged for 2 weeks) were exposed to (a) 3 mM Pb^{2+} and (b) 3 mM Cd^{2+} ions. (c) Representative TEM image of [+] AuNPs with their size distribution histogram shown in (d). Variation in the absorption of [+] - [-] Au nanoionic precipitates, formed from 11.4 ± 1.2 nm AuNPs, in the presence of (e) Pb^{2+} and (f) Cd^{2+} ions. A plasmon revival of ∼70 % was observed, as opposed to ∼85 % (with 5.5 nm AuNPs), upon addition of 3 mM Pb^{2+} ions. A possible reason for the lower plasmon revival could be the increase in the van der Waals attractions in nanoionic precipitates formed from 11.4 ± 1.2 nm AuNPs, making it difficult to break. More importantly, the system retained its selectivity towards Pb^{2+} over Cd^{2+} ions even with higher van der Waals, demonstrating the generality and flexibility of our identification protocol.

AuNPs, but with how well can they displace [+] AuNPs from [+] – [-] Au nanoionic precipitates (*'breaking of interactions'*). Figure 3.10a and b shows that neither [+]

nor [-] AuNPs possess selectivity towards M^{2+} ions. However, [+] – [-] Au nanoionic precipitate composed of such nonselective AuNPs demonstrate a selective turn on response (Figure 3.10c) towards Pb^{2+} over other M^{2+} ions used. This result was exciting and unprecedented because most of the protocols available in literature fail to differentiate between Pb^{2+} and Cd^{2+} without the use of analyte specific ligands.

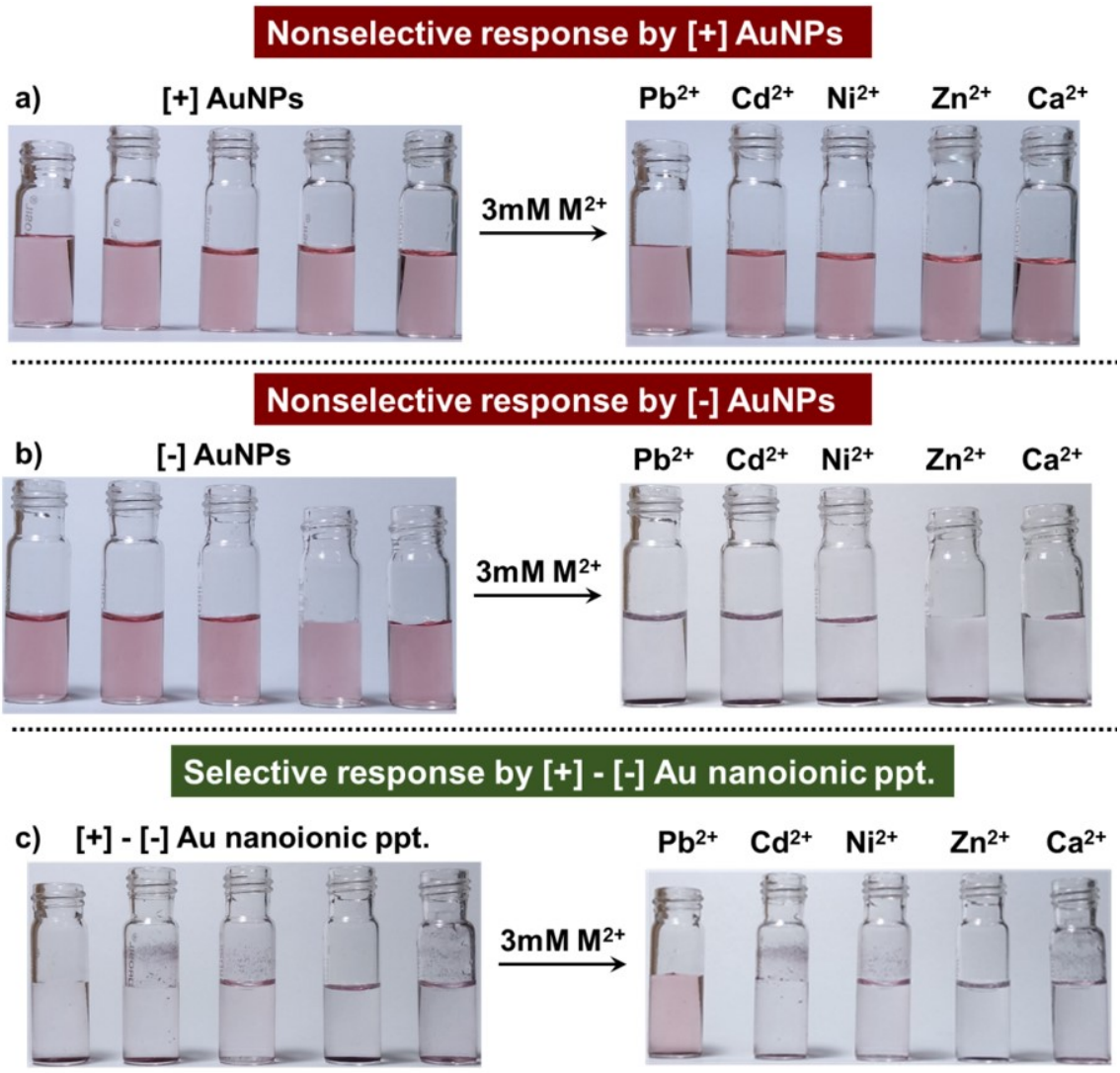


Figure 3.10: Optical photographs demonstrating the selectivity aspect of our identification protocol. Photographs showing the lack of ability of (a) [+] AuNPs, and (b) [-] AuNPs to selectively identify M^{2+} ions. [+] AuNPs demonstrate no color change in response to the addition of M^{2+} ions, while [-] AuNPs demonstrate a nonselective turn off response by invariably precipitating out from the solution, as shown in (b). (c) Nanoionic precipitates made from nonselective [+] and [-] AuNPs demonstrate selectivity towards Pb^{2+} ions by showing the release of plasmonic color (turn on response). In some of the vials, the precipitates are sticking to the sides of the vials.

3.4.2 Origins of the Selective Response

According to our hypothesis, the selectivity toward Pb^{2+} ions originates from the relative differences in the interaction strengths between [-] AuNPs and M^{2+} ion. To ascertain this, we have estimated the binding ability of various M^{2+} ions with [-] AuNP through two independent studies. First, titration experiments were performed between [-] AuNP and aliquots of different aggregation triggers ([+] AuNP, Pb^{2+}, Cd^{2+}, and Ca^{2+}; Figures 3.11, and 3.20). Figure 3.11b shows the variation in λ_{max} of [-] AuNP as a function of concentration of various aggregation triggers. The transition point (τ) that is estimated from the midpoint of the transition window signifies the minimum amount of titrant required to precipitate [-] AuNPs. The

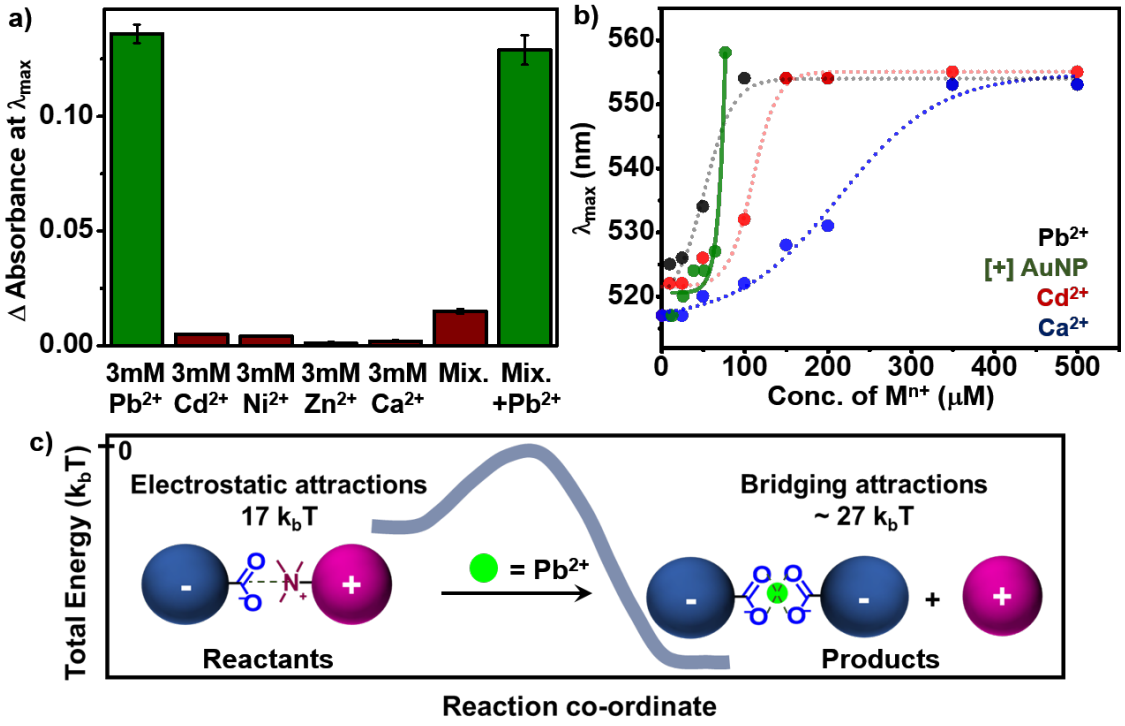


Figure 3.11: Selectivity of [+]–[-] Au nanoionic precipitates in identifying Pb^{2+} ions. (a) Effect of different M^{2+} ions and mixture of ions on the absorbance of [+]–[-] Au nanoionic precipitates. A revival of the plasmon band (∼85%) was observed only upon the addition of 3 mM Pb^{2+} ions, confirming the selective turn-on response. (b) Variation in the λ_{max} of [-] AuNP as a function of concentration of different aggregating triggers. The markers correspond to the experimental data, while the solid/dotted lines show the sigmoidal fits for the data. (c) Schematics showing the variation in energies of nanohybrid systems before and after the exposure to Pb^{2+} ions.

τ value describes the affinity between two interacting species, which is inversely proportional to (at least qualitatively) the magnitude of interaction energy.[45] The τ values for different aggregation triggers were estimated to be in the following order: $\tau(Pb^{2+}) \sim 50\ \mu M < \tau([+]\ AuNP) \sim 80\ \mu M < \tau(Cd^{2+}) \sim 110\ \mu M < \tau(Ca^{2+}) \sim 215\ \mu M$. The interaction energies between [-] AuNPs and M^{2+} ions, therefore, follow the reverse order: $Pb^{2+} > [+]$ AuNP $> Cd^{2+} > Ca^{2+}$. This series can now be read as a reactivity series, which is often used to summarize outcomes of a displacement reaction. The superior binding interactions between [-] AuNP and Pb^{2+} ions favor the displacement of [+] AuNP from nanoionic precipitates by Pb^{2+} ions, confirming our hypothesis. On the contrary, Cd^{2+} and Ca^{2+} ions failed to displace [+] AuNP from the nanoionic precipitates due to the higher τ values (and lower interaction energies). Even though there is an appreciable difference in the binding abilities of M^{2+} ions, the [-] AuNPs by itself fail to discriminate them. Interestingly, this difference in the binding abilities is good enough to preferentially break the [+] - [-] Au nanoionic precipitates, emphasizing the importance of our protocol.

Second, the interaction energies between NPs and M^{2+} ions were estimated using theoretical modeling of different interparticle interactions. In this model, we compared total energies of the nanohybrid systems before and after the exposure to different M^{2+} ions (Figure 3.11c).[14,46,47] The key forces holding the [+] - [-] Au nanoionic precipitates (reactants) are electrostatic and van der Waals interactions. Similarly, bridging and van der Waals interactions are the key forces responsible for [-] AuNP-M^{2+} complex formation (products). The detailed information about the process of modeling these interactions is given in section 3.7.3. Briefly, van der Waals attraction between two AuNPs in contact was modeled by using the Hamaker integral approximation[14] and was estimated to be $\sim 1.5 \times 10^{-21}$ J or $\sim 0.36 k_b T$. The electrostatic interactions between charged AuNPs in ionic solution ($c_s \sim 1$ mM)[47] were estimated by solving the electrostatic potential (ϕ) via thermodynamic integration.[47] We then solved the Poisson-Boltzmann equation (while accounting for '*charge regulation*') for two interacting spheres,[14,48] and the electrostatic attraction

energy between oppositely charged AuNP pair was estimated to be $\sim 6.2 \times 10^{-20}$ J or $\sim 17 k_b T$. This is in close agreement with the values reported by Grzybowski and co-workers.[14,49] The magnitude of the bridging interaction in the [-] AuNP-M^{2+} complex was estimated by modifying a reported equilibrium model of the cross-linking interactions[46] and using reported values for binding constants of Pb^{2+} and Cd^{2+} ions with acetate groups.[50,51] Based on the modeling studies, Pb^{2+} ions have the highest interaction energy with [-] AuNP ($\sim 1.1 \times 10^{-19}$ J or $\sim 27 k_b T$) followed by [+] AuNP ($\sim 6.2 \times 10^{-20}$ J or $\sim 17 k_b T$) and Cd^{2+} ions ($\sim 1.8 \times 10^{-20}$ J or $\sim 4.34 k_b T$). The estimated energy values reiterate superior interaction of Pb^{2+} ions with [-] AuNP and their ability to displace [+] AuNP from nanoionic precipitates, resulting in the desired selectivity.

3.4.3 Versatile and Tunable Identification Protocol

Next we discuss the versatility and tunability in the identification ability of Au nanoionic precipitates. The selectivity stems from the preferential breaking of electrostatic interactions in [+] - [-] Au nanoionic precipitates. Thus, a variation in the strength of electrostatic forces can, in principle, tune the selectivity and sensitivity for different analytes of interest. Moreover, the presence of [-] AuNP - Pb^{2+} precipitate in the final solution is undesirable, and can be circumvented by decreasing the strength of bridging attractions in the complex. Accordingly, the nanoionic precipitates were prepared by using heterogeneously charged [+/-] AuNP instead of homogeneously charged [-] AuNP (Figure 3.12a). The heterogeneously charged $[+/-]_9$ AuNP were synthesized by a place exchange reaction with 1:9 mixture of [+] and [-] ligands. The on-NP ratio of [+]:[-] was estimated to be 1:7 by using previously reported relative binding affinities of [+] and [-] ligands ($\frac{K_{[+]}}{K_{[-]}} = 1.2$). Here $K_{[+]}$ and $K_{[-]}$ are the equilibrium constants for the adsorption of [+] and [-] ligands onto AuNP, respectively.[12,29] Similar to the titration behavior of homogeneously charged AuNPs, the mixture of $[+/-]_9$ and [+] AuNPs precipitated sharply at the charge neutrality (Figures 3.12b, c). Interestingly, $\sim$70% of [+] AuNPs was sufficient to

form the [+] - [+/-]$_9$ Au nanoionic precipitates compared to [+] - [-] Au nanoionic precipitates. This confirms the decrease in the strength of electrostatic attractions in [+] - [+/-]$_9$ Au nanoionic precipitates. Consequently, the LOD for Pb^{2+} ion was improved to ~20 μM with [+] - [+/-]$_9$ Au nanoionic precipitates (green curve in

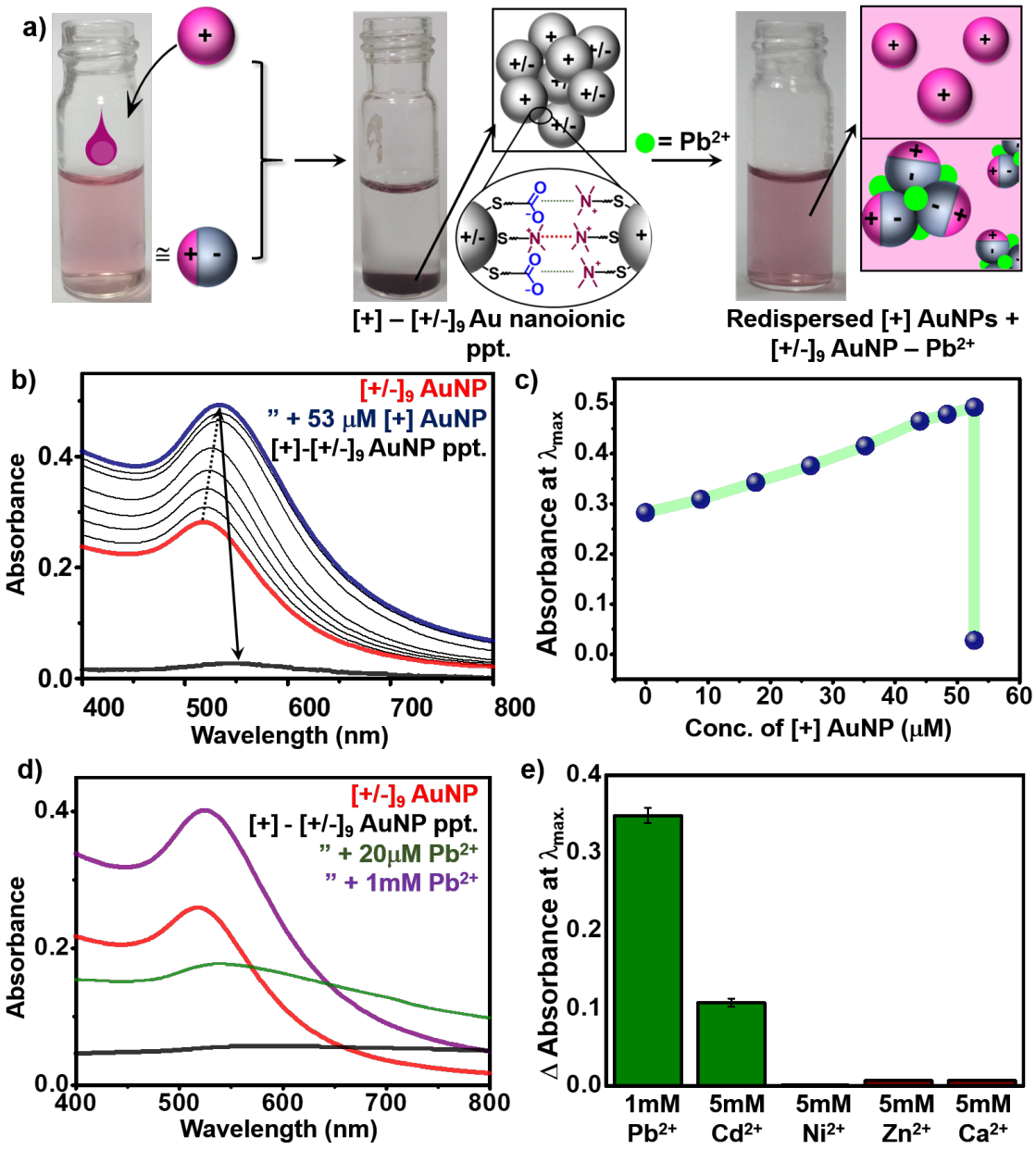


Figure 3.12: Versatility and tunability of Au nanoionic precipitates. (a) Scheme for the formation of [+] and [+/-]$_9$ Au nanoionic precipitates and amplification in the selective turn-on detection of Pb^{2+} ion. Variation in the (b) absorbance and (c) absorbance at λ_{max} of [+/-]$_9$ AuNP upon addition of [+] AuNP. (d) Redispersal of [+] and [+/-]$_9$ Au nanoionic precipitates in the presence of 1 mM Pb^{2+} ion (with LOD of 20 μM, green spectrum). (e) Selectivity of [+]–[+/-]$_9$ Au nanoionic precipitates in identifying Pb^{2+} and Cd^{2+} ions.

Figure 3.12d). The sensitivity of [+] - $[+/-]_9$ Au nanoionic precipitate is comparable to or even better than other reported NP systems with analyte nonspecific ligands (Figure 3.13). More importantly, the plasmon intensity of the redispersed nanoionic precipitates was higher than both of the individual $[+/-]_9$ and [+] AuNPs (close to the additive intensity; purple spectrum in Figure 3.12d). This confirms the complete redispersal of [+] - $[+/-]_9$ Au nanoionic precipitates in contrast to the partial redispersal of [+] - [-] Au nanoionic precipitates, which is an important requisite for an ideal detection system. In addition, 3 mM Cd^{2+} ions was also able to break the electrostatic interactions in [+] - $[+/-]_9$ Au nanoionic precipitates, with an ∼50% revival of plasmon band (Figure 3.12e). The decrease in the strength of the electrostatic tethers in [+] - $[+/-]_9$ Au nanoionic precipitates results in a turn-on response for Pb^{2+} and Cd^{2+} ions, demonstrating the tunability in the selectivity of toxic ions as per the demand. However, even 3 mM Ni^{2+}, Zn^{2+}, and Ca^{2+} ions failed to break the

M^{2+}	Nanoparticle System	Optical Sensitivity	Reference
Pb^{2+}	MUA – AuNPs	400 μM	*Nano Lett.* 2001, **1,** 165–167
Pb^{2+}	MUA – AuNPs	>50 μM	*Anal. Methods* 2016, **8,** 7232–7236
Pb^{2+}	Gallic Acid – AuNPs	5 -150 μM	*J. Phys. Chem. C* 2007, **111,** 12839–12847
Pb^{2+}	MUA – AuNPs	10 μM	*Sensors* 2012, **12,** 9467–9475
Pb^{2+}	MUA – AuNPs (Amino Acids)	2 - 50 μM	*ACS Appl. Mater. Interfaces* 2014, **6,** 18395–18400
Pb^{2+}	[+] – [-] Au nanoionic precipitate	1 mM	*Present work (ACS Appl. Nano Mater. 2019, **2**, 5625 - 5633)*
Pb^{2+}	[+] – $[+/-]_9$ AuNP nanoionic precipitate	20 μM	*Present work (ACS Appl. Nano Mater. 2019, **2**, 5625 - 5633)*

Figure 3.13: Fine-tuning of electrostatic interactions in the nanoionic precipitates leads to the improvement in the Pb^{2+} ion detection. Table comparing the sensitivity towards Pb^{2+} by AuNP systems with analyte non-specific ligands.

electrostatic interaction in [+] and $[+/-]_9$ Au nanoionic precipitates (Figures 3.12e, 3.21). It is worth mentioning that there are previous reports on the selective detection of Pb^{2+} and Cd^{2+} using metal NPs bearing analyte specific ligands.[52] However, the present work is conceptually different as it relies on the preferential breaking of electrostatic interactions in nanosystems deprived of analyte specific ligands.

The process of breaking of electrostatic interactions in [+] - $[+/-]_9$ Au nanoionic precipitates by Pb^{2+} ions was further monitored by using atomic force microscopy (AFM) and dynamic light scattering (DLS) studies. The presence of small and controlled AuNP aggregates (60-180 nm) was clearly visible in the AFM images presented in Figure 3.14. The 3D AFM height image shows the ripples demarcating the boundaries between the individual NPs constituting the aggregates (Figure

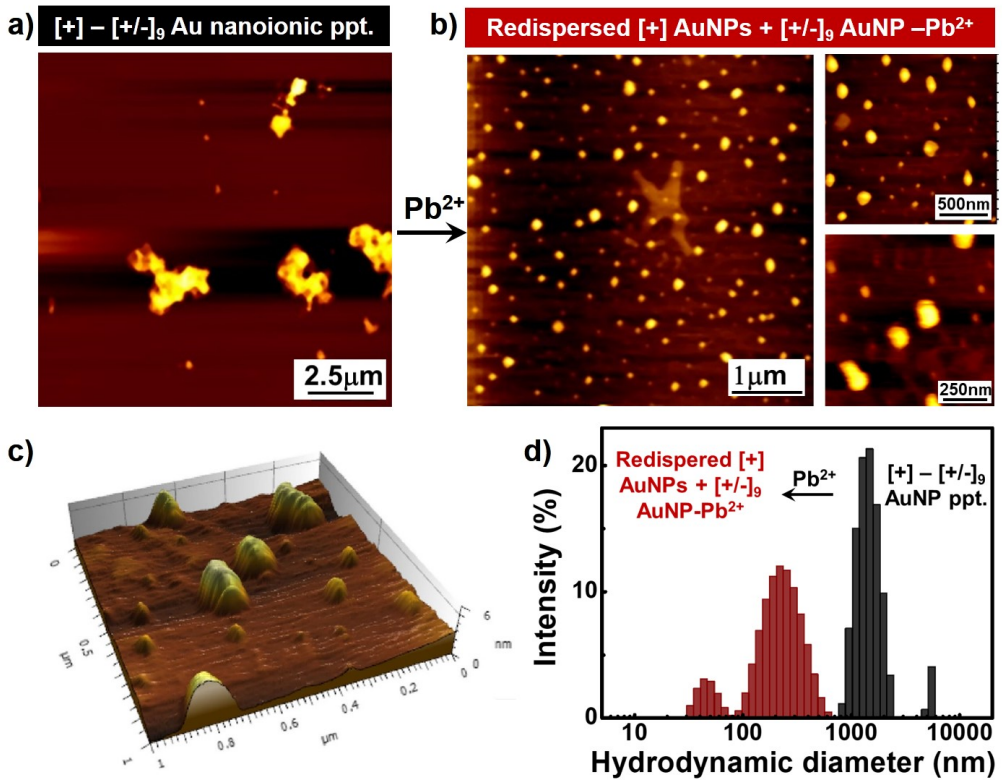


Figure 3.14: AFM and DLS studies for the breaking of electrostatic interactions in [+] - $[+/-]_9$ Au nanoionic precipitates by Pb^{2+} ions. AFM images of [+]–$[+/-]_9$ Au nanoionic precipitates (a) before and (b) after the addition of 1 mM Pb^{2+} ions. (c) 3D AFM height image showing the ripples demarcating the boundaries between the individual NPs constituting the aggregates. (d) DLS data showing the decrease in the hydrodynamic diameter from a few micrometers to 60–180 nm upon the addition of Pb^{2+} ions.

3.14c). The AFM results were well complemented by DLS studies, which confirms the breaking of [+] - $[+/-]_9$ Au nanoionic precipitates by Pb^{2+} to smaller aggregates (Figure 3.14d). Moreover, an ∼10 nm bathochromic shift in the λ_{max} of the redispersed sample confirms the formation of controlled aggregates between $[+/-]_9$ AuNP and Pb^{2+} ions (Figure 3.12d). Thus, the stability and dispersion of $[+/-]_9$ AuNP-Pb^{2+} complex is attributed to the perfect balance between attractive and repulsive interactions in the controlled aggregates, as reported previously.[12]

3.5 Conclusions

This work presents a straightforward, yet effective strategy to transform the commonly observed nonselective turn-off response of [-] AuNP into an attractive selective turn-on response for the identification of heavy metal ions. The difference in the abilities of various M^{2+} ions to break the interactions between the oppositely charged AuNPs (nanoionic precipitates) was chosen as the means of discrimination rather than the conventional method of forming an interaction. Among various M^{2+} ions tested, Pb^{2+} was solely able to break the electrostatic interactions in [+] - [-] Au nanoionic precipitates. The displaced [+] AuNPs imparted the characteristic wine-red color to the solution, resulting in a turn-on response. The favorable interaction energy for [-] AuNP-Pb^{2+} complexation is accountable for the discrimination of Pb^{2+} from other M^{2+} ions, including the Cd^{2+} ions. The fine-tuning of electrostatic interactions in the nanoionic precipitates helped in enhancing the Pb^{2+} ion sensitivity along with a complete redispersal of both the sets of AuNPs. The flexibility and tunability of identification were demonstrated by extending the selectivity toward the Cd^{2+} ion using [+] - $[+/-]_9$ Au nanoionic precipitates. The ability to control the electrostatic and bridging interactions was crucial in imparting selectivity to a nanohybrid system composed of constituents that are inherently nonselective. Despite having a lower sensitivity compared to the reported literature with analyte specific AuNPs, the present work demonstrates a unique strategy of selectively identifying heavy metal ions. The concept of breaking '*known*' strengths

of interactions through displacement reaction can help in ascertaining the strengths of '*unknown*' interactions, which can find far-reaching applications in fundamental as well as applied areas of nanoscience. In addition, the present demonstration of emergence of an inherently absent functionality in a system of interacting NPs will be of interest in the new emerging areas of out-of-equilibrium self-assembly and systems chemistry.

3.6 Future Directions

In the present chapter, we demonstrate that our fundamentally unique approach of using breaking of interactions as an identification protocol, could impart remarkable selectivity towards Pb^{2+} to a system, deprived of any analyte specific ligand (*emergence of a new property*). Furthermore, this strategy allowed us to transform a traditionally turn-off way of identification into a much more attractive turn-on means of identification. Also, a judicious control over the interparticle interactions allowed us to tune both the selectivity and sensitivity of our identification protocol. One can use this strategy of breaking known interactions to ascertain the strengths of unknown ones. After having demonstrated the potency of a control over interparticle interactions to create stimuli-responsive systems with (a) improved properties, and (b) inherently absent properties. We wanted to study whether a control over interactions can be used to create systems with *'life-like'* properties.

References

(1) Grzybowski, B. A.; Huck, W. T. S. *Nat. Nanotechnol.* **2016**, *11*, 585–592.

(2) Ashkenasy, G.; Hermans, T. M.; Otto, S.; Taylor, A. F. *Chem. Soc. Rev.* **2017**, *46*, 2543–2554.

(3) Grzelczak, M.; Liz-Marzán, L. M.; Klajn, R. *Chem. Soc. Rev.* **2019**, *48*, 1342–1361.

(4) Yan, Y.; Timonen, J. V. I.; Grzybowski, B. A. *Nat. Nanotechnol.* **2014**, *9*, 901–906.

(5) Zhao, H.; Sen, S.; Udayabhaskararao, T.; Sawczyk, M.; Kucanda, K.; Manna, D.; Kundu, P. K.; Lee, J.-W.; Kral, P.; Klajn, R. *Nat. Nanotechnol.* **2016**, *11*, 82–88.

(6) Li, D.; He, Q.; Cui, Y.; Li, J. *Chem. Mater.* **2007**, *19*, 412–417.

(7) Boekhoven, J.; Hendriksen, W. E.; Koper, G. J. M.; Eelkema, R.; Esch, J. H. v. *Science* **2015**, *349*, 1075–1079.

(8) Jain, A.; Dhiman, S.; Dhayani, A.; Vemula, P. K.; George, S. J. *Nat. Commun.* **2019**, *10*, 1–9.

(9) Kundu, P. K.; Samanta, D.; Leizrowice, R.; Margulis, B.; Zhao, H.; Börner, M.; Udayabhaskararao, T.; Manna, D.; Klajn, R. *Nat. Chem.* **2015**, *7*, 646–652.

(10) Klajn, R.; Wesson, P. J.; Bishop, K. J. M.; Grzybowski, B. A. *Angew. Chem. Int. Ed.* **2009**, *48*, 7035–7039.

(11) Leira-Iglesias, J.; Tassoni, A.; Adachi, T.; Stich, M.; Hermans, T. M. *Nat. Nanotechnol.* **2018**, *13*, 1021–1027.

(12) Rao, A.; Roy, S.; Unnikrishnan, M.; Bhosale, S. S.; Devatha, G.; Pillai, P. P. *Chem. Mater.* **2016**, *28*, 2348–2355.

(13) Batista, C. A. S.; Larson, R. G.; Kotov, N. A. *Science* **2015**, *350*, 1242477.

(14) Bishop, K. J. M.; Wilmer, C. E.; Soh, S.; Grzybowski, B. A. *Small* **2009**, *5*, 1600–1630.

(15) Min, Y.; Akbulut, M.; Kristiansen, K.; Golan, Y.; Israelachvili, J. *Nat. Mater.* **2008**, *7*, 527–538.

(16) Boles, M. A.; Engel, M.; Talapin, D. V. *Chem. Rev.* **2016**, *116*, 11220–11289.

(17) Taniguchi, Y.; Sazali, M. A. B.; Kobayashi, Y.; Arai, N.; Kawai, T.; Nakashima, T. *ACS Nano* **2017**, *11*, 9312–9320.

(18) Pramod, P.; Joseph, S. T. S.; Thomas, K. G. *J. Am. Chem. Soc.* **2007**, *129*, 6712–6713.

(19) Roy, S.; Rao, A.; Devatha, G.; Pillai, P. P. *ACS Catal.* **2017**, *7*, 7141–7145.

(20) Devatha, G.; Roy, S.; Rao, A.; Mallick, A.; Basu, S.; Pillai, P. P. *Chem. Sci.* **2017**, *8*, 3879–3884.

(21) Pillai, P. P.; Kowalczyk, B.; Kandere-Grzybowska, K.; Borkowska, M.; Grzybowski, B. A. *Angew. Chem., Int. Ed.* **2016**, *55*, 8610–8614.

(22) Chakraborty, A.; Dalal, C.; Jana, N. R. *Langmuir* **2018**, *34*, 13461–13471.

(23) Maity, A. R.; Saha, A.; Roy, A.; Jana, N. R. *ChemPlusChem* **2013**, *78*, 259–267.

(24) Mandal, K.; Jana, D.; Ghorai, B. K.; Jana, N. R. *New J. Chem.* **2018**, *42*, 5774–5784.

(25) Roy, S.; Roy, S.; Rao, A.; Devatha, G.; Pillai, P. P. *Chem. Mater.* **2018**, *30*, 8415–8419.

(26) Kim, Y.; Johnson, R. C.; Hupp, J. T. *Nano Lett.* **2001**, *1*, 165–167.

(27) Saha, K.; Agasti, S. S.; Kim, C.; Li, X.; Rotello, V. M. *Chem. Rev.* **2012**, *112*, 2739–2779.

(28) Devatha, G.; Rao, A.; Roy, S.; Pillai, P. P. *ACS Energy Lett.* **2019**, *4*, 1710–1716.

(29) Pillai, P. P.; Kowalczyk, B.; Pudlo, W. J.; Grzybowski, B. A. *J. Phys. Chem. C* **2016**, *120*, 4139–4144.

(30) Jeffery, G. H.; Bassett, J.; Mendham, J.; Denney, R. C., *Vogels Textbook Of Quantitative Chemical Analysis*, 5th ed.; Pearson Education India: 2006.

(31) Kowalczyk, B.; Walker, D. A.; Soh, S.; Grzybowski, B. A. *Angew. Chem., Int. Ed.* **2010**, *49*, 5737–5741.

(32) Liu, J.; Lu, Y. *J. Am. Chem. Soc.* **2003**, *125*, 6642–6643.

(33) Sokolov, S. V.; Katelhon, E.; Compton, R. G. *J. Phys. Chem. C* **2015**, *119*, 25093–25099.

(34) Storhoff, J. J.; Elghanian, R.; Mucic, R. C.; Mirkin, C. A.; Letsinger, R. L. *J. Am. Chem. Soc.* **1998**, *120*, 1959–1964.

(35) Yoosaf, K.; Ipe, B. I.; Suresh, C. H.; Thomas, K. G. *J. Phys. Chem. C* **2007**, *111*, 12839–12847.

(36) Liu, J.; Lu, Y. *J. Am. Chem. Soc.* **2004**, *126*, 12298–12305.

(37) Lee, J. H.; Wang, Z.; Liu, J.; Lu, Y. *J. Am. Chem. Soc.* **2008**, *130*, 14217–14226.

(38) Lin, S.-Y.; Wu, S.-H.; Chen, C.-h. *Angew. Chem., Int. Ed.* **2006**, *45*, 4948–4951.

(39) Liu, D.-B.; Qu, W.-S.; Chen, W.-W.; Zhang, W.; Wang, Z.; Jiang, X.-Y. *Anal. Chem.* **2010**, *82*, 9606–9610.

(40) Kalsin, A. M.; Kowalczyk, B.; Smoukov, S. K.; Klajn, R.; Grzybowski, B. A. *J. Am. Chem. Soc.* **2006**, *128*, 15046–15047.

(41) Jana, N. R.; Peng, X. *J. Am. Chem. Soc.* **2003**, *125*, 14280–14281.

(42) Pillai, P. P.; Huda, S.; Kowalczyk, B.; Grzybowski, B. A. *J. Am. Chem. Soc.* **2013**, *135*, 6392–6395.

(43) Kalsin, A. M.; Fialkowski, M.; Paszewski, M.; Smoukov, S. K.; Bishop, K. J. M.; Grzybowski, B. A. *Science* **2006**, *312*, 420–424.

(44) Nakanishi, H.; Deak, A.; Hollo, G.; Lagzi, I. *Angew. Chem., Int. Ed.* **2018**, *57*, 16062–16066.

(45) Eibling, M. J.; MacDermaid, C. M.; Qian, Z.; Lanci, C. J.; Park, S.-J.; Saven, J. G. *J. Am. Chem. Soc.* **2017**, *139*, 17811–17823.

(46) Wei, Y.; Bishop, K. J. M.; Kim, J.; Soh, S.; Grzybowski, B. A. *Angew. Chem., Int. Ed.* **2009**, *48*, 9477–9480.

(47) Bishop, K. J. M.; Kowalczyk, B.; Grzybowski, B. A. *J. Phys. Chem. B* **2009**, *113*, 1413–1417.

(48) Carnie, S. L.; Chan, D. Y. C.; Gunning, J. S. *Langmuir* **1994**, *10*, 2993–3009.

(49) Bishop, K. J. M.; Grzybowski, B. A. *ChemPhysChem* **2007**, *8*, 2171–2176.

(50) Gobom, S. *Nature* **1963**, *197*, 283–4.

(51) Ferrell, E.; Ridgion, J. M.; Riley, H. L. *J. Chem. Soc.* **1934**, 1440–7.

(52) Kumar, V. V.; Anthony, S. P. *Sens. Actuators B Chem.* **2014**, *191*, 31–36.

3.7 Appendix

3.7.1 Calculation of Concentrations of AuNPs

Concentration Calculation of [+] AuNPs

Absorbance of [+] AuNPs in cuvette: ∼0.20 - 0.24

We have, through ICP-MS studies, estimated that a solution having an absorbance of ∼0.61, has a concentration of ∼0.2 mM (in terms of Au atoms). Similar values are established in the literature as well.[1]

Concentration of [+] AuNPs in cuvette: **∼80.0 μM (in ∼3 mL)**

Concentration Calculation of [-] AuNPs

For the titration experiments, we added a total of ∼55 μLL of [-] AuNPs in different aliquots (from a∼10 times diluted solution) to get the nanoionic precipitate.

Concentration of diluted [-] AuNPs stock solution: ∼3.6 mM

Total volume of [-] AuNPs added to the cuvette: 55.0 μL

Concentration of [-] AuNPs in the cuvette: **∼65.0 μM (in ∼3 mL)**

3.7.2 Effect of Hg^{2+} Salts and Sn^{2+} Salts

Hg^{2+} ions are well known to desorb the thiols from the surface of NPs, leading to their precipitation.[2] Additionally, salts of Hg^{2+} ($HgCl_2$, $Hg(NO_3)_2$) and Sn^{2+} ($SnCl_2$, $SnSO_4$) require high amount of acid to achieve stable dispersion in water (pH 2-4).

Experiments Performed with Hg^{2+} Salts

It should be noted that an appreciable amount of acid was required to prepare the stock solution of $Hg(NO_3)_2$ in water. The addition of 3 mM of $Hg(NO_3)_2$ to [+] – [-] AuNP nanoionic precipitate resulted in the revival of plasmon band (magenta curve in Figure 3.15a). A closer examination revealed that the pH of the solution changed to ~2 upon addition of 3 mM of $Hg(NO_3)_2$. A similar breaking of electrostatic forces in [+] – [-] AuNP nanoionic precipitate was observed when a control experiment was performed by adding equivalent amount of acid (green curve in Figure 3.15a). This proves that the nanoionic precipitates are not stable at pH ~2, as mentioned before. Thus, the studies with $Hg(NO_3)_2$ failed to provide any conclusive evidence for the detection of Hg^{2+} ion by the nanoionic precipitates. Further, we used $HgCl_2$ as the source of Hg^{2+} ions. A lower amount of acid was required to solubilize $HgCl_2$ in water as compared to $Hg(NO_3)_2$. No appreciable revival of plasmon band was

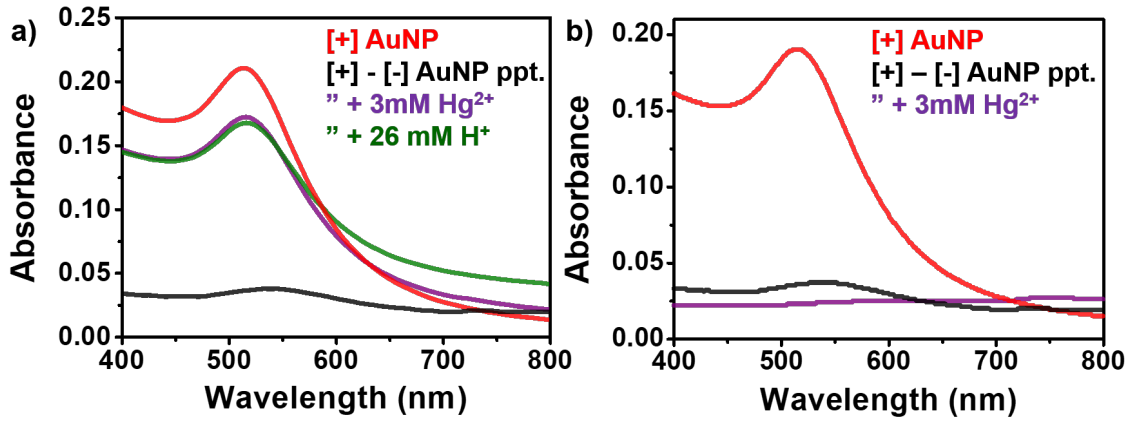


Figure 3.15: (a) Variation in the absorption of [+] – [-] Au nanoionic precipitates in the presence of 3 mM $Hg(NO_3)_2$ (magenta curve), and in the presence of equivalent amount of acid (green curve). (b) Absorption spectra of [+] – [-] Au nanoionic precipitates in the absence (black curve) and presence of 3 mM $HgCl_2$ (magenta curve).

observed upon the addition of 3 mM of $HgCl_2$ to [+] – [-] AuNP nanoionic precipitate (magenta curve in Figure 3.15b). The addition of 3 mM of $HgCl_2$ shifted the pH to $\sim$ 4, at which [+] – [-] AuNP nanoionic precipitate was found to retain its stability. Thus, it can be concluded that Hg^{2+} ions fail to break the electrostatic attraction in the [+] – [-] AuNP nanoionic precipitate.

Experiments Performed with Sn^{2+} Salts

The stock solution of $SnSO_4$ was prepared in water by the addition of $\sim$ 250 mM sulfuric acid. We also performed experiments with $SnCl_2$, which did not require addition of any acid for dissolution. But upon dissolution in water, $SnCl_2$ gives rise to the formation of HCl leading to a drift in the pH of the solution to $\sim$2.4. As in the case of $Hg(NO_3)_2$, we saw a revival in the plasmon color of the solution upon addition of 3 mM $SnSO_4$ and $SnCl_2$ to [+] – [-] Au nanoionic precipitates (magenta curves in Figure 3.16). Control experiments performed in the presence of equivalent amount of acid (pH$\sim$2.4) also showed a similar revival in the plasmon color, which again confirms the instability of [+] – [-] Au nanoionic precipitates under highly acidic condition. The studies with $SnSO_4$ and $SnCl_2$ failed to provide any conclusive evidence for the detection of Sn^{2+} ion by the nanoionic precipitates.

All the Hg^{2+} and Sn^{2+} salts that were available to us were unsuitable as they

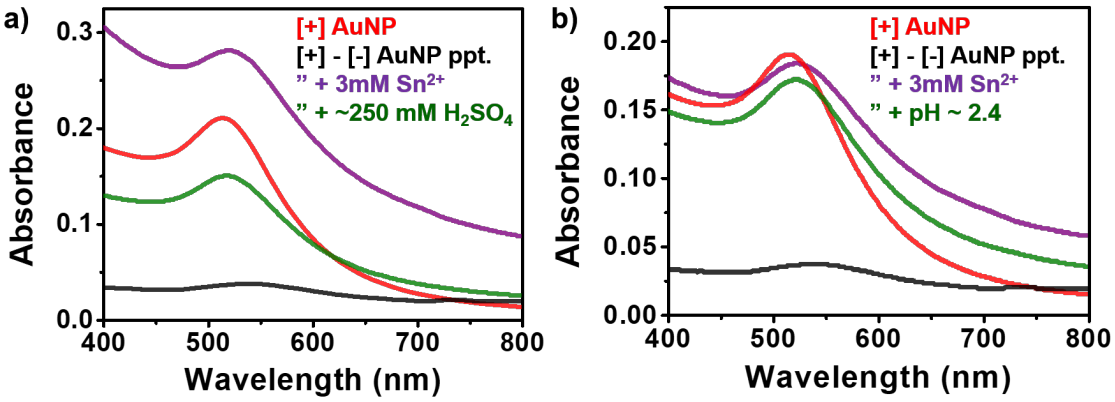


Figure 3.16: Variation in the absorption of [+] – [-] Au nanoionic precipitates in the presence of (a) 3 mM $SnSO_4$ (magenta curve), and in the presence of equivalent amount of acid (green curve). (b) 3 mM $SnCl_2$ (magenta curve), and in the presence of equivalent amount of acid (green curve).

shifted the pH of the solution to $\sim$ 2, at which the nanoionic precipitates were unstable.

3.7.3 Theoretical Model for the Origin of Selectivity

In order to rationalize the trends in the abilities of different M^{2+} ions to displace [+] AuNPs from the [+] – [-] Au nanoionic precipitates, stuck together by electrostatic tethers, we worked with well- established models for different interactions between NPs.[3–5] Specifically, the process of leaking out of [+] AuNPs from the nanoionic precipitates upon exposure to M^{2+} ions can be visualized to be stemming from van der Waals, electrostatics, and '*cation-specific*' bridging interactions. Here, the release of [+] AuNPs is similar to a displacement reaction, where M^{2+} tries to displace [+] AuNPs from the [+] – [-] Au nanoionic precipitates. In this model, we compare the total energies of NPs at contact before (energy of reactants) and after (energy of products) exposure to M^{2+} ions. We compute electrostatic and van der Waals interactions between [+] and [-] AuNPs to estimate the energy of the reactants and compare it with van der Waals as well as bridging interactions between [-] AuNPs and M^{2+} ions (energy of products). The detailed information about the process of modelling these interactions is described below:-

van der Waals interactions

The van der Waals (U_{vdW}) interactions originating from electromagnetic fluctuations due to ceaseless movements of positive and negative charges within the material is approximated using the Hamaker Integral Approximation.[3,5] In this approach, the interaction between the macroscopic objects (composed of many atoms/molecules) is estimated as a pairwise summation of the molecular interactions throughout the volumes of the two bodies.[3,5] According to this approach, the U_{vdW} between two spheres of radii R_c, separated by a distance d (where, $d = 2(R_c+\delta)$ and δ is the length of the ligands :1.63 nm for [-] and 1.90 nm for [+]) between the two centres is calculated by using the following equation:[3,5]-

$$U_{vdW} = -\frac{A}{3} \times \left[\frac{R_c^2}{d^2 - 4R_c^2} + \frac{R_c^2}{d^2} - \frac{1}{2} \ln \left(1 - \frac{4R_c^2}{d^2} \right) \right] \tag{3.1}$$

Here, $A = 4\times10^{-19}$ J,[5,6] is the Hamaker constant for gold across water. Upon

substituting the values of different parameters, the van der Waals energy (U_{vdW}) for [+] – [-] Au nanoionic precipitates (reactants) is summarized in the following table:-

Parameters	Value
A	4.0×10^{-19} J
R_c	3.0×10^{-9} m
$d = 2(R_c + \delta)$	9.45×10^{-9} m
$\mathbf{U}_{vdw}$	**-1.5×10^{-21} J ($\sim$0.36 k_bT)**

In order to calculate van der Waals energy (U_{vdW}) in products, where the gold cores are separated by d' = 2 × ($R_c + \delta + \lambda$). Here, λ = length of $COO^- — Pb^{2+}$ and $COO^- — Cd^{2+} \sim 0.25$nm.[7,8] Substituting the appropriate values in eqn. (9.1.1), van der Waals interaction between two [-] AuNPs bridged with Pb^{2+}/Cd^{2+}ions, 2 λ lengths apart, is:-

Parameters	Value
A	4.0×10^{-19} J
R_c	3.0×10^{-9} m
$d = 2(R_c + \delta)$	9.7×10^{-9} m
$\mathbf{U}_{vdw}$	**-1.55×10^{-21} J ($\sim$0.38 k_bT)**

Electrostatic Interactions

Electrostatic interaction energies between two NPs, which are in equilibrium with an electrolyte are derived from the appropriate electrostatic potentials, ϕ, via thermodynamic integration[9,10] and account for '*charge regulation*' at the NPs' surfaces; that is, for the equilibrium between counterions adsorbed onto the charged surfaces and those '*free*' in solution.[3] Briefly, the electrostatic potential around the NPs or the substrate is well approximated by the linearized Poisson-Boltzmann (PB) equation,

$$\nabla\varphi = \kappa^2\varphi \tag{3.2}$$

Here, $\kappa^{-1} = (\frac{\epsilon_0\epsilon k_bT}{2c_se^2})^{\frac{1}{2}}$ is the screening constant or Debye length, c_s is concentration of the monovalent ion, e is the fundamental charge of electron, ϵ_0 is the permittivity of vacuum, ϵ_0 is the dielectric constant of the solvent, k_b is Boltzmann's

constant, and T is the temperature. The adsorption equilibrium at a positively charged surface (here, TMA-coated NPs) presenting N_T positively charged groups, A^+, in a solution containing negatively charged counterions, B^-, is determined by the following equation

$$\frac{N_{A^+}c_{b^-}}{N_{AB}} = (\frac{1}{K_+})exp(\frac{e\varphi_s}{k_bT}) \tag{3.3}$$

Where, N_{A^+} and N_{AB} are the numbers of counterion-free and counterion-bound surface ligands ($N_{A^+} + N_{AB} = N_T$), c_{b^-} is the mole fraction of counterions in solution, K_+ is the free energy of ion dissociation in the absence of any external fields, and φ_s is the electrostatic potential at the surface. From this relation, the surface charge density, σ, may be expressed as,

$$\sigma = \frac{e\Gamma}{1 + (\frac{c_s}{K_+})exp(\frac{e\varphi_s}{k_bT})} \tag{3.4}$$

Where, $\Gamma = \frac{N_T}{4\pi R^2}$ is the surface density of charged headgroups $\sim 4.7\times10^{18}$ m^2.[11] Considering that the dielectric constant of the SAM ($\epsilon_p \sim 2$) is small compared to that of the solvent ($\epsilon \sim 80$ for water), the surface charge is related to the potential at the NP surface by,[3,6]

$$\sigma = -\epsilon_0\epsilon\nabla\varphi \tag{3.5}$$

Equating the two relations, 3.4 and 3.5, for σ provides the necessary boundary condition for a positively charged NP. The boundary condition for negatively charged NPs is derived in a similar fashion, where it is assumed that the free energy of ion desorption, $K_- = K_-$, (i.e., MUA^-/NMe_4^+ and TMA^+/Cl^- have similar interaction. energies)[12,13]

To obtain an analytic form for the interaction energy, it is necessary to make two additional simplifying assumptions. In addition to linearizing the PB equation (appropriate for dimensionless potentials, ($\frac{e\varphi_s}{k_bT} <= 2$), the boundary conditions at the NPs' surface are linearized about the potential of an isolated particle φ, such

that,[6,14]

$$-\epsilon_0\epsilon\nabla\varphi = S - C\varphi_\infty \tag{3.6}$$

Where, S = $\sigma(\varphi_\infty) - (\frac{\partial\sigma}{\partial\varphi})_\infty\varphi_\infty$, and after some algebra, the electrostatic interaction energybetween two like-charged NPs can be derived as[14]-

$$U_{es} = \pi\epsilon_0\epsilon R(\frac{(\varphi_2^\infty)^2 + (\varphi_1^\infty)^2}{2\Delta}ln(1-\Delta^2) + \frac{2\varphi_2^\infty\varphi_1^\infty}{\Delta}atanh(\Delta)) \tag{3.7}$$

Where, the coefficient $\Delta = \frac{C-\epsilon_0\epsilon\kappa}{C+\epsilon_0\epsilon\kappa}$ depends on C = -$(\frac{\partial\sigma}{\partial\varphi})_\infty$, which is the derivative of surface charge with respect to the potential of an isolated NP.

The substitution of appropriate values for different parameters in 3.7, gave us the magnitude of electrostatic attractions between [+] and [-] AuNPs, and is summarized in the following table-

Parameters	Value
K_+	0.06 mM[6]
c_s	1 mM[13]
φ	∼-50 mV
$\mathbf{U_{es}}$ **(+/-)**	**-6.2×10^{-20} J (∼17 k$_b$T)**

It must be noted that the interaction energy of ∼17 k_bT between [+] and [-] AuNPs is in close agreement with the reported values.[3,13]

Bridging Interactions

Irrespective of the specific nature of the linker molecule, the magnitude of crosslinking depends on the concentration of linkers in solution and on the energy of the bonds formed during crosslinking. These effects can be captured in a simple equilibrium model of the crosslinking interaction between two surfaces. In dilute solution, chemical potential of M^{2+} is given by,

$$\mu_s = \mu_s^0 + k_bTln(\chi) \tag{3.8}$$

Where, k_bT is the thermal energy, μ_s^0 and χ are the standard chemical potential

and mole fraction of M^{2+} ions respectively. The chemical potential, μ_1, of M^{2+} ions adsorbed onto a single AuNP surface, can be approximated using the following Langmuir-type isotherm[3,4]-

$$\mu_1 = \mu_1^0 + k_b T ln(\frac{\theta_1}{1-\theta_1}) \tag{3.9}$$

here, θ_1 is the fractional coverage of adsorbed M^{2+} ions on the surface.

This model assumes ideal solution behaviour and Langmuir-type adsorption equilibrium (no co-operative effects). At equilibrium, the chemical potentials for both, free M^{2+} ions in the solution as well as M^{2+} ions adsorbed onto the AuNP surface are equal, $\mu_s = \mu_1$, such that,

$$\frac{(\theta_1)^{eq}}{\chi(1-(\theta_1)^{eq})} = exp(\frac{-\epsilon}{k_b T}) \tag{3.10}$$

Here, $\epsilon = \mu_1^0$ - μ_s^0 is the energy of adsorption – assumed to be equal to the strength of one bond between COO^- and M^{2+} (For Pb^{2+}, $\epsilon(Pb^{2+}$ - $COO^-) = 2.21\times10^{-20}$ J,[15] while for Cd^{2+}, $\epsilon(Cd^{2+}$ - $COO^-) = 1.61\times10^{-20}$ J.[16] The free energy per unit area, f_1, of this model system may be calculated by thermodynamic integration from the reference state (θ=0) to the equilibrium state (θ=θ_{eq}).

$$f_1 = \Gamma \int_0^{\theta^{eq}\cdot} (\mu_s - \mu_1) d\theta = -\Gamma k_b T ln(1-\theta_1^{eq}) \tag{3.11}$$

Where, Γ is the maximum surface density of linkers adsorbed onto the surface, which will be equal to the number of [-] on AuNP surface (e.g., $\Gamma \sim 4.7 nm^{-2}$ for thiols on gold).[11]

To derive the free energy of crosslinking of two surfaces, we assume that the adsorbed M^{2+} binds to both surfaces, with fractional coverage θ_2 and chemical potential, μ_2. The chemical potential of the COO^- - M^{2+} crosslink joining two AuNPs is given by-

$$\mu_2 = \mu_2^0 + k_b T ln(\frac{\theta_2}{1-\theta_2}) \tag{3.12}$$

Thus, the free energy of formation (per unit area) for two cross-linked surfaces is given by-

$$f_1 = -\Gamma k_b T ln(1 - \theta_2^{eq}) \tag{3.13}$$

and the free energy of crosslinking, Δf, may be expressed as,

$$\Delta f = 2f_2 - f_1 = \Gamma k_b T ln(\frac{1 - \theta_2^{eq}}{(1 - \theta_1^{eq})^2})$$

$$= \Gamma k_b T ln(\frac{(1 + \chi exp(\frac{-\epsilon}{k_b T}))^2}{1 + \chi exp(\frac{-2\epsilon}{k_b T}}) \tag{3.14}$$

with $\mu_2^0 \sim$ 2 μ_1^0 (i.e., two bonds for each bound linker molecule).

Here, the second equality is derived directly from Equation 3.10. In the limit of very dilute linkers, such that $\chi << exp(\frac{-2\epsilon}{k_b T})$, this expression simplifies to Δf $\sim$ -$\Gamma k_b T \theta_2^{eq}$, in other words, the free energy is proportional to the number of crosslinker bridges formed at equilibrium. Equation 3.14 may then be used to estimate the free energy of the crosslinking, U_c, between two spherical particles (radii a_1 and a_2) using the Derjaguin approximation,[3,5]

$$U_c = \frac{2\pi a_1 a_2 \lambda}{(a_1 + a_2)} \Delta f \tag{3.15}$$

Here, λ is a characteristic length scale of molecular dimensions over which the crosslinking molecule can stretch (or compress) when bridging the two curved surfaces. Upon substituting the values of different parameters in eqn. 3.14 and 3.15, the magnitudes of U_c are summarized in the following table-

Parameters	Value
r	3.0×10^{-9} m
δ	2.5×10^{-10} m
$\mu(Pb^{2+})$	2.21×10^{-20} J[15]
$\mu(Cd^{2+})$	1.61×10^{-20} J[16]
$\mathbf{U}_{bridging}$ **(Pb^{2+})**	**-1.12$\times10^{-19}$ J ($\sim$27 k_bT)**
$\mathbf{U}_{bridging}$ **(Cd^{2+})**	**-1.8$\times10^{-20}$ J ($\sim$4 k_bT)**

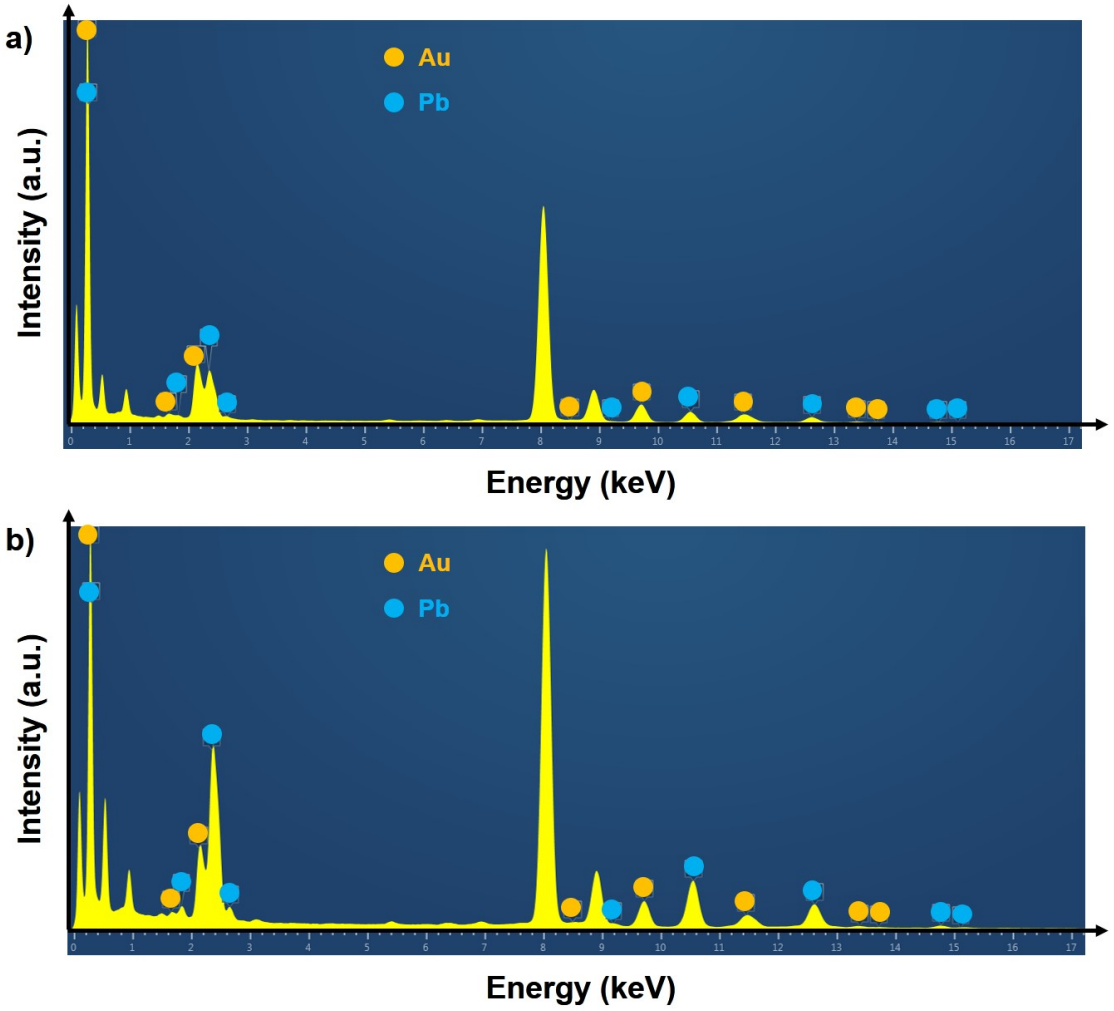


Figure 3.17: EDAX data (from TEM studies) showing the presence of both Au and Pb peaks in (a) the supernatant and (b) [-] AuNP - Pb^{2+} precipitates, after the breaking of [+] - [-] Au nanoionic precipitates by Pb^{2+} ions. The presence of Pb was observed in supernatant as well, which is due to the excess amount of Pb^{2+} ions that was used for the studies.

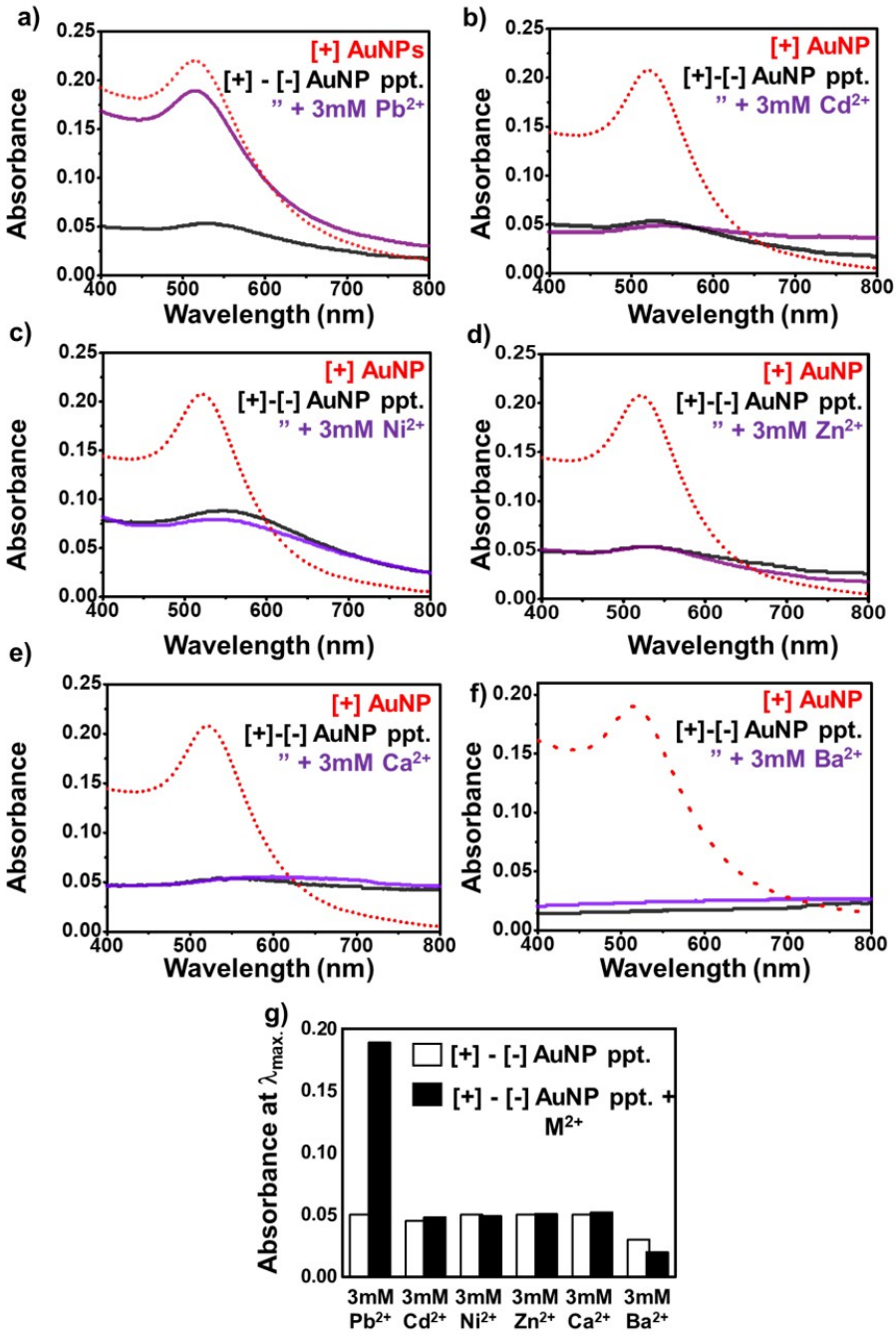


Figure 3.18: UV-Vis. absorption studies of [+] - [-] Au nanoionic precipitate before (shown in black) and after (shown in purple) the addition of (a) 3 mM Pb^{2+}, (b) 3 mM Cd^{2+}, (c) 3 mM Ni^{2+}, (d) 3 mM Zn^{2+}, (e) 3 mM Ca^{2+} (f) 3 mM Ba^{2+}. The negligible change in the absorption spectra shows the inability of these ions break the electrostatic attraction in [+] - [-] Au nanoionic precipitates, confirming the selectivity towards Pb^{2+} ions. The dotted spectra correspond to the plasmon band of the starting [+] AuNP solution, before forming the nanoionic precipitates. (g) Variation in absorbance at $\lambda_{max.}$ upon addition of different M^{2+} ions to [+] - [-] Au nanoionic precipitates. The appearance of plasmon peak after the addition of different M^{2+} (black spectrum) indicates redispersal of [+] AuNPs (turn-on response).

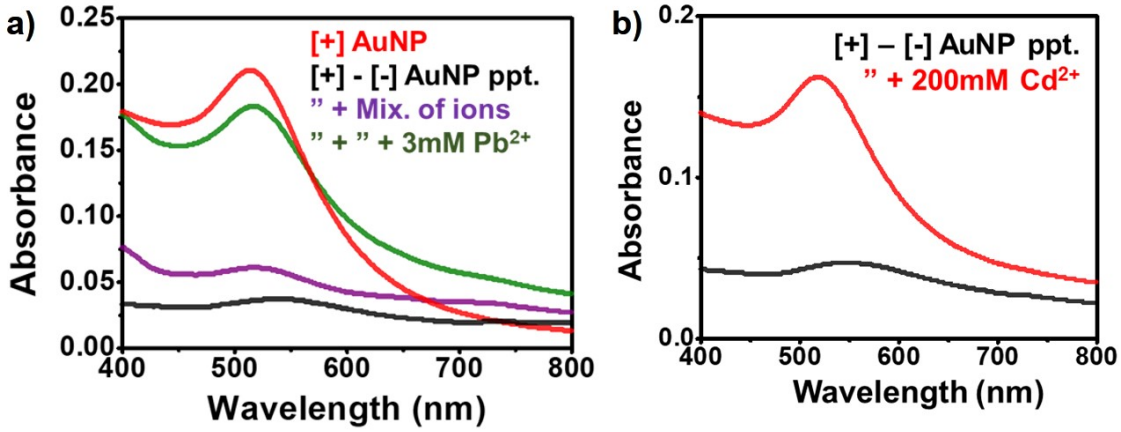


Figure 3.19: (a) Variation in the absorption of [+] - [-] Au nanoionic precipitates in the presence of mixtures of ions, with (shown in magenta) and without (shown in green) Pb^{2+} ions. (b) UV-Vis. absorption spectra of [+] - [-] Au nanoionic precipitate, before (shown in black) and after (shown in red) the addition of 200 mM of Cd^{2+} ions.

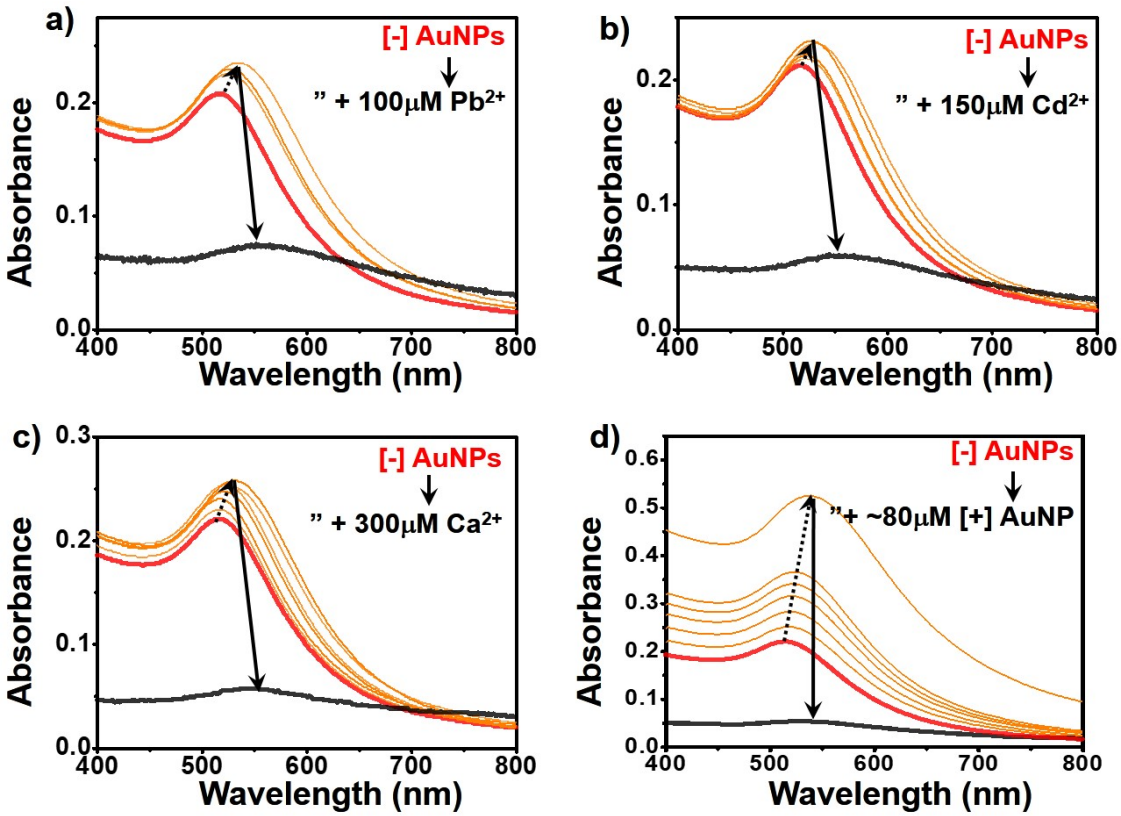


Figure 3.20: Variation in the UV-Vis. absorption spectrum of [-] AuNPs in the presence of different concentrations of (a) Pb^{2+}, (b) Cd^{2+}, (c) Ca^{2+}, (d) [+] AuNPs. The direction of variation is shown by arrows.

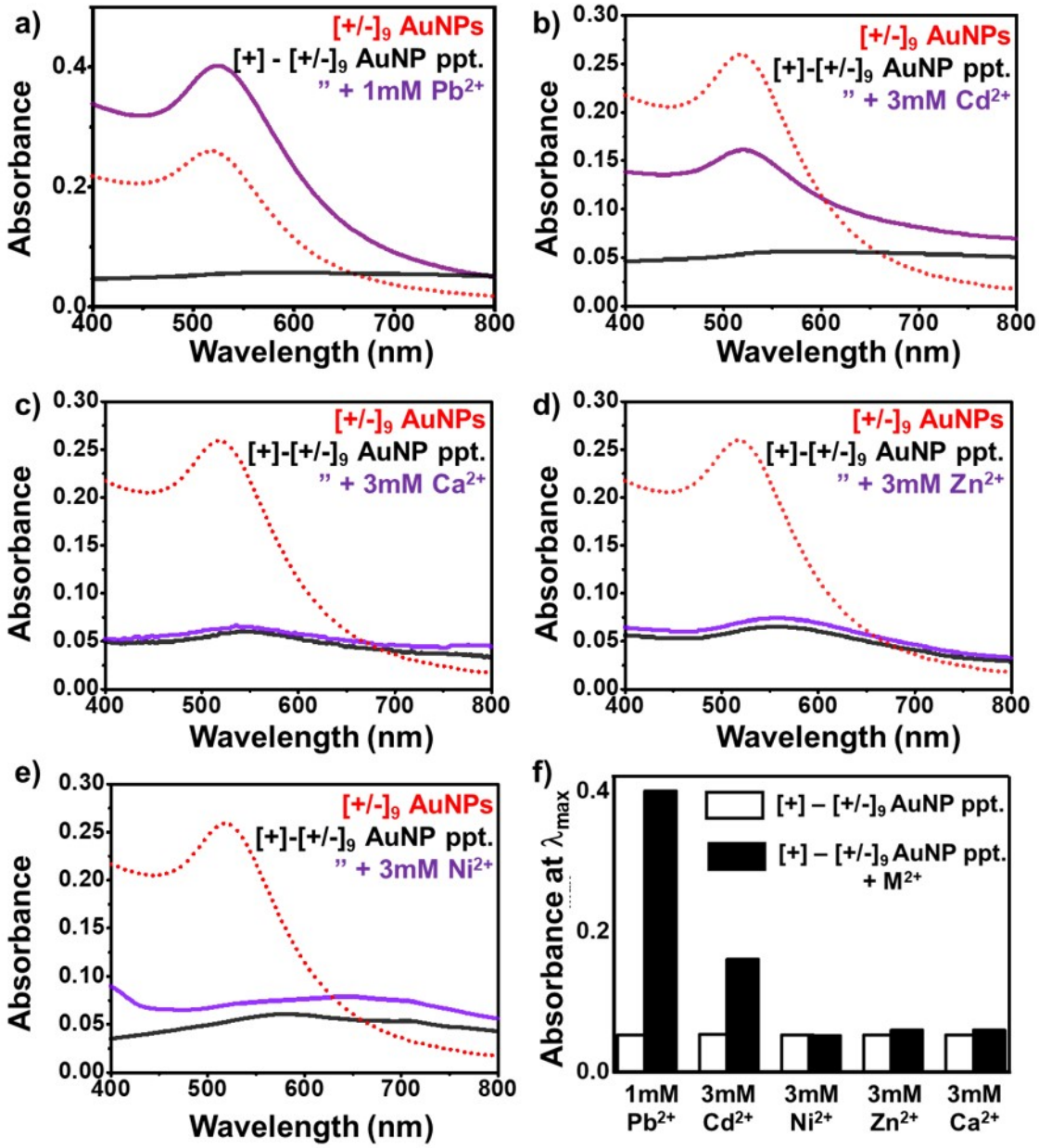


Figure 3.21: Variation in the UV-Vis. absorption spectra of [+] - $[+/-]_9$ Au nanoionic precipitate (shown in black) upon addition (shown in blue) of (a) 1 mM Pb^{2+}, (b) 3 mM Cd^{2+}, (c) 3 mM Ca^{2+}, (d) 3 mM Zn^{2+}, (e) 3 mM Ni^{2+}. The dotted spectra correspond to the plasmon band of heterogeneously charged $[+/-]_9$ AuNP before forming the nanoionic precipitates. (f) Variation in absorbance at $\lambda_{max.}$ upon addition of different M^{2+} ions to [+] - $[+/-]_9$ Au nanoionic precipitates. The appearance of plasmon peak after the addition of different M^{2+} (black spectrum) indicates the turn-on response. Out of the several ions tested, a revival of the plasmon band was observed upon the addition of both 1 mM Pb^{2+} and 3 mM Cd^{2+} ions.

References

(1) Kalsin, A. M.; Fialkowski, M.; Paszewski, M.; Smoukov, S. K.; Bishop, K. J. M.; Grzybowski, B. A. *Science* **2006**, *312*, 420–424.

(2) Liu, D.; Qu, W.; Chen, W.; Zhang, W.; Wang, Z.; Jiang, X. *Anal. Chem.* **2010**, *82*, 9606–9610.

(3) Bishop, K. J. M.; Wilmer, C. E.; Soh, S.; Grzybowski, B. A. *Small* **2009**, *5*, 1600–1630.

(4) Wei, Y.; Bishop, K. J. M.; Kim, J.; Soh, S.; Grzybowski, B. A. *Angew. Chem., Int. Ed.* **2009**, *48*, 9477–9480.

(5) Israelachvili, J. N., *Intermolecular and surface forces*; Academic press: 2015.

(6) Bishop, K. J. M.; Kowalczyk, B.; Grzybowski, B. A. *J. Phys. Chem. B* **2009**, *113*, 1413–1417.

(7) Catalano, J.; Murphy, A.; Yao, Y.; Yap, G. P.; Zumbulyadis, N.; Centeno, S. A.; Dybowski, C. *Dalton Trans.* **2015**, *44*, 2340–2347.

(8) Dakanali, M.; Kefalas, E.; Raptopoulou, C.; Terzis, A.; Mavromoustakos, T.; Salifoglou, A. *Inorg. Chem.* **2003**, *42*, 2531–2537.

(9) Verwey, E.; Overbeek, J. T. G. *Theory of the stability of lyophobic colloids. Elsevier, Amsterdam.* **1948**.

(10) Carnie, S. L.; Chan, D. Y. C.; Gunning, J. S. *Langmuir* **1994**, *10*, 2993–3009.

(11) Leff, D. V.; Ohara, P. C.; Heath, J. R.; Gelbart, W. M. *J. Phys. Chem.* **1995**, *99*, 7036–7041.

(12) Smoukov, S. K.; Bishop, K. J.; Kowalczyk, B.; Kalsin, A. M.; Grzybowski, B. A. *J. Am. Chem. Soc.* **2007**, *129*, 15623–15630.

(13) Bishop, K. J. M.; Grzybowski, B. A. *ChemPhysChem* **2007**, *8*, 2171–2176.

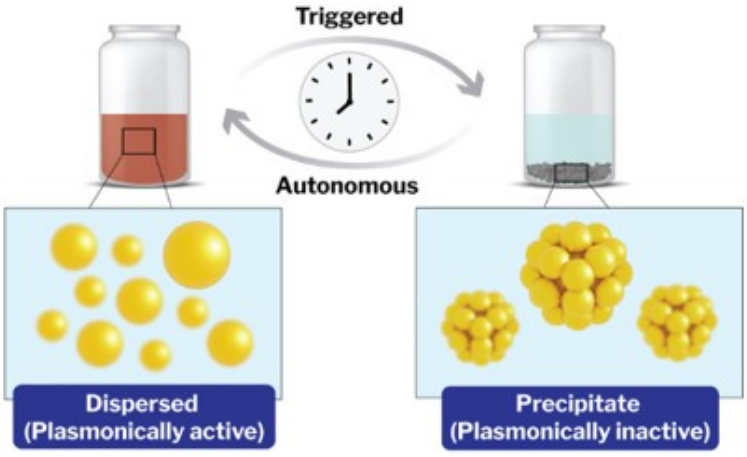
(14) Carnie, S. L.; Chan, D. Y.; Gunning, J. S. *Langmuir* **1994**, *10*, 2993–3009.

(15) Gobom, S. *Nature* **1963**, *197*, 283–284.

(16) Ferrell, E.; Ridgion, J.; Riley, H. *J. Chem. Soc.* **1934**, 1440–1447.

Chapter 4

Temporal Fluctuations in Interparticle Interactions Drive the Formation of Transiently Stable Precipitates



The answer is blowing in the wind

This chapter has been adapted from the following paper:- **Rao, A.**, Roy, S., Pillai, P. P.*, Temporal Fluctuations in Interparticle Interactions Drive the Formation of Transiently Stable Precipitates, ***Manuscript Submitted***

4.1 Abstract

Installing dynamic and transient behaviour in self-assembled systems is a promising strategy to give more *'life-like'* character to materials. Present work explores the pH dependence of electrostatic interactions to introduce temporal fluctuation in the strengths of interparticle forces, and choreograph a transient self-assembly response in plasmonic gold nanoparticles (AuNPs). The assembly process was triggered by the electrostatic attraction between positively charged AuNPs and an aggregating agent, ethylenediaminetetraacetic acid (EDTA). The disassembly step was based on the less explored ability of atmospheric components to transform a mundane mixture of chemicals into a dynamic, and active one. Under the influence of atmospheric CO_2, the autonomous changes in the pH and ionic strength of the solution weaken the aggregating ability of EDTA, thereby initiating the complete disassembly of [+] AuNP - EDTA precipitates. Thus, an uncommon observation of transient switching between complete precipitated and redispersed stages of plasmonic NPs is realized. The use of a non-destructive mode of autonomous disassembly, as opposed to the common way of chemical degradation, generates minimum amount of waste during the transient self-assembly process. Consequently, our strategy helped in achieving some of the desirable feats in the field of transient self-assembly like easy removal of waste, formation of a transiently stable precipitate state and negligible dampness of the redispersion response. These results demonstrate an original chemical strategy capable of introducing transientness to a system, which can act as a generic tool in creating the next generation of *complex matter*.

4.2 Introduction

Living systems have an extraordinary ability of creating *life* from simple precursors, by using highly reconfigurable and adaptive structures (active assemblies).[2,3] These assemblies are transient in nature, and are crucial for driving various cellular functions including those of microtubules and actin filaments.[4] Such structures have

inspired researchers to develop artificial systems with similar reconfigurability. A key strategic challenge in the construction of such active assemblies is the ability to introduce autonomous fluctuations in the strength of interparticle interactions.[5–15] Decades of research in the field of self-assembly has equipped chemists with the ability to regulate the interactions between molecular components in a pre-designed manner.[16–21] This skilful control over interactions has enabled researchers in the realization of intricate structures with fascinatingly complex design and functions.[16–21] Such structures, although interesting, are primarily static in nature, and hence differ noticeably from naturally occurring transient self-assemblies.[esch, 3,5,12] In order to push the field of self-assembly beyond the static domain, in a pioneering work, Grzybowski and co-workers used light as a trigger to introduce fluctuations in interparticle interactions for the transient assembly of gold nanoparticles (AuNPs) (see Figure 4.1).[1] Light is a *'neat'* trigger as it inflicts minimal chemical modifications, resulting in *'zero waste generation'*. Inspired by this, light was extensively used as

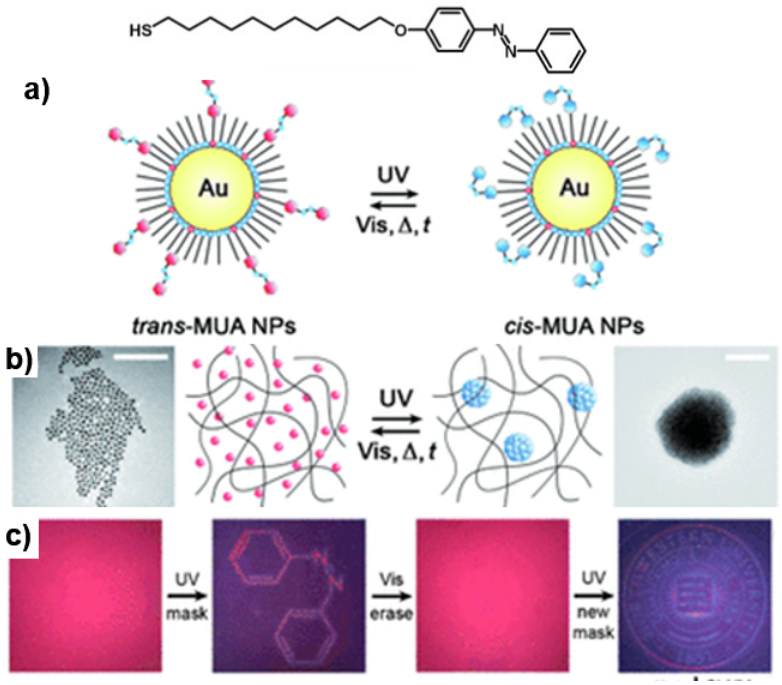


Figure 4.1: Light-driven dynamic self-assembly of AuNPs. (a) Schematic diagram showing the use of UV light to activate AuNPs capped with azobenzene thiol for the transient self-asssembly process. (b) TEM images and schematic representation of dispersed and assembled AuNPs. (c) Optical photographs of gel containing light-responsive AuNPs showing sequential writing and erasing cycles (Reproduced in part with permission from [1] Copyright 2009 John Wiley and Sons).

a trigger to impart temporal response to many molecular and nanoparticle systems, thereby taking the field of self-assembly beyond the static domain.[22–25] Another approach of creating active assemblies utilize chemical triggers to drive the formation of a transient self-assembly.[3,11–13]

In this direction, van Esch and co-workers have elegantly demonstrated the use of triggered esterification and autonomous de-esterification reactions to drive the formation of transiently stable molecular nanofibers (see Figure 4.2a).[26,27] This concept was later extended to different chemical triggers (like EDC, anhydride, redox reactions etc.) to drive transient self-assembly in several systems like polymers, colloids, gels etc.[28–33] In another class of transient self-assembly, the templating effects of a biofuel (ATP) was used to drive the formation of transiently stable structures like vesicles and 1-D supramolecular helixes (see Figure 4.2b) .[34–36] In a seminal work, Prins and co-workers developed a strategy where adenosine triphosphate (ATP) acted as a template to drive the self-assembly of a cationic surfactant into vesicles. The presence of potato apyrase in the system - an enzyme that catalyzes the hydrolysis of ATP – drove the gradual but spontaneous disassembly of vesicles that were stabilized by ATP. In another elegant strategy, George and coworkers utilized a well-known viologen redox chemistry for redox mediated transient morphological transitions. They employed, redox-mediated conformational changes in an amphiphilic foldamer (comprising viologen-pyranine charge transfer pair), to giving rise to two different nanostructures (see Figure 4.2e, f).[31] In an alternate approach, Walther and co-workers developed a generic protocol to drive a transient self-assembly response by using two antagonistic signals: a fast promoter (*for speedy assembly*) and a dormant deactivator (*for sluggish disassembly*)..[37] The upcoming challenge in the field is to translate similar chemically triggered transient assemblies to plasmonic nanoparticles for the effective realization of a transient functionality. Recent studies have used EDC and hydrazine based chemistries to drive the transient self-assembly of silicon and gold nanoparticles.[38–40] Noticeably, most of the reported studies involve the chemical degradation of activated monomers to drive

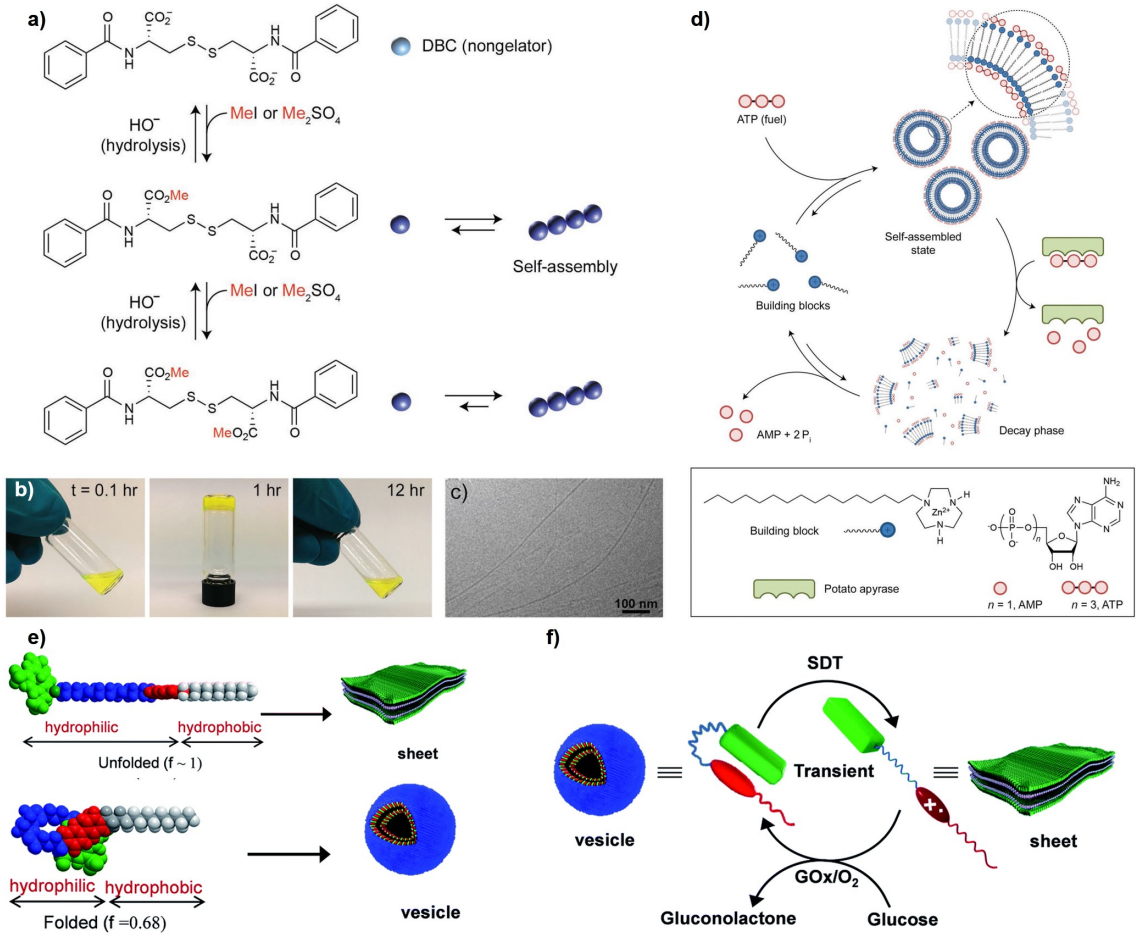


Figure 4.2: (a) Dynamic Self-Assembly by a covalent modification of the precursor. Here, methylation of a molecular gelator containg carboxylates is used for triggered self-assembly, and autonomous hydrolysis of the esters results in the formation of transient gels (adapted from [12, 27]). (b) Photographs of the solution showing the formation of transient gels. (c) cryogenic transmission electron microscopy (cryo-TEM) image of the assembled nanofibers (Reproduced in part with permission from [12] Copyright 2018 John Wiley and Sons). (d) Overview of ATP-driven dissipative self-assembly of vesicles. The schematic diagram shows the use of ATP to activate the assembly of a cationic surfactant, and potato apyrase for the deactivation, resulting in the formation of dynamic self-assembled vesicles (Reproduced in part with permission from [35] Copyright 2016 Springer Nature). (e) Schematic design of the amphiphilic foldamer in the folded and unfolded conformations, and their corresponding assemblies. (f) Schematic representation for the transient conformational response of the foldamer driven by a chemical trigger (figures are reproduced from an open access article [31]).

the disassembly process, resulting in an accumulation of waste that can adversely influence the transient self-assembly response.[7,12] One of the open challenges in the field is to develop new chemistries that produce minimum, or easily removable waste. Despite the rapid developments made in this area, most of the NP based systems do

not display the ideal and *'sought-after'* response of transiently switching between plasmoncially active and inactive states, with minimum dampness. It should be noted that, such a response has been realized with the help of light as a trigger.[23] A possible reason is the lack of formation of waste during light-triggered transient self-assemblies. However, the deactivation step during a chemical triggered self-assembly process, oftentimes results in the generation of waste. New chemistries, therefore, need to be developed where a transient self-assembly response can be realized with easily removable, or minimal waste production. A possible way of removing the interference by waste is its easy separation from the system. In this direction we thought of designing a protocol where NPs could precipitate out from the solution resulting in easy separation of the waste, amd improvements in the redispersion response.

In this regard, the present work exploits the temporal fluctuations in electrostatic interactions to drive the dynamic self-assembly of plasmonic AuNPs, ultimately resulting in the formation of transiently stable precipitates (see Figure 4.3). The assembly process was triggered through the electrostatic attraction between

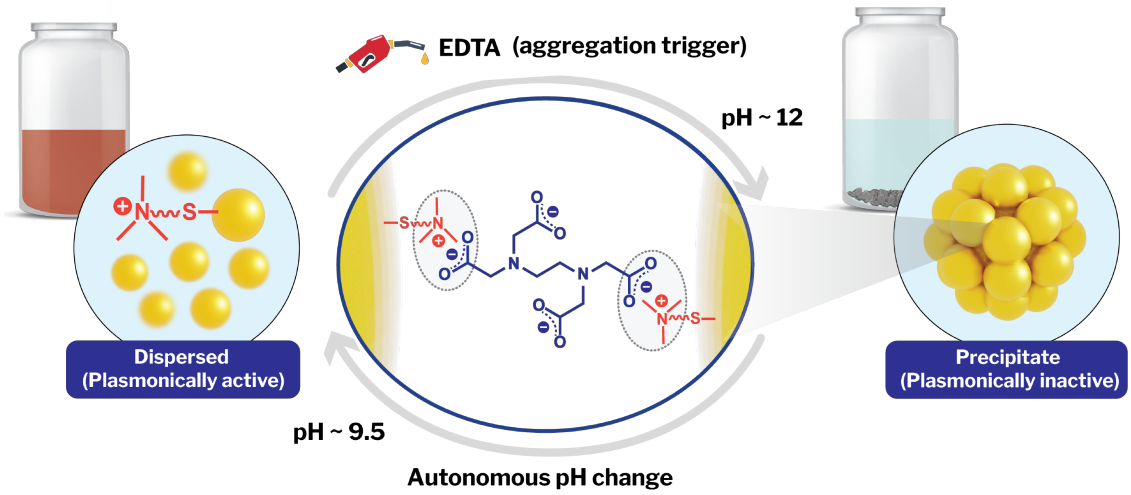


Figure 4.3: Schematics showing the concept of pH dependent temporal fluctuations of electrostatic interactions in driving the dynamic self-assembly in plasmonic [+] AuNPs. EDTA was used as the chemical trigger to bridge between [+] AuNPs and trigger the formation of NP precipitates at a high pH value (pH 12). An autonomous change in the pH and ionic strength of the solution resulted in the complete disassembly of [+] AuNPs - EDTA precipitates, and installed transientness in the self-assembly process.

positively charged AuNPs (bearing quaternary ammonium headgroup) and an aggregating agent EDTA (bearing carboxylate groups). The disassembly occurred due to the autonomous changes in the pH and ionic strength of the solution, under the influence of atmospheric CO_2. Both of these effects resulted in the complete breaking of electrostatic attractions that glued the [+] AuNP – EDTA precipitates. This is in stark contrast with most of the reported transient self-assembly systems, where a chemical degradation of the activated monomers is essential to drive the disassembly process.[7,11,13,41] Whereas, our strategy of non-destructive disassembly allowed the minimal accumulation of waste as well as a transient switching between completely precipitate and redispersed stages of plasmonic NPs. This paved way for the easy removal of waste generated, leading to the complete reversibility in transient self-assembly cycles without any noticeable loss of plasmon intensity (i.e. negligible damping). Our studies therefore, reveal the so far unknown ability of atmospheric components, to transform a mundane mixture of chemicals into a dynamically changing one – *a task usually accomplished with a network of chemical reactions.*

4.3 Experimental Section

4.3.1 Synthesis of AuNPs

We synthesized AuNPs according to an adapted literature protocol.[42–44] We have used $HAuCl_4.3H_2O$ as the gold precursor and a mixture of hydrazine monohydrate ($N_2H_4.H_2O$) and tetrabutylammonium borohydride (TBAB) as the reducing agent for AuNP synthesis. In a typical synthesis, we used a toluene solution (∼7 mL) of $HAuCl_4.3H_2O$ (23 mg, 0.058 mmol) containing 222 mg of DDA (1.2 mmol) and 277 mg (0.6 mmol) of DDAB. In order to completely solubilize the Au (III) salt, we sonicated the solution for ∼5 min (Solution A). We prepared another toluene solution (∼ 3mL) containing 56 mg of TBAB (0.22 mmol) and 110 mg of DDAB (0.24 mmol) (Solution B). Solution B was then rapidly injected to A, so as to ensure

the complete reduction of Au (III) salt. The resulting DDA-AuNP seeds were aged for ∼24 h. These NP seeds were then grown to form 5.5 ± 0.8 nm sized DDA-AuNPs. For this, we added 1 g of DDAB (2.2 mmol), 2.6 g of DDA (14 mmol), 224 mg of HAuCl4·3H2O (0.57 mmol), and 10 mL of seeds in 60 mL of toluene. This solution was reduced by the drop-wise addition (in ∼30 min) of 22 mL of a toluene solution containing 300 μL of $N_2H_4.H_2O$ and 3.5 mg of TBAB. The solution was left for overnight stirring to yield monodisperse 5.5 ± 0.8 nm sized DDA-AuNPs (Figure 4.4). DDA ligands were then place exchanged with TMA using the place exchange protocol given below.

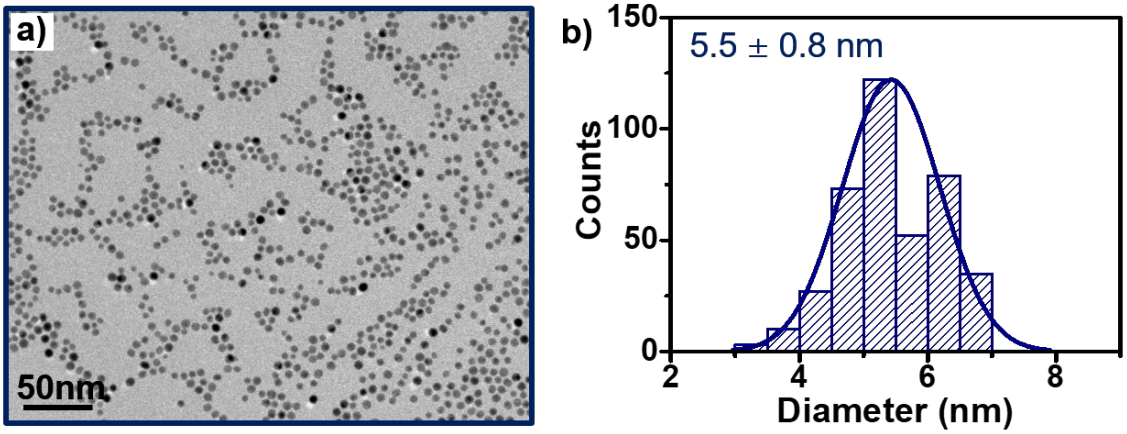


Figure 4.4: (a) Representative TEM image and (b) Size distribution histogram of [+] AuNPs. The average size was estimated to be 5.5 ± 0.8 nm. The size distribution was estimated from ∼ 400 NPs.

4.3.2 Place Exchange of AuNPs

In order to purify the prepared DDA capped AuNPs, ∼20 mL of the AuNP solution was quenched with ∼50 mL of methanol. This resulted in the formation of a black precipitate. The solution was then decanted and the DDA-AuNP precipitates were re-dissolved in 20 mL of toluene. We then added 10 mL of dichloromethane solution containing TMA (equal to the moles of Au (III) in the solution) to the toluene solution of DDA-AuNPs. This resulted in immediate precipitation of AuNPs, indicative of a successful place exchange of hydrophobic DDA with hydrophilic TMA ligand. This solution was kept undisturbed for ∼ 15 h for equilibration. Next, the super-

natant solution was decanted and the precipitates were washed with dichloromethane (3 × 50 mL) followed by acetone (1 × 50 mL). The precipitates were then dried, and re-dispersed in water to get [+] AuNPs (Figure 4.5).

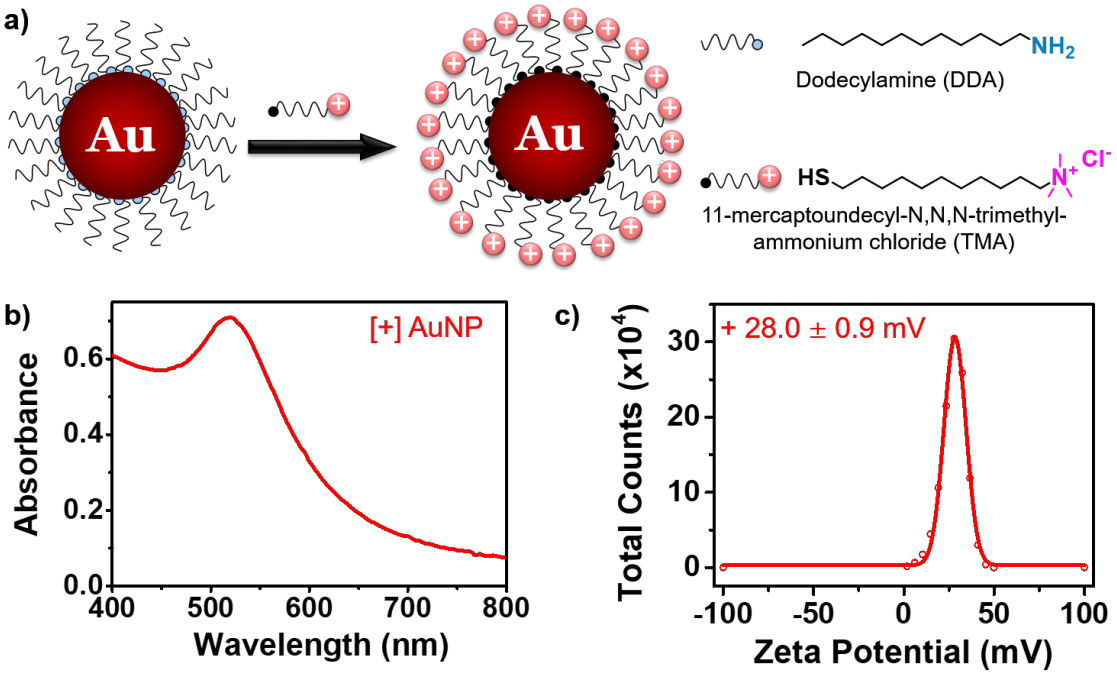


Figure 4.5: (a) Schematic illustration showing the place exchange of DDA ligands with TMA ([+]) to synthesize positively charged AuNPs ([+] AuNPs) (b) UV-Vis. absorption spectrum of [+] AuNPs showing the characteristic surface plasmon resonance peak at ∼520 nm (c) Zeta potential of [+] AuNPs confirming the positive surface charge.

4.3.3 Protocol for the Transient Self-Assembly of [+] AuNPs

All the transient self-assembly experiments were performed in an open vial, unless mentioned otherwise. Here, we used EDTA as a trigger for the self-assembly of [+] AuNPs, and atmospheric CO_2 for the spontaneous disassembly process. In a typical experiment, 10 μM EDTA was added to a dispersion of [+] AuNPs (∼60 μM in terms of Au atoms) and the system was left undisturbed for the formation of [+] AuNP- EDTA precipitates; triggered aggregation. These precipitates were then left exposed to the atmosphere for the autonomous redispersion cycle. Both the aggregation and redispersion responses were monitored with time dependent UV-Vis. absorption measurements. For the reversibility experiments in Figure 4.13, we

used ~7 μM of EDTA as the trigger since it showed faster transient self-assembly response.

4.4 Results and Discussion

4.4.1 Transient Self-Assembly of [+] AuNPs

The electrostatic interactions between quaternary ammonium and carboxylate group have been explored for a variety of self-assembly and sensing applications.[42,45,46] We aimed to explore the pH dependent fluctuations in such electrostatic attractions for the realization of a transient self-assembly in plasmonic AuNP system. Accordingly, 5.5 ± 0.8 nm sized AuNPs functionalized with non-ionizable 11-mercaptoundecyl-N,N,N-trimethyl-ammonium chloride ligands (TMA, [+]) were prepared using a place exchange protocol (see Figure 4.4, and 4.5).[42–44] Ethylenediaminetetraacetic acid (EDTA) bearing four carboxylate groups was used as the aggregating trigger to study the assembling properties of [+] AuNPs (see Figure 4.6a). The addition

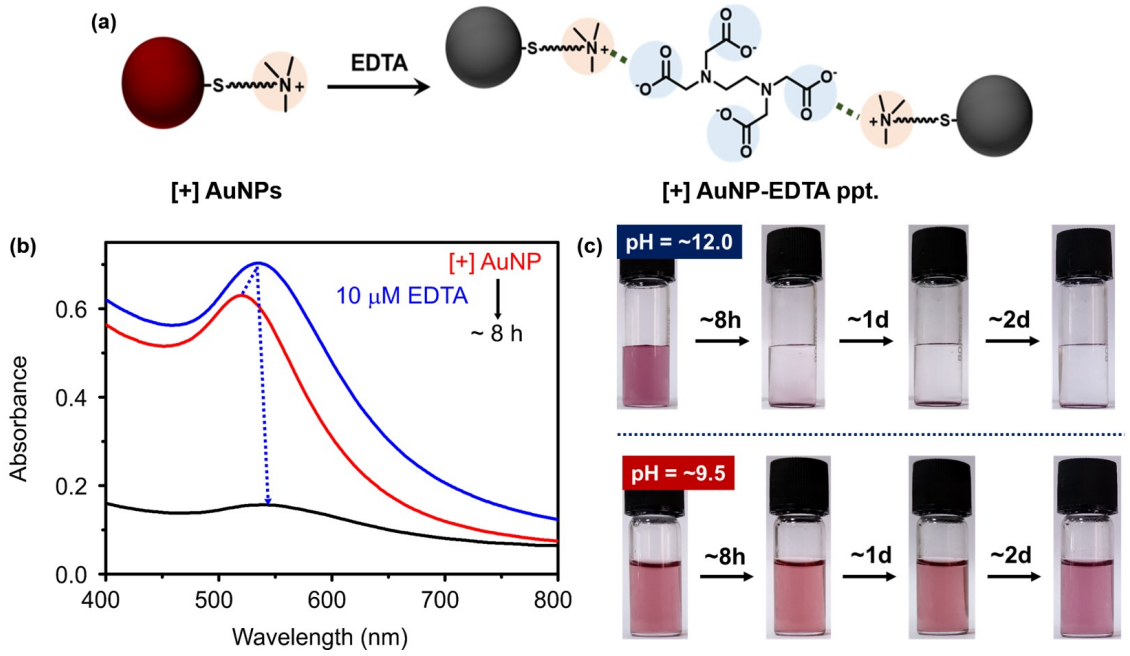


Figure 4.6: (a) Schematics and optical photographs showing the efficient bridging ability of EDTA at high pH values (~12), resulting in the precipitation of [+] AuNPs. Optical photographs showing the (a) efficient, and (b) inefficient bridging ability of EDTA at higher (~12) and lower pH values (~9.5), respectively.

of 10 μM EDTA to 60 μM [+] AuNPs (in terms of Au atoms) resulted in an immediate bathochromic shift of ∼15 nm. This along with a corresponding colour change from wine-red to blue confirms the plasmon coupling and aggregation of [+] AuNPs (blue curve in Figure 4.6b). The aggregates gradually precipitated from the solution, and ultimately settled at the bottom of the cuvette in ∼8 h (black curve in Figure 4.6b). It should be noted that EDTA could efficiently precipitate [+] AuNPs from the solution only at high pH values (pH ∼12). Whereas, no noticeable aggregation of [+] AuNPs was observed at lower pH values (pH ∼9.5), even after ∼2 days (see Figure 4.6c, 4.9a). This differential aggregation response is possibly because of the electrostatic repulsions between quaternary ammonium groups on [+] AuNPs and protonated amine on EDTA at lower pH values.[47] Having established that aggregation, and hence the stability of [+] AuNP – EDTA precipitates depend on the pH of the solution, we looked for autonomous ways of acidifying the solution. Such a decrease in the solution pH will destabilize the precipitates by deactivating the aggregating ability of EDTA, and resulting in an efficient release of [+] AuNPs from the precipitates (Figure 4.3). In our pursuit to autonomously acidify the solution, a serendipitous discovery was made where [+] AuNPs redispersed completely in ∼ 3 days, when kept in an open vial (see the green spectrum in Figure 4.7a, c). The redispersion of [+] AuNPs was accompanied with a decrease in the solution pH from ∼12 to ∼9.5, thereby confirming the decisive role of pH in breaking the [+] AuNP-EDTA precipitates. The transient switching between plasmonically active and inactive NP states was apparent from the changes in the optical photographs of the solution (optical photographs in Figure 4.7c). The UV-Vis changes associated with the triggered aggregation and autonomous disassembly were well complemented with Dynamic Light Scattering (DLS) studies. Here, the hydrodynamic diameter of [+] AuNPs increased from ∼8 nm to micron size upon the addition of 10 μM EDTA (Figure 4.7b), confirming the formation of [+] AuNP precipitates. The hydrodynamic diameter reverted to its initial value when the system autonomously redispersed after ∼3 days. Interestingly, there was no persistence of

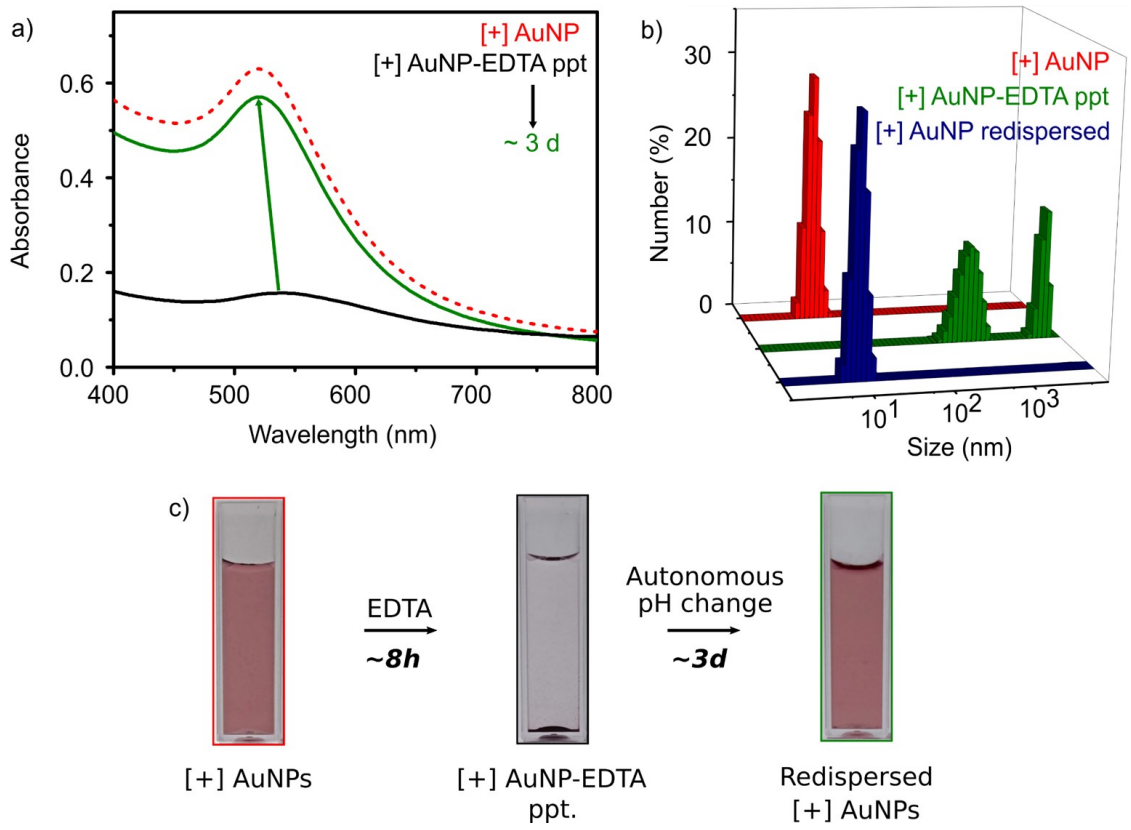


Figure 4.7: Transient self-assembly of plasmonic [+] AuNPs. (a) Variation in the absorption of ∼60 μM [+] AuNPs in the presence of ∼10 μM EDTA. (b) DLS plots confirming the assembly-disassembly process in [+] AuNPs. The hydrodynamic diameter of [+] AuNPs (shown in red) increased upon the addition of EDTA (shown in green), followed by an autonomous reversal to the initial value after ∼3 days (shown in blue). (c) Optical photographs of dispersed [+] AuNP, sedimented [+] AuNP-EDTA precipitates and completely re-dispersed [+] AuNPs, during a single transient self-assembly cycle.

aggregates in the solution, indicating a complete redispersal of the [+] AuNPs from the precipitates (blue spectrum in Figure 4.7b). The generality of our approach was proved by demonstrating similar transient self-assembly process in plasmonic NPs of varying size (∼11 nm AuNP) and core (∼5 nm [+] AgNPs (Figure 4.8). To the best of our knowledge, such a response of transiently switching between plasmonically active and inactive stages is scarce in the literature. In support of this, very recently, Boekhoven and co-workers witnessed the propensity of AuNP precipitates, once formed, to fall into a kinetically trapped state and become incapable of showing any transientness.[39]

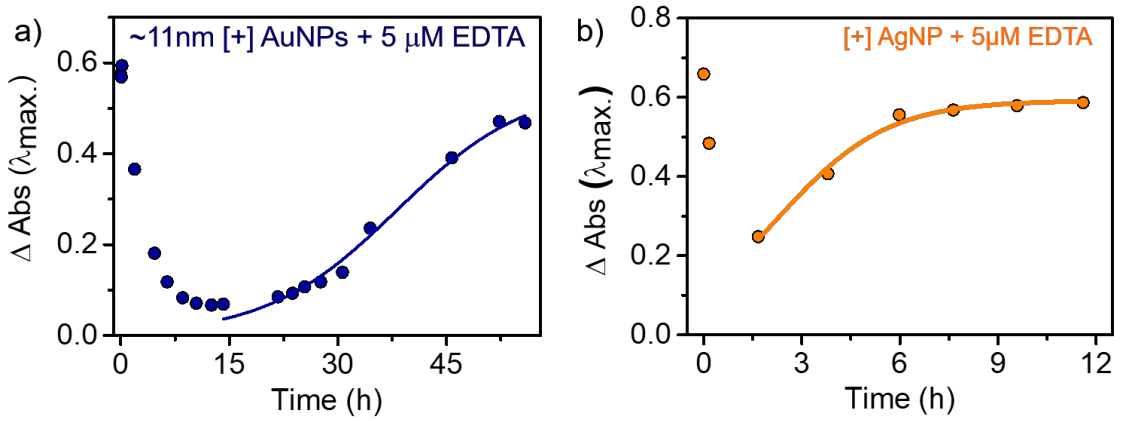


Figure 4.8: Variation in Δ Abs. at $\lambda_{max.}$ with time for (a) ∼11 nm [+] AuNPs, and (b) ∼5 nm [+] AgNPs upon the addition of ∼5 μM EDTA (trigger).

4.4.2 Mechanism of Transientness

In order to elucidate the mechanism of transientness, we performed a series of experiments under different conditions. Firstly, we tested the necessity of an open vial for the autonomous redispersion of [+] AuNPs. For this, the solution containing [+] AuNP – EDTA precipitates were incubated in a closed cuvette, and the redispersion process was monitored for several days. Surprisingly, no signs of re-dispersion of [+] AuNPs were observed even after ∼2 weeks, and the pH of the solution remained basic (∼12; Figure 4.9a). Upon performing similar experiments in an open vial, as mentioned in the previous section, the solution pH lowered to ∼ 9.5 and the precipitates redispersed within ∼3 days (Figure 4.9a). This indicates the necessity of components from the atmosphere for triggering the disassembly step, and to complete the transient self-assembly cycle. Later, we systematically exposed the solution containing [+] AuNP- EDTA precipitates to different atmospheric gases, and monitored the redispersion using UV- Vis. absorption studies. Within ∼15 mins of purging, CO_2 could completely break the [+] AuNP- EDTA precipitates and redipserse the plasmonic NPs to the solution (Figure 4.9b). On the contrary, other major atmosphere gases like N_2, O_2, and Ar failed to disassemble the precipitates even after ∼1 h of continuous purging (Figure 4.9b). The exclusivity of CO_2 to disassemble [+] AuNP-EDTA precipitates can be understood from its ability

to acidify an aqueous solution by forming carbonic acid (H_2CO_3).[48,49] Under these acidic conditions, the amine group on EDTA gets protonated and loses its ability to glue the [+] AuNPs together in the precipitate, thereby resulting in the spontaneous disassembly of the precipitates (*vide supra*).

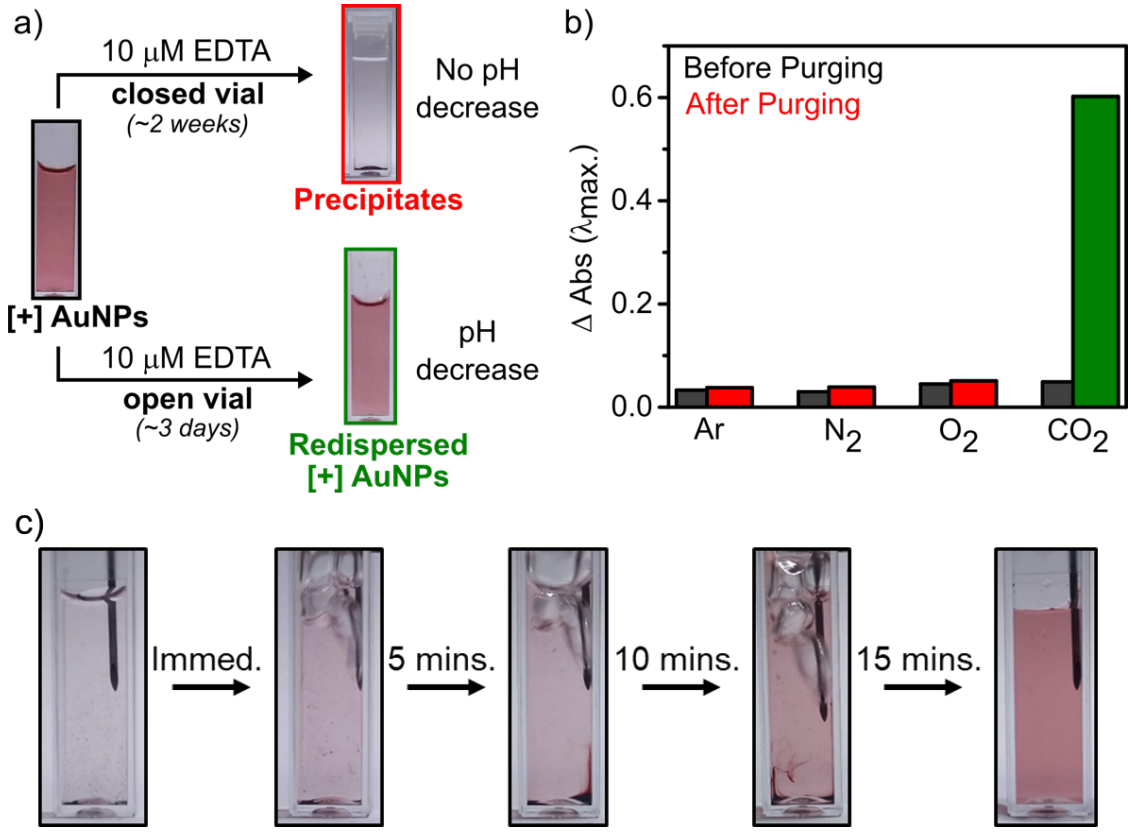


Figure 4.9: Mechanism of Transient Self-Assembly. (a) Optical photographs showing the necessity of open vial for the autonomous redispersion of [+] AuNPs from the precipitates. (b) Bar diagram showing the effect of bubbling of different atmospheric gases (for 1h) on the absorption of solution containing [+] AuNP-EDTA precipitates. Disassembly of [+] AuNP-EDTA precipitates and complete redispersal of plasmonic NPs was observed within ∼15 min of purging with CO_2. (c) Optical photographs clearly showing the redispersion of [+] AuNPs, upon purging a solution of [+] AuNP – EDTA precipitates with CO_2 for ∼15 mins.

In an unanticipated finding, contrasting outcomes were witnessed upon the exposure of [+] AuNPs to the same amount of chemical trigger through two different pathways. Specifically, we compared the responses of [+] AuNPs when 10 μM EDTA was added through single and multiple batches (4 × 2.5 μM). Care was taken during multiple additions so that each aliquot of EDTA trigger, on its own, didn't initiate the aggregation of [+] AuNPs. As shown before, the addition of 10 μM EDTA at

once resulted in the instantaneous decrease of the absorption intensity, followed by the complete precipitation of [+] AuNPs - assembling pathway (red curve in Figure 4.10). On the other hand, [+] AuNPs retained their colloidal stability when the same amount of EDTA was added through four aliquots of 2.5 μM each – non-assembling pathway (see blue curve in Figure 4.10). This intriguing observation can be rationalized by understanding the differential responses of precipitated and un-aggregated AuNPs towards the changes introduced by atmospheric CO_2. Under the action of atmospheric CO_2, EDTA consistently loses its aggregating ability because of the decrease in pH as well as an increase in the ionic strength of the solution. Both these factors hamper the activity of freshly added batch of EDTA, since the bridging is most potent at high pH (∼12) and low ionic strengths (as discussed in previous sections). As a consequence of these combined effects, the freshly added EDTA in batches fails to initiate the aggregation of [+] AuNPs. In order to validate the necessity of atmospheric CO_2 for installing the observed pathway dependence, multiple aliquots of EDTA (4 × 2.5 μM) was added to [+] AuNPs in a closed vial (so as to isolate the system from the effects of atmospheric CO_2). Interestingly,

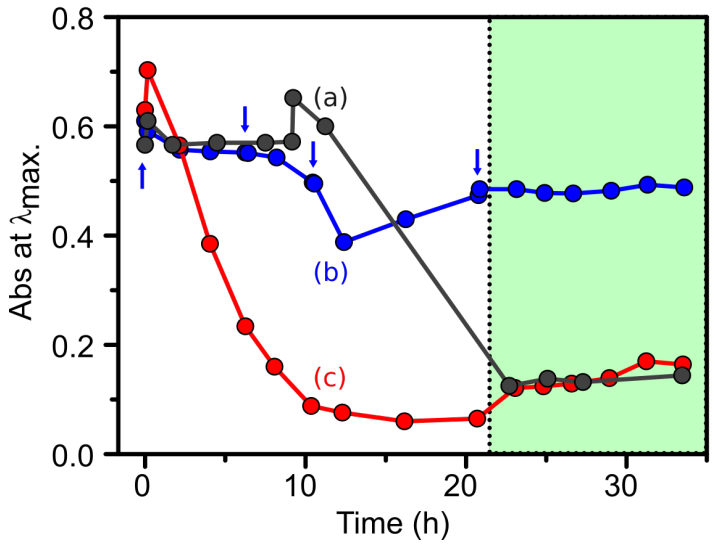


Figure 4.10: Pathway dependence in the transient self-assembly of [+] AuNPs. Variation in the absorbance of [+] AuNPs upon addition of 10 μM EDTA in (a) one batch (shown in red), (b) multiple batches in an open vial (shown in blue), and (c) multiple batches in a closed vial (shown in black). The green shaded portion corresponds to the same of EDTA in all the three systems, and arrows mark the addition of each aliquot additon.

the [+] AuNPs uniformly precipitated out of the solution similar, when multiple additions of EDTA were performed in a closed vial (curve green in Figure 4.10). These experiments further validate the necessity of atmospheric CO_2 in installing transientness to the system.

4.4.3 Tuneable Lifetime and Reversibility

A detailed understanding of various factors involved in the assembly-disassembly steps allowed us to tune the lifetime and reversibility of the transient self-assembly process. The weakening of the bridging ability of EDTA, upon autonomous pH change, was the key in the disassembly process. Hence, a variation in the amount of EDTA can be conveniently used to control the rate of disassembly, and ultimately the lifetime of AuNP precipitates. With this in mind, [+] AuNPs were exposed to varying concentrations of EDTA and the assembly-disassembly steps were monitored using UV-Vis studies (Figure 4.11). Addition of a small amount of EDTA (1 μM) failed to activate sufficient [+] AuNPs for the self-assembly process, as can be seen from the stagnant nature of the absorption intensity (red curve in Figure 4.11). Upon increasing the concentration of EDTA to 5 μM, an instantaneous decrease in

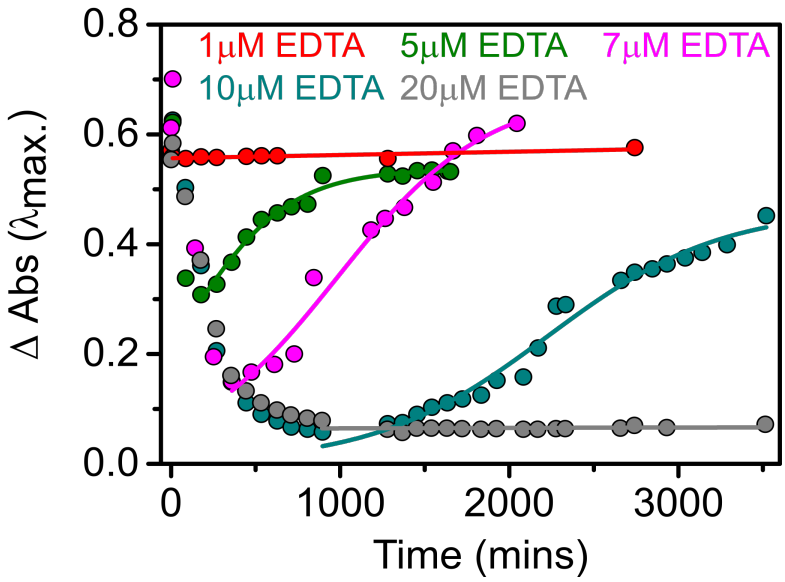


Figure 4.11: Variation in Δ Abs. at $\lambda_{max.}$ with time for [+] AuNPs upon the addition of different amounts of EDTA (trigger).

the absorption intensity was observed, indicating the onset of aggregation (green curve in Figure 4.11). This decrease in the absorption intensity ceased after ∼3 h and the system disassembled completely in ∼15 h, as it is clear from the reversal of absorbance to the original intensity. It should be noted that these aggregates, despite retaining their colloidal stability, exhibited noticeable differences in the absorption behaviour during the transient self-assembly cycle (Δ Abs. at $\lambda_{max.}$ = ∼0.25). A further increase in the amount of EDTA (∼7 μM) resulted in the formation of complete precipitates in ∼6 h, which disassembled completely in ∼34 h. Thus, the time taken to disassemble [+] AuNP–EDTA aggregates could be conveniently tuned from ∼3 days to ∼15 h by decreasing the amount of EDTA (Figure 4.11). Further, we could not only tune the lifetime but also the extent of assembly: formation of small aggregates vs precipitates. Alternatively, the lifetime of [+] AuNP– EDTA precipitates was reduced by increasing the surface area of the dish containing the precipitates. The rationale being that the redispersion of [+] AuNPs *(deactivation)* occurs under the influence of atmospheric CO_2, and an increase in the surface area of the container should enhance the dissolution of CO_2. Accordingly, the precipitates of [+] AuNPs were prepared with 10 μM EDTA and transferred to a petri-dish (∼3.5 cm in diameter). A dramatic reduction in the redispersion time from ∼3 days (in an open vial) to ∼4 h was observed when the disassembly step was carried out in a petri dish (Figures 4.12a, b).

Next we discuss the repeatability of the transient self-assembly process. Since the disassembly process was caused by an autonomous decrease in the pH and protonation of EDTA, a mere increase of pH back to ∼12 is expected to trigger the next cycle of aggregation. However, no signs of aggregation were observed by increasing the pH of the solution. Similarly, fuelling with a fresh batch of basic solution of EDTA (∼7 μM) also failed to trigger the aggregation process. To our surprise, the aggregation and precipitation of [+] AuNPs was only observed after the addition of higher amounts of EDTA (∼ 1.5 times more compared to the first cycle). The disassembly process occurred similar to the first cycle, with a plasmon recov-

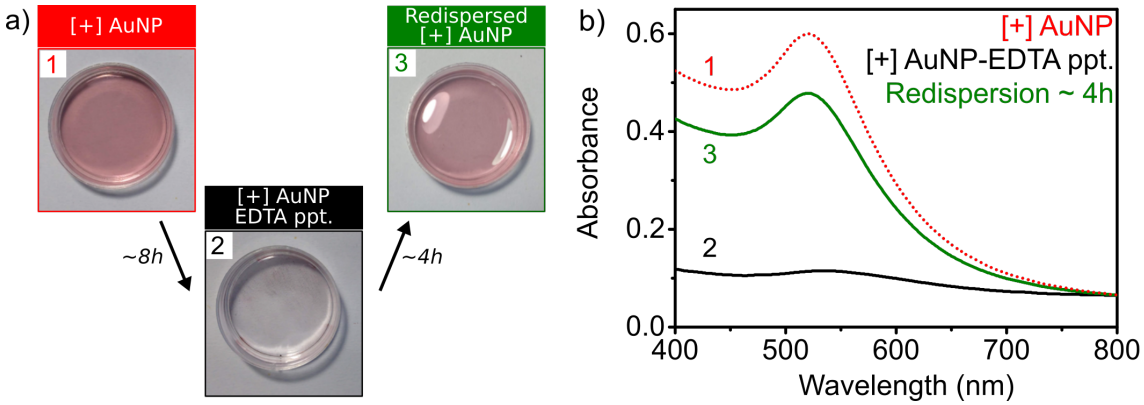


Figure 4.12: (a) Optical photographs of the petri-dish containing [+] AuNPs, [+] AuNP – EDTA precipitate, and redispersed [+] AuNPs marked as 1, 2, and 3 respectively. (b) Variation in the absorption of [+] AuNPs in the presence of 10 μM EDTA. Here, the redispersion process was carried out in a petri-dish, resulting in the faster disassembly of [+] AuNP – EDTA precipitates (in ~4 h).

ery of ~95 % (Figure 4.13). It was also observed that the concentration of EDTA had to be continuously increased for each subsequent cycle to efficiently carry out reversibility studies (Figure 4.13). This curious observation of demand for higher trigger for each cycle can be rationalized in the following fashion. The aggregation between EDTA and [+] AuNPs is primarily through electrostatic attractions, and the strength of which will depend on the ionic strength of the solution. In the present system, spontaneous acidification and decrease in pH of the solution was observed during the autonomous disassembly of [+] AuNP– EDTA precipitates. The

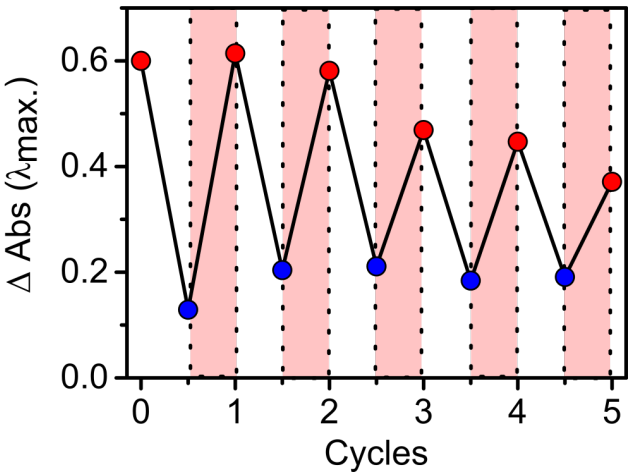


Figure 4.13: Five complete cycles of transient self-assembly of [+] AuNPs with increasing concentrations of EDTA for each subsequent cycle. We observe a consistent dampening of the redispersion response due to interference from the waste

produced carbonic acid can react with the base already present in the solution to form a salt ($NaHCO_3$), thereby increasing the ionic strength of the solution.[17,50,51] Consequently, the electrostatic attractions will be weaker in the next assembling cycle, demanding for higher amounts of EDTA. In a similar way, the presence of high salt affects the disassembly process as well. Here, for subsequent cycles, noticeable losses in the absorption intensity of redispersed [+] AuNPs was observed (only ∼60 % of plasmon intensity was retained at the end of the 5^{th} cycle; Figure 4.13). It has been established in literature that the waste generated during the process of transient self-assembly interferes with the redispersion process, resulting in a damped response – *a longstanding challenge in the field.*[7,12] An obvious way out is to separate the waste from the disassembled monomers, without affecting the transientness.

4.4.4 Easy Removal of Waste

The next objective was to minimize the interference of the waste produced during the disassembly step, and overcome the damped response observed during the re-

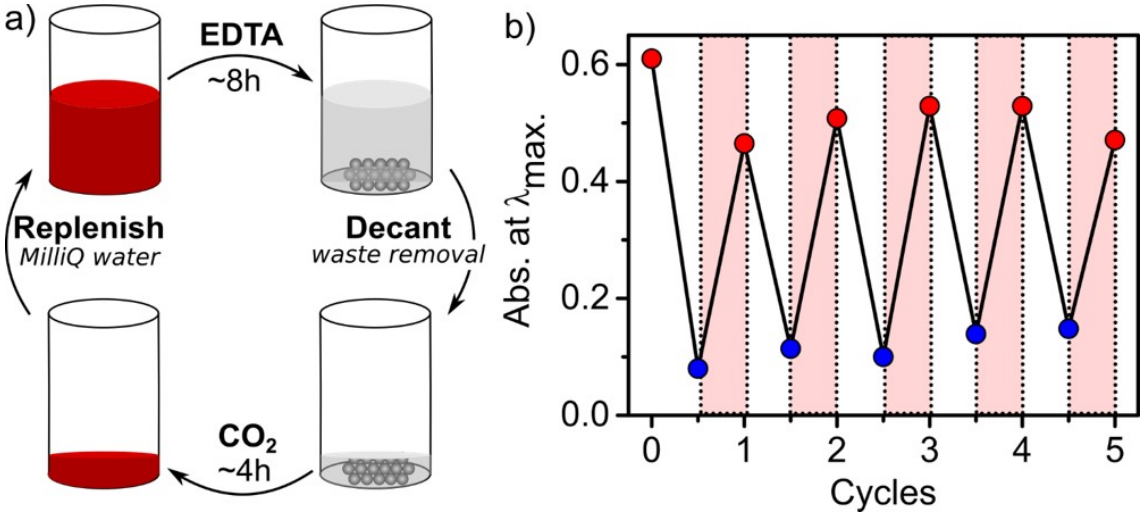


Figure 4.14: Easy waste removal and improved reversibility. (a) Schematics showing the sequence of steps undertaken to study the effect of solution volume on the disassembly of [+] AuNP-EDTA precipitates. The formation of complete precipitate allowed the easy removal of waste through simple de-cantation. (b) Five complete cycles of transient self-assembly of [+] AuNPs, where the redispersion was undertaken in a smaller volume (∼100 μL). Lowering of the solution volume resulted in a faster disassembly of [+] AuNP – EDTA precipitates (∼4 h), along with minimal loss of plasmon intensity during each cycle.

versibility studies. In the present system, salt ($NaHCO_3$) is the main waste produced that adversely affects the transient self-assembly process in subsequent cycles. To our advantage, the complete sedimentation of NP precipitate (formed during the assembling stage) allowed the possibility of easy removal of waste through a simple decantation of the supernatant (Figure 4.14a). Moreover, lowering the solution volume of NP precipitate also helped in minimizing the amount of salt (waste) produced in subsequent autonomous disassembly step. In a typical recyclability experiment, the redispersion of [+] AuNP precipitates was performed in lower volumes by decanting the supernatant solution. A dramatic decrease in the lifetime of [+] AuNP precipitates was observed from ∼3 days (in 3 mL solution) to ∼4 h, when the redispersion was performed at a lower volume (∼100 μL). This is because lower amounts of CO_2 will now be required to change the pH of a ∼ 100 μL solution and switch off the electrostatic attractions, compared to that of 3 mL solution. In order to perform the next cycle of transient self-assembly, the redispersed [+] AuNPs was replenished with MilliQ water to the initial volume of 3 mL, and refuelled with ∼10 μM EDTA. It is worth mentioning that exactly the same of amount of EDTA, as used in the first cycle, was enough to get an immediate aggregation of [+] AuNPs in subsequent cycles (Note: no noticeable aggregation was observed with similar concentrations of EDTA, when the recyclability experiment was performed without removing the waste from the system). The aggregated [+] AuNPs then subsequently precipitated from the solution, indicating negligible interference from the waste on the triggered assembly process. Again, the volume of [+] AuNPs precipitates was reduced by decantation to minimize the waste generated from the second cycle of redispersion. Furthermore, the redispersed [+] AuNPs showed negligible losses in the plasmon intensity, indicating minimal interference from the waste during the autonomous disassembly process. Similar reversibility cycles were performed for at least 5 times, without much dampening in redispersion response (∼95 % of plasmon intensity was retained in each cycles) – *a feat scarcely seen in literature* (Figure 4.14). Thus, our original chemical strategy of transiently switching between dispersed and completely

precipitate states allowed us to conveniently isolate the activated AuNPs from the excess trigger and waste, without compromising the dynamic self-assembly process.

4.5 Conclusion

The pH and ionic strength dependence of electrostatic interactions was explored to introduce temporal fluctuations in the strengths of interparticle interactions and choreograph a transient self-assembly response in plasmonic NPs. The bridging and aggregating ability of EDTA with quaternary ammonium groups was used to trigger the aggregation in [+] AuNP. The observation of pH dependent aggregation of [+] AuNP with EDTA was decisive, as it revealed the factors influencing the stability of NP precipitates and hinted towards an autonomous pathway for the disassembly process. The autonomous changes in the pH and ionic strength values, under the influence of atmospheric CO_2, resulted in the complete breaking of electrostatic attractions that glued the [+] AuNP - EDTA precipitates. This is in stark contrast with most of the reported transient self-assembly systems, where a chemical degradation of the activated monomers is required to drive the disassembly process. Strikingly, the present use of temporal fluctuation in electrostatic interactions allowed the realization of transient switching between completely precipitate and redispersed stages of plasmonic NPs. The self-assembly process in [+] AuNP – EDTA system exhibits all the key characteristics of transient behaviour like triggered assembly, autonomous disassembly, tuneable aggregate lifetime (from days to hours), and pathway dependence in aggregation. On top of this, our strategy of using non-destructive ways for disassembly helped in achieving some of the desirable feats in the field of dynamic self-assembly like easy removal of waste, formation of a transiently stable precipitate state and negligible dampness of the redispersion response. The next logical step will be to impart distinct NP functionalities to such temporal self-assembly process, for which the capability of forming transiently stable NP precipitates will be advantageous.

4.6 Future Directions

In the present chapter, we demonstrate a new strategy to control over interparticle interactions to choreograph a transient self-assembly response. Here, we reveal the *so-far-unknown* ability of atmospheric components to transform a mundane mixture of chemicals into a dynamically changing one - a task usually accomplished with a network of chemical reactions. In future, one can couple functions like catalysis to the two distinct states so as to realize dynamic functions.

References

(1) Klajn, R.; Wesson, P. J.; Bishop, K. J. M.; Grzybowski, B. A. *Angew. Chem. Int. Ed.* **2009**, *48*, 7035–7039.

(2) Karsenti, E. *Nat. Rev. Mol. Cell Biol.* **2008**, *9*, 255–262.

(3) Grzybowski, B. A.; Huck, W. T. S. *Nat. Nanotechnol.* **2016**, *11*, 585–592.

(4) Hess, H.; L. Ross, J. *Chem. Soc. Rev.* **2017**, *46*, 5570–5587.

(5) Fialkowski, M.; Bishop, K. J. M.; Klajn, R.; Smoukov, S. K.; Campbell, C. J.; Grzybowski, B. A. *J. Phys. Chem. B* **2006**, *110*, 2482–2496.

(6) Sorrenti, A.; Leira-Iglesias, J.; Markvoort, A. J.; de Greef, T. F. A.; Hermans, T. M. *Chem. Soc. Rev.* **2017**, *46*, 5476–5490.

(7) Ashkenasy, G.; Hermans, T. M.; Otto, S.; Taylor, A. F. *Chem. Soc. Rev.* **2017**, *46*, 2543–2554.

(8) Frederick Ludlow, R.; Otto, S. *Chem. Soc. Rev.* **2008**, *37*, 101–108.

(9) Grzelczak, M. *J. Colloid Interface Sci.* **2019**, *537*, 269–279.

(10) Grzybowski, B. A.; Fitzner, K.; Paczesny, J.; Granick, S. *Chem. Soc. Rev.* **2017**, *46*, 5647–5678.

(11) Della Sala, F.; Neri, S.; Maiti, S.; Chen, J. L.-Y.; Prins, L. J. *Curr. Opin. Biotech.* **2017**, *46*, 27–33.

(12) De, S.; Klajn, R. *Adv. Mater.* **2018**, *30*, 1706750.

(13) Ragazzon, G.; Prins, L. J. *Nat. Nanotechnol.* **2018**, *13*, 882–889.

(14) Merindol, R.; Walther, A. *Chem. Soc. Rev.* **2017**, *46*, 5588–5619.

(15) Heinen, L.; Walther, A. *Soft Matter* **2015**, *11*, 7857–7866.

(16) Batista, C. A. S.; Larson, R. G.; Kotov, N. A. *Science* **2015**, *350*, 1242477.

(17) Bishop, K. J. M.; Wilmer, C. E.; Soh, S.; Grzybowski, B. A. *Small* **2009**, *5*, 1600–1630.

(18) Grzelczak, M.; Vermant, J.; Furst, E. M.; Liz-Marzán, L. M. *ACS Nano* **2010**, *4*, 3591–3605.

(19) Boles, M. A.; Engel, M.; Talapin, D. V. *Chem. Rev.* **2016**, *116*, 11220–11289.

(20) Taniguchi, Y.; Sazali, M. A. B.; Kobayashi, Y.; Arai, N.; Kawai, T.; Nakashima, T. *ACS Nano* **2017**, *11*, 9312–9320.

(21) Merg, A. D.; Zhou, Y.; Smith, A. M.; Millstone, J. E.; Rosi, N. L. *ChemNanoMat* **2017**, *3*, 745–749.

(22) Klajn, R. *Chem. Soc. Rev.* **2013**, *43*, 148–184.

(23) Klajn, R.; Bishop, K. J. M.; Grzybowski, B. A. *Proc. Natl. Acad. Sci.* **2007**, *104*, 10305–10309.

(24) Lutz, J.-F.; Lehn, J.-M.; Meijer, E. W.; Matyjaszewski, K. *Nat. Rev. Mater.* **2016**, *1*, 1–14.

(25) Bian, T.; Chu, Z.; Klajn, R. *Adv. Mater.* **2019**, 1905866.

(26) Boekhoven, J.; Brizard, A. M.; Kowlgi, K. N. K.; Koper, G. J. M.; Eelkema, R.; van Esch, J. H. *Angew. Chem. Int. Ed.* **2010**, *49*, 4825–4828.

(27) Boekhoven, J.; Hendriksen, W. E.; Koper, G. J. M.; Eelkema, R.; Esch, J. H. v. *Science* **2015**, *349*, 1075–1079.

(28) Kariyawasam, L. S.; Hartley, C. S. *J. Am. Chem. Soc.* **2017**, *139*, 11949–11955.

(29) Tena-Solsona, M.; Wanzke, C.; Riess, B.; Bausch, A. R.; Boekhoven, J. *Nat. Commun.* **2018**, *9*, 1–8.

(30) Leira-Iglesias, J.; Tassoni, A.; Adachi, T.; Stich, M.; Hermans, T. M. *Nat. Nanotechnol.* **2018**, *13*, 1021–1027.

(31) Jalani, K.; Dhiman, S.; Jain, A.; George, S. J. *Chem. Sci.* **2017**, *8*, 6030–6036.

(32) Van Ravensteijn, B. G. P.; Hendriksen, W. E.; Eelkema, R.; van Esch, J. H.; Kegel, W. K. *J. Am. Chem. Soc.* **2017**, *139*, 9763–9766.

(33) Debnath, S.; Roy, S.; Ulijn, R. V. *J. Am. Chem. Soc.* **2013**, *135*, 16789–16792.

(34) Dhiman, S.; Jain, A.; Kumar, M.; George, S. J. *J. Am. Chem. Soc.* **2017**, *139*, 16568–16575.

(35) Maiti, S.; Fortunati, I.; Ferrante, C.; Scrimin, P.; Prins, L. J. *Nat. Chem.* **2016**, *8*, 725–731.

(36) Dhiman, S.; Sarkar, A.; George, S. J. *RSC Adv.* **2018**, *8*, 18913–18925.

(37) Heuser, T.; Steppert, A.-K.; Molano Lopez, C.; Zhu, B.; Walther, A. *Nano Lett.* **2015**, *15*, 2213–2219.

(38) Grötsch, R. K.; Angı, A.; Mideksa, Y. G.; Wanzke, C.; Tena-Solsona, M.; Feige, M. J.; Rieger, B.; Boekhoven, J. *Angew. Chem. Int. Ed.* **2018**, *57*, 14608–14612.

(39) Grötsch, R. K.; Wanzke, C.; Speckbacher, M.; Angı, A.; Rieger, B.; Boekhoven, J. *J. Am. Chem. Soc.* **2019**, *141*, 9872–9878.

(40) Sawczyk, M.; Klajn, R. *J. Am. Chem. Soc.* **2017**, *139*, 17973–17978.

(41) Rossum, S. A. P. v.; Tena-Solsona, M.; Esch, J. H. v.; Eelkema, R.; Boekhoven, J. *Chem. Soc. Rev.* **2017**, *46*, 5519–5535.

(42) Rao, A.; Roy, S.; Unnikrishnan, M.; Bhosale, S. S.; Devatha, G.; Pillai, P. P. *Chem. Mater.* **2016**, *28*, 2348–2355.

(43) Jana, N. R.; Peng, X. *J. Am. Chem. Soc.* **2003**, *125*, 14280–14281.

(44) Pillai, P. P.; Huda, S.; Kowalczyk, B.; Grzybowski, B. A. *J. Am. Chem. Soc.* **2013**, *135*, 6392–6395.

(45) Santos, E. C. S.; Santos, T. C. d.; Fernandes, T. S.; Jorge, F. L.; Nascimento, V.; Madriaga, V. G. C.; Cordeiro, P. S.; Checca, N. R.; Costa, N. M. D.; Pinto, L. F. R.; Ronconi, C. M. *J. Mater. Chem. B* **2020**, DOI: 10.1039/C9TB00946A.

(46) Méric, R.; Vigneron, J.-P.; Lehn, J.-M. *J. Chem. Soc., Chem. Commun.* **1993**, *0*, 129–131.

(47) Jeffery, G. H.; Bassett, J.; Mendham, J.; Denney, R. C., *Vogels Textbook Of Quantitative Chemical Analysis*, 5th ed.; Pearson Education India: 2006.

(48) Angulo-Pachón, C. A.; Miravet, J. F. *Chem. Commun.* **2016**, *52*, 5398–5401.

(49) Lee, J.-W.; Klajn, R. *Chem. Commun.* **2015**, *51*, 2036–2039.

(50) Roy, S.; Rao, A.; Devatha, G.; Pillai, P. P. *ACS Catal.* **2017**, *7*, 7141–7145.

(51) Devatha, G.; Roy, S.; Rao, A.; Mallick, A.; Basu, S.; Pillai, P. P. *Chem. Sci.* **2017**, *8*, 3879–3884.

List of Publications

Included in thesis

1. **Rao, A.**, Roy, S., Unnikrishnan, M., Bhosale, S. S., Devatha, G., and Pillai, P. P., Regulation of Interparticle Forces Reveals Controlled Aggregation in Charged Nanoparticles. *Chem. Mater.* **2016**, *28*, 2348 − 2355.

2. **Rao, A.**, Kumar, G. S., Roy, S., Ajesh, T. R., Devatha, G., Pillai, P. P., Turn-On Selectivity in Inherently Nonselective Gold Nanoparticles for Pb^{2+} Detection by Preferential Breaking of Interparticle Interactions, *ACS Appl. Nano Mater.* **2019**, *2*, 5625 − 5633.

3. **Rao, A.**, Roy, S., Pillai, P. P., Realization of Transiently Stable Precipitates in the Fuel-Driven Dynamic Self-Assembly of Plasmonic Nanoparticles, ***Manuscript Submitted***.

Not included in thesis:

1. Devatha, G., Roy, S., **Rao, A.**, Mallick, A., Basu, S., Pillai, P. P., Electrostatically Driven Resonance Energy Transfer in 'Cationic' Biocompatible Indium Phosphide Quantum Dots. *Chem. Sci.* **2017**, *8*, 3879 − 3884.

2. Roy, S., **Rao, A.**, Devatha, G., Pillai, P. P., Revealing the Role of Electrostatics in Gold − Nanoparticle − Catalyzed Reduction of Charged Substrates. *ACS Catal.* **2017**, *7*, 7141 − 7145.

3. Xavier, J. A. M., Devatha, G., Roy, S., **Rao, A.**, Pillai, P. P., Electrostatically Regulated Photoinduced Electron Transfer in 'Cationic' Eco-friendly CuInS2/ZnS Quantum Dots in Water. *J. Mater. Chem. A* **2018**, *6*, 22248 − 22255.

4. Roy, S., Roy, S., **Rao, A.**, Devatha, G., Pillai P. P., Precise Nanoparticle −

Reactant Interaction Outplays Ligand Poisoning in Visible−Light Photocatalysis. *Chem. Mater.* **2018**, *30*, 8415 − 8419.

5. Chakraborty, I.; Roy, S.; Devatha, G.; **Rao, A.**; Pillai, P. P., InP/ZnS Quantum Dots as Efficient Visible-Light Photocatalysts for Redox and Carbon-Carbon Coupling Reaction. *Chem. Mater.* **2019**, *31*, 2258 − 2262.

6. Devatha, G., **Rao, A.**, Roy, S. Pillai, P. P., Förster Resonance Energy Transfer Regulated Multicolor Photopatterning from Single Quantum Dot Nanohybrid Films. *ACS Energy Lett.* **2019**, *4*, 1710 − 1716.

List of Conferences Attended

- Presented a poster titled *"Regulating Interparticle Forces Reveal Phenomenon of Controlled Aggregation in Charged Nanoparticles"*in **International Conference on Nanoscience and Technology (ICONSAT)** held at **IISER-Pune** in March 2016.

- Attended **DST School on Nanoscience and Nanotechnology** at **CeNS Bengaluru** from 23rd October to 3rd November 2017.

- Attended the Conference **Innovations in Frontier Chemistry (IFC)** 2018 held at **IISER Pune** from May 8th – 9th.

- Gave a talk titled *"Emergence of Selectivity in Inherently Nonselective Gold Nanoparticles by Controlling Interparticle Interactions"* in **Chemsymphoria (in-house Symposium)** at **IISER Pune** in July 2018.

- Attended **Indo-US Workshop on Soft Matter (IUWSM-2018)** at **IIT Roorkee** from December 9 - 11, 2018.

- Presented a poster as well as short 90 second talk at **Alexander von Humboldt (AvH) Kolleg** titled *"Regulating Nanoscale Forces to Control Macroscale Functions"* from January 31 to February 2, 2019 at **Kashid**.

- Presented a poster at **Gordon Research Conference on Self-Assembly and Supramolecular Chemistry** titled *"Regulating Nanoscale Forces to Control Macroscale Functions"* from May 19 - 24, 2019.

Regulation of Interparticle Forces Reveals Controlled Aggregation in Charged Nanoparticles

Author: Anish Rao, Soumendu Roy, Mahima Unnikrishnan, et al
Publication: Chemistry of Materials
Publisher: American Chemical Society
Date: Apr 1, 2016

PERMISSION/LICENSE IS GRANTED FOR YOUR ORDER AT NO CHARGE

This type of permission/license, instead of the standard Terms & Conditions, is sent to you because no fee is being charged for your order. Please note the following:

- Permission is granted for your request in both print and electronic formats, and translations.
- If figures and/or tables were requested, they may be adapted or used in part.
- Please print this page for your records and send a copy of it to your publisher/graduate school.
- Appropriate credit for the requested material should be given as follows: "Reprinted (adapted) with permission from (COMPLETE REFERENCE CITATION). Copyright (YEAR) American Chemical Society." Insert appropriate information in place of the capitalized words.
- One-time permission is granted only for the use specified in your request. No additional uses are granted (such as derivative works or other editions). For any other uses, please submit a new request.

BACK CLOSE WINDOW

Turn-On Selectivity in Inherently Nonselective Gold Nanoparticles for Pb2 Detection by Preferential Breaking of Interparticle Interactions

Author: Anish Rao, Govind Sasi Kumar, Soumendu Roy, et al

Publication: ACS Applied Nano Materials

Publisher: American Chemical Society

Date: Sep 1, 2019

PERMISSION/LICENSE IS GRANTED FOR YOUR ORDER AT NO CHARGE

This type of permission/license, instead of the standard Terms & Conditions, is sent to you because no fee is being charged for your order. Please note the following:

- Permission is granted for your request in both print and electronic formats, and translations.
- If figures and/or tables were requested, they may be adapted or used in part.
- Please print this page for your records and send a copy of it to your publisher/graduate school.
- Appropriate credit for the requested material should be given as follows: "Reprinted (adapted) with permission from (COMPLETE REFERENCE CITATION). Copyright (YEAR) American Chemical Society." Insert appropriate information in place of the capitalized words.
- One-time permission is granted only for the use specified in your request. No additional uses are granted (such as derivative works or other editions). For any other uses, please submit a new request.

BACK CLOSE WINDOW

Chapter 5

Copyright Forms

SPRINGER NATURE LICENSE
TERMS AND CONDITIONS

Feb 29, 2020

This Agreement between Indian Institute of Science Education and Research (IISER) Pune -- Anish Rao ("You") and Springer Nature ("Springer Nature") consists of your license details and the terms and conditions provided by Springer Nature and Copyright Clearance Center.

License Number	4778060097352
License date	Feb 29, 2020
Licensed Content Publisher	Springer Nature
Licensed Content Publication	Nature Materials
Licensed Content Title	Kinetically driven self assembly of highly ordered nanoparticle monolayers
Licensed Content Author	Terry P. Bigioni et al
Licensed Content Date	Mar 19, 2006
Type of Use	Thesis/Dissertation
Requestor type	academic/university or research institute
Format	print and electronic
Portion	figures/tables/illustrations
Number of figures/tables/illustrations	2
High-res required	no

Will you be translating?	no
Circulation/distribution	1 - 29
Author of this Springer Nature content	no
Title	Regulating Interparticle Interactions to Control the Spatio-Temporal Assembly of Gold Nanoparticles
Institution name	n/a
Expected presentation date	Mar 2020
Order reference number	Chapter 1, [41]
Portions	Figure 1, Figure 2
Requestor Location	Indian Institute of Science Education and Research (IISER) Pune IISER New Hostel 2, IISER Campus, Pashan Pune, Maharashtra 411008 India Attn: Indian Institute of Science Education and Research (IISER) Pune
Total	0.00 USD

Terms and Conditions

Springer Nature Customer Service Centre GmbH
Terms and Conditions

This agreement sets out the terms and conditions of the licence (the **Licence**) between you and **Springer Nature Customer Service Centre GmbH** (the **Licensor**). By clicking 'accept' and completing the transaction for the material (**Licensed Material**), you also confirm your acceptance of these terms and conditions.

1. Grant of License

1. 1. The Licensor grants you a personal, non-exclusive, non-transferable, world-wide licence to reproduce the Licensed Material for the purpose specified in your order only. Licences are granted for the specific use requested in the order and for no other use, subject to the conditions below.

1. 2. The Licensor warrants that it has, to the best of its knowledge, the rights to license reuse of the Licensed Material. However, you should ensure that the material you are requesting is original to the Licensor and does not carry the copyright of another entity (as credited in the published version).

1. 3. If the credit line on any part of the material you have requested indicates that it was reprinted or adapted with permission from another source, then you should also seek permission from that source to reuse the material.

2. Scope of Licence

2. 1. You may only use the Licensed Content in the manner and to the extent permitted by these Ts&Cs and any applicable laws.

2. 2. A separate licence may be required for any additional use of the Licensed Material, e.g. where a licence has been purchased for print only use, separate permission must be obtained for electronic re-use. Similarly, a licence is only valid in the language selected and does not apply for editions in other languages unless additional translation rights have been granted separately in the licence. Any content owned by third parties are expressly excluded from the licence.

2. 3. Similarly, rights for additional components such as custom editions and derivatives require additional permission and may be subject to an additional fee. Please apply to Journalpermissions@springernature.com/bookpermissions@springernature.com for these rights.

2. 4. Where permission has been granted **free of charge** for material in print, permission may also be granted for any electronic version of that work, provided that the material is incidental to your work as a whole and that the electronic version is essentially equivalent to, or substitutes for, the print version.

2. 5. An alternative scope of licence may apply to signatories of the STM Permissions Guidelines, as amended from time to time.

3. Duration of Licence

3. 1. A licence for is valid from the date of purchase ('Licence Date') at the end of the relevant period in the below table:

Scope of Licence	Duration of Licence
Post on a website	12 months
Presentations	12 months
Books and journals	Lifetime of the edition in the language purchased

4. Acknowledgement

4. 1. The Licensor's permission must be acknowledged next to the Licenced Material in print. In electronic form, this acknowledgement must be visible at the same time as the figures/tables/illustrations or abstract, and must be hyperlinked to the journal/book's homepage. Our required acknowledgement format is in the Appendix below.

5. Restrictions on use

5. 1. Use of the Licensed Material may be permitted for incidental promotional use and minor editing privileges e.g. minor adaptations of single figures, changes of format, colour and/or style where the adaptation is credited as set out in Appendix 1 below. Any other changes including but not limited to, cropping, adapting, omitting material that affect the meaning, intention or moral rights of the author are strictly prohibited.

5. 2. You must not use any Licensed Material as part of any design or trademark.

5. 3. Licensed Material may be used in Open Access Publications (OAP) before publication by Springer Nature, but any Licensed Material must be removed from OAP sites prior to final publication.

6. Ownership of Rights

6. 1. Licensed Material remains the property of either Licensor or the relevant third party and any rights not explicitly granted herein are expressly reserved.

7. Warranty

IN NO EVENT SHALL LICENSOR BE LIABLE TO YOU OR ANY OTHER PARTY OR ANY OTHER PERSON OR FOR ANY SPECIAL, CONSEQUENTIAL, INCIDENTAL OR INDIRECT DAMAGES, HOWEVER CAUSED, ARISING OUT OF OR IN CONNECTION WITH THE DOWNLOADING, VIEWING OR USE OF THE MATERIALS REGARDLESS OF THE FORM OF ACTION, WHETHER FOR BREACH OF CONTRACT, BREACH OF WARRANTY, TORT, NEGLIGENCE, INFRINGEMENT OR OTHERWISE (INCLUDING, WITHOUT LIMITATION, DAMAGES BASED ON LOSS OF PROFITS, DATA, FILES, USE, BUSINESS OPPORTUNITY OR CLAIMS OF THIRD PARTIES), AND
WHETHER OR NOT THE PARTY HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES. THIS LIMITATION SHALL APPLY NOTWITHSTANDING ANY FAILURE OF ESSENTIAL PURPOSE OF ANY LIMITED REMEDY PROVIDED HEREIN.

8. Limitations

8. 1. ***BOOKS ONLY:***Where **'reuse in a dissertation/thesis'** has been selected the following terms apply: Print rights of the final author's accepted manuscript (for clarity, NOT the published version) for up to 100 copies, electronic rights for use only on a personal website or institutional repository as defined by the Sherpa guideline (www.sherpa.ac.uk/romeo/).

9. Termination and Cancellation

9. 1. Licences will expire after the period shown in Clause 3 (above).

9. 2. Licensee reserves the right to terminate the Licence in the event that payment is not received in full or if there has been a breach of this agreement by you.

Appendix 1 — Acknowledgements:

For Journal Content:
Reprinted by permission from [**the Licensor**]: [**Journal Publisher** (e.g. Nature/Springer/Palgrave)] [**JOURNAL NAME**] [**REFERENCE CITATION** (Article name, Author(s) Name), [**COPYRIGHT**] (year of publication)

For **Advance Online Publication papers:**
Reprinted by permission from [**the Licensor**]: [**Journal Publisher** (e.g. Nature/Springer/Palgrave)] [**JOURNAL NAME**] [**REFERENCE CITATION** (Article name, Author(s) Name), [**COPYRIGHT**] (year of publication), advance online publication, day month year (doi: 10.1038/sj.[JOURNAL ACRONYM].)

For Adaptations/Translations:
Adapted/Translated by permission from [**the Licensor**]: [**Journal Publisher** (e.g. Nature/Springer/Palgrave)] [**JOURNAL NAME**] [**REFERENCE CITATION** (Article name, Author(s) Name), [**COPYRIGHT**] (year of publication)

Note: For any republication from the British Journal of Cancer, the following credit line style applies:

Reprinted/adapted/translated by permission from [**the Licensor**]: on behalf of Cancer Research UK: : [**Journal Publisher** (e.g. Nature/Springer/Palgrave)] [**JOURNAL NAME**] [**REFERENCE CITATION** (Article name, Author(s) Name), [**COPYRIGHT**] (year of publication)

For **Advance Online Publication** papers:
Reprinted by permission from The [**the Licensor**]: on behalf of Cancer Research UK: [**Journal Publisher** (e.g. Nature/Springer/Palgrave)] [**JOURNAL NAME**] [**REFERENCE CITATION** (Article name, Author(s) Name), [**COPYRIGHT**] (year of publication), advance online publication, day month year (doi: 10.1038/sj.[JOURNAL ACRONYM])

For Book content:
Reprinted/adapted by permission from [**the Licensor**]: [**Book Publisher** (e.g. Palgrave Macmillan, Springer etc) [**Book Title**] by [**Book author**(s)] [**COPYRIGHT**] (year of publication)

Other Conditions:

Version 1.2

Questions? customercare@copyright.com or +1-855-239-3415 (toll free in the US) or +1-978-646-2777.

SPRINGER NATURE LICENSE
TERMS AND CONDITIONS

Feb 29, 2020

This Agreement between Indian Institute of Science Education and Research (IISER) Pune -- Anish Rao ("You") and Springer Nature ("Springer Nature") consists of your license details and the terms and conditions provided by Springer Nature and Copyright Clearance Center.

License Number	4778040009659
License date	Feb 29, 2020
Licensed Content Publisher	Springer Nature
Licensed Content Publication	Nature Reviews Materials
Licensed Content Title	Crystal engineering with DNA
Licensed Content Author	Christine R. Laramy et al
Licensed Content Date	Feb 18, 2019
Type of Use	Thesis/Dissertation
Requestor type	academic/university or research institute
Format	print and electronic
Portion	figures/tables/illustrations
Number of figures/tables/illustrations	2
High-res required	no

Will you be translating?	no
Circulation/distribution	1 - 29
Author of this Springer Nature content	no
Title	Regulating Interparticle Interactions to Control the Spatio-Temporal Assembly of Gold Nanoparticles
Institution name	n/a
Expected presentation date	Mar 2020
Order reference number	Chapter 1, [18]
Portions	Figure 1, Figure 4
Requestor Location	Indian Institute of Science Education and Research (IISER) Pune IISER New Hostel 2, IISER Campus, Pashan Pune, Maharashtra 411008 India Attn: Indian Institute of Science Education and Research (IISER) Pune
Total	0.00 USD

Terms and Conditions

Springer Nature Customer Service Centre GmbH
Terms and Conditions

This agreement sets out the terms and conditions of the licence (the **Licence**) between you and **Springer Nature Customer Service Centre GmbH** (the **Licensor**). By clicking 'accept' and completing the transaction for the material (**Licensed Material**), you also confirm your acceptance of these terms and conditions.

1. Grant of License

1. 1. The Licensor grants you a personal, non-exclusive, non-transferable, world-wide licence to reproduce the Licensed Material for the purpose specified in your order only. Licences are granted for the specific use requested in the order and for no other use, subject to the conditions below.

1. 2. The Licensor warrants that it has, to the best of its knowledge, the rights to license reuse of the Licensed Material. However, you should ensure that the material you are requesting is original to the Licensor and does not carry the copyright of another entity (as credited in the published version).

1. 3. If the credit line on any part of the material you have requested indicates that it was reprinted or adapted with permission from another source, then you should also seek permission from that source to reuse the material.

2. Scope of Licence

2. 1. You may only use the Licensed Content in the manner and to the extent permitted by these Ts&Cs and any applicable laws.

2. 2. A separate licence may be required for any additional use of the Licensed Material, e.g. where a licence has been purchased for print only use, separate permission must be obtained for electronic re-use. Similarly, a licence is only valid in the language selected and does not apply for editions in other languages unless additional translation rights have been granted separately in the licence. Any content owned by third parties are expressly excluded from the licence.

2. 3. Similarly, rights for additional components such as custom editions and derivatives require additional permission and may be subject to an additional fee. Please apply to Journalpermissions@springernature.com/bookpermissions@springernature.com for these rights.

2. 4. Where permission has been granted **free of charge** for material in print, permission may also be granted for any electronic version of that work, provided that the material is incidental to your work as a whole and that the electronic version is essentially equivalent to, or substitutes for, the print version.

2. 5. An alternative scope of licence may apply to signatories of the STM Permissions Guidelines, as amended from time to time.

3. Duration of Licence

3. 1. A licence for is valid from the date of purchase ('Licence Date') at the end of the relevant period in the below table:

Scope of Licence	Duration of Licence
Post on a website	12 months
Presentations	12 months
Books and journals	Lifetime of the edition in the language purchased

4. Acknowledgement

4. 1. The Licensor's permission must be acknowledged next to the Licenced Material in print. In electronic form, this acknowledgement must be visible at the same time as the figures/tables/illustrations or abstract, and must be hyperlinked to the journal/book's homepage. Our required acknowledgement format is in the Appendix below.

5. Restrictions on use

5. 1. Use of the Licensed Material may be permitted for incidental promotional use and minor editing privileges e.g. minor adaptations of single figures, changes of format, colour and/or style where the adaptation is credited as set out in Appendix 1 below. Any other changes including but not limited to, cropping, adapting, omitting material that affect the meaning, intention or moral rights of the author are strictly prohibited.

5. 2. You must not use any Licensed Material as part of any design or trademark.

5. 3. Licensed Material may be used in Open Access Publications (OAP) before publication by Springer Nature, but any Licensed Material must be removed from OAP sites prior to final publication.

6. Ownership of Rights

6. 1. Licensed Material remains the property of either Licensor or the relevant third party and any rights not explicitly granted herein are expressly reserved.

7. Warranty

IN NO EVENT SHALL LICENSOR BE LIABLE TO YOU OR ANY OTHER PARTY OR ANY OTHER PERSON OR FOR ANY SPECIAL, CONSEQUENTIAL, INCIDENTAL OR INDIRECT DAMAGES, HOWEVER CAUSED, ARISING OUT OF OR IN CONNECTION WITH THE DOWNLOADING, VIEWING OR USE OF THE MATERIALS REGARDLESS OF THE FORM OF ACTION, WHETHER FOR BREACH OF CONTRACT, BREACH OF WARRANTY, TORT, NEGLIGENCE, INFRINGEMENT OR OTHERWISE (INCLUDING, WITHOUT LIMITATION, DAMAGES BASED ON LOSS OF PROFITS, DATA, FILES, USE, BUSINESS OPPORTUNITY OR CLAIMS OF THIRD PARTIES), AND
WHETHER OR NOT THE PARTY HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES. THIS LIMITATION SHALL APPLY NOTWITHSTANDING ANY FAILURE OF ESSENTIAL PURPOSE OF ANY LIMITED REMEDY PROVIDED HEREIN.

8. Limitations

8. 1. ***BOOKS ONLY:***Where **'reuse in a dissertation/thesis'** has been selected the following terms apply: Print rights of the final author's accepted manuscript (for clarity, NOT the published version) for up to 100 copies, electronic rights for use only on a personal website or institutional repository as defined by the Sherpa guideline (www.sherpa.ac.uk/romeo/).

9. Termination and Cancellation

9. 1. Licences will expire after the period shown in Clause 3 (above).

9. 2. Licensee reserves the right to terminate the Licence in the event that payment is not received in full or if there has been a breach of this agreement by you.

Appendix 1 — Acknowledgements:

For Journal Content:
Reprinted by permission from [**the Licensor**]: [**Journal Publisher** (e.g. Nature/Springer/Palgrave)] [**JOURNAL NAME**] [**REFERENCE CITATION** (Article name, Author(s) Name), [**COPYRIGHT**] (year of publication)

For **Advance Online Publication papers:**
Reprinted by permission from [**the Licensor**]: [**Journal Publisher** (e.g. Nature/Springer/Palgrave)] [**JOURNAL NAME**] [**REFERENCE CITATION** (Article name, Author(s) Name), [**COPYRIGHT**] (year of publication), advance online publication, day month year (doi: 10.1038/sj.[JOURNAL ACRONYM].)

For Adaptations/Translations:
Adapted/Translated by permission from [**the Licensor**]: [**Journal Publisher** (e.g. Nature/Springer/Palgrave)] [**JOURNAL NAME**] [**REFERENCE CITATION** (Article name, Author(s) Name), [**COPYRIGHT**] (year of publication)

Note: For any republication from the British Journal of Cancer, the following credit line style applies:

Reprinted/adapted/translated by permission from [**the Licensor**]: on behalf of Cancer Research UK: : [**Journal Publisher** (e.g. Nature/Springer/Palgrave)] [**JOURNAL NAME**] [**REFERENCE CITATION** (Article name, Author(s) Name), [**COPYRIGHT**] (year of publication)

For **Advance Online Publication** papers:
Reprinted by permission from The [**the Licensor**]: on behalf of Cancer Research UK: [**Journal Publisher** (e.g. Nature/Springer/Palgrave)] [**JOURNAL NAME**] [**REFERENCE CITATION** (Article name, Author(s) Name), [**COPYRIGHT**] (year of publication), advance online publication, day month year (doi: 10.1038/sj.[JOURNAL ACRONYM])

For Book content:
Reprinted/adapted by permission from [**the Licensor**]: [**Book Publisher** (e.g. Palgrave Macmillan, Springer etc) [**Book Title**] by [**Book author**(s)] [**COPYRIGHT**] (year of publication)

Other Conditions:

Version 1.2

Questions? customercare@copyright.com or +1-855-239-3415 (toll free in the US) or +1-978-646-2777.

THE AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE LICENSE TERMS AND CONDITIONS

Feb 29, 2020

This Agreement between Indian Institute of Science Education and Research (IISER) Pune -- Anish Rao ("You") and The American Association for the Advancement of Science ("The American Association for the Advancement of Science") consists of your license details and the terms and conditions provided by The American Association for the Advancement of Science and Copyright Clearance Center.

License Number	4778040501108
License date	Feb 29, 2020
Licensed Content Publisher	The American Association for the Advancement of Science
Licensed Content Publication	Science
Licensed Content Title	Self-assembly of magnetite nanocubes into helical superstructures
Licensed Content Author	Gurvinder Singh,Henry Chan,Artem Baskin,Elijah Gelman,Nikita Repnin,Petr Král,Rafal Klajn
Licensed Content Date	Sep 5, 2014
Licensed Content Volume	345
Licensed Content Issue	6201
Volume number	345
Issue number	6201

Type of Use	Thesis / Dissertation
Requestor type	Scientist/individual at a research institution
Format	Print and electronic
Portion	Figure
Number of figures/tables	1
Title	Regulating Interparticle Interactions to Control the Spatio-Temporal Assembly of Gold Nanoparticles
Institution name	n/a
Expected presentation date	Mar 2020
Order reference number	Chapter 1, [23]
Portions	Figure 2
Requestor Location	Indian Institute of Science Education and Research (IISER) Pune IISER New Hostel 2, IISER Campus, Pashan Pune, Maharashtra 411008 India Attn: Indian Institute of Science Education and Research (IISER) Pune
Total	0.00 USD

Terms and Conditions

American Association for the Advancement of Science TERMS AND CONDITIONS

Regarding your request, we are pleased to grant you non-exclusive, non-transferable permission, to republish the AAAS material identified above in your work identified above, subject to the terms and conditions herein. We must be contacted for permission for any uses other than those specifically identified in your request above.

The following credit line must be printed along with the AAAS material: "From [Full Reference Citation]. Reprinted with permission from AAAS."

All required credit lines and notices must be visible any time a user accesses any part of the AAAS material and must appear on any printed copies and authorized user might make.

This permission does not apply to figures / photos / artwork or any other content or materials included in your work that are credited to non-AAAS sources. If the requested material is sourced to or references non-AAAS sources, you must obtain authorization from that source as well before using that material. You agree to hold harmless and indemnify AAAS against any claims arising from your use of any content in your work that is credited to non-AAAS sources.

If the AAAS material covered by this permission was published in Science during the years 1974 - 1994, you must also obtain permission from the author, who may grant or withhold permission, and who may or may not charge a fee if permission is granted. See original article for author's address. This condition does not apply to news articles.

The AAAS material may not be modified or altered except that figures and tables may be modified with permission from the author. Author permission for any such changes must be secured prior to your use.

Whenever possible, we ask that electronic uses of the AAAS material permitted herein include a hyperlink to the original work on AAAS's website (hyperlink may be embedded in the reference citation).

AAAS material reproduced in your work identified herein must not account for more than 30% of the total contents of that work.

AAAS must publish the full paper prior to use of any text.

AAAS material must not imply any endorsement by the American Association for the Advancement of Science.

This permission is not valid for the use of the AAAS and/or Science logos.

AAAS makes no representations or warranties as to the accuracy of any information contained in the AAAS material covered by this permission, including any warranties of

merchantability or fitness for a particular purpose.

If permission fees for this use are waived, please note that AAAS reserves the right to charge for reproduction of this material in the future.

Permission is not valid unless payment is received within sixty (60) days of the issuance of this permission. If payment is not received within this time period then all rights granted herein shall be revoked and this permission will be considered null and void.

In the event of breach of any of the terms and conditions herein or any of CCC's Billing and Payment terms and conditions, all rights granted herein shall be revoked and this permission will be considered null and void.

AAAS reserves the right to terminate this permission and all rights granted herein at its discretion, for any purpose, at any time. In the event that AAAS elects to terminate this permission, you will have no further right to publish, publicly perform, publicly display, distribute or otherwise use any matter in which the AAAS content had been included, and all fees paid hereunder shall be fully refunded to you. Notification of termination will be sent to the contact information as supplied by you during the request process and termination shall be immediate upon sending the notice. Neither AAAS nor CCC shall be liable for any costs, expenses, or damages you may incur as a result of the termination of this permission, beyond the refund noted above.

This Permission may not be amended except by written document signed by both parties.

The terms above are applicable to all permissions granted for the use of AAAS material. Below you will find additional conditions that apply to your particular type of use.

FOR A THESIS OR DISSERTATION
If you are using figure(s)/table(s), permission is granted for use in print and electronic versions of your dissertation or thesis. A full text article may be used in print versions only of a dissertation or thesis.

Permission covers the distribution of your dissertation or thesis on demand by ProQuest / UMI, provided the AAAS material covered by this permission remains in situ.

If you are an Original Author on the AAAS article being reproduced, please refer to your License to Publish for rules on reproducing your paper in a dissertation or thesis.

FOR JOURNALS:
Permission covers both print and electronic versions of your journal article, however the AAAS material may not be used in any manner other than within the context of your article.

FOR BOOKS/TEXTBOOKS:
If this license is to reuse figures/tables, then permission is granted for non-exclusive world rights in all languages in both print and electronic formats (electronic formats are defined below).

If this license is to reuse a text excerpt or a full text article, then permission is granted for non-exclusive world rights in English only. You have the option of securing either print or electronic rights or both, but electronic rights are not automatically granted and do garner additional fees. Permission for translations of text excerpts or full text articles into other languages must be obtained separately.

Licenses granted for use of AAAS material in electronic format books/textbooks are valid only in cases where the electronic version is equivalent to or substitutes for the print version of the book/textbook. The AAAS material reproduced as permitted herein must remain in situ and must not be exploited separately (for example, if permission covers the use of a full text article, the article may not be offered for access or for purchase as a stand-alone unit), except in the case of permitted textbook companions as noted below.

You must include the following notice in any electronic versions, either adjacent to the reprinted AAAS material or in the terms and conditions for use of your electronic products: "Readers may view, browse, and/or download material for temporary copying purposes only, provided these uses are for noncommercial personal purposes. Except as provided by law, this material may not be further reproduced, distributed, transmitted, modified, adapted, performed, displayed, published, or sold in whole or in part, without prior written permission from the publisher."

If your book is an academic textbook, permission covers the following companions to your textbook, provided such companions are distributed only in conjunction with your textbook at no additional cost to the user:

- Password-protected website
- Instructor's image CD/DVD and/or PowerPoint resource
- Student CD/DVD

All companions must contain instructions to users that the AAAS material may be used for non-commercial, classroom purposes only. Any other uses require the prior written permission from AAAS.

If your license is for the use of AAAS Figures/Tables, then the electronic rights granted herein permit use of the Licensed Material in any Custom Databases that you distribute the electronic versions of your textbook through, so long as the Licensed Material remains within the context of a chapter of the title identified in your request and cannot be downloaded by a user as an independent image file.

Rights also extend to copies/files of your Work (as described above) that you are required to provide for use by the visually and/or print disabled in compliance with state and federal laws.

This permission only covers a single edition of your work as identified in your request.

FOR NEWSLETTERS:
Permission covers print and/or electronic versions, provided the AAAS material reproduced as permitted herein remains in situ and is not exploited separately (for example, if permission covers the use of a full text article, the article may not be offered for access or for purchase as a stand-alone unit)

FOR ANNUAL REPORTS:
Permission covers print and electronic versions provided the AAAS material reproduced as permitted herein remains in situ and is not exploited separately (for example, if permission covers the use of a full text article, the article may not be offered for access or for purchase as a stand-alone unit)

FOR PROMOTIONAL/MARKETING USES:
Permission covers the use of AAAS material in promotional or marketing pieces such as information packets, media kits, product slide kits, brochures, or flyers limited to a single print run. The AAAS Material may not be used in any manner which implies endorsement or promotion by the American Association for the Advancement of Science (AAAS) or Science of any product or service. AAAS does not permit the reproduction of its name, logo or text on promotional literature.

If permission to use a full text article is permitted, The Science article covered by this permission must not be altered in any way. No additional printing may be set onto an article copy other than the copyright credit line required above. Any alterations must be approved in advance and in writing by AAAS. This includes, but is not limited to, the placement of sponsorship identifiers, trademarks, logos, rubber stamping or self-adhesive stickers onto the article copies.

Additionally, article copies must be a freestanding part of any information package (i.e. media kit) into which they are inserted. They may not be physically attached to anything, such as an advertising insert, or have anything attached to them, such as a sample product. Article copies must be easily removable from any kits or informational packages in which they are used. The only exception is that article copies may be inserted into three-ring binders.

FOR CORPORATE INTERNAL USE:
The AAAS material covered by this permission may not be altered in any way. No additional printing may be set onto an article copy other than the required credit line. Any alterations must be approved in advance and in writing by AAAS. This includes, but is not limited to the placement of sponsorship identifiers, trademarks, logos, rubber stamping or self-adhesive stickers onto article copies.

If you are making article copies, copies are restricted to the number indicated in your request and must be distributed only to internal employees for internal use.

If you are using AAAS Material in Presentation Slides, the required credit line must be visible on the slide where the AAAS material will be reprinted

If you are using AAAS Material on a CD, DVD, Flash Drive, or the World Wide Web, you must include the following notice in any electronic versions, either adjacent to the reprinted AAAS material or in the terms and conditions for use of your electronic products: "Readers may view, browse, and/or download material for temporary copying purposes only, provided these uses are for noncommercial personal purposes. Except as provided by law, this material may not be further reproduced, distributed, transmitted, modified, adapted, performed, displayed, published, or sold in whole or in part, without prior written permission from the publisher." Access to any such CD, DVD, Flash Drive or Web page must be restricted to your organization's employees only.

FOR CME COURSE and SCIENTIFIC SOCIETY MEETINGS:
Permission is restricted to the particular Course, Seminar, Conference, or Meeting indicated in your request. If this license covers a text excerpt or a Full Text Article, access to the reprinted AAAS material must be restricted to attendees of your event only (if you have been granted electronic rights for use of a full text article on your website, your website must be password protected, or access restricted so that only attendees can access the content on your site).

If you are using AAAS Material on a CD, DVD, Flash Drive, or the World Wide Web, you must include the following notice in any electronic versions, either adjacent to the reprinted AAAS material or in the terms and conditions for use of your electronic products: "Readers may view, browse, and/or download material for temporary copying purposes only, provided these uses are for noncommercial personal purposes. Except as provided by law, this material may not be further reproduced, distributed, transmitted, modified, adapted, performed, displayed, published, or sold in whole or in part, without prior written permission from the publisher."

FOR POLICY REPORTS:
These rights are granted only to non-profit organizations and/or government agencies. Permission covers print and electronic versions of a report, provided the required credit line appears in both versions and provided the AAAS material reproduced as permitted herein remains in situ and is not exploited separately.

FOR CLASSROOM PHOTOCOPIES:
Permission covers distribution in print copy format only. Article copies must be freestanding and not part of a course pack. They may not be physically attached to anything or have anything attached to them.

FOR COURSEPACKS OR COURSE WEBSITES:
These rights cover use of the AAAS material in one class at one institution. Permission is valid only for a single semester after which the AAAS material must be removed from the Electronic Course website, unless new permission is obtained for an additional semester. If the material is to be distributed online, access must be restricted to students and instructors enrolled in that particular course by some means of password or access control.

FOR WEBSITES:
You must include the following notice in any electronic versions, either adjacent to the reprinted AAAS material or in the terms and conditions for use of your electronic products: "Readers may view, browse, and/or download material for temporary copying purposes only, provided these uses are for noncommercial personal purposes. Except as provided by law, this material may not be further reproduced, distributed, transmitted, modified, adapted, performed, displayed, published, or sold in whole or in part, without prior written permission from the publisher."

Permissions for the use of Full Text articles on third party websites are granted on a case by case basis and only in cases where access to the AAAS Material is restricted by some means of password or access control. Alternately, an E-Print may be purchased through our reprints department (brocheleau@rockwaterinc.com).

REGARDING FULL TEXT ARTICLE USE ON THE WORLD WIDE WEB IF YOU ARE AN 'ORIGINAL AUTHOR' OF A SCIENCE PAPER

If you chose "Original Author" as the Requestor Type, you are warranting that you are one of authors listed on the License Agreement as a "Licensed content author" or that you are acting on that author's behalf to use the Licensed content in a new work that one of the authors listed on the License Agreement as a "Licensed content author" has written.

Original Authors may post the 'Accepted Version' of their full text article on their personal or on their University website and not on any other website. The 'Accepted Version' is the version of the paper accepted for publication by AAAS including changes resulting from peer review but prior to AAAS's copy editing and production (in other words not the AAAS published version).

FOR MOVIES / FILM / TELEVISION:
Permission is granted to use, record, film, photograph, and/or tape the AAAS material in connection with your program/film and in any medium your program/film may be shown or heard, including but not limited to broadcast and cable television, radio, print, world wide web, and videocassette.

The required credit line should run in the program/film's end credits.

FOR MUSEUM EXHIBITIONS:
Permission is granted to use the AAAS material as part of a single exhibition for the duration of that exhibit. Permission for use of the material in promotional materials for the exhibit must be cleared separately with AAAS (please contact us at permissions@aaas.org).

FOR TRANSLATIONS:
Translation rights apply only to the language identified in your request summary above.

The following disclaimer must appear with your translation, on the first page of the article, after the credit line: "This translation is not an official translation by AAAS staff, nor is it endorsed by AAAS as accurate. In crucial matters, please refer to the official English-language version originally published by AAAS."

FOR USE ON A COVER:
Permission is granted to use the AAAS material on the cover of a journal issue, newsletter issue, book, textbook, or annual report in print and electronic formats provided the AAAS material reproduced as permitted herein remains in situ and is not exploited separately

By using the AAAS Material identified in your request, you agree to abide by all the terms and conditions herein.

Questions about these terms can be directed to the AAAS Permissions department permissions@aaas.org.

Other Terms and Conditions:

v 2

SPRINGER NATURE LICENSE
TERMS AND CONDITIONS

Feb 29, 2020

This Agreement between Indian Institute of Science Education and Research (IISER) Pune -- Anish Rao ("You") and Springer Nature ("Springer Nature") consists of your license details and the terms and conditions provided by Springer Nature and Copyright Clearance Center.

License Number	4778050259469
License date	Feb 29, 2020
Licensed Content Publisher	Springer Nature
Licensed Content Publication	Nature
Licensed Content Title	Lock and key colloids
Licensed Content Author	S. Sacanna et al
Licensed Content Date	Mar 25, 2010
Type of Use	Thesis/Dissertation
Requestor type	academic/university or research institute
Format	print and electronic
Portion	figures/tables/illustrations
Number of figures/tables/illustrations	1
High-res required	no

Will you be translating?	no
Circulation/distribution	1 - 29
Author of this Springer Nature content	no
Title	Regulating Interparticle Interactions to Control the Spatio-Temporal Assembly of Gold Nanoparticles
Institution name	n/a
Expected presentation date	Mar 2020
Order reference number	Chapter 1, [26]
Portions	Figure 2
Requestor Location	Indian Institute of Science Education and Research (IISER) Pune IISER New Hostel 2, IISER Campus, Pashan Pune, Maharashtra 411008 India Attn: Indian Institute of Science Education and Research (IISER) Pune
Total	0.00 USD

Terms and Conditions

Springer Nature Customer Service Centre GmbH
Terms and Conditions

This agreement sets out the terms and conditions of the licence (the **Licence**) between you and **Springer Nature Customer Service Centre GmbH** (the **Licensor**). By clicking 'accept' and completing the transaction for the material (**Licensed Material**), you also confirm your acceptance of these terms and conditions.

1. Grant of License

1. 1. The Licensor grants you a personal, non-exclusive, non-transferable, world-wide licence to reproduce the Licensed Material for the purpose specified in your order only. Licences are granted for the specific use requested in the order and for no other use, subject to the conditions below.

1. 2. The Licensor warrants that it has, to the best of its knowledge, the rights to license reuse of the Licensed Material. However, you should ensure that the material you are requesting is original to the Licensor and does not carry the copyright of another entity (as credited in the published version).

1. 3. If the credit line on any part of the material you have requested indicates that it was reprinted or adapted with permission from another source, then you should also seek permission from that source to reuse the material.

2. Scope of Licence

2. 1. You may only use the Licensed Content in the manner and to the extent permitted by these Ts&Cs and any applicable laws.

2. 2. A separate licence may be required for any additional use of the Licensed Material, e.g. where a licence has been purchased for print only use, separate permission must be obtained for electronic re-use. Similarly, a licence is only valid in the language selected and does not apply for editions in other languages unless additional translation rights have been granted separately in the licence. Any content owned by third parties are expressly excluded from the licence.

2. 3. Similarly, rights for additional components such as custom editions and derivatives require additional permission and may be subject to an additional fee. Please apply to Journalpermissions@springernature.com/bookpermissions@springernature.com for these rights.

2. 4. Where permission has been granted **free of charge** for material in print, permission may also be granted for any electronic version of that work, provided that the material is incidental to your work as a whole and that the electronic version is essentially equivalent to, or substitutes for, the print version.

2. 5. An alternative scope of licence may apply to signatories of the STM Permissions Guidelines, as amended from time to time.

3. Duration of Licence

3. 1. A licence for is valid from the date of purchase ('Licence Date') at the end of the relevant period in the below table:

Scope of Licence	Duration of Licence
Post on a website	12 months
Presentations	12 months
Books and journals	Lifetime of the edition in the language purchased

4. Acknowledgement

4. 1. The Licensor's permission must be acknowledged next to the Licenced Material in print. In electronic form, this acknowledgement must be visible at the same time as the figures/tables/illustrations or abstract, and must be hyperlinked to the journal/book's homepage. Our required acknowledgement format is in the Appendix below.

5. Restrictions on use

5. 1. Use of the Licensed Material may be permitted for incidental promotional use and minor editing privileges e.g. minor adaptations of single figures, changes of format, colour and/or style where the adaptation is credited as set out in Appendix 1 below. Any other changes including but not limited to, cropping, adapting, omitting material that affect the meaning, intention or moral rights of the author are strictly prohibited.

5. 2. You must not use any Licensed Material as part of any design or trademark.

5. 3. Licensed Material may be used in Open Access Publications (OAP) before publication by Springer Nature, but any Licensed Material must be removed from OAP sites prior to final publication.

6. Ownership of Rights

6. 1. Licensed Material remains the property of either Licensor or the relevant third party and any rights not explicitly granted herein are expressly reserved.

7. Warranty

IN NO EVENT SHALL LICENSOR BE LIABLE TO YOU OR ANY OTHER PARTY OR ANY OTHER PERSON OR FOR ANY SPECIAL, CONSEQUENTIAL, INCIDENTAL OR INDIRECT DAMAGES, HOWEVER CAUSED, ARISING OUT OF OR IN CONNECTION WITH THE DOWNLOADING, VIEWING OR USE OF THE MATERIALS REGARDLESS OF THE FORM OF ACTION, WHETHER FOR BREACH OF CONTRACT, BREACH OF WARRANTY, TORT, NEGLIGENCE, INFRINGEMENT OR OTHERWISE (INCLUDING, WITHOUT LIMITATION, DAMAGES BASED ON LOSS OF PROFITS, DATA, FILES, USE, BUSINESS OPPORTUNITY OR CLAIMS OF THIRD PARTIES), AND
WHETHER OR NOT THE PARTY HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES. THIS LIMITATION SHALL APPLY NOTWITHSTANDING ANY FAILURE OF ESSENTIAL PURPOSE OF ANY LIMITED REMEDY PROVIDED HEREIN.

8. Limitations

8. 1. ***BOOKS ONLY:***Where **'reuse in a dissertation/thesis'** has been selected the following terms apply: Print rights of the final author's accepted manuscript (for clarity, NOT the published version) for up to 100 copies, electronic rights for use only on a personal website or institutional repository as defined by the Sherpa guideline (www.sherpa.ac.uk/romeo/).

9. Termination and Cancellation

9. 1. Licences will expire after the period shown in Clause 3 (above).

9. 2. Licensee reserves the right to terminate the Licence in the event that payment is not received in full or if there has been a breach of this agreement by you.

Appendix 1 — Acknowledgements:

For Journal Content:
Reprinted by permission from [**the Licensor**]: [**Journal Publisher** (e.g. Nature/Springer/Palgrave)] [**JOURNAL NAME**] [**REFERENCE CITATION** (Article name, Author(s) Name), [**COPYRIGHT**] (year of publication)

For **Advance Online Publication papers:**
Reprinted by permission from [**the Licensor**]: [**Journal Publisher** (e.g. Nature/Springer/Palgrave)] [**JOURNAL NAME**] [**REFERENCE CITATION** (Article name, Author(s) Name), [**COPYRIGHT**] (year of publication), advance online publication, day month year (doi: 10.1038/sj.[JOURNAL ACRONYM].)

For Adaptations/Translations:
Adapted/Translated by permission from [**the Licensor**]: [**Journal Publisher** (e.g. Nature/Springer/Palgrave)] [**JOURNAL NAME**] [**REFERENCE CITATION** (Article name, Author(s) Name), [**COPYRIGHT**] (year of publication)

Note: For any republication from the British Journal of Cancer, the following credit line style applies:

Reprinted/adapted/translated by permission from [**the Licensor**]: on behalf of Cancer Research UK: : [**Journal Publisher** (e.g. Nature/Springer/Palgrave)] [**JOURNAL NAME**] [**REFERENCE CITATION** (Article name, Author(s) Name), [**COPYRIGHT**] (year of publication)

For **Advance Online Publication** papers:
Reprinted by permission from The [**the Licensor**]: on behalf of Cancer Research UK: [**Journal Publisher** (e.g. Nature/Springer/Palgrave)] [**JOURNAL NAME**] [**REFERENCE CITATION** (Article name, Author(s) Name), [**COPYRIGHT**] (year of publication), advance online publication, day month year (doi: 10.1038/sj.[JOURNAL ACRONYM])

For Book content:
Reprinted/adapted by permission from [**the Licensor**]: [**Book Publisher** (e.g. Palgrave Macmillan, Springer etc) [**Book Title**] by [**Book author**(s)] [**COPYRIGHT**] (year of publication)

Other Conditions:

Version 1.2

Questions? customercare@copyright.com or +1-855-239-3415 (toll free in the US) or +1-978-646-2777.

AIP PUBLISHING LICENSE
TERMS AND CONDITIONS

Feb 29, 2020

This Agreement between Indian Institute of Science Education and Research (IISER) Pune -- Anish Rao ("You") and AIP Publishing ("AIP Publishing") consists of your license details and the terms and conditions provided by AIP Publishing and Copyright Clearance Center.

License Number	4778050481545
License date	Feb 29, 2020
Licensed Content Publisher	AIP Publishing
Licensed Content Publication	Journal of Chemical Physics
Licensed Content Title	Binding kinetics of lock and key colloids
Licensed Content Author	Laura Colón-Meléndez, Daniel J. Beltran-Villegas, Greg van Anders, et al
Licensed Content Date	May 7, 2015
Licensed Content Volume	142
Licensed Content Issue	17
Type of Use	Thesis/Dissertation
Requestor type	Student
Format	Print and electronic

Portion	Figure/Table
Number of figures/tables	1
Title	Regulating Interparticle Interactions to Control the Spatio-Temporal Assembly of Gold Nanoparticles
Institution name	n/a
Expected presentation date	Mar 2020
Order reference number	Chapter 1, [28]
Portions	Figure 1
Requestor Location	Indian Institute of Science Education and Research (IISER) Pune IISER New Hostel 2, IISER Campus, Pashan Pune, Maharashtra 411008 India Attn: Indian Institute of Science Education and Research (IISER) Pune
Total	0.00 USD

Terms and Conditions

AIP Publishing -- Terms and Conditions: Permissions Uses

AIP Publishing hereby grants to you the non-exclusive right and license to use and/or distribute the Material according to the use specified in your order, on a one-time basis, for the specified term, with a maximum distribution equal to the number that you have ordered. Any links or other content accompanying the Material are not the subject of this license.

1. You agree to include the following copyright and permission notice with the reproduction of the Material:"Reprinted from [FULL CITATION], with the permission of AIP Publishing." For an article, the credit line and permission notice must be printed on the first page of the article or book chapter. For photographs, covers, or tables, the notice may appear with the Material, in a footnote, or in the reference list.
2. If you have licensed reuse of a figure, photograph, cover, or table, it is your responsibility to ensure that the material is original to AIP Publishing and does not contain the copyright of another entity, and that the copyright notice of the figure, photograph, cover, or table does not indicate that it was reprinted by AIP Publishing, with permission, from another source. Under no circumstances does AIP Publishing purport or intend to grant permission to reuse material to which it does not hold

appropriate rights.
You may not alter or modify the Material in any manner. You may translate the Material into another language only if you have licensed translation rights. You may not use the Material for promotional purposes.

3. The foregoing license shall not take effect unless and until AIP Publishing or its agent, Copyright Clearance Center, receives the Payment in accordance with Copyright Clearance Center Billing and Payment Terms and Conditions, which are incorporated herein by reference.
4. AIP Publishing or Copyright Clearance Center may, within two business days of granting this license, revoke the license for any reason whatsoever, with a full refund payable to you. Should you violate the terms of this license at any time, AIP Publishing, or Copyright Clearance Center may revoke the license with no refund to you. Notice of such revocation will be made using the contact information provided by you. Failure to receive such notice will not nullify the revocation.
5. AIP Publishing makes no representations or warranties with respect to the Material. You agree to indemnify and hold harmless AIP Publishing, and their officers, directors, employees or agents from and against any and all claims arising out of your use of the Material other than as specifically authorized herein.
6. The permission granted herein is personal to you and is not transferable or assignable without the prior written permission of AIP Publishing. This license may not be amended except in a writing signed by the party to be charged.
7. If purchase orders, acknowledgments or check endorsements are issued on any forms containing terms and conditions which are inconsistent with these provisions, such inconsistent terms and conditions shall be of no force and effect. This document, including the CCC Billing and Payment Terms and Conditions, shall be the entire agreement between the parties relating to the subject matter hereof.

This Agreement shall be governed by and construed in accordance with the laws of the State of New York. Both parties hereby submit to the jurisdiction of the courts of New York County for purposes of resolving any disputes that may arise hereunder.

V1.2

Questions? customercare@copyright.com or +1-855-239-3415 (toll free in the US) or +1-978-646-2777.

THE AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE LICENSE TERMS AND CONDITIONS

Feb 29, 2020

This Agreement between Indian Institute of Science Education and Research (IISER) Pune -- Anish Rao ("You") and The American Association for the Advancement of Science ("The American Association for the Advancement of Science") consists of your license details and the terms and conditions provided by The American Association for the Advancement of Science and Copyright Clearance Center.

License Number	4778050765048
License date	Feb 29, 2020
Licensed Content Publisher	The American Association for the Advancement of Science
Licensed Content Publication	Science
Licensed Content Title	Predictive Self-Assembly of Polyhedra into Complex Structures
Licensed Content Author	Pablo F. Damasceno,Michael Engel,Sharon C. Glotzer
Licensed Content Date	Jul 27, 2012
Licensed Content Volume	337
Licensed Content Issue	6093
Volume number	337
Issue number	6093

Type of Use	Thesis / Dissertation
Requestor type	Scientist/individual at a research institution
Format	Print and electronic
Portion	Figure
Number of figures/tables	2
Title	Regulating Interparticle Interactions to Control the Spatio-Temporal Assembly of Gold Nanoparticles
Institution name	n/a
Expected presentation date	Mar 2020
Order reference number	Chapter 1, [29]
Portions	Figure 1, Figure 4
Requestor Location	Indian Institute of Science Education and Research (IISER) Pune IISER New Hostel 2, IISER Campus, Pashan Pune, Maharashtra 411008 India Attn: Indian Institute of Science Education and Research (IISER) Pune
Total	0.00 USD

Terms and Conditions

American Association for the Advancement of Science TERMS AND CONDITIONS

Regarding your request, we are pleased to grant you non-exclusive, non-transferable permission, to republish the AAAS material identified above in your work identified above, subject to the terms and conditions herein. We must be contacted for permission for any uses other than those specifically identified in your request above.

The following credit line must be printed along with the AAAS material: "From [Full Reference Citation]. Reprinted with permission from AAAS."

All required credit lines and notices must be visible any time a user accesses any part of the AAAS material and must appear on any printed copies and authorized user might make.

This permission does not apply to figures / photos / artwork or any other content or materials included in your work that are credited to non-AAAS sources. If the requested material is sourced to or references non-AAAS sources, you must obtain authorization from that source as well before using that material. You agree to hold harmless and indemnify AAAS against any claims arising from your use of any content in your work that is credited to non-AAAS sources.

If the AAAS material covered by this permission was published in Science during the years 1974 - 1994, you must also obtain permission from the author, who may grant or withhold permission, and who may or may not charge a fee if permission is granted. See original article for author's address. This condition does not apply to news articles.

The AAAS material may not be modified or altered except that figures and tables may be modified with permission from the author. Author permission for any such changes must be secured prior to your use.

Whenever possible, we ask that electronic uses of the AAAS material permitted herein include a hyperlink to the original work on AAAS's website (hyperlink may be embedded in the reference citation).

AAAS material reproduced in your work identified herein must not account for more than 30% of the total contents of that work.

AAAS must publish the full paper prior to use of any text.

AAAS material must not imply any endorsement by the American Association for the Advancement of Science.

This permission is not valid for the use of the AAAS and/or Science logos.

AAAS makes no representations or warranties as to the accuracy of any information contained in the AAAS material covered by this permission, including any warranties of

merchantability or fitness for a particular purpose.

If permission fees for this use are waived, please note that AAAS reserves the right to charge for reproduction of this material in the future.

Permission is not valid unless payment is received within sixty (60) days of the issuance of this permission. If payment is not received within this time period then all rights granted herein shall be revoked and this permission will be considered null and void.

In the event of breach of any of the terms and conditions herein or any of CCC's Billing and Payment terms and conditions, all rights granted herein shall be revoked and this permission will be considered null and void.

AAAS reserves the right to terminate this permission and all rights granted herein at its discretion, for any purpose, at any time. In the event that AAAS elects to terminate this permission, you will have no further right to publish, publicly perform, publicly display, distribute or otherwise use any matter in which the AAAS content had been included, and all fees paid hereunder shall be fully refunded to you. Notification of termination will be sent to the contact information as supplied by you during the request process and termination shall be immediate upon sending the notice. Neither AAAS nor CCC shall be liable for any costs, expenses, or damages you may incur as a result of the termination of this permission, beyond the refund noted above.

This Permission may not be amended except by written document signed by both parties.

The terms above are applicable to all permissions granted for the use of AAAS material. Below you will find additional conditions that apply to your particular type of use.

FOR A THESIS OR DISSERTATION
If you are using figure(s)/table(s), permission is granted for use in print and electronic versions of your dissertation or thesis. A full text article may be used in print versions only of a dissertation or thesis.

Permission covers the distribution of your dissertation or thesis on demand by ProQuest / UMI, provided the AAAS material covered by this permission remains in situ.

If you are an Original Author on the AAAS article being reproduced, please refer to your License to Publish for rules on reproducing your paper in a dissertation or thesis.

FOR JOURNALS:
Permission covers both print and electronic versions of your journal article, however the AAAS material may not be used in any manner other than within the context of your article.

FOR BOOKS/TEXTBOOKS:
If this license is to reuse figures/tables, then permission is granted for non-exclusive world rights in all languages in both print and electronic formats (electronic formats are defined below).

If this license is to reuse a text excerpt or a full text article, then permission is granted for non-exclusive world rights in English only. You have the option of securing either print or electronic rights or both, but electronic rights are not automatically granted and do garner additional fees. Permission for translations of text excerpts or full text articles into other languages must be obtained separately.

Licenses granted for use of AAAS material in electronic format books/textbooks are valid only in cases where the electronic version is equivalent to or substitutes for the print version of the book/textbook. The AAAS material reproduced as permitted herein must remain in situ and must not be exploited separately (for example, if permission covers the use of a full text article, the article may not be offered for access or for purchase as a stand-alone unit), except in the case of permitted textbook companions as noted below.

You must include the following notice in any electronic versions, either adjacent to the reprinted AAAS material or in the terms and conditions for use of your electronic products: "Readers may view, browse, and/or download material for temporary copying purposes only, provided these uses are for noncommercial personal purposes. Except as provided by law, this material may not be further reproduced, distributed, transmitted, modified, adapted, performed, displayed, published, or sold in whole or in part, without prior written permission from the publisher."

If your book is an academic textbook, permission covers the following companions to your textbook, provided such companions are distributed only in conjunction with your textbook at no additional cost to the user:

- Password-protected website
- Instructor's image CD/DVD and/or PowerPoint resource
- Student CD/DVD

All companions must contain instructions to users that the AAAS material may be used for non-commercial, classroom purposes only. Any other uses require the prior written permission from AAAS.

If your license is for the use of AAAS Figures/Tables, then the electronic rights granted herein permit use of the Licensed Material in any Custom Databases that you distribute the electronic versions of your textbook through, so long as the Licensed Material remains within the context of a chapter of the title identified in your request and cannot be downloaded by a user as an independent image file.

Rights also extend to copies/files of your Work (as described above) that you are required to provide for use by the visually and/or print disabled in compliance with state and federal laws.

This permission only covers a single edition of your work as identified in your request.

FOR NEWSLETTERS:
Permission covers print and/or electronic versions, provided the AAAS material reproduced as permitted herein remains in situ and is not exploited separately (for example, if permission covers the use of a full text article, the article may not be offered for access or for purchase as a stand-alone unit)

FOR ANNUAL REPORTS:
Permission covers print and electronic versions provided the AAAS material reproduced as permitted herein remains in situ and is not exploited separately (for example, if permission covers the use of a full text article, the article may not be offered for access or for purchase as a stand-alone unit)

FOR PROMOTIONAL/MARKETING USES:
Permission covers the use of AAAS material in promotional or marketing pieces such as information packets, media kits, product slide kits, brochures, or flyers limited to a single print run. The AAAS Material may not be used in any manner which implies endorsement or promotion by the American Association for the Advancement of Science (AAAS) or Science of any product or service. AAAS does not permit the reproduction of its name, logo or text on promotional literature.

If permission to use a full text article is permitted, The Science article covered by this permission must not be altered in any way. No additional printing may be set onto an article copy other than the copyright credit line required above. Any alterations must be approved in advance and in writing by AAAS. This includes, but is not limited to, the placement of sponsorship identifiers, trademarks, logos, rubber stamping or self-adhesive stickers onto the article copies.

Additionally, article copies must be a freestanding part of any information package (i.e. media kit) into which they are inserted. They may not be physically attached to anything, such as an advertising insert, or have anything attached to them, such as a sample product. Article copies must be easily removable from any kits or informational packages in which they are used. The only exception is that article copies may be inserted into three-ring binders.

FOR CORPORATE INTERNAL USE:
The AAAS material covered by this permission may not be altered in any way. No additional printing may be set onto an article copy other than the required credit line. Any alterations must be approved in advance and in writing by AAAS. This includes, but is not limited to the placement of sponsorship identifiers, trademarks, logos, rubber stamping or self-adhesive stickers onto article copies.

If you are making article copies, copies are restricted to the number indicated in your request and must be distributed only to internal employees for internal use.

If you are using AAAS Material in Presentation Slides, the required credit line must be visible on the slide where the AAAS material will be reprinted

If you are using AAAS Material on a CD, DVD, Flash Drive, or the World Wide Web, you must include the following notice in any electronic versions, either adjacent to the reprinted AAAS material or in the terms and conditions for use of your electronic products: "Readers may view, browse, and/or download material for temporary copying purposes only, provided these uses are for noncommercial personal purposes. Except as provided by law, this material may not be further reproduced, distributed, transmitted, modified, adapted, performed, displayed, published, or sold in whole or in part, without prior written permission from the publisher." Access to any such CD, DVD, Flash Drive or Web page must be restricted to your organization's employees only.

FOR CME COURSE and SCIENTIFIC SOCIETY MEETINGS:
Permission is restricted to the particular Course, Seminar, Conference, or Meeting indicated in your request. If this license covers a text excerpt or a Full Text Article, access to the reprinted AAAS material must be restricted to attendees of your event only (if you have been granted electronic rights for use of a full text article on your website, your website must be password protected, or access restricted so that only attendees can access the content on your site).

If you are using AAAS Material on a CD, DVD, Flash Drive, or the World Wide Web, you must include the following notice in any electronic versions, either adjacent to the reprinted AAAS material or in the terms and conditions for use of your electronic products: "Readers may view, browse, and/or download material for temporary copying purposes only, provided these uses are for noncommercial personal purposes. Except as provided by law, this material may not be further reproduced, distributed, transmitted, modified, adapted, performed, displayed, published, or sold in whole or in part, without prior written permission from the publisher."

FOR POLICY REPORTS:
These rights are granted only to non-profit organizations and/or government agencies. Permission covers print and electronic versions of a report, provided the required credit line appears in both versions and provided the AAAS material reproduced as permitted herein remains in situ and is not exploited separately.

FOR CLASSROOM PHOTOCOPIES:
Permission covers distribution in print copy format only. Article copies must be freestanding and not part of a course pack. They may not be physically attached to anything or have anything attached to them.

FOR COURSEPACKS OR COURSE WEBSITES:
These rights cover use of the AAAS material in one class at one institution. Permission is valid only for a single semester after which the AAAS material must be removed from the Electronic Course website, unless new permission is obtained for an additional semester. If the material is to be distributed online, access must be restricted to students and instructors enrolled in that particular course by some means of password or access control.

FOR WEBSITES:
You must include the following notice in any electronic versions, either adjacent to the reprinted AAAS material or in the terms and conditions for use of your electronic products: "Readers may view, browse, and/or download material for temporary copying purposes only, provided these uses are for noncommercial personal purposes. Except as provided by law, this material may not be further reproduced, distributed, transmitted, modified, adapted, performed, displayed, published, or sold in whole or in part, without prior written permission from the publisher."

Permissions for the use of Full Text articles on third party websites are granted on a case by case basis and only in cases where access to the AAAS Material is restricted by some means of password or access control. Alternately, an E-Print may be purchased through our reprints department (brocheleau@rockwaterinc.com).

REGARDING FULL TEXT ARTICLE USE ON THE WORLD WIDE WEB IF YOU ARE AN 'ORIGINAL AUTHOR' OF A SCIENCE PAPER

If you chose "Original Author" as the Requestor Type, you are warranting that you are one of authors listed on the License Agreement as a "Licensed content author" or that you are acting on that author's behalf to use the Licensed content in a new work that one of the authors listed on the License Agreement as a "Licensed content author" has written.

Original Authors may post the 'Accepted Version' of their full text article on their personal or on their University website and not on any other website. The 'Accepted Version' is the version of the paper accepted for publication by AAAS including changes resulting from peer review but prior to AAAS's copy editing and production (in other words not the AAAS published version).

FOR MOVIES / FILM / TELEVISION:
Permission is granted to use, record, film, photograph, and/or tape the AAAS material in connection with your program/film and in any medium your program/film may be shown or heard, including but not limited to broadcast and cable television, radio, print, world wide web, and videocassette.

The required credit line should run in the program/film's end credits.

FOR MUSEUM EXHIBITIONS:
Permission is granted to use the AAAS material as part of a single exhibition for the duration of that exhibit. Permission for use of the material in promotional materials for the exhibit must be cleared separately with AAAS (please contact us at permissions@aaas.org).

FOR TRANSLATIONS:
Translation rights apply only to the language identified in your request summary above.

The following disclaimer must appear with your translation, on the first page of the article, after the credit line: "This translation is not an official translation by AAAS staff, nor is it endorsed by AAAS as accurate. In crucial matters, please refer to the official English-language version originally published by AAAS."

FOR USE ON A COVER:
Permission is granted to use the AAAS material on the cover of a journal issue, newsletter issue, book, textbook, or annual report in print and electronic formats provided the AAAS material reproduced as permitted herein remains in situ and is not exploited separately

By using the AAAS Material identified in your request, you agree to abide by all the terms and conditions herein.

Questions about these terms can be directed to the AAAS Permissions department permissions@aaas.org.

Other Terms and Conditions:

v 2

THE AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE LICENSE TERMS AND CONDITIONS

Feb 29, 2020

This Agreement between Indian Institute of Science Education and Research (IISER) Pune -- Anish Rao ("You") and The American Association for the Advancement of Science ("The American Association for the Advancement of Science") consists of your license details and the terms and conditions provided by The American Association for the Advancement of Science and Copyright Clearance Center.

License Number	4778051204905
License date	Feb 29, 2020
Licensed Content Publisher	The American Association for the Advancement of Science
Licensed Content Publication	Science
Licensed Content Title	Self-Assembly and Mineralization of Peptide-Amphiphile Nanofibers
Licensed Content Author	Jeffrey D. Hartgerink,Elia Beniash,Samuel I. Stupp
Licensed Content Date	Nov 23, 2001
Licensed Content Volume	294
Licensed Content Issue	5547
Volume number	294
Issue number	5547

Type of Use	Thesis / Dissertation
Requestor type	Scientist/individual at a research institution
Format	Print and electronic
Portion	Figure
Number of figures/tables	2
Title	Regulating Interparticle Interactions to Control the Spatio-Temporal Assembly of Gold Nanoparticles
Institution name	n/a
Expected presentation date	Mar 2020
Order reference number	Chapter 1, [38]
Portions	Figure 1, Figure 2
Requestor Location	Indian Institute of Science Education and Research (IISER) Pune IISER New Hostel 2, IISER Campus, Pashan Pune, Maharashtra 411008 India Attn: Indian Institute of Science Education and Research (IISER) Pune
Total	0.00 USD

Terms and Conditions

American Association for the Advancement of Science TERMS AND CONDITIONS

Regarding your request, we are pleased to grant you non-exclusive, non-transferable permission, to republish the AAAS material identified above in your work identified above, subject to the terms and conditions herein. We must be contacted for permission for any uses other than those specifically identified in your request above.

The following credit line must be printed along with the AAAS material: "From [Full Reference Citation]. Reprinted with permission from AAAS."

All required credit lines and notices must be visible any time a user accesses any part of the AAAS material and must appear on any printed copies and authorized user might make.

This permission does not apply to figures / photos / artwork or any other content or materials included in your work that are credited to non-AAAS sources. If the requested material is sourced to or references non-AAAS sources, you must obtain authorization from that source as well before using that material. You agree to hold harmless and indemnify AAAS against any claims arising from your use of any content in your work that is credited to non-AAAS sources.

If the AAAS material covered by this permission was published in Science during the years 1974 - 1994, you must also obtain permission from the author, who may grant or withhold permission, and who may or may not charge a fee if permission is granted. See original article for author's address. This condition does not apply to news articles.

The AAAS material may not be modified or altered except that figures and tables may be modified with permission from the author. Author permission for any such changes must be secured prior to your use.

Whenever possible, we ask that electronic uses of the AAAS material permitted herein include a hyperlink to the original work on AAAS's website (hyperlink may be embedded in the reference citation).

AAAS material reproduced in your work identified herein must not account for more than 30% of the total contents of that work.

AAAS must publish the full paper prior to use of any text.

AAAS material must not imply any endorsement by the American Association for the Advancement of Science.

This permission is not valid for the use of the AAAS and/or Science logos.

AAAS makes no representations or warranties as to the accuracy of any information contained in the AAAS material covered by this permission, including any warranties of

merchantability or fitness for a particular purpose.

If permission fees for this use are waived, please note that AAAS reserves the right to charge for reproduction of this material in the future.

Permission is not valid unless payment is received within sixty (60) days of the issuance of this permission. If payment is not received within this time period then all rights granted herein shall be revoked and this permission will be considered null and void.

In the event of breach of any of the terms and conditions herein or any of CCC's Billing and Payment terms and conditions, all rights granted herein shall be revoked and this permission will be considered null and void.

AAAS reserves the right to terminate this permission and all rights granted herein at its discretion, for any purpose, at any time. In the event that AAAS elects to terminate this permission, you will have no further right to publish, publicly perform, publicly display, distribute or otherwise use any matter in which the AAAS content had been included, and all fees paid hereunder shall be fully refunded to you. Notification of termination will be sent to the contact information as supplied by you during the request process and termination shall be immediate upon sending the notice. Neither AAAS nor CCC shall be liable for any costs, expenses, or damages you may incur as a result of the termination of this permission, beyond the refund noted above.

This Permission may not be amended except by written document signed by both parties.

The terms above are applicable to all permissions granted for the use of AAAS material. Below you will find additional conditions that apply to your particular type of use.

FOR A THESIS OR DISSERTATION
If you are using figure(s)/table(s), permission is granted for use in print and electronic versions of your dissertation or thesis. A full text article may be used in print versions only of a dissertation or thesis.

Permission covers the distribution of your dissertation or thesis on demand by ProQuest / UMI, provided the AAAS material covered by this permission remains in situ.

If you are an Original Author on the AAAS article being reproduced, please refer to your License to Publish for rules on reproducing your paper in a dissertation or thesis.

FOR JOURNALS:
Permission covers both print and electronic versions of your journal article, however the AAAS material may not be used in any manner other than within the context of your article.

FOR BOOKS/TEXTBOOKS:
If this license is to reuse figures/tables, then permission is granted for non-exclusive world rights in all languages in both print and electronic formats (electronic formats are defined below).

If this license is to reuse a text excerpt or a full text article, then permission is granted for non-exclusive world rights in English only. You have the option of securing either print or electronic rights or both, but electronic rights are not automatically granted and do garner additional fees. Permission for translations of text excerpts or full text articles into other languages must be obtained separately.

Licenses granted for use of AAAS material in electronic format books/textbooks are valid only in cases where the electronic version is equivalent to or substitutes for the print version of the book/textbook. The AAAS material reproduced as permitted herein must remain in situ and must not be exploited separately (for example, if permission covers the use of a full text article, the article may not be offered for access or for purchase as a stand-alone unit), except in the case of permitted textbook companions as noted below.

You must include the following notice in any electronic versions, either adjacent to the reprinted AAAS material or in the terms and conditions for use of your electronic products: "Readers may view, browse, and/or download material for temporary copying purposes only, provided these uses are for noncommercial personal purposes. Except as provided by law, this material may not be further reproduced, distributed, transmitted, modified, adapted, performed, displayed, published, or sold in whole or in part, without prior written permission from the publisher."

If your book is an academic textbook, permission covers the following companions to your textbook, provided such companions are distributed only in conjunction with your textbook at no additional cost to the user:

- Password-protected website
- Instructor's image CD/DVD and/or PowerPoint resource
- Student CD/DVD

All companions must contain instructions to users that the AAAS material may be used for non-commercial, classroom purposes only. Any other uses require the prior written permission from AAAS.

If your license is for the use of AAAS Figures/Tables, then the electronic rights granted herein permit use of the Licensed Material in any Custom Databases that you distribute the electronic versions of your textbook through, so long as the Licensed Material remains within the context of a chapter of the title identified in your request and cannot be downloaded by a user as an independent image file.

Rights also extend to copies/files of your Work (as described above) that you are required to provide for use by the visually and/or print disabled in compliance with state and federal laws.

This permission only covers a single edition of your work as identified in your request.

FOR NEWSLETTERS:
Permission covers print and/or electronic versions, provided the AAAS material reproduced as permitted herein remains in situ and is not exploited separately (for example, if permission covers the use of a full text article, the article may not be offered for access or for purchase as a stand-alone unit)

FOR ANNUAL REPORTS:
Permission covers print and electronic versions provided the AAAS material reproduced as permitted herein remains in situ and is not exploited separately (for example, if permission covers the use of a full text article, the article may not be offered for access or for purchase as a stand-alone unit)

FOR PROMOTIONAL/MARKETING USES:
Permission covers the use of AAAS material in promotional or marketing pieces such as information packets, media kits, product slide kits, brochures, or flyers limited to a single print run. The AAAS Material may not be used in any manner which implies endorsement or promotion by the American Association for the Advancement of Science (AAAS) or Science of any product or service. AAAS does not permit the reproduction of its name, logo or text on promotional literature.

If permission to use a full text article is permitted, The Science article covered by this permission must not be altered in any way. No additional printing may be set onto an article copy other than the copyright credit line required above. Any alterations must be approved in advance and in writing by AAAS. This includes, but is not limited to, the placement of sponsorship identifiers, trademarks, logos, rubber stamping or self-adhesive stickers onto the article copies.

Additionally, article copies must be a freestanding part of any information package (i.e. media kit) into which they are inserted. They may not be physically attached to anything, such as an advertising insert, or have anything attached to them, such as a sample product. Article copies must be easily removable from any kits or informational packages in which they are used. The only exception is that article copies may be inserted into three-ring binders.

FOR CORPORATE INTERNAL USE:
The AAAS material covered by this permission may not be altered in any way. No additional printing may be set onto an article copy other than the required credit line. Any alterations must be approved in advance and in writing by AAAS. This includes, but is not limited to the placement of sponsorship identifiers, trademarks, logos, rubber stamping or self-adhesive stickers onto article copies.

If you are making article copies, copies are restricted to the number indicated in your request and must be distributed only to internal employees for internal use.

If you are using AAAS Material in Presentation Slides, the required credit line must be visible on the slide where the AAAS material will be reprinted

If you are using AAAS Material on a CD, DVD, Flash Drive, or the World Wide Web, you must include the following notice in any electronic versions, either adjacent to the reprinted AAAS material or in the terms and conditions for use of your electronic products: "Readers may view, browse, and/or download material for temporary copying purposes only, provided these uses are for noncommercial personal purposes. Except as provided by law, this material may not be further reproduced, distributed, transmitted, modified, adapted, performed, displayed, published, or sold in whole or in part, without prior written permission from the publisher." Access to any such CD, DVD, Flash Drive or Web page must be restricted to your organization's employees only.

FOR CME COURSE and SCIENTIFIC SOCIETY MEETINGS:
Permission is restricted to the particular Course, Seminar, Conference, or Meeting indicated in your request. If this license covers a text excerpt or a Full Text Article, access to the reprinted AAAS material must be restricted to attendees of your event only (if you have been granted electronic rights for use of a full text article on your website, your website must be password protected, or access restricted so that only attendees can access the content on your site).

If you are using AAAS Material on a CD, DVD, Flash Drive, or the World Wide Web, you must include the following notice in any electronic versions, either adjacent to the reprinted AAAS material or in the terms and conditions for use of your electronic products: "Readers may view, browse, and/or download material for temporary copying purposes only, provided these uses are for noncommercial personal purposes. Except as provided by law, this material may not be further reproduced, distributed, transmitted, modified, adapted, performed, displayed, published, or sold in whole or in part, without prior written permission from the publisher."

FOR POLICY REPORTS:
These rights are granted only to non-profit organizations and/or government agencies. Permission covers print and electronic versions of a report, provided the required credit line appears in both versions and provided the AAAS material reproduced as permitted herein remains in situ and is not exploited separately.

FOR CLASSROOM PHOTOCOPIES:
Permission covers distribution in print copy format only. Article copies must be freestanding and not part of a course pack. They may not be physically attached to anything or have anything attached to them.

FOR COURSEPACKS OR COURSE WEBSITES:
These rights cover use of the AAAS material in one class at one institution. Permission is valid only for a single semester after which the AAAS material must be removed from the Electronic Course website, unless new permission is obtained for an additional semester. If the material is to be distributed online, access must be restricted to students and instructors enrolled in that particular course by some means of password or access control.

FOR WEBSITES:
You must include the following notice in any electronic versions, either adjacent to the reprinted AAAS material or in the terms and conditions for use of your electronic products: "Readers may view, browse, and/or download material for temporary copying purposes only, provided these uses are for noncommercial personal purposes. Except as provided by law, this material may not be further reproduced, distributed, transmitted, modified, adapted, performed, displayed, published, or sold in whole or in part, without prior written permission from the publisher."

Permissions for the use of Full Text articles on third party websites are granted on a case by case basis and only in cases where access to the AAAS Material is restricted by some means of password or access control. Alternately, an E-Print may be purchased through our reprints department (brocheleau@rockwaterinc.com).

REGARDING FULL TEXT ARTICLE USE ON THE WORLD WIDE WEB IF YOU ARE AN 'ORIGINAL AUTHOR' OF A SCIENCE PAPER

If you chose "Original Author" as the Requestor Type, you are warranting that you are one of authors listed on the License Agreement as a "Licensed content author" or that you are acting on that author's behalf to use the Licensed content in a new work that one of the authors listed on the License Agreement as a "Licensed content author" has written.

Original Authors may post the 'Accepted Version' of their full text article on their personal or on their University website and not on any other website. The 'Accepted Version' is the version of the paper accepted for publication by AAAS including changes resulting from peer review but prior to AAAS's copy editing and production (in other words not the AAAS published version).

FOR MOVIES / FILM / TELEVISION:
Permission is granted to use, record, film, photograph, and/or tape the AAAS material in connection with your program/film and in any medium your program/film may be shown or heard, including but not limited to broadcast and cable television, radio, print, world wide web, and videocassette.

The required credit line should run in the program/film's end credits.

FOR MUSEUM EXHIBITIONS:
Permission is granted to use the AAAS material as part of a single exhibition for the duration of that exhibit. Permission for use of the material in promotional materials for the exhibit must be cleared separately with AAAS (please contact us at permissions@aaas.org).

FOR TRANSLATIONS:
Translation rights apply only to the language identified in your request summary above.

The following disclaimer must appear with your translation, on the first page of the article, after the credit line: "This translation is not an official translation by AAAS staff, nor is it endorsed by AAAS as accurate. In crucial matters, please refer to the official English-language version originally published by AAAS."

FOR USE ON A COVER:
Permission is granted to use the AAAS material on the cover of a journal issue, newsletter issue, book, textbook, or annual report in print and electronic formats provided the AAAS material reproduced as permitted herein remains in situ and is not exploited separately

By using the AAAS Material identified in your request, you agree to abide by all the terms and conditions herein.

Questions about these terms can be directed to the AAAS Permissions department permissions@aaas.org.

Other Terms and Conditions:

v 2

SPRINGER NATURE LICENSE
TERMS AND CONDITIONS

Feb 29, 2020

This Agreement between Indian Institute of Science Education and Research (IISER) Pune -- Anish Rao ("You") and Springer Nature ("Springer Nature") consists of your license details and the terms and conditions provided by Springer Nature and Copyright Clearance Center.

License Number	4778060097352
License date	Feb 29, 2020
Licensed Content Publisher	Springer Nature
Licensed Content Publication	Nature Materials
Licensed Content Title	Kinetically driven self assembly of highly ordered nanoparticle monolayers
Licensed Content Author	Terry P. Bigioni et al
Licensed Content Date	Mar 19, 2006
Type of Use	Thesis/Dissertation
Requestor type	academic/university or research institute
Format	print and electronic
Portion	figures/tables/illustrations
Number of figures/tables/illustrations	2
High-res required	no

Will you be translating?	no
Circulation/distribution	1 - 29
Author of this Springer Nature content	no
Title	Regulating Interparticle Interactions to Control the Spatio-Temporal Assembly of Gold Nanoparticles
Institution name	n/a
Expected presentation date	Mar 2020
Order reference number	Chapter 1, [41]
Portions	Figure 1, Figure 2
Requestor Location	Indian Institute of Science Education and Research (IISER) Pune IISER New Hostel 2, IISER Campus, Pashan Pune, Maharashtra 411008 India Attn: Indian Institute of Science Education and Research (IISER) Pune
Total	0.00 USD

Terms and Conditions

Springer Nature Customer Service Centre GmbH
Terms and Conditions

This agreement sets out the terms and conditions of the licence (the **Licence**) between you and **Springer Nature Customer Service Centre GmbH** (the **Licensor**). By clicking 'accept' and completing the transaction for the material (**Licensed Material**), you also confirm your acceptance of these terms and conditions.

1. Grant of License

1. 1. The Licensor grants you a personal, non-exclusive, non-transferable, world-wide licence to reproduce the Licensed Material for the purpose specified in your order only. Licences are granted for the specific use requested in the order and for no other use, subject to the conditions below.

1. 2. The Licensor warrants that it has, to the best of its knowledge, the rights to license reuse of the Licensed Material. However, you should ensure that the material you are requesting is original to the Licensor and does not carry the copyright of another entity (as credited in the published version).

1. 3. If the credit line on any part of the material you have requested indicates that it was reprinted or adapted with permission from another source, then you should also seek permission from that source to reuse the material.

2. Scope of Licence

2. 1. You may only use the Licensed Content in the manner and to the extent permitted by these Ts&Cs and any applicable laws.

2. 2. A separate licence may be required for any additional use of the Licensed Material, e.g. where a licence has been purchased for print only use, separate permission must be obtained for electronic re-use. Similarly, a licence is only valid in the language selected and does not apply for editions in other languages unless additional translation rights have been granted separately in the licence. Any content owned by third parties are expressly excluded from the licence.

2. 3. Similarly, rights for additional components such as custom editions and derivatives require additional permission and may be subject to an additional fee. Please apply to Journalpermissions@springernature.com/bookpermissions@springernature.com for these rights.

2. 4. Where permission has been granted **free of charge** for material in print, permission may also be granted for any electronic version of that work, provided that the material is incidental to your work as a whole and that the electronic version is essentially equivalent to, or substitutes for, the print version.

2. 5. An alternative scope of licence may apply to signatories of the STM Permissions Guidelines, as amended from time to time.

3. Duration of Licence

3. 1. A licence for is valid from the date of purchase ('Licence Date') at the end of the relevant period in the below table:

Scope of Licence	Duration of Licence
Post on a website	12 months
Presentations	12 months
Books and journals	Lifetime of the edition in the language purchased

4. Acknowledgement

4. 1. The Licensor's permission must be acknowledged next to the Licenced Material in print. In electronic form, this acknowledgement must be visible at the same time as the figures/tables/illustrations or abstract, and must be hyperlinked to the journal/book's homepage. Our required acknowledgement format is in the Appendix below.

5. Restrictions on use

5. 1. Use of the Licensed Material may be permitted for incidental promotional use and minor editing privileges e.g. minor adaptations of single figures, changes of format, colour and/or style where the adaptation is credited as set out in Appendix 1 below. Any other changes including but not limited to, cropping, adapting, omitting material that affect the meaning, intention or moral rights of the author are strictly prohibited.

5. 2. You must not use any Licensed Material as part of any design or trademark.

5. 3. Licensed Material may be used in Open Access Publications (OAP) before publication by Springer Nature, but any Licensed Material must be removed from OAP sites prior to final publication.

6. Ownership of Rights

6. 1. Licensed Material remains the property of either Licensor or the relevant third party and any rights not explicitly granted herein are expressly reserved.

7. Warranty

IN NO EVENT SHALL LICENSOR BE LIABLE TO YOU OR ANY OTHER PARTY OR ANY OTHER PERSON OR FOR ANY SPECIAL, CONSEQUENTIAL, INCIDENTAL OR INDIRECT DAMAGES, HOWEVER CAUSED, ARISING OUT OF OR IN CONNECTION WITH THE DOWNLOADING, VIEWING OR USE OF THE MATERIALS REGARDLESS OF THE FORM OF ACTION, WHETHER FOR BREACH OF CONTRACT, BREACH OF WARRANTY, TORT, NEGLIGENCE, INFRINGEMENT OR OTHERWISE (INCLUDING, WITHOUT LIMITATION, DAMAGES BASED ON LOSS OF PROFITS, DATA, FILES, USE, BUSINESS OPPORTUNITY OR CLAIMS OF THIRD PARTIES), AND
WHETHER OR NOT THE PARTY HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES. THIS LIMITATION SHALL APPLY NOTWITHSTANDING ANY FAILURE OF ESSENTIAL PURPOSE OF ANY LIMITED REMEDY PROVIDED HEREIN.

8. Limitations

8. 1. ***BOOKS ONLY:***Where **'reuse in a dissertation/thesis'** has been selected the following terms apply: Print rights of the final author's accepted manuscript (for clarity, NOT the published version) for up to 100 copies, electronic rights for use only on a personal website or institutional repository as defined by the Sherpa guideline (www.sherpa.ac.uk/romeo/).

9. Termination and Cancellation

9. 1. Licences will expire after the period shown in Clause 3 (above).

9. 2. Licensee reserves the right to terminate the Licence in the event that payment is not received in full or if there has been a breach of this agreement by you.

Appendix 1 — Acknowledgements:

For Journal Content:
Reprinted by permission from [**the Licensor**]: [**Journal Publisher** (e.g. Nature/Springer/Palgrave)] [**JOURNAL NAME**] [**REFERENCE CITATION** (Article name, Author(s) Name), [**COPYRIGHT**] (year of publication)

For **Advance Online Publication papers:**
Reprinted by permission from [**the Licensor**]: [**Journal Publisher** (e.g. Nature/Springer/Palgrave)] [**JOURNAL NAME**] [**REFERENCE CITATION** (Article name, Author(s) Name), [**COPYRIGHT**] (year of publication), advance online publication, day month year (doi: 10.1038/sj.[JOURNAL ACRONYM].)

For Adaptations/Translations:
Adapted/Translated by permission from [**the Licensor**]: [**Journal Publisher** (e.g. Nature/Springer/Palgrave)] [**JOURNAL NAME**] [**REFERENCE CITATION** (Article name, Author(s) Name), [**COPYRIGHT**] (year of publication)

Note: For any republication from the British Journal of Cancer, the following credit line style applies:

Reprinted/adapted/translated by permission from [**the Licensor**]: on behalf of Cancer Research UK: : [**Journal Publisher** (e.g. Nature/Springer/Palgrave)] [**JOURNAL NAME**] [**REFERENCE CITATION** (Article name, Author(s) Name), [**COPYRIGHT**] (year of publication)

For **Advance Online Publication** papers:
Reprinted by permission from The [**the Licensor**]: on behalf of Cancer Research UK: [**Journal Publisher** (e.g. Nature/Springer/Palgrave)] [**JOURNAL NAME**] [**REFERENCE CITATION** (Article name, Author(s) Name), [**COPYRIGHT**] (year of publication), advance online publication, day month year (doi: 10.1038/sj.[JOURNAL ACRONYM])

For Book content:
Reprinted/adapted by permission from [**the Licensor**]: [**Book Publisher** (e.g. Palgrave Macmillan, Springer etc) [**Book Title**] by [**Book author**(s)] [**COPYRIGHT**] (year of publication)

Other Conditions:

Version 1.2

Questions? customercare@copyright.com or +1-855-239-3415 (toll free in the US) or +1-978-646-2777.

SPRINGER NATURE LICENSE
TERMS AND CONDITIONS

Feb 29, 2020

This Agreement between Indian Institute of Science Education and Research (IISER) Pune -- Anish Rao ("You") and Springer Nature ("Springer Nature") consists of your license details and the terms and conditions provided by Springer Nature and Copyright Clearance Center.

License Number	4778060879885
License date	Feb 29, 2020
Licensed Content Publisher	Springer Nature
Licensed Content Publication	Nature
Licensed Content Title	Structural diversity in binary nanoparticle superlattices
Licensed Content Author	Elena V. Shevchenko et al
Licensed Content Date	Jan 5, 2006
Type of Use	Thesis/Dissertation
Requestor type	academic/university or research institute
Format	print and electronic
Portion	figures/tables/illustrations
Number of figures/tables/illustrations	1
High-res required	no

Will you be translating?	no
Circulation/distribution	1 - 29
Author of this Springer Nature content	no
Title	Regulating Interparticle Interactions to Control the Spatio-Temporal Assembly of Gold Nanoparticles
Institution name	n/a
Expected presentation date	Mar 2020
Order reference number	Chapter 1, [42]
Portions	Figure 1
Requestor Location	Indian Institute of Science Education and Research (IISER) Pune IISER New Hostel 2, IISER Campus, Pashan Pune, Maharashtra 411008 India Attn: Indian Institute of Science Education and Research (IISER) Pune
Total	0.00 USD

Terms and Conditions

Springer Nature Customer Service Centre GmbH
Terms and Conditions

This agreement sets out the terms and conditions of the licence (the **Licence**) between you and **Springer Nature Customer Service Centre GmbH** (the **Licensor**). By clicking 'accept' and completing the transaction for the material (**Licensed Material**), you also confirm your acceptance of these terms and conditions.

1. Grant of License

1. 1. The Licensor grants you a personal, non-exclusive, non-transferable, world-wide licence to reproduce the Licensed Material for the purpose specified in your order only. Licences are granted for the specific use requested in the order and for no other use, subject to the conditions below.

1. 2. The Licensor warrants that it has, to the best of its knowledge, the rights to license reuse of the Licensed Material. However, you should ensure that the material you are requesting is original to the Licensor and does not carry the copyright of another entity (as credited in the published version).

1. 3. If the credit line on any part of the material you have requested indicates that it was reprinted or adapted with permission from another source, then you should also seek permission from that source to reuse the material.

2. Scope of Licence

2. 1. You may only use the Licensed Content in the manner and to the extent permitted by these Ts&Cs and any applicable laws.

2. 2. A separate licence may be required for any additional use of the Licensed Material, e.g. where a licence has been purchased for print only use, separate permission must be obtained for electronic re-use. Similarly, a licence is only valid in the language selected and does not apply for editions in other languages unless additional translation rights have been granted separately in the licence. Any content owned by third parties are expressly excluded from the licence.

2. 3. Similarly, rights for additional components such as custom editions and derivatives require additional permission and may be subject to an additional fee. Please apply to Journalpermissions@springernature.com/bookpermissions@springernature.com for these rights.

2. 4. Where permission has been granted **free of charge** for material in print, permission may also be granted for any electronic version of that work, provided that the material is incidental to your work as a whole and that the electronic version is essentially equivalent to, or substitutes for, the print version.

2. 5. An alternative scope of licence may apply to signatories of the STM Permissions Guidelines, as amended from time to time.

3. Duration of Licence

3. 1. A licence for is valid from the date of purchase ('Licence Date') at the end of the relevant period in the below table:

Scope of Licence	Duration of Licence
Post on a website	12 months
Presentations	12 months
Books and journals	Lifetime of the edition in the language purchased

4. Acknowledgement

4. 1. The Licensor's permission must be acknowledged next to the Licenced Material in print. In electronic form, this acknowledgement must be visible at the same time as the figures/tables/illustrations or abstract, and must be hyperlinked to the journal/book's homepage. Our required acknowledgement format is in the Appendix below.

5. Restrictions on use

5. 1. Use of the Licensed Material may be permitted for incidental promotional use and minor editing privileges e.g. minor adaptations of single figures, changes of format, colour and/or style where the adaptation is credited as set out in Appendix 1 below. Any other changes including but not limited to, cropping, adapting, omitting material that affect the meaning, intention or moral rights of the author are strictly prohibited.

5. 2. You must not use any Licensed Material as part of any design or trademark.

5. 3. Licensed Material may be used in Open Access Publications (OAP) before publication by Springer Nature, but any Licensed Material must be removed from OAP sites prior to final publication.

6. Ownership of Rights

6. 1. Licensed Material remains the property of either Licensor or the relevant third party and any rights not explicitly granted herein are expressly reserved.

7. Warranty

IN NO EVENT SHALL LICENSOR BE LIABLE TO YOU OR ANY OTHER PARTY OR ANY OTHER PERSON OR FOR ANY SPECIAL, CONSEQUENTIAL, INCIDENTAL OR INDIRECT DAMAGES, HOWEVER CAUSED, ARISING OUT OF OR IN CONNECTION WITH THE DOWNLOADING, VIEWING OR USE OF THE MATERIALS REGARDLESS OF THE FORM OF ACTION, WHETHER FOR BREACH OF CONTRACT, BREACH OF WARRANTY, TORT, NEGLIGENCE, INFRINGEMENT OR OTHERWISE (INCLUDING, WITHOUT LIMITATION, DAMAGES BASED ON LOSS OF PROFITS, DATA, FILES, USE, BUSINESS OPPORTUNITY OR CLAIMS OF THIRD PARTIES), AND
WHETHER OR NOT THE PARTY HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES. THIS LIMITATION SHALL APPLY NOTWITHSTANDING ANY FAILURE OF ESSENTIAL PURPOSE OF ANY LIMITED REMEDY PROVIDED HEREIN.

8. Limitations

8. 1. ***BOOKS ONLY:***Where **'reuse in a dissertation/thesis'** has been selected the following terms apply: Print rights of the final author's accepted manuscript (for clarity, NOT the published version) for up to 100 copies, electronic rights for use only on a personal website or institutional repository as defined by the Sherpa guideline (www.sherpa.ac.uk/romeo/).

9. Termination and Cancellation

9. 1. Licences will expire after the period shown in Clause 3 (above).

9. 2. Licensee reserves the right to terminate the Licence in the event that payment is not received in full or if there has been a breach of this agreement by you.

Appendix 1 — Acknowledgements:

For Journal Content:
Reprinted by permission from [**the Licensor**]: [**Journal Publisher** (e.g. Nature/Springer/Palgrave)] [**JOURNAL NAME**] [**REFERENCE CITATION** (Article name, Author(s) Name), [**COPYRIGHT**] (year of publication)

For **Advance Online Publication papers:**
Reprinted by permission from [**the Licensor**]: [**Journal Publisher** (e.g. Nature/Springer/Palgrave)] [**JOURNAL NAME**] [**REFERENCE CITATION** (Article name, Author(s) Name), [**COPYRIGHT**] (year of publication), advance online publication, day month year (doi: 10.1038/sj.[JOURNAL ACRONYM].)

For Adaptations/Translations:
Adapted/Translated by permission from [**the Licensor**]: [**Journal Publisher** (e.g. Nature/Springer/Palgrave)] [**JOURNAL NAME**] [**REFERENCE CITATION** (Article name, Author(s) Name), [**COPYRIGHT**] (year of publication)

Note: For any republication from the British Journal of Cancer, the following credit line style applies:

Reprinted/adapted/translated by permission from [**the Licensor**]: on behalf of Cancer Research UK: : [**Journal Publisher** (e.g. Nature/Springer/Palgrave)] [**JOURNAL NAME**] [**REFERENCE CITATION** (Article name, Author(s) Name), [**COPYRIGHT**] (year of publication)

For **Advance Online Publication** papers:
Reprinted by permission from The [**the Licensor**]: on behalf of Cancer Research UK: [**Journal Publisher** (e.g. Nature/Springer/Palgrave)] [**JOURNAL NAME**] [**REFERENCE CITATION** (Article name, Author(s) Name), [**COPYRIGHT**] (year of publication), advance online publication, day month year (doi: 10.1038/sj.[JOURNAL ACRONYM])

For Book content:
Reprinted/adapted by permission from [**the Licensor**]: [**Book Publisher** (e.g. Palgrave Macmillan, Springer etc) [**Book Title**] by [**Book author**(s)] [**COPYRIGHT**] (year of publication)

Other Conditions:

Version 1.2

Questions? customercare@copyright.com or +1-855-239-3415 (toll free in the US) or +1-978-646-2777.

THE AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE LICENSE TERMS AND CONDITIONS

Feb 29, 2020

This Agreement between Indian Institute of Science Education and Research (IISER) Pune -- Anish Rao ("You") and The American Association for the Advancement of Science ("The American Association for the Advancement of Science") consists of your license details and the terms and conditions provided by The American Association for the Advancement of Science and Copyright Clearance Center.

License Number	4778061198865
License date	Feb 29, 2020
Licensed Content Publisher	The American Association for the Advancement of Science
Licensed Content Publication	Science
Licensed Content Title	Tunable porous nanoallotropes prepared by post-assembly etching of binary nanoparticle superlattices
Licensed Content Author	Thumu Udayabhaskararao,Thomas Altantzis,Lothar Houben,Marc Coronado-Puchau,Judith Langer,Ronit Popovitz-Biro,Luis M. Liz-Marzán,Lela Vuković,Petr Král,Sara Bals,Rafal Klajn
Licensed Content Date	Oct 27, 2017
Licensed Content Volume	358
Licensed Content Issue	6362

Volume number	358
Issue number	6362
Type of Use	Thesis / Dissertation
Requestor type	Scientist/individual at a research institution
Format	Print and electronic
Portion	Figure
Number of figures/tables	2
Title	Regulating Interparticle Interactions to Control the Spatio-Temporal Assembly of Gold Nanoparticles
Institution name	n/a
Expected presentation date	Mar 2020
Order reference number	Chapter 1, [43]
Portions	Figure 1, Figure 4
Requestor Location	Indian Institute of Science Education and Research (IISER) Pune IISER New Hostel 2, IISER Campus, Pashan Pune, Maharashtra 411008 India Attn: Indian Institute of Science Education and Research (IISER) Pune
Total	0.00 USD

Terms and Conditions

American Association for the Advancement of Science TERMS AND CONDITIONS

Regarding your request, we are pleased to grant you non-exclusive, non-transferable permission, to republish the AAAS material identified above in your work identified above, subject to the terms and conditions herein. We must be contacted for permission for any uses other than those specifically identified in your request above.

The following credit line must be printed along with the AAAS material: "From [Full Reference Citation]. Reprinted with permission from AAAS."

All required credit lines and notices must be visible any time a user accesses any part of the AAAS material and must appear on any printed copies and authorized user might make.

This permission does not apply to figures / photos / artwork or any other content or materials included in your work that are credited to non-AAAS sources. If the requested material is sourced to or references non-AAAS sources, you must obtain authorization from that source as well before using that material. You agree to hold harmless and indemnify AAAS against any claims arising from your use of any content in your work that is credited to non-AAAS sources.

If the AAAS material covered by this permission was published in Science during the years 1974 - 1994, you must also obtain permission from the author, who may grant or withhold permission, and who may or may not charge a fee if permission is granted. See original article for author's address. This condition does not apply to news articles.

The AAAS material may not be modified or altered except that figures and tables may be modified with permission from the author. Author permission for any such changes must be secured prior to your use.

Whenever possible, we ask that electronic uses of the AAAS material permitted herein include a hyperlink to the original work on AAAS's website (hyperlink may be embedded in the reference citation).

AAAS material reproduced in your work identified herein must not account for more than 30% of the total contents of that work.

AAAS must publish the full paper prior to use of any text.

AAAS material must not imply any endorsement by the American Association for the Advancement of Science.

This permission is not valid for the use of the AAAS and/or Science logos.

AAAS makes no representations or warranties as to the accuracy of any information contained in the AAAS material covered by this permission, including any warranties of

merchantability or fitness for a particular purpose.

If permission fees for this use are waived, please note that AAAS reserves the right to charge for reproduction of this material in the future.

Permission is not valid unless payment is received within sixty (60) days of the issuance of this permission. If payment is not received within this time period then all rights granted herein shall be revoked and this permission will be considered null and void.

In the event of breach of any of the terms and conditions herein or any of CCC's Billing and Payment terms and conditions, all rights granted herein shall be revoked and this permission will be considered null and void.

AAAS reserves the right to terminate this permission and all rights granted herein at its discretion, for any purpose, at any time. In the event that AAAS elects to terminate this permission, you will have no further right to publish, publicly perform, publicly display, distribute or otherwise use any matter in which the AAAS content had been included, and all fees paid hereunder shall be fully refunded to you. Notification of termination will be sent to the contact information as supplied by you during the request process and termination shall be immediate upon sending the notice. Neither AAAS nor CCC shall be liable for any costs, expenses, or damages you may incur as a result of the termination of this permission, beyond the refund noted above.

This Permission may not be amended except by written document signed by both parties.

The terms above are applicable to all permissions granted for the use of AAAS material. Below you will find additional conditions that apply to your particular type of use.

FOR A THESIS OR DISSERTATION
If you are using figure(s)/table(s), permission is granted for use in print and electronic versions of your dissertation or thesis. A full text article may be used in print versions only of a dissertation or thesis.

Permission covers the distribution of your dissertation or thesis on demand by ProQuest / UMI, provided the AAAS material covered by this permission remains in situ.

If you are an Original Author on the AAAS article being reproduced, please refer to your License to Publish for rules on reproducing your paper in a dissertation or thesis.

FOR JOURNALS:
Permission covers both print and electronic versions of your journal article, however the AAAS material may not be used in any manner other than within the context of your article.

FOR BOOKS/TEXTBOOKS:
If this license is to reuse figures/tables, then permission is granted for non-exclusive world rights in all languages in both print and electronic formats (electronic formats are defined below).

If this license is to reuse a text excerpt or a full text article, then permission is granted for non-exclusive world rights in English only. You have the option of securing either print or electronic rights or both, but electronic rights are not automatically granted and do garner additional fees. Permission for translations of text excerpts or full text articles into other languages must be obtained separately.

Licenses granted for use of AAAS material in electronic format books/textbooks are valid only in cases where the electronic version is equivalent to or substitutes for the print version of the book/textbook. The AAAS material reproduced as permitted herein must remain in situ and must not be exploited separately (for example, if permission covers the use of a full text article, the article may not be offered for access or for purchase as a stand-alone unit), except in the case of permitted textbook companions as noted below.

You must include the following notice in any electronic versions, either adjacent to the reprinted AAAS material or in the terms and conditions for use of your electronic products: "Readers may view, browse, and/or download material for temporary copying purposes only, provided these uses are for noncommercial personal purposes. Except as provided by law, this material may not be further reproduced, distributed, transmitted, modified, adapted, performed, displayed, published, or sold in whole or in part, without prior written permission from the publisher."

If your book is an academic textbook, permission covers the following companions to your textbook, provided such companions are distributed only in conjunction with your textbook at no additional cost to the user:

- Password-protected website
- Instructor's image CD/DVD and/or PowerPoint resource
- Student CD/DVD

All companions must contain instructions to users that the AAAS material may be used for non-commercial, classroom purposes only. Any other uses require the prior written permission from AAAS.

If your license is for the use of AAAS Figures/Tables, then the electronic rights granted herein permit use of the Licensed Material in any Custom Databases that you distribute the electronic versions of your textbook through, so long as the Licensed Material remains within the context of a chapter of the title identified in your request and cannot be downloaded by a user as an independent image file.

Rights also extend to copies/files of your Work (as described above) that you are required to provide for use by the visually and/or print disabled in compliance with state and federal laws.

This permission only covers a single edition of your work as identified in your request.

FOR NEWSLETTERS:
Permission covers print and/or electronic versions, provided the AAAS material reproduced as permitted herein remains in situ and is not exploited separately (for example, if permission covers the use of a full text article, the article may not be offered for access or for purchase as a stand-alone unit)

FOR ANNUAL REPORTS:
Permission covers print and electronic versions provided the AAAS material reproduced as permitted herein remains in situ and is not exploited separately (for example, if permission covers the use of a full text article, the article may not be offered for access or for purchase as a stand-alone unit)

FOR PROMOTIONAL/MARKETING USES:
Permission covers the use of AAAS material in promotional or marketing pieces such as information packets, media kits, product slide kits, brochures, or flyers limited to a single print run. The AAAS Material may not be used in any manner which implies endorsement or promotion by the American Association for the Advancement of Science (AAAS) or Science of any product or service. AAAS does not permit the reproduction of its name, logo or text on promotional literature.

If permission to use a full text article is permitted, The Science article covered by this permission must not be altered in any way. No additional printing may be set onto an article copy other than the copyright credit line required above. Any alterations must be approved in advance and in writing by AAAS. This includes, but is not limited to, the placement of sponsorship identifiers, trademarks, logos, rubber stamping or self-adhesive stickers onto the article copies.

Additionally, article copies must be a freestanding part of any information package (i.e. media kit) into which they are inserted. They may not be physically attached to anything, such as an advertising insert, or have anything attached to them, such as a sample product. Article copies must be easily removable from any kits or informational packages in which they are used. The only exception is that article copies may be inserted into three-ring binders.

FOR CORPORATE INTERNAL USE:
The AAAS material covered by this permission may not be altered in any way. No additional printing may be set onto an article copy other than the required credit line. Any alterations must be approved in advance and in writing by AAAS. This includes, but is not

limited to the placement of sponsorship identifiers, trademarks, logos, rubber stamping or self-adhesive stickers onto article copies.

If you are making article copies, copies are restricted to the number indicated in your request and must be distributed only to internal employees for internal use.

If you are using AAAS Material in Presentation Slides, the required credit line must be visible on the slide where the AAAS material will be reprinted

If you are using AAAS Material on a CD, DVD, Flash Drive, or the World Wide Web, you must include the following notice in any electronic versions, either adjacent to the reprinted AAAS material or in the terms and conditions for use of your electronic products: "Readers may view, browse, and/or download material for temporary copying purposes only, provided these uses are for noncommercial personal purposes. Except as provided by law, this material may not be further reproduced, distributed, transmitted, modified, adapted, performed, displayed, published, or sold in whole or in part, without prior written permission from the publisher." Access to any such CD, DVD, Flash Drive or Web page must be restricted to your organization's employees only.

FOR CME COURSE and SCIENTIFIC SOCIETY MEETINGS:
Permission is restricted to the particular Course, Seminar, Conference, or Meeting indicated in your request. If this license covers a text excerpt or a Full Text Article, access to the reprinted AAAS material must be restricted to attendees of your event only (if you have been granted electronic rights for use of a full text article on your website, your website must be password protected, or access restricted so that only attendees can access the content on your site).

If you are using AAAS Material on a CD, DVD, Flash Drive, or the World Wide Web, you must include the following notice in any electronic versions, either adjacent to the reprinted AAAS material or in the terms and conditions for use of your electronic products: "Readers may view, browse, and/or download material for temporary copying purposes only, provided these uses are for noncommercial personal purposes. Except as provided by law, this material may not be further reproduced, distributed, transmitted, modified, adapted, performed, displayed, published, or sold in whole or in part, without prior written permission from the publisher."

FOR POLICY REPORTS:
These rights are granted only to non-profit organizations and/or government agencies. Permission covers print and electronic versions of a report, provided the required credit line appears in both versions and provided the AAAS material reproduced as permitted herein remains in situ and is not exploited separately.

FOR CLASSROOM PHOTOCOPIES:
Permission covers distribution in print copy format only. Article copies must be freestanding and not part of a course pack. They may not be physically attached to anything or have anything attached to them.

FOR COURSEPACKS OR COURSE WEBSITES:
These rights cover use of the AAAS material in one class at one institution. Permission is valid only for a single semester after which the AAAS material must be removed from the Electronic Course website, unless new permission is obtained for an additional semester. If the material is to be distributed online, access must be restricted to students and instructors enrolled in that particular course by some means of password or access control.

FOR WEBSITES:
You must include the following notice in any electronic versions, either adjacent to the reprinted AAAS material or in the terms and conditions for use of your electronic products: "Readers may view, browse, and/or download material for temporary copying purposes only, provided these uses are for noncommercial personal purposes. Except as provided by law,

this material may not be further reproduced, distributed, transmitted, modified, adapted, performed, displayed, published, or sold in whole or in part, without prior written permission from the publisher."

Permissions for the use of Full Text articles on third party websites are granted on a case by case basis and only in cases where access to the AAAS Material is restricted by some means of password or access control. Alternately, an E-Print may be purchased through our reprints department (brocheleau@rockwaterinc.com).

REGARDING FULL TEXT ARTICLE USE ON THE WORLD WIDE WEB IF YOU ARE AN 'ORIGINAL AUTHOR' OF A SCIENCE PAPER

If you chose "Original Author" as the Requestor Type, you are warranting that you are one of authors listed on the License Agreement as a "Licensed content author" or that you are acting on that author's behalf to use the Licensed content in a new work that one of the authors listed on the License Agreement as a "Licensed content author" has written.

Original Authors may post the 'Accepted Version' of their full text article on their personal or on their University website and not on any other website. The 'Accepted Version' is the version of the paper accepted for publication by AAAS including changes resulting from peer review but prior to AAAS's copy editing and production (in other words not the AAAS published version).

FOR MOVIES / FILM / TELEVISION:
Permission is granted to use, record, film, photograph, and/or tape the AAAS material in connection with your program/film and in any medium your program/film may be shown or heard, including but not limited to broadcast and cable television, radio, print, world wide web, and videocassette.

The required credit line should run in the program/film's end credits.

FOR MUSEUM EXHIBITIONS:
Permission is granted to use the AAAS material as part of a single exhibition for the duration of that exhibit. Permission for use of the material in promotional materials for the exhibit must be cleared separately with AAAS (please contact us at permissions@aaas.org).

FOR TRANSLATIONS:
Translation rights apply only to the language identified in your request summary above.

The following disclaimer must appear with your translation, on the first page of the article, after the credit line: "This translation is not an official translation by AAAS staff, nor is it endorsed by AAAS as accurate. In crucial matters, please refer to the official English-language version originally published by AAAS."

FOR USE ON A COVER:
Permission is granted to use the AAAS material on the cover of a journal issue, newsletter issue, book, textbook, or annual report in print and electronic formats provided the AAAS material reproduced as permitted herein remains in situ and is not exploited separately

By using the AAAS Material identified in your request, you agree to abide by all the terms and conditions herein.

Questions about these terms can be directed to the AAAS Permissions department permissions@aaas.org.

Other Terms and Conditions:

v 2

Questions? customercare@copyright.com or +1-855-239-3415 (toll free in the US) or +1-978-646-2777.

SPRINGER NATURE LICENSE
TERMS AND CONDITIONS

Feb 29, 2020

This Agreement between Indian Institute of Science Education and Research (IISER) Pune -- Anish Rao ("You") and Springer Nature ("Springer Nature") consists of your license details and the terms and conditions provided by Springer Nature and Copyright Clearance Center.

License Number	4778101193376
License date	Feb 29, 2020
Licensed Content Publisher	Springer Nature
Licensed Content Publication	Nature Chemistry
Licensed Content Title	Dynamic hook-and-eye nanoparticle sponges
Licensed Content Author	Rafal Klajn et al
Licensed Content Date	Nov 15, 2009
Type of Use	Thesis/Dissertation
Requestor type	academic/university or research institute
Format	print and electronic
Portion	figures/tables/illustrations
Number of figures/tables/illustrations	1
High-res required	no

Will you be translating?	no
Circulation/distribution	1 - 29
Author of this Springer Nature content	no
Title	Regulating Interparticle Interactions to Control the Spatio-Temporal Assembly of Gold Nanoparticles
Institution name	n/a
Expected presentation date	Mar 2020
Order reference number	Chapter 1, [47]
Portions	Figure 4
Requestor Location	Indian Institute of Science Education and Research (IISER) Pune IISER New Hostel 2, IISER Campus, Pashan Pune, Maharashtra 411008 India Attn: Indian Institute of Science Education and Research (IISER) Pune
Total	0.00 USD

Terms and Conditions

Springer Nature Customer Service Centre GmbH
Terms and Conditions

This agreement sets out the terms and conditions of the licence (the **Licence**) between you and **Springer Nature Customer Service Centre GmbH** (the **Licensor**). By clicking 'accept' and completing the transaction for the material (**Licensed Material**), you also confirm your acceptance of these terms and conditions.

1. Grant of License

1. 1. The Licensor grants you a personal, non-exclusive, non-transferable, world-wide licence to reproduce the Licensed Material for the purpose specified in your order only. Licences are granted for the specific use requested in the order and for no other use, subject to the conditions below.

1. 2. The Licensor warrants that it has, to the best of its knowledge, the rights to license reuse of the Licensed Material. However, you should ensure that the material you are requesting is original to the Licensor and does not carry the copyright of another entity (as credited in the published version).

1. 3. If the credit line on any part of the material you have requested indicates that it was reprinted or adapted with permission from another source, then you should also seek permission from that source to reuse the material.

2. Scope of Licence

2. 1. You may only use the Licensed Content in the manner and to the extent permitted by these Ts&Cs and any applicable laws.

2. 2. A separate licence may be required for any additional use of the Licensed Material, e.g. where a licence has been purchased for print only use, separate permission must be obtained for electronic re-use. Similarly, a licence is only valid in the language selected and does not apply for editions in other languages unless additional translation rights have been granted separately in the licence. Any content owned by third parties are expressly excluded from the licence.

2. 3. Similarly, rights for additional components such as custom editions and derivatives require additional permission and may be subject to an additional fee. Please apply to Journalpermissions@springernature.com/bookpermissions@springernature.com for these rights.

2. 4. Where permission has been granted **free of charge** for material in print, permission may also be granted for any electronic version of that work, provided that the material is incidental to your work as a whole and that the electronic version is essentially equivalent to, or substitutes for, the print version.

2. 5. An alternative scope of licence may apply to signatories of the STM Permissions Guidelines, as amended from time to time.

3. Duration of Licence

3. 1. A licence for is valid from the date of purchase ('Licence Date') at the end of the relevant period in the below table:

Scope of Licence	Duration of Licence
Post on a website	12 months
Presentations	12 months
Books and journals	Lifetime of the edition in the language purchased

4. Acknowledgement

4. 1. The Licensor's permission must be acknowledged next to the Licenced Material in print. In electronic form, this acknowledgement must be visible at the same time as the figures/tables/illustrations or abstract, and must be hyperlinked to the journal/book's homepage. Our required acknowledgement format is in the Appendix below.

5. Restrictions on use

5. 1. Use of the Licensed Material may be permitted for incidental promotional use and minor editing privileges e.g. minor adaptations of single figures, changes of format, colour and/or style where the adaptation is credited as set out in Appendix 1 below. Any other changes including but not limited to, cropping, adapting, omitting material that affect the meaning, intention or moral rights of the author are strictly prohibited.

5. 2. You must not use any Licensed Material as part of any design or trademark.

5. 3. Licensed Material may be used in Open Access Publications (OAP) before publication by Springer Nature, but any Licensed Material must be removed from OAP sites prior to final publication.

6. Ownership of Rights

6. 1. Licensed Material remains the property of either Licensor or the relevant third party and any rights not explicitly granted herein are expressly reserved.

7. Warranty

IN NO EVENT SHALL LICENSOR BE LIABLE TO YOU OR ANY OTHER PARTY OR ANY OTHER PERSON OR FOR ANY SPECIAL, CONSEQUENTIAL, INCIDENTAL OR INDIRECT DAMAGES, HOWEVER CAUSED, ARISING OUT OF OR IN CONNECTION WITH THE DOWNLOADING, VIEWING OR USE OF THE MATERIALS REGARDLESS OF THE FORM OF ACTION, WHETHER FOR BREACH OF CONTRACT, BREACH OF WARRANTY, TORT, NEGLIGENCE, INFRINGEMENT OR OTHERWISE (INCLUDING, WITHOUT LIMITATION, DAMAGES BASED ON LOSS OF PROFITS, DATA, FILES, USE, BUSINESS OPPORTUNITY OR CLAIMS OF THIRD PARTIES), AND
WHETHER OR NOT THE PARTY HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES. THIS LIMITATION SHALL APPLY NOTWITHSTANDING ANY FAILURE OF ESSENTIAL PURPOSE OF ANY LIMITED REMEDY PROVIDED HEREIN.

8. Limitations

8. 1. ***BOOKS ONLY:***Where **'reuse in a dissertation/thesis'** has been selected the following terms apply: Print rights of the final author's accepted manuscript (for clarity, NOT the published version) for up to 100 copies, electronic rights for use only on a personal website or institutional repository as defined by the Sherpa guideline (www.sherpa.ac.uk/romeo/).

9. Termination and Cancellation

9. 1. Licences will expire after the period shown in Clause 3 (above).

9. 2. Licensee reserves the right to terminate the Licence in the event that payment is not received in full or if there has been a breach of this agreement by you.

Appendix 1 — Acknowledgements:

For Journal Content:
Reprinted by permission from [**the Licensor**]: [**Journal Publisher** (e.g. Nature/Springer/Palgrave)] [**JOURNAL NAME**] [**REFERENCE CITATION** (Article name, Author(s) Name), [**COPYRIGHT**] (year of publication)

For **Advance Online Publication papers:**
Reprinted by permission from [**the Licensor**]: [**Journal Publisher** (e.g. Nature/Springer/Palgrave)] [**JOURNAL NAME**] [**REFERENCE CITATION** (Article name, Author(s) Name), [**COPYRIGHT**] (year of publication), advance online publication, day month year (doi: 10.1038/sj.[JOURNAL ACRONYM].)

For Adaptations/Translations:
Adapted/Translated by permission from [**the Licensor**]: [**Journal Publisher** (e.g. Nature/Springer/Palgrave)] [**JOURNAL NAME**] [**REFERENCE CITATION** (Article name, Author(s) Name), [**COPYRIGHT**] (year of publication)

Note: For any republication from the British Journal of Cancer, the following credit line style applies:

Reprinted/adapted/translated by permission from [**the Licensor**]: on behalf of Cancer Research UK: : [**Journal Publisher** (e.g. Nature/Springer/Palgrave)] [**JOURNAL NAME**] [**REFERENCE CITATION** (Article name, Author(s) Name), [**COPYRIGHT**] (year of publication)

For **Advance Online Publication** papers:
Reprinted by permission from The [**the Licensor**]: on behalf of Cancer Research UK: [**Journal Publisher** (e.g. Nature/Springer/Palgrave)] [**JOURNAL NAME**] [**REFERENCE CITATION** (Article name, Author(s) Name), [**COPYRIGHT**] (year of publication), advance online publication, day month year (doi: 10.1038/sj.[JOURNAL ACRONYM])

For Book content:
Reprinted/adapted by permission from [**the Licensor**]: [**Book Publisher** (e.g. Palgrave Macmillan, Springer etc) [**Book Title**] by [**Book author**(s)] [**COPYRIGHT**] (year of publication)

Other Conditions:

Version 1.2

Questions? customercare@copyright.com or +1-855-239-3415 (toll free in the US) or +1-978-646-2777.

Photoswitchable Catalysis Mediated by Dynamic Aggregation of Nanoparticles

Author: Yanhu Wei, Shuangbing Han, Jiwon Kim, et al
Publication: Journal of the American Chemical Society
Publisher: American Chemical Society
Date: Aug 1, 2010

PERMISSION/LICENSE IS GRANTED FOR YOUR ORDER AT NO CHARGE

This type of permission/license, instead of the standard Terms & Conditions, is sent to you because no fee is being charged for your order. Please note the following:

- Permission is granted for your request in both print and electronic formats, and translations.
- If figures and/or tables were requested, they may be adapted or used in part.
- Please print this page for your records and send a copy of it to your publisher/graduate school.
- Appropriate credit for the requested material should be given as follows: "Reprinted (adapted) with permission from (COMPLETE REFERENCE CITATION). Copyright (YEAR) American Chemical Society." Insert appropriate information in place of the capitalized words.
- One-time permission is granted only for the use specified in your request. No additional uses are granted (such as derivative works or other editions). For any other uses, please submit a new request.

If credit is given to another source for the material you requested, permission must be obtained from that source.

BACK CLOSE WINDOW

SPRINGER NATURE LICENSE
TERMS AND CONDITIONS

Feb 29, 2020

This Agreement between Indian Institute of Science Education and Research (IISER) Pune -- Anish Rao ("You") and Springer Nature ("Springer Nature") consists of your license details and the terms and conditions provided by Springer Nature and Copyright Clearance Center.

License Number	4778110314179
License date	Feb 29, 2020
Licensed Content Publisher	Springer Nature
Licensed Content Publication	Nature Nanotechnology
Licensed Content Title	Reversible trapping and reaction acceleration within dynamically self-assembling nanoflasks
Licensed Content Author	Hui Zhao et al
Licensed Content Date	Nov 23, 2015
Type of Use	Thesis/Dissertation
Requestor type	academic/university or research institute
Format	print and electronic
Portion	figures/tables/illustrations
Number of figures/tables/illustrations	4
High-res required	no

Will you be translating?	no
Circulation/distribution	1 - 29
Author of this Springer Nature content	no
Title	Regulating Interparticle Interactions to Control the Spatio-Temporal Assembly of Gold Nanoparticles
Institution name	n/a
Expected presentation date	Mar 2020
Order reference number	Chapter 1, [50]
Portions	Figure 4
Requestor Location	Indian Institute of Science Education and Research (IISER) Pune IISER New Hostel 2, IISER Campus, Pashan Pune, Maharashtra 411008 India Attn: Indian Institute of Science Education and Research (IISER) Pune
Total	0.00 USD

Terms and Conditions

Springer Nature Customer Service Centre GmbH
Terms and Conditions

This agreement sets out the terms and conditions of the licence (the **Licence**) between you and **Springer Nature Customer Service Centre GmbH** (the **Licensor**). By clicking 'accept' and completing the transaction for the material (**Licensed Material**), you also confirm your acceptance of these terms and conditions.

1. Grant of License

1. 1. The Licensor grants you a personal, non-exclusive, non-transferable, world-wide licence to reproduce the Licensed Material for the purpose specified in your order only. Licences are granted for the specific use requested in the order and for no other use, subject to the conditions below.

1. 2. The Licensor warrants that it has, to the best of its knowledge, the rights to license reuse of the Licensed Material. However, you should ensure that the material you are requesting is original to the Licensor and does not carry the copyright of another entity (as credited in the published version).

1. 3. If the credit line on any part of the material you have requested indicates that it was reprinted or adapted with permission from another source, then you should also seek permission from that source to reuse the material.

2. Scope of Licence

2. 1. You may only use the Licensed Content in the manner and to the extent permitted by these Ts&Cs and any applicable laws.

2. 2. A separate licence may be required for any additional use of the Licensed Material, e.g. where a licence has been purchased for print only use, separate permission must be obtained for electronic re-use. Similarly, a licence is only valid in the language selected and does not apply for editions in other languages unless additional translation rights have been granted separately in the licence. Any content owned by third parties are expressly excluded from the licence.

2. 3. Similarly, rights for additional components such as custom editions and derivatives require additional permission and may be subject to an additional fee. Please apply to Journalpermissions@springernature.com/bookpermissions@springernature.com for these rights.

2. 4. Where permission has been granted **free of charge** for material in print, permission may also be granted for any electronic version of that work, provided that the material is incidental to your work as a whole and that the electronic version is essentially equivalent to, or substitutes for, the print version.

2. 5. An alternative scope of licence may apply to signatories of the STM Permissions Guidelines, as amended from time to time.

3. Duration of Licence

3. 1. A licence for is valid from the date of purchase ('Licence Date') at the end of the relevant period in the below table:

Scope of Licence	Duration of Licence
Post on a website	12 months
Presentations	12 months
Books and journals	Lifetime of the edition in the language purchased

4. Acknowledgement

4. 1. The Licensor's permission must be acknowledged next to the Licenced Material in print. In electronic form, this acknowledgement must be visible at the same time as the figures/tables/illustrations or abstract, and must be hyperlinked to the journal/book's homepage. Our required acknowledgement format is in the Appendix below.

5. Restrictions on use

5. 1. Use of the Licensed Material may be permitted for incidental promotional use and minor editing privileges e.g. minor adaptations of single figures, changes of format, colour and/or style where the adaptation is credited as set out in Appendix 1 below. Any other changes including but not limited to, cropping, adapting, omitting material that affect the meaning, intention or moral rights of the author are strictly prohibited.

5. 2. You must not use any Licensed Material as part of any design or trademark.

5. 3. Licensed Material may be used in Open Access Publications (OAP) before publication by Springer Nature, but any Licensed Material must be removed from OAP sites prior to final publication.

6. Ownership of Rights

6. 1. Licensed Material remains the property of either Licensor or the relevant third party and any rights not explicitly granted herein are expressly reserved.

7. Warranty

IN NO EVENT SHALL LICENSOR BE LIABLE TO YOU OR ANY OTHER PARTY OR ANY OTHER PERSON OR FOR ANY SPECIAL, CONSEQUENTIAL, INCIDENTAL OR INDIRECT DAMAGES, HOWEVER CAUSED, ARISING OUT OF OR IN CONNECTION WITH THE DOWNLOADING, VIEWING OR USE OF THE MATERIALS REGARDLESS OF THE FORM OF ACTION, WHETHER FOR BREACH OF CONTRACT, BREACH OF WARRANTY, TORT, NEGLIGENCE, INFRINGEMENT OR OTHERWISE (INCLUDING, WITHOUT LIMITATION, DAMAGES BASED ON LOSS OF PROFITS, DATA, FILES, USE, BUSINESS OPPORTUNITY OR CLAIMS OF THIRD PARTIES), AND
WHETHER OR NOT THE PARTY HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES. THIS LIMITATION SHALL APPLY NOTWITHSTANDING ANY FAILURE OF ESSENTIAL PURPOSE OF ANY LIMITED REMEDY PROVIDED HEREIN.

8. Limitations

8. 1. ***BOOKS ONLY:***Where **'reuse in a dissertation/thesis'** has been selected the following terms apply: Print rights of the final author's accepted manuscript (for clarity, NOT the published version) for up to 100 copies, electronic rights for use only on a personal website or institutional repository as defined by the Sherpa guideline (www.sherpa.ac.uk/romeo/).

9. Termination and Cancellation

9. 1. Licences will expire after the period shown in Clause 3 (above).

9. 2. Licensee reserves the right to terminate the Licence in the event that payment is not received in full or if there has been a breach of this agreement by you.

Appendix 1 — Acknowledgements:

For Journal Content:
Reprinted by permission from [**the Licensor**]: [**Journal Publisher** (e.g. Nature/Springer/Palgrave)] [**JOURNAL NAME**] [**REFERENCE CITATION** (Article name, Author(s) Name), [**COPYRIGHT**] (year of publication)

For **Advance Online Publication papers:**
Reprinted by permission from [**the Licensor**]: [**Journal Publisher** (e.g. Nature/Springer/Palgrave)] [**JOURNAL NAME**] [**REFERENCE CITATION** (Article name, Author(s) Name), [**COPYRIGHT**] (year of publication), advance online publication, day month year (doi: 10.1038/sj.[JOURNAL ACRONYM].)

For Adaptations/Translations:
Adapted/Translated by permission from [**the Licensor**]: [**Journal Publisher** (e.g. Nature/Springer/Palgrave)] [**JOURNAL NAME**] [**REFERENCE CITATION** (Article name, Author(s) Name), [**COPYRIGHT**] (year of publication)

Note: For any republication from the British Journal of Cancer, the following credit line style applies:

Reprinted/adapted/translated by permission from [**the Licensor**]: on behalf of Cancer Research UK: : [**Journal Publisher** (e.g. Nature/Springer/Palgrave)] [**JOURNAL NAME**] [**REFERENCE CITATION** (Article name, Author(s) Name), [**COPYRIGHT**] (year of publication)

For **Advance Online Publication** papers:
Reprinted by permission from The [**the Licensor**]: on behalf of Cancer Research UK: [**Journal Publisher** (e.g. Nature/Springer/Palgrave)] [**JOURNAL NAME**] [**REFERENCE CITATION** (Article name, Author(s) Name), [**COPYRIGHT**] (year of publication), advance online publication, day month year (doi: 10.1038/sj.[JOURNAL ACRONYM])

For Book content:
Reprinted/adapted by permission from [**the Licensor**]: [**Book Publisher** (e.g. Palgrave Macmillan, Springer etc) [**Book Title**] by [**Book author**(s)] [**COPYRIGHT**] (year of publication)

Other Conditions:

Version 1.2

Questions? customercare@copyright.com or +1-855-239-3415 (toll free in the US) or +1-978-646-2777.

JOHN WILEY AND SONS LICENSE
TERMS AND CONDITIONS

Feb 29, 2020

This Agreement between Indian Institute of Science Education and Research (IISER) Pune -- Anish Rao ("You") and John Wiley and Sons ("John Wiley and Sons") consists of your license details and the terms and conditions provided by John Wiley and Sons and Copyright Clearance Center.

License Number	4778270980598
License date	Feb 29, 2020
Licensed Content Publisher	John Wiley and Sons
Licensed Content Publication	Angewandte Chemie International Edition
Licensed Content Title	Writing Self-Erasing Images using Metastable Nanoparticle “Inks”
Licensed Content Author	Rafal Klajn, Paul J. Wesson, Kyle J. M. Bishop, et al
Licensed Content Date	Sep 1, 2009
Licensed Content Volume	48
Licensed Content Issue	38
Licensed Content Pages	5
Type of use	Dissertation/Thesis
Requestor type	University/Academic
Format	Print and electronic

Portion	Figure/table
Number of figures/tables	3
Original Wiley figure/table number(s)	Figure 1, Figure 2, Figure 3
Will you be translating?	No
Title of your thesis / dissertation	Regulating Interparticle Interactions to Control the Spatio-Temporal Assembly of Gold Nanoparticles
Expected completion date	Mar 2020
Expected size (number of pages)	180
Requestor Location	Indian Institute of Science Education and Research (IISER) Pune IISER New Hostel 2, IISER Campus, Pashan Pune, Maharashtra 411008 India Attn: Indian Institute of Science Education and Research (IISER) Pune
Publisher Tax ID	EU826007151
Total	0.00 USD
Terms and Conditions	

TERMS AND CONDITIONS

Terms and Conditions

- The materials you have requested permission to reproduce or reuse (the "Wiley Materials") are protected by copyright.

- You are hereby granted a personal, non-exclusive, non-sub licensable (on a stand-alone basis), non-transferable, worldwide, limited license to reproduce the Wiley Materials for the purpose specified in the licensing process. This license, **and any CONTENT (PDF or image file) purchased as part of your order,** is for a one-time use only and limited to any maximum distribution number specified in the license. The first instance of republication or reuse granted by this license must be completed within two years of the date of the grant of this license (although copies prepared before the end date may be distributed thereafter). The Wiley Materials shall not be used in any other manner or for any other purpose, beyond what is granted in the license. Permission is granted subject to an appropriate acknowledgement given to the author, title of the material/book/journal and the publisher. You shall also duplicate the copyright notice that appears in the Wiley publication in your use of the Wiley Material. Permission is also granted on the understanding that nowhere in the text is a previously published source acknowledged for all or part of this Wiley Material. Any third party content is expressly excluded from this permission.

- With respect to the Wiley Materials, all rights are reserved. Except as expressly granted by the terms of the license, no part of the Wiley Materials may be copied, modified, adapted (except for minor reformatting required by the new Publication), translated, reproduced, transferred or distributed, in any form or by any means, and no derivative works may be made based on the Wiley Materials without the prior permission of the respective copyright owner.**For STM Signatory Publishers clearing permission under the terms of the STM Permissions Guidelines only, the terms of the license are extended to include subsequent editions and for editions in other languages, provided such editions are for the work as a whole in situ and does not involve the separate exploitation of the permitted figures or extracts,** You may not alter, remove or suppress in any manner any copyright, trademark or other notices displayed by the Wiley Materials. You may not license, rent, sell, loan, lease, pledge, offer as security, transfer or assign the Wiley Materials on a stand-alone basis, or any of the rights granted to you hereunder to any other person.

- The Wiley Materials and all of the intellectual property rights therein shall at all times remain the exclusive property of John Wiley & Sons Inc, the Wiley Companies, or their respective licensors, and your interest therein is only that of having possession of and the right to reproduce the Wiley Materials pursuant to Section 2 herein during the continuance of this Agreement. You agree that you own no right, title or interest in or to the Wiley Materials or any of the intellectual property rights therein. You shall have no rights hereunder other than the license as provided for above in Section 2. No right, license or interest to any trademark, trade name, service mark or other branding ("Marks") of WILEY or its licensors is granted hereunder, and you agree that you shall not assert any such right, license or interest with respect thereto

- NEITHER WILEY NOR ITS LICENSORS MAKES ANY WARRANTY OR REPRESENTATION OF ANY KIND TO YOU OR ANY THIRD PARTY, EXPRESS, IMPLIED OR STATUTORY, WITH RESPECT TO THE MATERIALS OR THE ACCURACY OF ANY INFORMATION CONTAINED IN THE MATERIALS, INCLUDING, WITHOUT LIMITATION, ANY IMPLIED WARRANTY OF MERCHANTABILITY, ACCURACY, SATISFACTORY QUALITY, FITNESS FOR A PARTICULAR PURPOSE, USABILITY, INTEGRATION OR NON-INFRINGEMENT AND ALL SUCH WARRANTIES ARE HEREBY EXCLUDED BY WILEY AND ITS LICENSORS AND WAIVED

BY YOU.

- WILEY shall have the right to terminate this Agreement immediately upon breach of this Agreement by you.

- You shall indemnify, defend and hold harmless WILEY, its Licensors and their respective directors, officers, agents and employees, from and against any actual or threatened claims, demands, causes of action or proceedings arising from any breach of this Agreement by you.

- IN NO EVENT SHALL WILEY OR ITS LICENSORS BE LIABLE TO YOU OR ANY OTHER PARTY OR ANY OTHER PERSON OR ENTITY FOR ANY SPECIAL, CONSEQUENTIAL, INCIDENTAL, INDIRECT, EXEMPLARY OR PUNITIVE DAMAGES, HOWEVER CAUSED, ARISING OUT OF OR IN CONNECTION WITH THE DOWNLOADING, PROVISIONING, VIEWING OR USE OF THE MATERIALS REGARDLESS OF THE FORM OF ACTION, WHETHER FOR BREACH OF CONTRACT, BREACH OF WARRANTY, TORT, NEGLIGENCE, INFRINGEMENT OR OTHERWISE (INCLUDING, WITHOUT LIMITATION, DAMAGES BASED ON LOSS OF PROFITS, DATA, FILES, USE, BUSINESS OPPORTUNITY OR CLAIMS OF THIRD PARTIES), AND WHETHER OR NOT THE PARTY HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES. THIS LIMITATION SHALL APPLY NOTWITHSTANDING ANY FAILURE OF ESSENTIAL PURPOSE OF ANY LIMITED REMEDY PROVIDED HEREIN.

- Should any provision of this Agreement be held by a court of competent jurisdiction to be illegal, invalid, or unenforceable, that provision shall be deemed amended to achieve as nearly as possible the same economic effect as the original provision, and the legality, validity and enforceability of the remaining provisions of this Agreement shall not be affected or impaired thereby.

- The failure of either party to enforce any term or condition of this Agreement shall not constitute a waiver of either party's right to enforce each and every term and condition of this Agreement. No breach under this agreement shall be deemed waived or excused by either party unless such waiver or consent is in writing signed by the party granting such waiver or consent. The waiver by or consent of a party to a breach of any provision of this Agreement shall not operate or be construed as a waiver of or consent to any other or subsequent breach by such other party.

- This Agreement may not be assigned (including by operation of law or otherwise) by you without WILEY's prior written consent.

- Any fee required for this permission shall be non-refundable after thirty (30) days from receipt by the CCC.

- These terms and conditions together with CCC's Billing and Payment terms and conditions (which are incorporated herein) form the entire agreement between you and WILEY concerning this licensing transaction and (in the absence of fraud) supersedes all prior agreements and representations of the parties, oral or written. This Agreement may not be amended except in writing signed by both parties. This Agreement shall be binding upon and inure to the benefit of the parties' successors, legal representatives, and authorized assigns.

- In the event of any conflict between your obligations established by these terms and conditions and those established by CCC's Billing and Payment terms and conditions, these terms and conditions shall prevail.

- WILEY expressly reserves all rights not specifically granted in the combination of (i) the license details provided by you and accepted in the course of this licensing transaction, (ii) these terms and conditions and (iii) CCC's Billing and Payment terms and conditions.

- This Agreement will be void if the Type of Use, Format, Circulation, or Requestor Type was misrepresented during the licensing process.

- This Agreement shall be governed by and construed in accordance with the laws of the State of New York, USA, without regards to such state's conflict of law rules. Any legal action, suit or proceeding arising out of or relating to these Terms and Conditions or the breach thereof shall be instituted in a court of competent jurisdiction in New York County in the State of New York in the United States of America and each party hereby consents and submits to the personal jurisdiction of such court, waives any objection to venue in such court and consents to service of process by registered or certified mail, return receipt requested, at the last known address of such party.

WILEY OPEN ACCESS TERMS AND CONDITIONS

Wiley Publishes Open Access Articles in fully Open Access Journals and in Subscription journals offering Online Open. Although most of the fully Open Access journals publish open access articles under the terms of the Creative Commons Attribution (CC BY) License only, the subscription journals and a few of the Open Access Journals offer a choice of Creative Commons Licenses. The license type is clearly identified on the article.

The Creative Commons Attribution License

The Creative Commons Attribution License (CC-BY) allows users to copy, distribute and transmit an article, adapt the article and make commercial use of the article. The CC-BY license permits commercial and non-

Creative Commons Attribution Non-Commercial License

The Creative Commons Attribution Non-Commercial (CC-BY-NC)License permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.(see below)

Creative Commons Attribution-Non-Commercial-NoDerivs License

The Creative Commons Attribution Non-Commercial-NoDerivs License (CC-BY-NC-ND) permits use, distribution and reproduction in any medium, provided the original work is properly cited, is not used for commercial purposes and no modifications or adaptations are made. (see below)

Use by commercial "for-profit" organizations

Use of Wiley Open Access articles for commercial, promotional, or marketing purposes requires further explicit permission from Wiley and will be subject to a fee.

Further details can be found on Wiley Online Library
http://olabout.wiley.com/WileyCDA/Section/id-410895.html

Other Terms and Conditions:

v1.10 Last updated September 2015

Questions? customercare@copyright.com or +1-855-239-3415 (toll free in the US) or +1-978-646-2777.

THE AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE LICENSE TERMS AND CONDITIONS

Feb 29, 2020

This Agreement between Indian Institute of Science Education and Research (IISER) Pune -- Anish Rao ("You") and The American Association for the Advancement of Science ("The American Association for the Advancement of Science") consists of your license details and the terms and conditions provided by The American Association for the Advancement of Science and Copyright Clearance Center.

License Number	4778110997248
License date	Feb 29, 2020
Licensed Content Publisher	The American Association for the Advancement of Science
Licensed Content Publication	Science
Licensed Content Title	Transient assembly of active materials fueled by a chemical reaction
Licensed Content Author	Job Boekhoven,Wouter E. Hendriksen,Ger J. M. Koper,Rienk Eelkema,Jan H. van Esch
Licensed Content Date	Sep 4, 2015
Licensed Content Volume	349
Licensed Content Issue	6252
Volume number	349
Issue number	6252

Type of Use	Thesis / Dissertation
Requestor type	Scientist/individual at a research institution
Format	Print and electronic
Portion	Figure
Number of figures/tables	1
Title	Regulating Interparticle Interactions to Control the Spatio-Temporal Assembly of Gold Nanoparticles
Institution name	n/a
Expected presentation date	Mar 2020
Order reference number	Chapter 1, [55]
Portions	Figure 1
Requestor Location	Indian Institute of Science Education and Research (IISER) Pune IISER New Hostel 2, IISER Campus, Pashan Pune, Maharashtra 411008 India Attn: Indian Institute of Science Education and Research (IISER) Pune
Total	0.00 USD

Terms and Conditions

American Association for the Advancement of Science TERMS AND CONDITIONS

Regarding your request, we are pleased to grant you non-exclusive, non-transferable permission, to republish the AAAS material identified above in your work identified above, subject to the terms and conditions herein. We must be contacted for permission for any uses other than those specifically identified in your request above.

The following credit line must be printed along with the AAAS material: "From [Full Reference Citation]. Reprinted with permission from AAAS."

All required credit lines and notices must be visible any time a user accesses any part of the AAAS material and must appear on any printed copies and authorized user might make.

This permission does not apply to figures / photos / artwork or any other content or materials included in your work that are credited to non-AAAS sources. If the requested material is sourced to or references non-AAAS sources, you must obtain authorization from that source as well before using that material. You agree to hold harmless and indemnify AAAS against any claims arising from your use of any content in your work that is credited to non-AAAS sources.

If the AAAS material covered by this permission was published in Science during the years 1974 - 1994, you must also obtain permission from the author, who may grant or withhold permission, and who may or may not charge a fee if permission is granted. See original article for author's address. This condition does not apply to news articles.

The AAAS material may not be modified or altered except that figures and tables may be modified with permission from the author. Author permission for any such changes must be secured prior to your use.

Whenever possible, we ask that electronic uses of the AAAS material permitted herein include a hyperlink to the original work on AAAS's website (hyperlink may be embedded in the reference citation).

AAAS material reproduced in your work identified herein must not account for more than 30% of the total contents of that work.

AAAS must publish the full paper prior to use of any text.

AAAS material must not imply any endorsement by the American Association for the Advancement of Science.

This permission is not valid for the use of the AAAS and/or Science logos.

AAAS makes no representations or warranties as to the accuracy of any information contained in the AAAS material covered by this permission, including any warranties of

merchantability or fitness for a particular purpose.

If permission fees for this use are waived, please note that AAAS reserves the right to charge for reproduction of this material in the future.

Permission is not valid unless payment is received within sixty (60) days of the issuance of this permission. If payment is not received within this time period then all rights granted herein shall be revoked and this permission will be considered null and void.

In the event of breach of any of the terms and conditions herein or any of CCC's Billing and Payment terms and conditions, all rights granted herein shall be revoked and this permission will be considered null and void.

AAAS reserves the right to terminate this permission and all rights granted herein at its discretion, for any purpose, at any time. In the event that AAAS elects to terminate this permission, you will have no further right to publish, publicly perform, publicly display, distribute or otherwise use any matter in which the AAAS content had been included, and all fees paid hereunder shall be fully refunded to you. Notification of termination will be sent to the contact information as supplied by you during the request process and termination shall be immediate upon sending the notice. Neither AAAS nor CCC shall be liable for any costs, expenses, or damages you may incur as a result of the termination of this permission, beyond the refund noted above.

This Permission may not be amended except by written document signed by both parties.

The terms above are applicable to all permissions granted for the use of AAAS material. Below you will find additional conditions that apply to your particular type of use.

FOR A THESIS OR DISSERTATION
If you are using figure(s)/table(s), permission is granted for use in print and electronic versions of your dissertation or thesis. A full text article may be used in print versions only of a dissertation or thesis.

Permission covers the distribution of your dissertation or thesis on demand by ProQuest / UMI, provided the AAAS material covered by this permission remains in situ.

If you are an Original Author on the AAAS article being reproduced, please refer to your License to Publish for rules on reproducing your paper in a dissertation or thesis.

FOR JOURNALS:
Permission covers both print and electronic versions of your journal article, however the AAAS material may not be used in any manner other than within the context of your article.

FOR BOOKS/TEXTBOOKS:
If this license is to reuse figures/tables, then permission is granted for non-exclusive world rights in all languages in both print and electronic formats (electronic formats are defined below).

If this license is to reuse a text excerpt or a full text article, then permission is granted for non-exclusive world rights in English only. You have the option of securing either print or electronic rights or both, but electronic rights are not automatically granted and do garner additional fees. Permission for translations of text excerpts or full text articles into other languages must be obtained separately.

Licenses granted for use of AAAS material in electronic format books/textbooks are valid only in cases where the electronic version is equivalent to or substitutes for the print version of the book/textbook. The AAAS material reproduced as permitted herein must remain in situ and must not be exploited separately (for example, if permission covers the use of a full text article, the article may not be offered for access or for purchase as a stand-alone unit), except in the case of permitted textbook companions as noted below.

You must include the following notice in any electronic versions, either adjacent to the reprinted AAAS material or in the terms and conditions for use of your electronic products: "Readers may view, browse, and/or download material for temporary copying purposes only, provided these uses are for noncommercial personal purposes. Except as provided by law, this material may not be further reproduced, distributed, transmitted, modified, adapted, performed, displayed, published, or sold in whole or in part, without prior written permission from the publisher."

If your book is an academic textbook, permission covers the following companions to your textbook, provided such companions are distributed only in conjunction with your textbook at no additional cost to the user:

- Password-protected website
- Instructor's image CD/DVD and/or PowerPoint resource
- Student CD/DVD

All companions must contain instructions to users that the AAAS material may be used for non-commercial, classroom purposes only. Any other uses require the prior written permission from AAAS.

If your license is for the use of AAAS Figures/Tables, then the electronic rights granted herein permit use of the Licensed Material in any Custom Databases that you distribute the electronic versions of your textbook through, so long as the Licensed Material remains within the context of a chapter of the title identified in your request and cannot be downloaded by a user as an independent image file.

Rights also extend to copies/files of your Work (as described above) that you are required to provide for use by the visually and/or print disabled in compliance with state and federal laws.

This permission only covers a single edition of your work as identified in your request.

FOR NEWSLETTERS:
Permission covers print and/or electronic versions, provided the AAAS material reproduced as permitted herein remains in situ and is not exploited separately (for example, if permission covers the use of a full text article, the article may not be offered for access or for purchase as a stand-alone unit)

FOR ANNUAL REPORTS:
Permission covers print and electronic versions provided the AAAS material reproduced as permitted herein remains in situ and is not exploited separately (for example, if permission covers the use of a full text article, the article may not be offered for access or for purchase as a stand-alone unit)

FOR PROMOTIONAL/MARKETING USES:
Permission covers the use of AAAS material in promotional or marketing pieces such as information packets, media kits, product slide kits, brochures, or flyers limited to a single print run. The AAAS Material may not be used in any manner which implies endorsement or promotion by the American Association for the Advancement of Science (AAAS) or Science of any product or service. AAAS does not permit the reproduction of its name, logo or text on promotional literature.

If permission to use a full text article is permitted, The Science article covered by this permission must not be altered in any way. No additional printing may be set onto an article copy other than the copyright credit line required above. Any alterations must be approved in advance and in writing by AAAS. This includes, but is not limited to, the placement of sponsorship identifiers, trademarks, logos, rubber stamping or self-adhesive stickers onto the article copies.

Additionally, article copies must be a freestanding part of any information package (i.e. media kit) into which they are inserted. They may not be physically attached to anything, such as an advertising insert, or have anything attached to them, such as a sample product. Article copies must be easily removable from any kits or informational packages in which they are used. The only exception is that article copies may be inserted into three-ring binders.

FOR CORPORATE INTERNAL USE:
The AAAS material covered by this permission may not be altered in any way. No additional printing may be set onto an article copy other than the required credit line. Any alterations must be approved in advance and in writing by AAAS. This includes, but is not limited to the placement of sponsorship identifiers, trademarks, logos, rubber stamping or self-adhesive stickers onto article copies.

If you are making article copies, copies are restricted to the number indicated in your request and must be distributed only to internal employees for internal use.

If you are using AAAS Material in Presentation Slides, the required credit line must be visible on the slide where the AAAS material will be reprinted

If you are using AAAS Material on a CD, DVD, Flash Drive, or the World Wide Web, you must include the following notice in any electronic versions, either adjacent to the reprinted AAAS material or in the terms and conditions for use of your electronic products: "Readers may view, browse, and/or download material for temporary copying purposes only, provided these uses are for noncommercial personal purposes. Except as provided by law, this material may not be further reproduced, distributed, transmitted, modified, adapted, performed, displayed, published, or sold in whole or in part, without prior written permission from the publisher." Access to any such CD, DVD, Flash Drive or Web page must be restricted to your organization's employees only.

FOR CME COURSE and SCIENTIFIC SOCIETY MEETINGS:
Permission is restricted to the particular Course, Seminar, Conference, or Meeting indicated in your request. If this license covers a text excerpt or a Full Text Article, access to the reprinted AAAS material must be restricted to attendees of your event only (if you have been granted electronic rights for use of a full text article on your website, your website must be password protected, or access restricted so that only attendees can access the content on your site).

If you are using AAAS Material on a CD, DVD, Flash Drive, or the World Wide Web, you must include the following notice in any electronic versions, either adjacent to the reprinted AAAS material or in the terms and conditions for use of your electronic products: "Readers may view, browse, and/or download material for temporary copying purposes only, provided these uses are for noncommercial personal purposes. Except as provided by law, this material may not be further reproduced, distributed, transmitted, modified, adapted, performed, displayed, published, or sold in whole or in part, without prior written permission from the publisher."

FOR POLICY REPORTS:
These rights are granted only to non-profit organizations and/or government agencies. Permission covers print and electronic versions of a report, provided the required credit line appears in both versions and provided the AAAS material reproduced as permitted herein remains in situ and is not exploited separately.

FOR CLASSROOM PHOTOCOPIES:
Permission covers distribution in print copy format only. Article copies must be freestanding and not part of a course pack. They may not be physically attached to anything or have anything attached to them.

FOR COURSEPACKS OR COURSE WEBSITES:
These rights cover use of the AAAS material in one class at one institution. Permission is valid only for a single semester after which the AAAS material must be removed from the Electronic Course website, unless new permission is obtained for an additional semester. If the material is to be distributed online, access must be restricted to students and instructors enrolled in that particular course by some means of password or access control.

FOR WEBSITES:
You must include the following notice in any electronic versions, either adjacent to the reprinted AAAS material or in the terms and conditions for use of your electronic products: "Readers may view, browse, and/or download material for temporary copying purposes only, provided these uses are for noncommercial personal purposes. Except as provided by law, this material may not be further reproduced, distributed, transmitted, modified, adapted, performed, displayed, published, or sold in whole or in part, without prior written permission from the publisher."

Permissions for the use of Full Text articles on third party websites are granted on a case by case basis and only in cases where access to the AAAS Material is restricted by some means of password or access control. Alternately, an E-Print may be purchased through our reprints department (brocheleau@rockwaterinc.com).

REGARDING FULL TEXT ARTICLE USE ON THE WORLD WIDE WEB IF YOU ARE AN 'ORIGINAL AUTHOR' OF A SCIENCE PAPER

If you chose "Original Author" as the Requestor Type, you are warranting that you are one of authors listed on the License Agreement as a "Licensed content author" or that you are acting on that author's behalf to use the Licensed content in a new work that one of the authors listed on the License Agreement as a "Licensed content author" has written.

Original Authors may post the 'Accepted Version' of their full text article on their personal or on their University website and not on any other website. The 'Accepted Version' is the version of the paper accepted for publication by AAAS including changes resulting from peer review but prior to AAAS's copy editing and production (in other words not the AAAS published version).

FOR MOVIES / FILM / TELEVISION:
Permission is granted to use, record, film, photograph, and/or tape the AAAS material in connection with your program/film and in any medium your program/film may be shown or heard, including but not limited to broadcast and cable television, radio, print, world wide web, and videocassette.

The required credit line should run in the program/film's end credits.

FOR MUSEUM EXHIBITIONS:
Permission is granted to use the AAAS material as part of a single exhibition for the duration of that exhibit. Permission for use of the material in promotional materials for the exhibit must be cleared separately with AAAS (please contact us at permissions@aaas.org).

FOR TRANSLATIONS:
Translation rights apply only to the language identified in your request summary above.

The following disclaimer must appear with your translation, on the first page of the article, after the credit line: "This translation is not an official translation by AAAS staff, nor is it endorsed by AAAS as accurate. In crucial matters, please refer to the official English-language version originally published by AAAS."

FOR USE ON A COVER:
Permission is granted to use the AAAS material on the cover of a journal issue, newsletter issue, book, textbook, or annual report in print and electronic formats provided the AAAS material reproduced as permitted herein remains in situ and is not exploited separately

By using the AAAS Material identified in your request, you agree to abide by all the terms and conditions herein.

Questions about these terms can be directed to the AAAS Permissions department permissions@aaas.org.

Other Terms and Conditions:

v 2

SPRINGER NATURE LICENSE
TERMS AND CONDITIONS

Feb 29, 2020

This Agreement between Indian Institute of Science Education and Research (IISER) Pune -- Anish Rao ("You") and Springer Nature ("Springer Nature") consists of your license details and the terms and conditions provided by Springer Nature and Copyright Clearance Center.

License Number	4778111216181
License date	Feb 29, 2020
Licensed Content Publisher	Springer Nature
Licensed Content Publication	Nature Chemistry
Licensed Content Title	Dissipative self-assembly of vesicular nanoreactors
Licensed Content Author	Subhabrata Maiti et al
Licensed Content Date	May 2, 2016
Type of Use	Thesis/Dissertation
Requestor type	academic/university or research institute
Format	print and electronic
Portion	figures/tables/illustrations
Number of figures/tables/illustrations	1
High-res required	no

Will you be translating?	no
Circulation/distribution	1 - 29
Author of this Springer Nature content	no
Title	Regulating Interparticle Interactions to Control the Spatio-Temporal Assembly of Gold Nanoparticles
Institution name	n/a
Expected presentation date	Mar 2020
Order reference number	Chapter 1, [61]
Portions	Figure 1
Requestor Location	Indian Institute of Science Education and Research (IISER) Pune IISER New Hostel 2, IISER Campus, Pashan Pune, Maharashtra 411008 India Attn: Indian Institute of Science Education and Research (IISER) Pune
Total	0.00 USD

Terms and Conditions

Springer Nature Customer Service Centre GmbH
Terms and Conditions

This agreement sets out the terms and conditions of the licence (the **Licence**) between you and **Springer Nature Customer Service Centre GmbH** (the **Licensor**). By clicking 'accept' and completing the transaction for the material (**Licensed Material**), you also confirm your acceptance of these terms and conditions.

1. Grant of License

1. 1. The Licensor grants you a personal, non-exclusive, non-transferable, world-wide licence to reproduce the Licensed Material for the purpose specified in your order only. Licences are granted for the specific use requested in the order and for no other use, subject to the conditions below.

1. 2. The Licensor warrants that it has, to the best of its knowledge, the rights to license reuse of the Licensed Material. However, you should ensure that the material you are requesting is original to the Licensor and does not carry the copyright of another entity (as credited in the published version).

1. 3. If the credit line on any part of the material you have requested indicates that it was reprinted or adapted with permission from another source, then you should also seek permission from that source to reuse the material.

2. Scope of Licence

2. 1. You may only use the Licensed Content in the manner and to the extent permitted by these Ts&Cs and any applicable laws.

2. 2. A separate licence may be required for any additional use of the Licensed Material, e.g. where a licence has been purchased for print only use, separate permission must be obtained for electronic re-use. Similarly, a licence is only valid in the language selected and does not apply for editions in other languages unless additional translation rights have been granted separately in the licence. Any content owned by third parties are expressly excluded from the licence.

2. 3. Similarly, rights for additional components such as custom editions and derivatives require additional permission and may be subject to an additional fee. Please apply to Journalpermissions@springernature.com/bookpermissions@springernature.com for these rights.

2. 4. Where permission has been granted **free of charge** for material in print, permission may also be granted for any electronic version of that work, provided that the material is incidental to your work as a whole and that the electronic version is essentially equivalent to, or substitutes for, the print version.

2. 5. An alternative scope of licence may apply to signatories of the STM Permissions Guidelines, as amended from time to time.

3. Duration of Licence

3. 1. A licence for is valid from the date of purchase ('Licence Date') at the end of the relevant period in the below table:

Scope of Licence	Duration of Licence
Post on a website	12 months
Presentations	12 months
Books and journals	Lifetime of the edition in the language purchased

4. Acknowledgement

4. 1. The Licensor's permission must be acknowledged next to the Licenced Material in print. In electronic form, this acknowledgement must be visible at the same time as the figures/tables/illustrations or abstract, and must be hyperlinked to the journal/book's homepage. Our required acknowledgement format is in the Appendix below.

5. Restrictions on use

5. 1. Use of the Licensed Material may be permitted for incidental promotional use and minor editing privileges e.g. minor adaptations of single figures, changes of format, colour and/or style where the adaptation is credited as set out in Appendix 1 below. Any other changes including but not limited to, cropping, adapting, omitting material that affect the meaning, intention or moral rights of the author are strictly prohibited.

5. 2. You must not use any Licensed Material as part of any design or trademark.

5. 3. Licensed Material may be used in Open Access Publications (OAP) before publication by Springer Nature, but any Licensed Material must be removed from OAP sites prior to final publication.

6. Ownership of Rights

6. 1. Licensed Material remains the property of either Licensor or the relevant third party and any rights not explicitly granted herein are expressly reserved.

7. Warranty

IN NO EVENT SHALL LICENSOR BE LIABLE TO YOU OR ANY OTHER PARTY OR ANY OTHER PERSON OR FOR ANY SPECIAL, CONSEQUENTIAL, INCIDENTAL OR INDIRECT DAMAGES, HOWEVER CAUSED, ARISING OUT OF OR IN CONNECTION WITH THE DOWNLOADING, VIEWING OR USE OF THE MATERIALS REGARDLESS OF THE FORM OF ACTION, WHETHER FOR BREACH OF CONTRACT, BREACH OF WARRANTY, TORT, NEGLIGENCE, INFRINGEMENT OR OTHERWISE (INCLUDING, WITHOUT LIMITATION, DAMAGES BASED ON LOSS OF PROFITS, DATA, FILES, USE, BUSINESS OPPORTUNITY OR CLAIMS OF THIRD PARTIES), AND
WHETHER OR NOT THE PARTY HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES. THIS LIMITATION SHALL APPLY NOTWITHSTANDING ANY FAILURE OF ESSENTIAL PURPOSE OF ANY LIMITED REMEDY PROVIDED HEREIN.

8. Limitations

8. 1. ***BOOKS ONLY:***Where **'reuse in a dissertation/thesis'** has been selected the following terms apply: Print rights of the final author's accepted manuscript (for clarity, NOT the published version) for up to 100 copies, electronic rights for use only on a personal website or institutional repository as defined by the Sherpa guideline (www.sherpa.ac.uk/romeo/).

9. Termination and Cancellation

9. 1. Licences will expire after the period shown in Clause 3 (above).

9. 2. Licensee reserves the right to terminate the Licence in the event that payment is not received in full or if there has been a breach of this agreement by you.

Appendix 1 — Acknowledgements:

For Journal Content:
Reprinted by permission from [**the Licensor**]: [**Journal Publisher** (e.g. Nature/Springer/Palgrave)] [**JOURNAL NAME**] [**REFERENCE CITATION** (Article name, Author(s) Name), [**COPYRIGHT**] (year of publication)

For **Advance Online Publication papers:**
Reprinted by permission from [**the Licensor**]: [**Journal Publisher** (e.g. Nature/Springer/Palgrave)] [**JOURNAL NAME**] [**REFERENCE CITATION** (Article name, Author(s) Name), [**COPYRIGHT**] (year of publication), advance online publication, day month year (doi: 10.1038/sj.[JOURNAL ACRONYM].)

For Adaptations/Translations:
Adapted/Translated by permission from [**the Licensor**]: [**Journal Publisher** (e.g. Nature/Springer/Palgrave)] [**JOURNAL NAME**] [**REFERENCE CITATION** (Article name, Author(s) Name), [**COPYRIGHT**] (year of publication)

Note: For any republication from the British Journal of Cancer, the following credit line style applies:

Reprinted/adapted/translated by permission from [**the Licensor**]: on behalf of Cancer Research UK: : [**Journal Publisher** (e.g. Nature/Springer/Palgrave)] [**JOURNAL NAME**] [**REFERENCE CITATION** (Article name, Author(s) Name), [**COPYRIGHT**] (year of publication)

For **Advance Online Publication** papers:
Reprinted by permission from The [**the Licensor**]: on behalf of Cancer Research UK: [**Journal Publisher** (e.g. Nature/Springer/Palgrave)] [**JOURNAL NAME**] [**REFERENCE CITATION** (Article name, Author(s) Name), [**COPYRIGHT**] (year of publication), advance online publication, day month year (doi: 10.1038/sj.[JOURNAL ACRONYM])

For Book content:
Reprinted/adapted by permission from [**the Licensor**]: [**Book Publisher** (e.g. Palgrave Macmillan, Springer etc) [**Book Title**] by [**Book author**(s)] [**COPYRIGHT**] (year of publication)

Other Conditions:

Version 1.2

Questions? customercare@copyright.com or +1-855-239-3415 (toll free in the US) or +1-978-646-2777.

Gold Nanoparticle-Based Sensing of "Spectroscopically Silent" Heavy Metal Ions

Author: Youngjin Kim, Robert C. Johnson, Joseph T. Hupp

Publication: Nano Letters

Publisher: American Chemical Society

Date: Apr 1, 2001

PERMISSION/LICENSE IS GRANTED FOR YOUR ORDER AT NO CHARGE

This type of permission/license, instead of the standard Terms & Conditions, is sent to you because no fee is being charged for your order. Please note the following:

- Permission is granted for your request in both print and electronic formats, and translations.
- If figures and/or tables were requested, they may be adapted or used in part.
- Please print this page for your records and send a copy of it to your publisher/graduate school.
- Appropriate credit for the requested material should be given as follows: "Reprinted (adapted) with permission from (COMPLETE REFERENCE CITATION). Copyright (YEAR) American Chemical Society." Insert appropriate information in place of the capitalized words.
- One-time permission is granted only for the use specified in your request. No additional uses are granted (such as derivative works or other editions). For any other uses, please submit a new request.

If credit is given to another source for the material you requested, permission must be obtained from that source.

BACK CLOSE WINDOW

JOHN WILEY AND SONS LICENSE
TERMS AND CONDITIONS

Feb 28, 2020

This Agreement between Indian Institute of Science Education and Research (IISER) Pune -- Anish Rao ("You") and John Wiley and Sons ("John Wiley and Sons") consists of your license details and the terms and conditions provided by John Wiley and Sons and Copyright Clearance Center.

License Number	4777681075372
License date	Feb 28, 2020
Licensed Content Publisher	John Wiley and Sons
Licensed Content Publication	Angewandte Chemie International Edition
Licensed Content Title	Colorimetric Detection of Mercuric Ion (Hg2) in Aqueous Media using DNA-Functionalized Gold Nanoparticles
Licensed Content Author	Chad A. Mirkin, Min Su Han, Jae-Seung Lee
Licensed Content Date	May 16, 2007
Licensed Content Volume	46
Licensed Content Issue	22
Licensed Content Pages	4
Type of use	Dissertation/Thesis
Requestor type	University/Academic

JOHN WILEY AND SONS LICENSE
TERMS AND CONDITIONS

Feb 28, 2020

This Agreement between Indian Institute of Science Education and Research (IISER) Pune -- Anish Rao ("You") and John Wiley and Sons ("John Wiley and Sons") consists of your license details and the terms and conditions provided by John Wiley and Sons and Copyright Clearance Center.

License Number	4777700200111
License date	Feb 28, 2020
Licensed Content Publisher	John Wiley and Sons
Licensed Content Publication	Angewandte Chemie International Edition
Licensed Content Title	Nanoparticle Supracrystals and Layered Supracrystals as Chemical Amplifiers
Licensed Content Author	Bartosz A. Grzybowski, Siowling Soh, David A. Walker, et al
Licensed Content Date	Jul 28, 2010
Licensed Content Volume	49
Licensed Content Issue	33
Licensed Content Pages	5
Type of use	Dissertation/Thesis
Requestor type	University/Academic

Format	Print and electronic
Portion	Figure/table
Number of figures/tables	2
Original Wiley figure/table number(s)	Figure 2, Figure 3
Will you be translating?	No
Title of your thesis / dissertation	Regulating Interparticle Interactions to Control the Spatio-Temporal Assembly of Gold Nanoparticles
Expected completion date	Mar 2020
Expected size (number of pages)	180
Requestor Location	Indian Institute of Science Education and Research (IISER) Pune IISER New Hostel 2, IISER Campus, Pashan Pune, Maharashtra 411008 India Attn: Indian Institute of Science Education and Research (IISER) Pune
Publisher Tax ID	EU826007151
Total	0.00 USD

Terms and Conditions

TERMS AND CONDITIONS

This copyrighted material is owned by or exclusively licensed to John Wiley & Sons, Inc. or one of its group companies (each a"Wiley Company") or handled on behalf of a society with which a Wiley Company has exclusive publishing rights in relation to a particular work (collectively "WILEY"). By clicking "accept" in connection with completing this licensing transaction, you agree that the following terms and conditions apply to this transaction (along with the billing and payment terms and conditions established by the Copyright Clearance Center Inc., ("CCC's Billing and Payment terms and conditions"), at the time that you opened your RightsLink account (these are available at any time at http://myaccount.copyright.com).

Terms and Conditions

- The materials you have requested permission to reproduce or reuse (the "Wiley Materials") are protected by copyright.

- You are hereby granted a personal, non-exclusive, non-sub licensable (on a stand-alone basis), non-transferable, worldwide, limited license to reproduce the Wiley Materials for the purpose specified in the licensing process. This license, **and any CONTENT (PDF or image file) purchased as part of your order,** is for a one-time use only and limited to any maximum distribution number specified in the license. The first instance of republication or reuse granted by this license must be completed within two years of the date of the grant of this license (although copies prepared before the end date may be distributed thereafter). The Wiley Materials shall not be used in any other manner or for any other purpose, beyond what is granted in the license. Permission is granted subject to an appropriate acknowledgement given to the author, title of the material/book/journal and the publisher. You shall also duplicate the copyright notice that appears in the Wiley publication in your use of the Wiley Material. Permission is also granted on the understanding that nowhere in the text is a previously published source acknowledged for all or part of this Wiley Material. Any third party content is expressly excluded from this permission.

- With respect to the Wiley Materials, all rights are reserved. Except as expressly granted by the terms of the license, no part of the Wiley Materials may be copied, modified, adapted (except for minor reformatting required by the new Publication), translated, reproduced, transferred or distributed, in any form or by any means, and no derivative works may be made based on the Wiley Materials without the prior permission of the respective copyright owner.**For STM Signatory Publishers clearing permission under the terms of the STM Permissions Guidelines only, the terms of the license are extended to include subsequent editions and for editions in other languages, provided such editions are for the work as a whole in situ and does not involve the separate exploitation of the permitted figures or extracts,** You may not alter, remove or suppress in any manner any copyright, trademark or other notices displayed by the Wiley Materials. You may not license, rent, sell, loan, lease, pledge, offer as security, transfer or assign the Wiley Materials on a stand-alone basis, or any of the rights granted to you hereunder to any other person.

- The Wiley Materials and all of the intellectual property rights therein shall at all times remain the exclusive property of John Wiley & Sons Inc, the Wiley Companies, or their respective licensors, and your interest therein is only that of having possession of and the right to reproduce the Wiley Materials pursuant to Section 2 herein during the continuance of this Agreement. You agree that you own no right, title or interest in or to the Wiley Materials or any of the intellectual property rights therein. You shall have no rights hereunder other than the license as provided for above in Section 2. No right, license or interest to any trademark, trade name, service mark or other branding ("Marks") of WILEY or its licensors is granted hereunder, and you agree that you shall not assert any such right, license or interest with respect thereto

- NEITHER WILEY NOR ITS LICENSORS MAKES ANY WARRANTY OR REPRESENTATION OF ANY KIND TO YOU OR ANY THIRD PARTY, EXPRESS, IMPLIED OR STATUTORY, WITH RESPECT TO THE MATERIALS OR THE ACCURACY OF ANY INFORMATION CONTAINED IN THE MATERIALS, INCLUDING, WITHOUT LIMITATION, ANY IMPLIED WARRANTY OF MERCHANTABILITY, ACCURACY, SATISFACTORY QUALITY, FITNESS FOR A PARTICULAR PURPOSE, USABILITY, INTEGRATION OR NON-INFRINGEMENT AND ALL SUCH WARRANTIES ARE HEREBY EXCLUDED BY WILEY AND ITS LICENSORS AND WAIVED

BY YOU.

- WILEY shall have the right to terminate this Agreement immediately upon breach of this Agreement by you.

- You shall indemnify, defend and hold harmless WILEY, its Licensors and their respective directors, officers, agents and employees, from and against any actual or threatened claims, demands, causes of action or proceedings arising from any breach of this Agreement by you.

- IN NO EVENT SHALL WILEY OR ITS LICENSORS BE LIABLE TO YOU OR ANY OTHER PARTY OR ANY OTHER PERSON OR ENTITY FOR ANY SPECIAL, CONSEQUENTIAL, INCIDENTAL, INDIRECT, EXEMPLARY OR PUNITIVE DAMAGES, HOWEVER CAUSED, ARISING OUT OF OR IN CONNECTION WITH THE DOWNLOADING, PROVISIONING, VIEWING OR USE OF THE MATERIALS REGARDLESS OF THE FORM OF ACTION, WHETHER FOR BREACH OF CONTRACT, BREACH OF WARRANTY, TORT, NEGLIGENCE, INFRINGEMENT OR OTHERWISE (INCLUDING, WITHOUT LIMITATION, DAMAGES BASED ON LOSS OF PROFITS, DATA, FILES, USE, BUSINESS OPPORTUNITY OR CLAIMS OF THIRD PARTIES), AND WHETHER OR NOT THE PARTY HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES. THIS LIMITATION SHALL APPLY NOTWITHSTANDING ANY FAILURE OF ESSENTIAL PURPOSE OF ANY LIMITED REMEDY PROVIDED HEREIN.

- Should any provision of this Agreement be held by a court of competent jurisdiction to be illegal, invalid, or unenforceable, that provision shall be deemed amended to achieve as nearly as possible the same economic effect as the original provision, and the legality, validity and enforceability of the remaining provisions of this Agreement shall not be affected or impaired thereby.

- The failure of either party to enforce any term or condition of this Agreement shall not constitute a waiver of either party's right to enforce each and every term and condition of this Agreement. No breach under this agreement shall be deemed waived or excused by either party unless such waiver or consent is in writing signed by the party granting such waiver or consent. The waiver by or consent of a party to a breach of any provision of this Agreement shall not operate or be construed as a waiver of or consent to any other or subsequent breach by such other party.

- This Agreement may not be assigned (including by operation of law or otherwise) by you without WILEY's prior written consent.

- Any fee required for this permission shall be non-refundable after thirty (30) days from receipt by the CCC.

- These terms and conditions together with CCC's Billing and Payment terms and conditions (which are incorporated herein) form the entire agreement between you and WILEY concerning this licensing transaction and (in the absence of fraud) supersedes all prior agreements and representations of the parties, oral or written. This Agreement may not be amended except in writing signed by both parties. This Agreement shall be binding upon and inure to the benefit of the parties' successors, legal representatives, and authorized assigns.

- In the event of any conflict between your obligations established by these terms and conditions and those established by CCC's Billing and Payment terms and conditions, these terms and conditions shall prevail.

- WILEY expressly reserves all rights not specifically granted in the combination of (i) the license details provided by you and accepted in the course of this licensing transaction, (ii) these terms and conditions and (iii) CCC's Billing and Payment terms and conditions.

- This Agreement will be void if the Type of Use, Format, Circulation, or Requestor Type was misrepresented during the licensing process.

- This Agreement shall be governed by and construed in accordance with the laws of the State of New York, USA, without regards to such state's conflict of law rules. Any legal action, suit or proceeding arising out of or relating to these Terms and Conditions or the breach thereof shall be instituted in a court of competent jurisdiction in New York County in the State of New York in the United States of America and each party hereby consents and submits to the personal jurisdiction of such court, waives any objection to venue in such court and consents to service of process by registered or certified mail, return receipt requested, at the last known address of such party.

WILEY OPEN ACCESS TERMS AND CONDITIONS

Wiley Publishes Open Access Articles in fully Open Access Journals and in Subscription journals offering Online Open. Although most of the fully Open Access journals publish open access articles under the terms of the Creative Commons Attribution (CC BY) License only, the subscription journals and a few of the Open Access Journals offer a choice of Creative Commons Licenses. The license type is clearly identified on the article.

The Creative Commons Attribution License

The Creative Commons Attribution License (CC-BY) allows users to copy, distribute and transmit an article, adapt the article and make commercial use of the article. The CC-BY license permits commercial and non-

Creative Commons Attribution Non-Commercial License

The Creative Commons Attribution Non-Commercial (CC-BY-NC)License permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.(see below)

Creative Commons Attribution-Non-Commercial-NoDerivs License

The Creative Commons Attribution Non-Commercial-NoDerivs License (CC-BY-NC-ND) permits use, distribution and reproduction in any medium, provided the original work is properly cited, is not used for commercial purposes and no modifications or adaptations are made. (see below)

Use by commercial "for-profit" organizations

Use of Wiley Open Access articles for commercial, promotional, or marketing purposes requires further explicit permission from Wiley and will be subject to a fee.

Further details can be found on Wiley Online Library
http://olabout.wiley.com/WileyCDA/Section/id-410895.html

Other Terms and Conditions:

v1.10 Last updated September 2015

Questions? customercare@copyright.com or +1-855-239-3415 (toll free in the US) or +1-978-646-2777.

A Colorimetric Lead Biosensor Using DNAzyme-Directed Assembly of Gold Nanoparticles

Author: Juewen Liu, Yi Lu
Publication: Journal of the American Chemical Society
Publisher: American Chemical Society
Date: Jun 1, 2003

PERMISSION/LICENSE IS GRANTED FOR YOUR ORDER AT NO CHARGE

This type of permission/license, instead of the standard Terms & Conditions, is sent to you because no fee is being charged for your order. Please note the following:

- Permission is granted for your request in both print and electronic formats, and translations.
- If figures and/or tables were requested, they may be adapted or used in part.
- Please print this page for your records and send a copy of it to your publisher/graduate school.
- Appropriate credit for the requested material should be given as follows: "Reprinted (adapted) with permission from (COMPLETE REFERENCE CITATION). Copyright (YEAR) American Chemical Society." Insert appropriate information in place of the capitalized words.
- One-time permission is granted only for the use specified in your request. No additional uses are granted (such as derivative works or other editions). For any other uses, please submit a new request.

If credit is given to another source for the material you requested, permission must be obtained from that source.

BACK CLOSE WINDOW