# Biochemical and Structural Characterization of Nucleotide Hydrolysis and DNA Binding by McrB – an atypical AAA+ GTPase

A Thesis

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Indian Institute of Science Education and Research Pune in partial fulfilment of the requirements for the BS-MS Dual Degree Programme

by

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Pratima Singh

# Certificate

This is to certify that this dissertation entitled **Biochemical and Structural Characterization of Nucleotide Hydrolysis and DNA Binding by McrB – an atypical AAA+ GTPase** towards the partial fulfilment of the BS-MS dual degree programme at the Indian Institute of Science Education and Research, Pune represents study/work carried out by **Pratima Singh** at Indian Institute of Science Education and Research under the supervision of **Dr. Saikrishnan Kayarat**, Associate Professor, Department of Biology, during the academic year 2019-2020.

Dr. Saikrishnan Kayarat

Committee:

Dr. Saikrishnan Kayarat

Dr. M. S. Madhusudhan

This thesis is dedicated to Science.

# Declaration

I hereby declare that the matter embodied in the report entitled **Biochemical and Structural Characterization of Nucleotide Hydrolysis and DNA Binding by McrB – an atypical AAA+ GTPase** are the results of the work carried out by me at the Department of Biology, Indian Institute of Science Education and Research, Pune, under the supervision of Dr. Saikrishnan Kayarat and the same has not been submitted elsewhere for any other degree

Pratima Singh

Date: 22/06/2020

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# Abstract

Methylated Cytosine Restriction B and C (McrBC) is a modification-dependent restriction enzyme from Escherichia coli K-12. It identifies the DNA recognition sequence RmC and restricts any DNA having at least two $R^mC$ sites separated by 40-3000 bp. The enzyme is made of two subunits – McrB, which has a DNA binding domain and a AAA+ (extended ATPases Associated with various cellular Activities) motor that is uniquely employed in hydrolyzing GTP rather than ATP, and the endonuclease McrC. The subunits assemble together to form a functional tetradecameric ($2McrB_6McrC$) complex in presence of GTP. The DNA binding domain of McrB specifically binds to DNA with the RmC recognition site and feeds it to McrC for endonucleolytic cleavage. McrB thus serves as a model system to understand how ring-like motors, in particular, those involved in DNA metabolism, function. The mechanisms of GTP hydrolysis and DNA binding for nucleolytic cleavage by McrB is unknown. As part of my research project, I plan to carry out the biochemical and structural characterization of McrB and McrBC and their mutants to dissect the mechanistic details of GTP hydrolysis and DNA binding. Towards this, we have generated a family of mutants that differentially affect GTP binding, the oligomeric assembly, and the GTPase activity of McrB. The results suggest that McrB has a canonical arginine-finger, MBR349 and a non-canonical sensor-II, McrBR348. Mutating other residues like MBE298, MBR347 and MBD343 which lie in the vicinity of nucleotide binding pocket affect nucleotide binding as well as oligomerization of McrB. To our surprise, proposed DNA loop binding mutants, $McrB^{R253A}$ and $McrB^{R254A}$ also affected the nucleotide binding which suggests an allosteric regulation of nucleotide binding.

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*And to my parents and brothers, who set me off on the road to this MSc a long time ago.*

Chapter 1

# Methylated Cytosine Restriction B and C (McrBC) – a modification dependent restriction enzyme

## 1.1 General Introduction

Restriction-Modification (RM) the system was developed by bacteria to cope up with the incessant threat posed by bacteriophage. As the name suggests, the RM system is a two-component system: a restriction subunit and a modification subunit. The modification subunit marks (modifies) the self DNA to distinguish it from any non-self DNA. The restriction subunit identifies the marked DNA as self and cleaves any foreign DNA (Meselson et.al, 1972). Conversely in the race of evolution phages also developed the modified DNA against which bacteria counter-evolved a Modification Dependent Restriction (MDR) system. MDR system lacks the cognate modification unit and hence self DNA is identified as unmarked but any modified DNA will be acknowledged as non-self and will be cleaved off. Methylated Cytosine Restriction B and C (McrBC) is one such MDR system. It recognizes DNA with 5'-[A/G] $^{m}$C-3', where $^{m}$C can be 5-methyl cytidine, 4-methyl cytidine or 5-hydroxymethyl cytidine, and severs it (Stewart et al., 2000).

## 1.2 Discovery of McrBC

In 1952, Luria and his colleagues observed that phages that were cultured in bacteria lacking UDP-glucose or functional glucosyltransferase were incompetent to infect certain *Escherichia coli* strains. The responsible factors were then named as Rgl (Restriction of glucose-less DNA) A and B (Hattman & Fukasawa, 1963). McrBC was later termed when transforming methylated or methyltransferase genes posed a challenge in *E. coli* K-12 (Raleigh & Wilson, 1986).

## 1.3 The functional McrBC complex

McrBC is a two-component system. The functional complex, as shown in Figure 1, is a hetero-oligomer of a 2 hexameric McrB rings bridged by a dimer of McrC. Together, the tetradecameric McrBC complex can restrict a DNA with two 5'-[A/G] mC-3' sites separated by 40-3000bp (Sutherland et al., 1992; Nirwan et al., 2018).

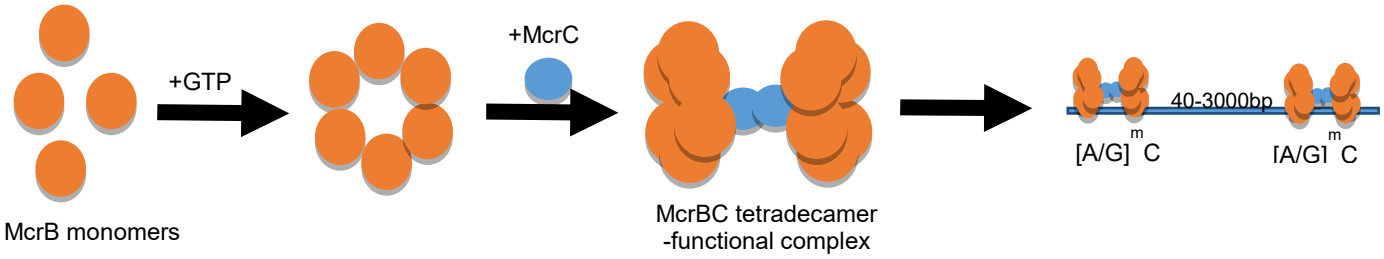



***Figure 1: Schematic of the model proposed for assembly of functional McrBC complex in presence of GTP***

### 1.3.1 **McrB**

McrB is a 54kDa bi-domain protein with its N-terminal being the target recognition subdomain that identifies and loads the protein on the specific site and the C-terminal being the GTPase.The N-terminal of the protein is known to have an SRA-like but a novel fold (Sukackaite et al., 2012). While the C-terminal GTPase is classified under AAA+ (extended ATPases Associated with various cellular Activities) (Sutherland et al., 1992; Neuwald et al., 1999). AAA+ family of proteins are known to use the energy obtained by hydrolyzing nucleoside triphosphates (majorly ATP) to carry out mechanical work like pulling a peptide chain in a tunnel for proteolysis, membrane fusion, DNA replication, etc. These proteins typically form a hexameric ring-like structure in the presence or absence of nucleotide and the hydrolysis is more often regulated mostly by substrates or adapter proteins in some cases to conserve energy (Ades, 2006).

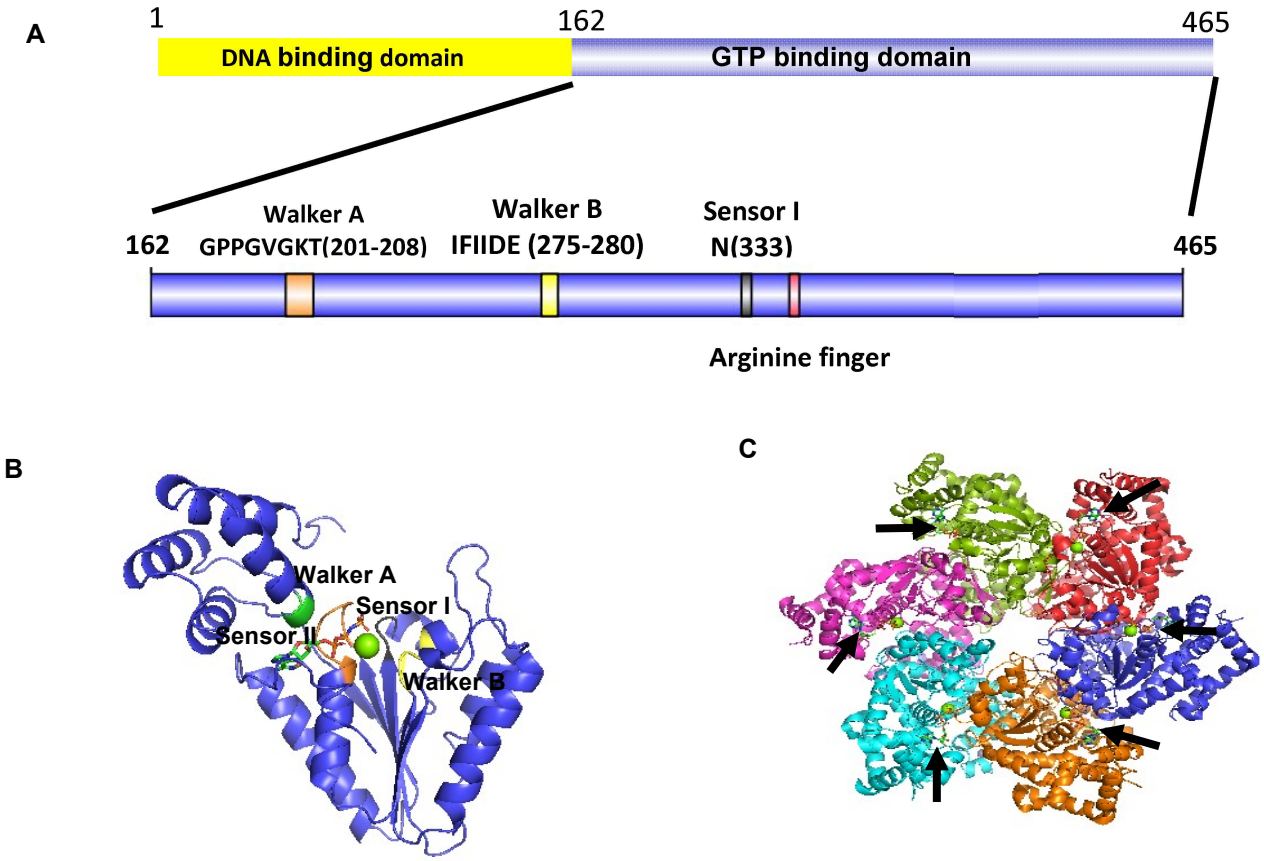



***Figure 2: (A) Schematic representing domains of McrB and the conserved motifs with their positions. (B) A representative of AAA+ family monomer showing orientation of conserved motifs around the bound nucleotide, PDB structure: 1D2N. (C) A AAA+ hexameric ring with bound nucleotide (indicated by black arrows), PDB structure: 1SVM.***

Regardless of the miscellaneous functions they perform, they all harbor strongly conserved motifs and structural fold which defines the characteristic features of the AAA+ family. The N-terminal, α-β-α subdomain forms the wedge-like structure over which α-helical C-terminal subdomain rests like a lid. Among all the most important motifs of the AAA+ family are Walker A and Walker B. Walker A motif, also known as

phosphate-loop interacts with the phosphates of bound nucleotide. The consensus sequence for P-loop is GxxxxGK[S/T]. Mutating residues of this motif, predominantly K has shown to affect nucleotide binding. The acidic residues of the Walker B motif are found to be more important for nucleotide hydrolysis rather than binding. The aspartate of the hhhhDE motif (h=hydrophobic amino acid) coordinates $Mg^{+2}$ ion and the glutamate activates the water molecule for nucleotide hydrolysis. Another polar residue acts in concert with the glutamate to activate the water molecule, this is called the sensor I. Sensor I interacts with the γ-phosphate of the bound ATP. Mutation in the polar residue impairs the nucleotide hydrolysis suggesting that the Walker B glutamate on its own is not sufficient to activate the water molecule. Another residue - an arginine, similar to arginine-finger of the GTPase activator proteins interact with the nucleotide in *trans.* Mutating this residue has resulted in nucleotide binding and hydrolysis defects. Lastly, the C-terminal helical domain also shows substantial motion throughout a nucleotide hydrolysis cycle. The lid has conserved arginine residue that directly interacts with the phosphate group of the bound nucleotide hence responding conformational changes following the stage of nucleotide hydrolysis (Wendler et.al, 2012; Sauer & Baker, 2011; Hanson et al., 2005). All these interactions together position the nucleotide at the interface of two protomers leading to oligomerization of AAA+ domains (in most cases, the formation of a hexamer) in the presence or absence of nucleotide.

### 1.3.2 **McrC**

McrC is a 39kDa protein that deciphers the endonuclease activity to the complex. It is also a bi-domain protein with no homolog for the N-terminal subdomain however the C-terminal is homologous to the classical Type II endonuclease fold. It has the characteristic PD…[D/E]xK motif non-canonically as $TD_{244}…D_{257}AK$ (Pieper & Pingoud, 2002). McrC is proposed to be dimeric in a solution similar to Type II endonucleases.


***Figure 3: Schematic representing the conserved motif of McrC***

Although McrBC shares similarities with well-studied systems, it is unique in numerous ways. McrB is the only known AAA+ protein to hydrolyze GTP instead of ATP. Secondly, it has a very low intrinsic GTPase activity which is not stimulated the substrate DNA unlike other members of the family. The cognate protein McrC is known to stimulate the GTPase activity of McrB ~30 folds or more. Also interestingly, McrB, unlike other AAA+ proteins, shows dependence on nucleotide for oligomerization (Nirwan et al., 2018). These features make McrBC an interesting system to explore. We obtained a structure of McrBΔN (McrB without the N-terminal 161 residues) in complex with McrC at 3.6 Å resolution which helped us identify the residues important for nucleotide hydrolysis. My study focuses on understanding the role of these residues to decipher the mechanism of action of the functional McrBC complex.

Chapter 2

# Characterization of Mutatnts in the Nucleotide Binding Pocket

## 2.1 Introduction

Multiple sequence alignment of McrB with other representatives of AAA+ family proteins showed three consecutive arginines (McrBR347, McrBR348, and MCRBR349) aligning close to the canonical arginine finger. We carried out a mutagenesis study on the role of each arginine. Here I will discuss the effect of mutating McrBR348 and McrBR349 to alanine. McrB$^{R347A}$ will be discussed in the next chapter.

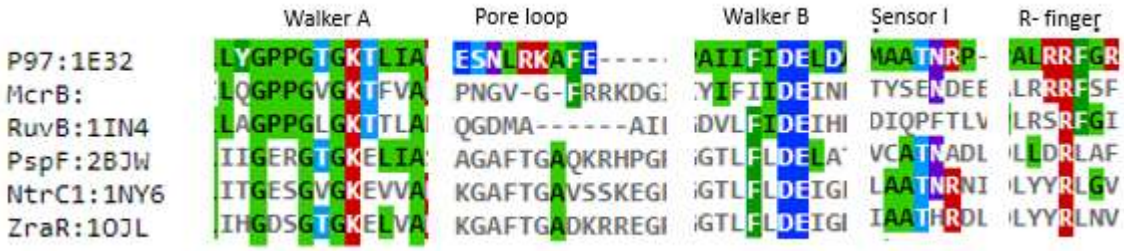



***Figure 4: Multiple sequence alignment of representatives of AAA+ superfamily with McrB (obtained using Clustal omega (Sievers et al., 2011)). Shown here are only the representative motifs conserved across the AAA+ domains***

## 2.2 Materials and methods

### 2.2.1 Cloning and Protein Purification

A restriction-free cloning method was used to obtain respective mutations. The table lists the primers used to introduce each mutation. The clones thus obtained were fully sequenced to confirm mutations. The proteins were purified using two-step purification: An affinity column chromatography followed by ion-exchange column chromatography. Following is the list of primers used to incorporate respective mutations (Nirwan et al., 2018).

Table1: Table of primers for mutants McrB$^{E298A}$, McrB$^{R348A}$ and McrB$^{R349A}$

| Mutations | Primers used to incorporate mutation (5'->3') | |
|---|---|---|
| | Forward primer | Reverse primer |
| McrB$^{E298A}$ | TAATACGACTCACTATAGGG | CGTTTATCATGCGCCATTAACATCATC |
| McrB$^{R348A}$ | TAATACGACTCACTATAGGG | GAAAATCGCGCGCGTAGGGCATAGTC |
| McrB$^{R349A}$ | TAATACGACTCACTATAGGG | GAAAGAAAACGCTCTGCGTAGGGC |

### 2.2.2 Size Exclusion Column Chromatography- To study binding and oligomerization in presence of Nucleotide

In view of the fact that the nucleotide-binding pocket is at the interface of two protomers, oligomerization was studied as a measure of nucleotide-binding. To study the oligomerization of proteins 18 µM McrB or its mutants were injected in both the presence and absence of 4.5 µM McrC. The protomers were mixed in the presence of 2.5 mM GTP and 5mM $MgCl_2$ at 4 °C in buffer (50 mM Tris-HCl pH 8, 100 mM NaCl, 1 mM DTT). 400 µl of this preformed mix was then loaded on
The oligomerization of McrB and its mutants 24 ml Superdex200 10/300 GL (GE Life Sciences) SEC column and 24 ml Superose6 10/300 GL SEC column (GE Life Sciences) in the absence and presence of McrC respectively. Both the columns were pre-equilibrated with 50 mM Tris-HCl pH 8, 100 mM NaCl, 1 mM DTT, 5 mM $MgCl_2$ and 0.1 mM GTP.

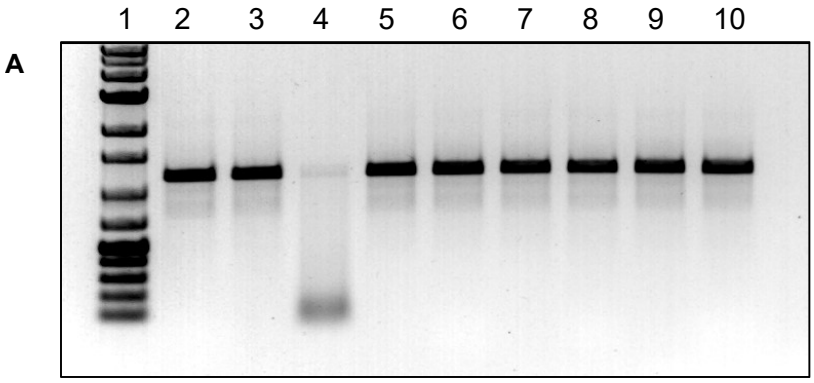
### 2.2.3 NADH-coupled GTPase assay

GTPase activity of McrB and its mutants was assessed using NADH-coupled GTPase assay (Nørby, 1988). 0.8 µM of McrB or its mutants and 0.2 µM of McrC were mixed in 200 µl reaction buffer which consists of 1 mM GTP (Jena Bioscience), 0.6 mM NADH (Sigma-Aldrich), 1 mM phosphoenolpyruvate, and 2 U each of pyruvate kinase (Sigma-Aldrich) and lactate dehydrogenase (Sigma-Aldrich) at 25 °C. Absorption at 340 nm was read at an interval of 10s for 3000s using a Varioscan plate reader.

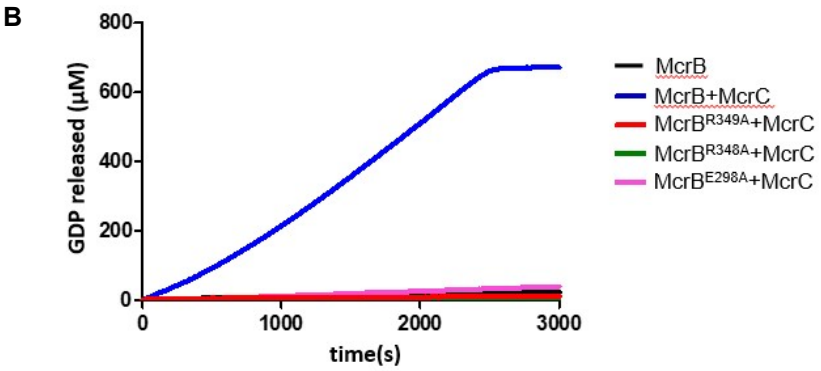
### 2.2.4 DNA cleavage assay

Substrate DNA was PCR amplified T7-Forward (5'-TAATACGACTCACTATAGGG-3') and T7-Reverse (5'-GCTAGTTATTGCTCAGCGG-3') as primers against pHISMcrBΔN plasmid. 5-methyl-deoxycytidine instead of deoxycytidine was used in the dNTP mix to obtain a 1127bp long fully-methylated substrate. The substrate was then purified using the Qiagen PCR Purification Kit. Nuclease activity of 50nM McrBC and the mutants was assessed at 37 °C. 75ng of the substrate DNA was incubated with protein complex for 3600s in presence of 10 mM Tris-Cl pH 8, 50 mM KCl, 5 mM MgCl2, 1 mM DTT and 1 mM GTP (Jena bioscience). The reaction was then quenched using 2 µl 6× STES buffer (40% Sucrose, 0.2 M Tris-Cl pH 7.5, 40 mM EDTA, 1% SDS) followed by heating at 65 °C for 10 min. The cleaved substrate was resolved at 110 V for 45 min on a 0.8% agarose gel containing 2 µg/ml ethidium bromide. The resolved DNA was imaged on E-Gel TM Imager System (Invitrogen Life Technologies).

## 2.3 Results and Discussion




Lane 1: Ladder
Lane 2: DNA + GTP
Lane 3: DNA + McrB+McrC
Lane 4: DNA + McrB+McrC +GTP
Lane 5: DNA + $McrB^{E298A}$+McrC
Lane 6: DNA + $McrB^{E298A}$+McrC +GTP
Lane 7: DNA + $McrB^{R348A}$+McrC
Lane 8: DNA + $McrB^{R348A}$+McrC +GTP
Lane 9: DNA + $McrB^{R349A}$+McrC
Lane 10: DNA + $McrB^{R349A}$+McrC +GTP



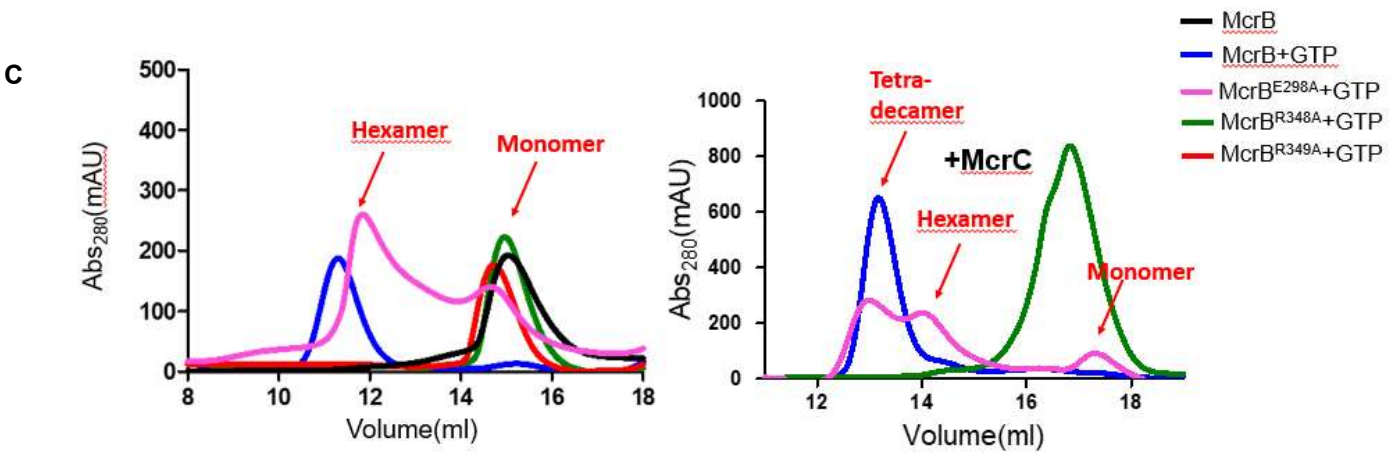




***Figure 5:** (A) Nuclease activity check of McrB and its mutants (**$McrB^{E298A}$**, **$MCRB^{R348A}$** and **$MCRB^{R349A}$**) in complex with McrC. (B) Comparison of the GTP hydrolysis by McrB and its mutants when stimulated by McrC (C)Size exclusion chromatographic profile of McrB and its mutants in the presence of GTP using Superdex 200 column (the red arrows mark the expected positions of monomeric and hexameric McrB) and in the presence of McrC and GTP using Superose 6 column(the red arrows mark the expected positions of monomeric and hexameric McrB and tetradecameric McrBC complex).*

Mutagenesis study divulged that each arginine was catalytically important. $MCRB^{R348A}$ and $MCRB^{R349A}$ failed to cleave a specific substrate or hydrolyze GTP. Since arginine finger, like in the case of ecHslU (Song et al.,2000), has shown to affect nucleotide-binding and thus oligomerization we examined if the mutants can bind to GTP. $MCRB^{R348A}$ and $MCRB^{R349A}$ did not oligomerize in presence of nucleotide and thus were nucleotide-binding deficient. These results made more sense when we looked at the structure. The MCRBR349 was interacting with the γ-

phosphate in trans, like the canonical arginine finger. However, there was no cis-acting sensor II instead McrBR348-trans is in the vicinity of β- and γ-phosphates. Hence, we propose that McrBR348-trans as sensor II. MCRBE298 interacts and positions McrBR348-trans. Mutating MCRBE298 to alanine caused an unstable oligomerization of $MCRB^{E298A}$ protomers. These unstable oligomers were incompetent in hydrolyzing the nucleotide and cleaving the DNA.

Chapter 3

# Residues important for McrB hexamer stabilization

## 3.1 Introduction

A study in 1999 by Alfred Pingoud and group showed that McrB$^{R347A}$ and McrB$^{D343A}$ failed to bind nucleotide but were capable of cleaving the substrate DNA. As discussed in chapter 2 we tried mutating McrBR347A. In order to understand the behavior of these mutants we also mutated McrBD343 to alanine.

## 3.2 Materials and methods

Same as Chapter 2. Following is the list of primers used to incorporate respective mutation.

Table2: Table of primers for mutants McrB$^{R347A}$ and McrB$^{D343A}$

| Mutations | Primers used to incorporate mutation (5'->3') | |
|---|---|---|
| | Forward primer | Reverse primer |
| McrB$^{R347A}$ | TAATACGACTCACTATAGGG | GAAAATCGTCTCGCTAGGGCATAGTC |
| McrB$^{D343A}$ | TAATACGACTCACTATAGGG | GCGTAGGGCATACGCAACAACGGCC |

## 3.3 Results and Discussion

McrB$^{R347A}$ efficiently cleaved DNA but did not oligomerize in the presence of GTP, consistent with the previous study. But when we looked at the GTPase activity of McrB$^{R347A}$C, it was significantly affected but was able to hydrolyze nucleotide. So we assessed the oligomerization of McrB$^{R347A}$ in presence of McrC. Surprisingly McrB$^{R347A}$ formed a tetradecameric complex in presence of McrC. Hence we concluded that McrB$^{R347A}$ can bind to nucleotide to form unstable hexamers which are stabilized by McrC.McrB$^{D343A}$ was a very unstable protein and precipitated soonest. However, we could see McrB$^{D343A}$ hexamerising in the presence of nucleotide contradictory to the previous study (Data not shown). We also looked at the catalytic activity of McrB$^{D343A}$ where it fails to hydrolyze nucleotide efficiently or cleave DNA. But this can also be because the protein was unstable at 25°C and 37°C.

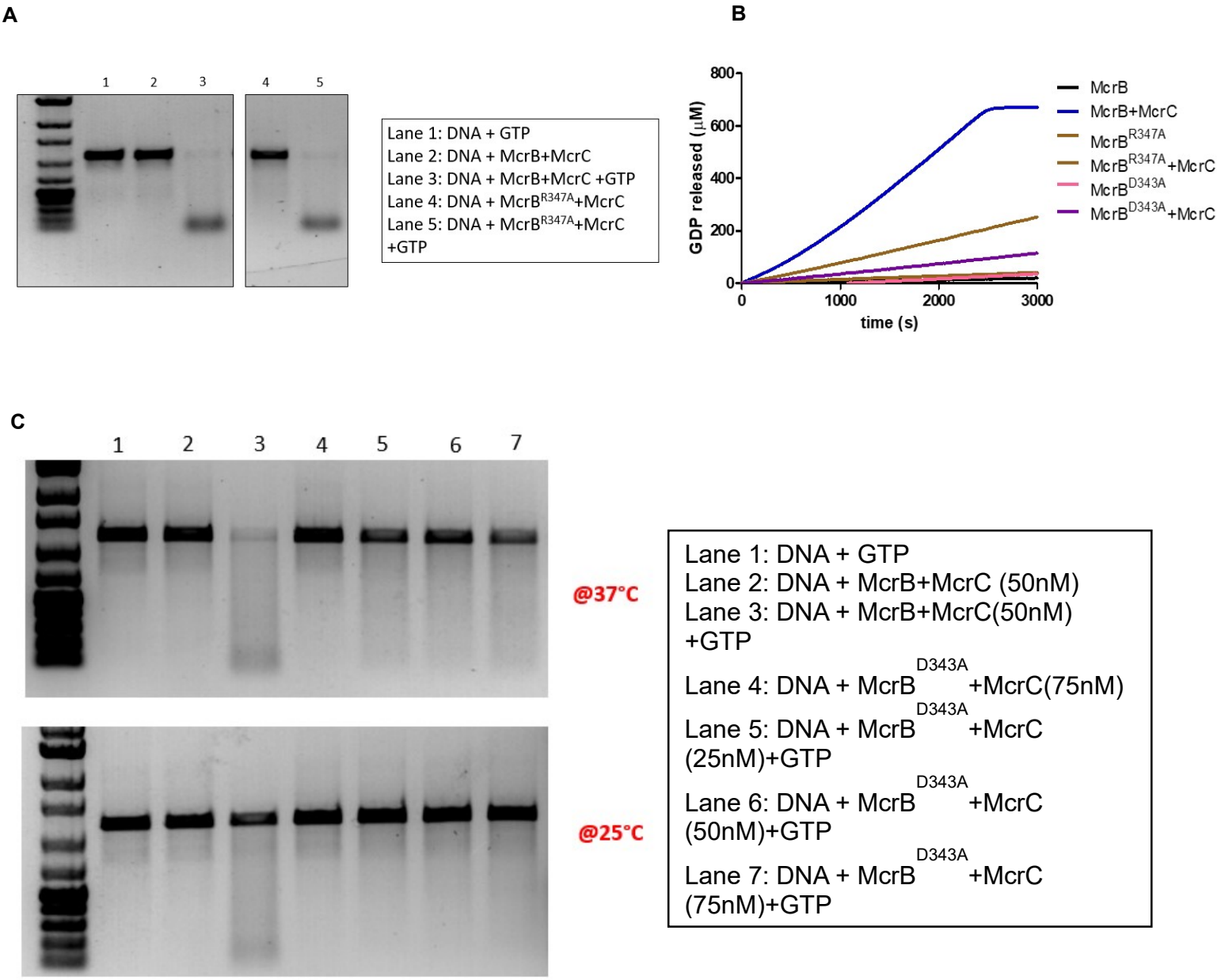



***Figure 6:** (A) Nuclease activity check of McrB and **McrB**$^{R347A}$ in complex with McrC. (B) Comparison of the GTP hydrolysis by McrB, **McrB**$^{R347A}$ and **McrB**$^{D343A}$ when stimulated by McrC (C) Nuclease activity check of McrB and **McrB**$^{D343A}$ in complex with McrC at 37°C and 25°C*

Chapter 4

# How does the McrB hexameric ring interact with the substrate DNA?

## 4.1 Introduction

McrB is classified under the clade VI of AAA+ superfamily. Members of this family oligomerize to form closed ring-like hexamers towards the center of which a loop protrudes. This, so-called pore loop is known to interact with the substrates and pull them in the central pore of the hexameric ring. Multiple sequence alignment and secondary structure prediction suggested GVGFRRKDG (249-257) as the potential pore loop for McrB. Here, RRK (253-255) is a positive stretch of amino acids that might interact with DNA. The previous study in the lab shows that when these positively charged residues of the loop R253 R254 and K255 were together mutated to alanine (RRK), the mutant did not bind to nucleotide to form higher-order oligomers, although mutating only K255 to alanine did not affect the catalytic activity of McrB. Here I will discuss the effect of mutating the other two arginines.

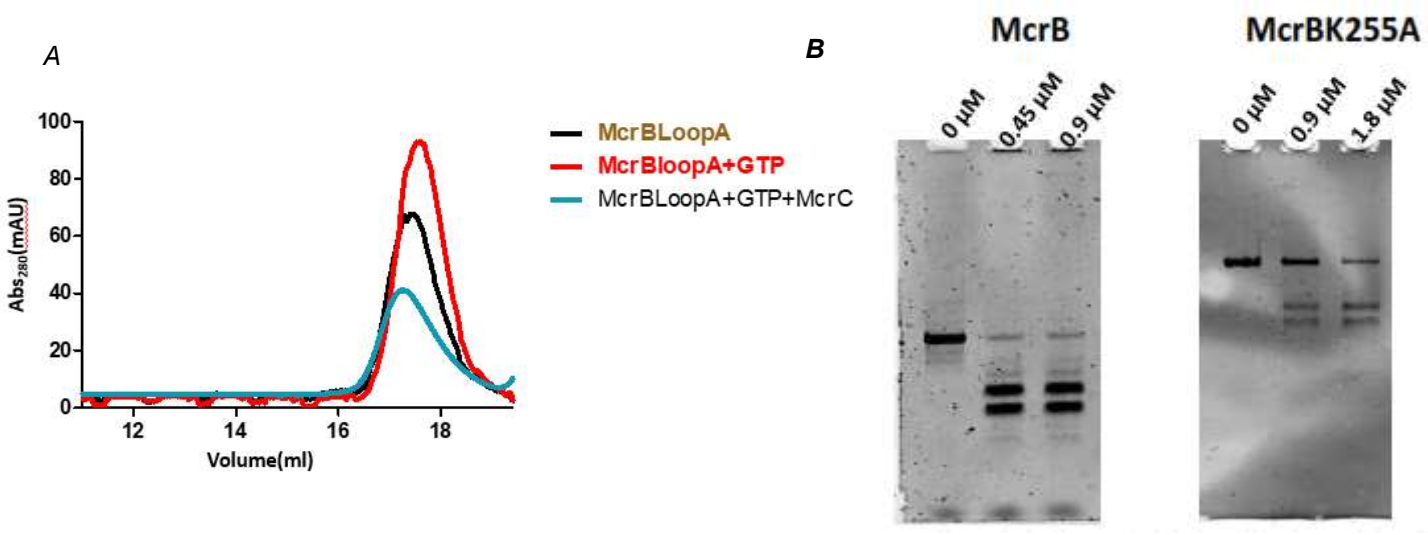



***Figure 7: Adapted from Neha Nirwan's Thesis(A) Size Exclusion column chromatographic profile of McrB and McrBRRK i.e. McrBLoopA mutant in presence and absence of McrC on Superdex200 24ml column (B) Nuclease activity check of McrB and McrB$^{K255A}$ in complex with McrC with 114bp long specific substrate***

## 4.2 Materials and methods

Same as Chapter 2. Following is the list of primers used to incorporate respective mutation.

Table3: Table of primers for mutants McrB$^{R253A}$ and McrB$^{R254A}$

| Mutations | Primers used to incorporate mutation (5'->3') | |
|---|---|---|
| | Forward primer | Reverse primer |
| McrB$^{R253A}$ | TAATACGACTCACTATAGGG | GCCGTCTTTACGCGCGAAGCCGACGCC |
| McrB$^{R254A}$ | TAATACGACTCACTATAGGG | GCCGTCTTTCGCTCGGAAGCCG |

## 4.3 **Results and Discussion**

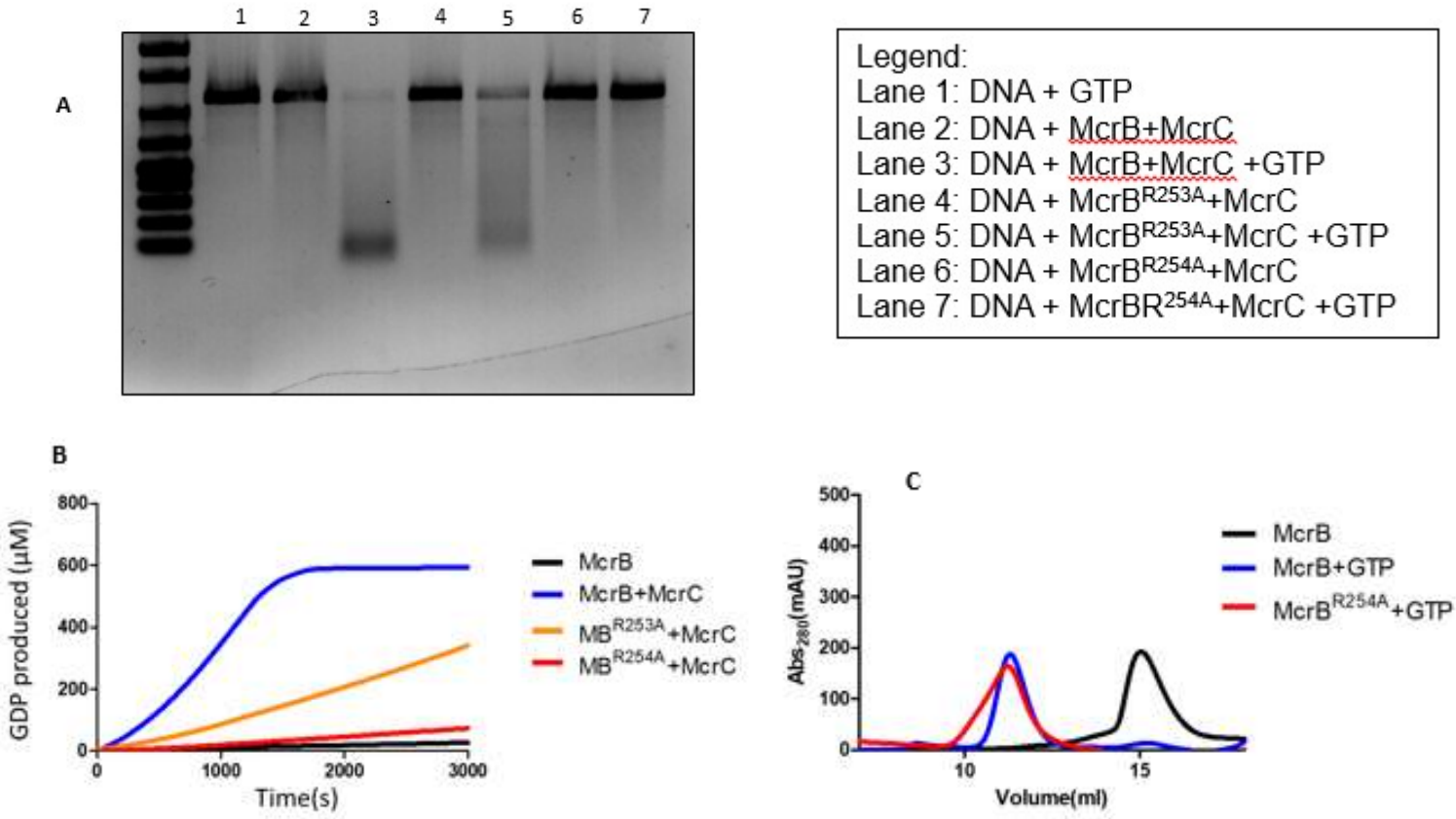



***Figure 8. (A) 0.8% Agarose Gel showing nuclease activity of proposed-pore loop mutants. (B)Comparison of GTP hydrolysis by McrB, McrB$^{R253A}$ and McrB$^{R254A}$ in presence of McrC. (C) Size exclusion column chromatogram. The oligomerization of each McrB and McrB$^{R254A}$ mutant in presence of GTP.***

Mutating McrBR253 and McrBR254 to alanine showed differential effects. McrB$^{R253A}$ was capable of cleaving the specific substrate although less efficiently. Also, the GTPase activity of McrB$^{R253A}$ was significantly affected. Mutating McrBR254 to alanine abolished the nuclease activity of the McrB$^{R254A}$C complex. A similar effect was observed on the rate of nucleotide hydrolysis for the mutant. Although not hydrolyzing, the size exclusion chromatography of McrB$^{R254A}$ shows that it binds to GTP and oligomerizes to form stable hexamer.

Chapter 5

# Status of other Clones

Table4: Status of uncharacterized clones

| Residues mutated | Name of the Primer Used | Sequence of Primer Used(5'->3') | Status of cloning | Sequencing status | Characterisation |
|---|---|---|---|---|---|
| McrB_Zrar_Nterm | Zrar_Nterm_F | GAGTGTTATTCCACCTATGGGCAGCCATATGATTGGCAGCAGCCCTGAAACCACAATAGAGACGATAC | + | Fully sequenced | No expression |
| McrB$^{F178P}$ | MBF178P_F | GCGTTAAATGATTTGCCGATCCCTGAAACCAC | + | Fully sequenced | |
| McrB$^{F178G}$ | MBF178G_F | GCGTTAAATGATTTGGGCATCCCTGAAACCAC | + | Fully sequenced | |
| McrB$^{I179G}$ | MBI179G_F | GCGTTAAATGATTTGTTTGGCCCTGAAACCACAA | + | Fully sequenced | |
| McrB$^{R212AR213A}$ | MBR212AR213A_F | ACCTTTGTTGCAGCGGCGCTGGCTTACTTGC | + | Mutation sequenced | - |
| McrB$^{K222A}$ | MBK222A_F | GACAGGAGAAGCGGCTCCGCAACGC | + | - | - |
| McrB$^{R246A}$ | MBR246A_F | ACAGGGCTATGCGCCGAATGGCGTCGGC | + | Mutation sequenced | - |
| McrB$^{K288A}$ | MBK288A_F | GCCAATCTCAGTGCGGTATTTGGCG | + | - | - |
| McrB$^{E239A}$ | MBE239A F | CTTATAGCTATGCGGATTTTATACAGGGC | + | Fully sequenced | + |
| McrB$^{R253AK255A}$ | MBR253AK255A_R | AAATATGCCGTCCGCACGCGCGAAGCCGAC | + | Fully sequenced | - |
| McrB$^{H299A}$ | MBH299A_R | CCTCGTTTATCCGCTTCCATTAACATCATCACTTCGCC | + | Fully sequenced | - |
| McrB$^{D300A}$ | MBD300A_R | CACCTCGTTTCGCATGTTCCATTAACATC | + | Fully sequenced | - |
| McrB$^{K301A}$ | McrBK301A_R | GTTTTCACCTCGCGCATCATGTTCCAT | + | Fully sequenced | - |
| McrB$^{R337A}$ | McrBR337A_R | CCAGAGACGCATCGGCAGTATTCATTAAACCG | + | Fully sequenced | + |
| McrB$^{Y344A}$ | MBY344A_R | GCGTAGGGCCGCGTCAACAACGGCC | + | Fully sequenced | + |
| McrB$^{H407A}$ | MBH407A_R | CACAGCAGAAGTAACTCGCCCCAATGCGG | + | Fully sequenced | - |
| McrB$^{S408A}$ | MBS408A_R | CACAGCAGAAGTACGCATGCCCAATGCGG | + | Fully sequenced | - |
| McrB$^{E439A}$ | McrBE439A_R | CAAAGAAATACGCTTCGAGTAAAGGGGC | + | Fully sequenced | - |
| | | | | | |
| McrC 20dN | MC20dN_F | AAGAAGGAGATATACATATGTATTTACAGGAAATTAAGCA | + | Fully sequenced | + |
| McrC$^{D257A}$ | MC D257A R | GTATTTGGCCGCAACGATAAGTATTTTTTCTGATGAGCG | + | Fully sequenced | + |

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