Rebuttal Letter

I would like to thank my TAC examiner for his valuable comments. The following are my responses to the comments, in the same order in which they have been given.

1. All figures mentioned in the text are now referred to by the figure number.

2. Unqualified terms and phrases have been removed/amended, as far as possible.

3. Magnified plots of the step wise relaxation, from the representative curves Fig. 3.1 and Fig. 3.4 have been added.

4. The points raised by the TAC examiner are valid possibilities. In fact, these are of concern to us with regard to interpreting the causes for steps, particularly in cell membranes since there appears to be at least two kinds of steps - one with a characteristic 'saw-tooth' pattern and the other preceded by a plateau. We have therefore been careful while interpreting the origins of these steps. Relaxation in cell membranes can be a complicated phenomenon and possibilities range from a membrane-only response to unfolding of tethered membrane proteins. In fact, the 'saw-tooth' patterns seen in few of the steps in Fig. 3.1 have previously been observed in AFM-induced protein unfolding experiments (Aggarwal et al. (2011) J. Biol.Chem. 286:28056-28065). These possibilities have now been included in the thesis (see pages 17, 31).

5. The occurence of steps in the force plots can be represented in two ways: (1) as step probability (fraction of force curves with at least one step wise relaxation), which shows an increasing trend with pulling rate, and (2) no. of steps per force curve. The variation in step probability with pulling rate for cell membranes and supported bilayers has been presented in this thesis and is quite consistent between experiments (see Fig. 3.6f in the revised thesis). The plot of the no. of steps per force curve vs pulling rate very often shows a flat trend with increasing pulling rates. Since this form of data representations does not fully consider the entire data set on force curves, we have not reported it in the thesis.

6. Figs. 3.2, 4.1, 4.2 and 4.3 in the earlier version of the thesis were from referred papers and have now been removed from the thesis.

7. In Fig. 3.13, the left most figure visually does show an onset (inflection) closer to 1 μm/s than to 10 μm/s. However, this is not the most rigorous method to estimate an onset. One way to reliably estimate the onset is to fit straight lines to the two parts of the trend with different slopes and assign the point of intersection of these straight lines as the onset. This method of analysis, when applied to data in the left most panel of Fig. 3.13, does show an onset of 1.1 μm/s. We have now mentioned the onsets for each data set analyzed in the manner described above.

8. We have found it difficult to estimate relaxation times accurtely since the relaxation data cannot be fitted robustly to any obvious equations (exponential or (t/(1+t)). For this reason, we prefer to show the raw data for relaxation times in cell membranes and supported bilayers. This point has now been discussed in the revised thesis (see pages 26, 27, 31).