Supporting Data:

The interconnection of salt induced hydrophobic compaction and secondary structure formation depends on solution conditions: revisiting early events of protein folding at single molecule resolution.

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Figure S1: The effect of sodium perchlorate on the secondary structure of Cytc-TMR at pH 2 monitored by far-UV CD spectroscopy. Far-UV CD spectra of Cytc-TMR at pH 7.5 and pH 2 are shown by black square and red circle respectively. Far-UV CD spectrum of Cytc-TMR in the presence of 126 mM sodium perchlorate at pH 2 is shown by blue triangles. A large decrease in the ellipticity has been observed at pH 2 suggesting acid induced unfolding of the protein. Addition of sodium perchlorate leads to an increase in the ellipticity suggesting partial refolding of the protein.

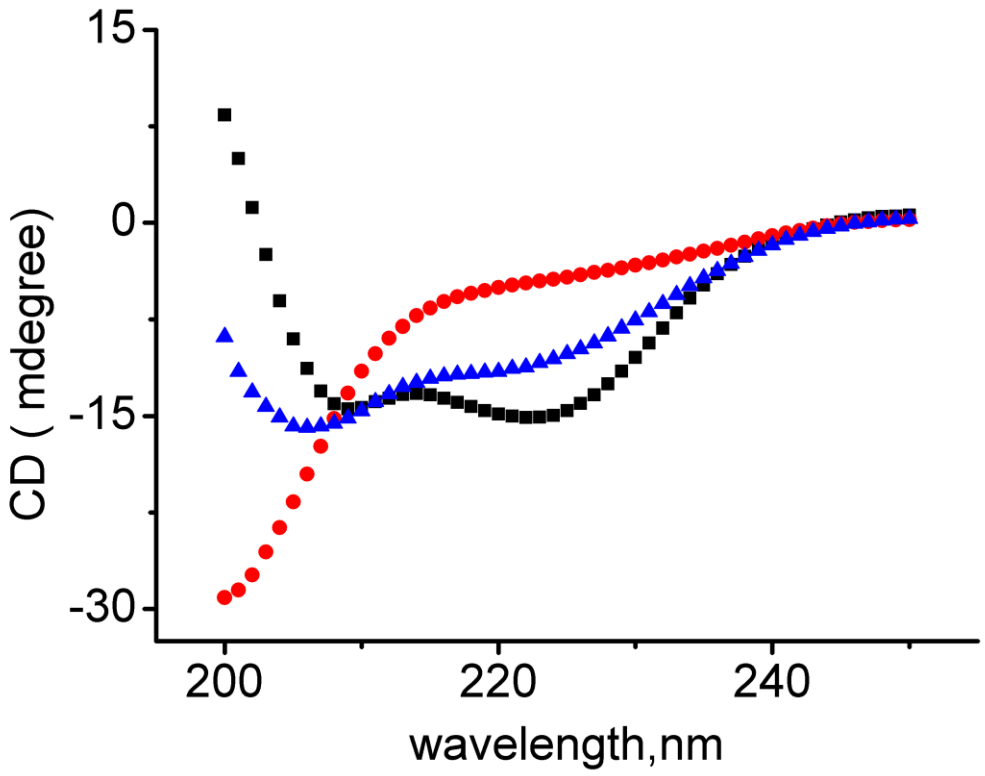

Figure S2: The autocorrelation function obtained with free TMR and its fit (red lines drawn through the data) to (a) Equation 1 and (b) Equation 2. The residual distributions of the fits are shown at the bottom of the figures. The use of equation 1 has been found appropriate to fit the autocorrelation function data obtained with the free dye. Addition of an extra exponential component (Equation 2) does not improve the fit. These experiments have been carried out at pH 2.

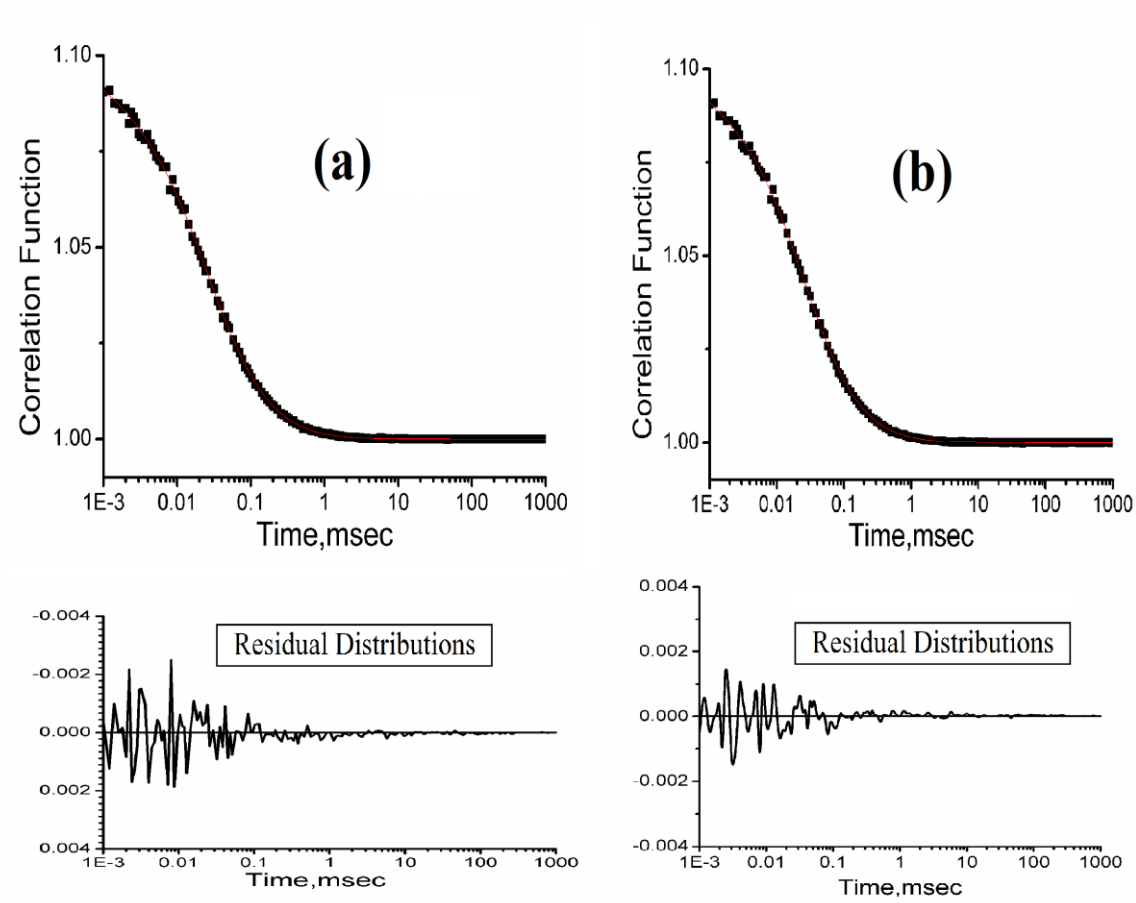

Figure S3: The variation of τ_R obtained from the FCS experiments with Cytc-TMR at pH 2 with pinhole diameters and laser powers**.** The values of τ_R do not show any dependence either with pinhole diameters (in the absence (a) and presence of $NaClO_4$ (b)) and laser power (in the absence (c) and presence of $NaClO_4$ (d)).

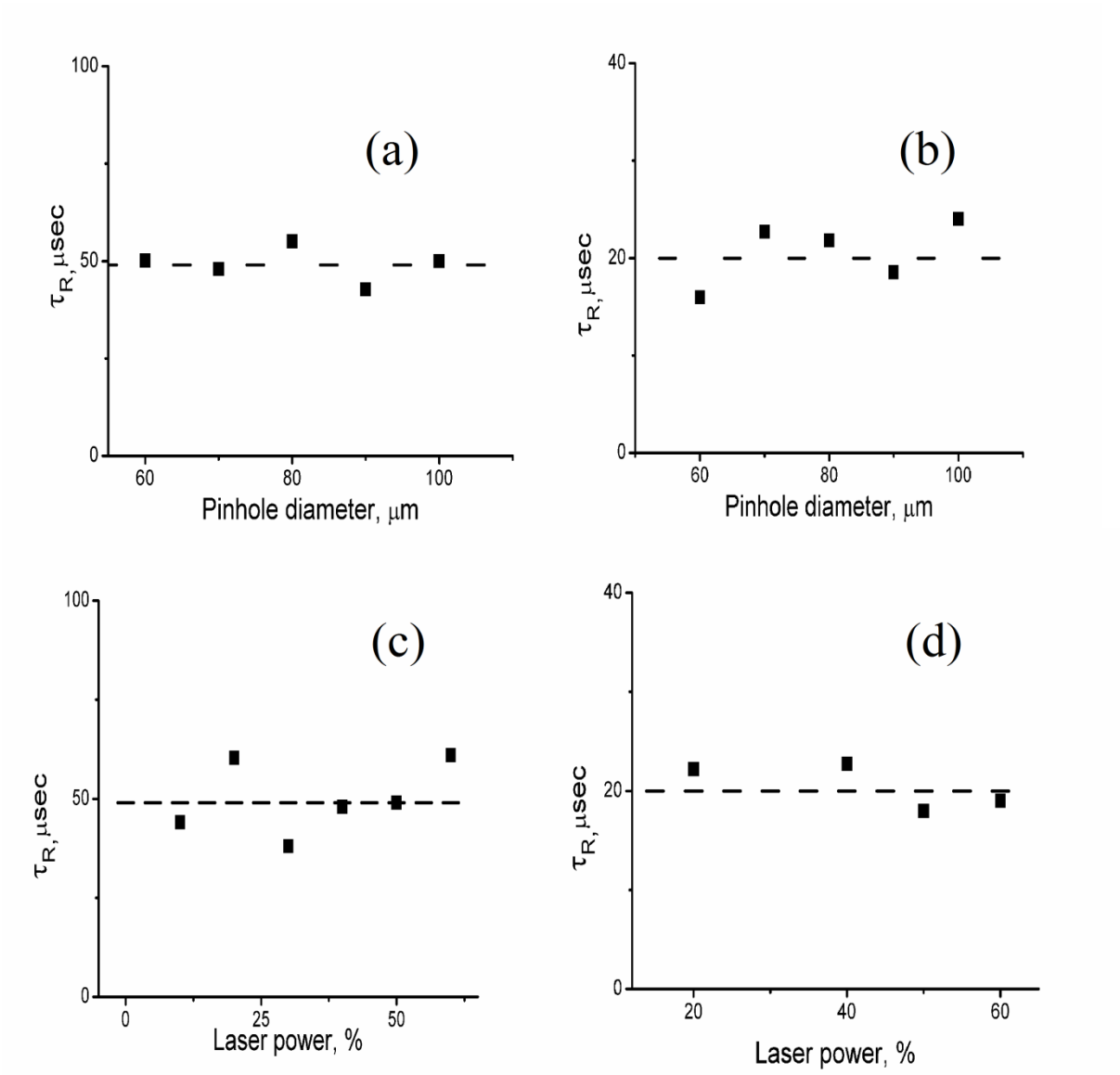

Figure S4: The variation of F obtained from the FCS experiments with Cytc-TMR at pH 2 with pinhole diameters and laser powers**.** The values of F do not show any dependence either with pinhole diameters (in the absence (a) and presence of $NaClO_4$ (b)) and laser power (in the absence (c) and presence of $NaClO_4$ (d)).

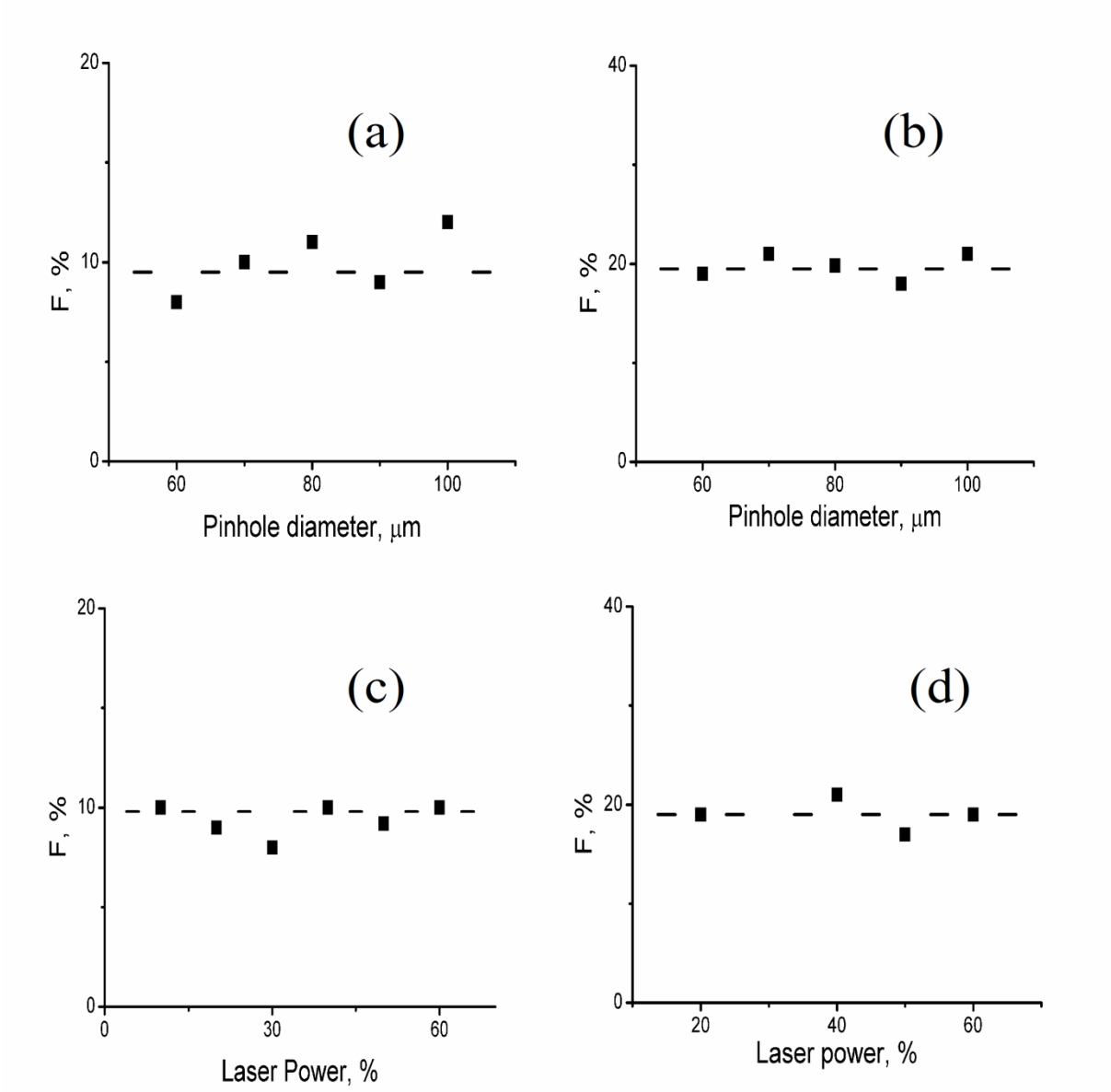

Figure S5: Mass spectrum of the cyanogen bromide treated fragment of cytochrome c. A single species of the mass 3139 D has been observed by the mass spectral analysis.

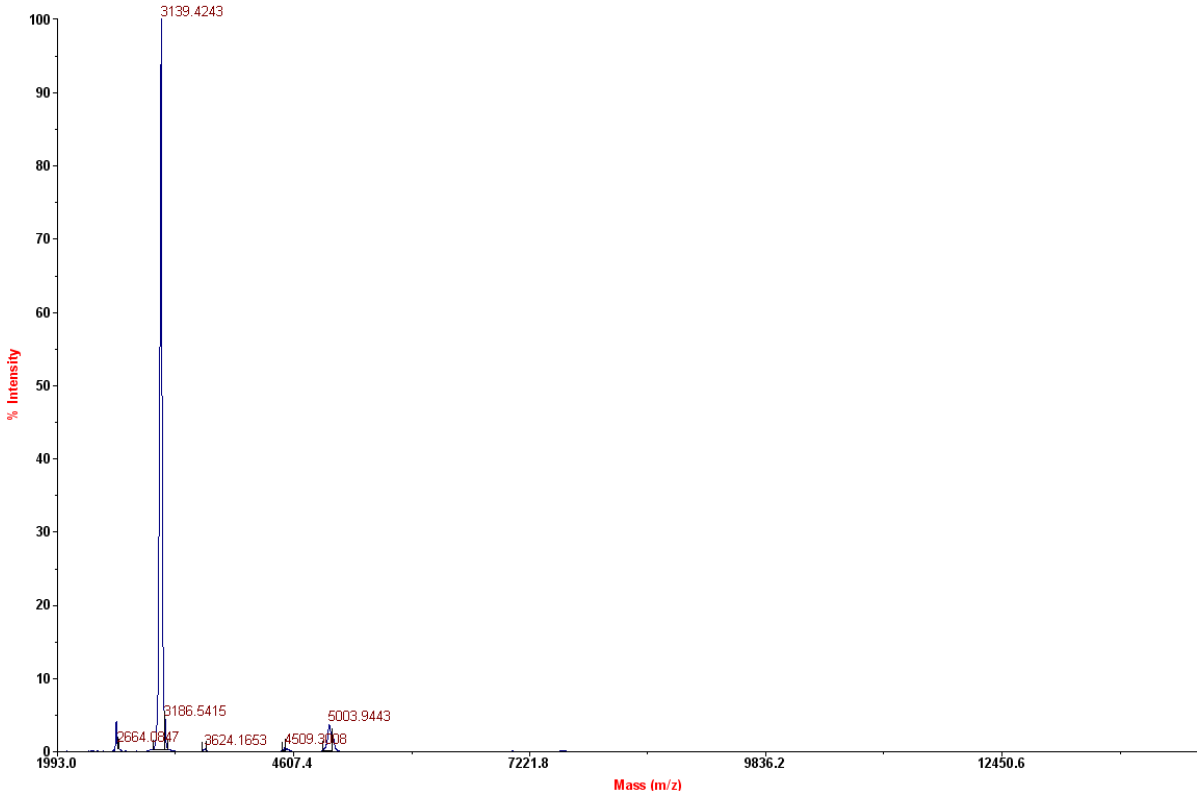

Figure S6: UV-visible absorption spectra of the full length (black) and cyanogen bromide treated Cytc-TMR (red) in 20mM phosphate buffer at pH 7.5. The absorbance band at 550 nm is found to be present for both the samples which suggest that both the full length protein and cyanogen bromide treated fragment contain the external label, TMR. The cyanogen bromide treated fragment, however, lacks the Soret absorbance at 409 nm indicating the absence of heme in the cyanogen bromide treated fragment.

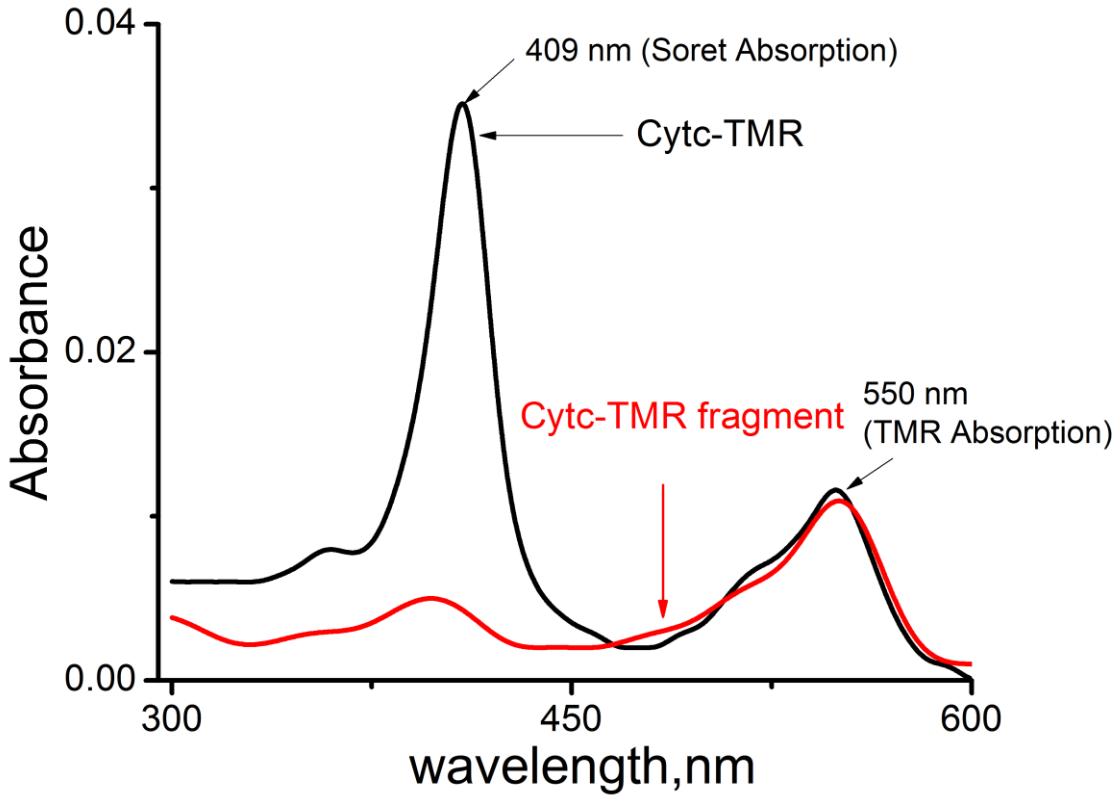

Figure S7: Fluorescence emission spectra of the full length Cytc-TMR (red) and cyanogen bromide treated Cytc-TMR fragment (black) in 20mM phosphate buffer at pH 7.5. The samples are excited at 540 nm for the TMR excitation. Both the full length protein and the cyanogen bromide treated fragment contain the external label, TMR although the fragment lacks the heme group. The presence of heme group in the full length Cytc-TMR results in significant quenching of the TMR fluorescence (red). Since the fragmented Cytc-TMR does not contain the heme group, its quenching effect on the TMR fluorescence is also absent which yields a large increase in the fluorescence intensity (black).

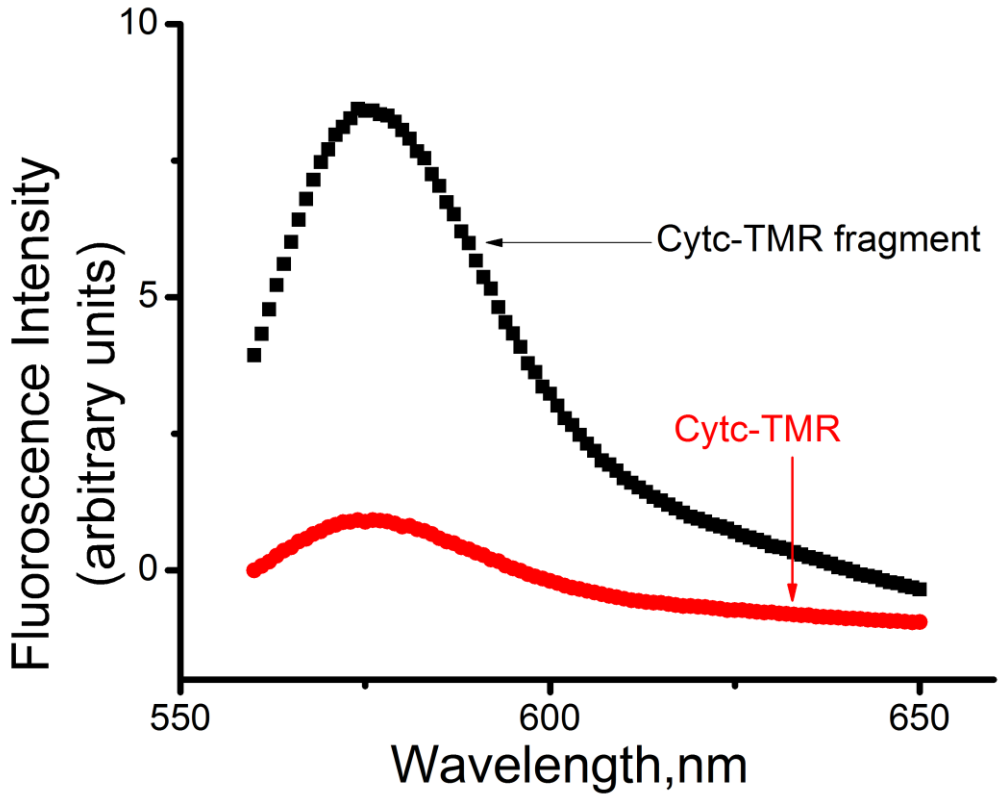

Figure S8: A typical correlation function obtained with cyanogen bromide treated Cytc-TMR fragment at pH 2 and its fit to Equation 1. The correlation function could be fit successfully to Equation 1 as judged by the residual distribution analyses (shown at the bottom). Addition of an extra component (τ_R) does not improve the fit (not shown).

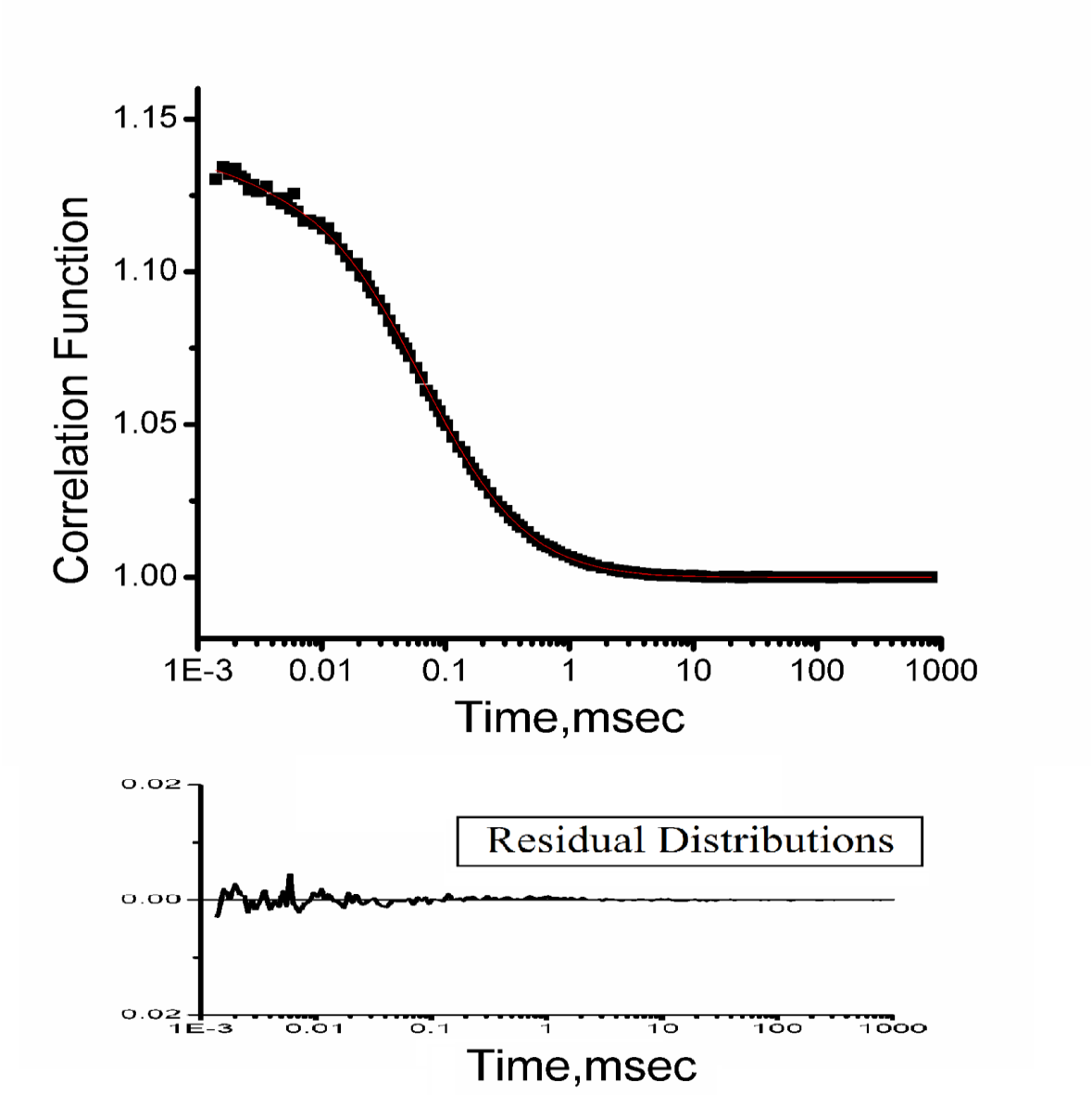

Figure S9: Far UV-CD spectra of cytochrome c in presence of (a) 0M urea (b) 1M urea (c) 3M urea (d) 4M urea, in the presence (red) and absence of 160 mM sodium perchlorate (black). Far-UV CD data below 210 nm are unreliable as a result of the high sample absorbance. All these experiments have been carried out in 20mM sodium phosphate buffer at pH 2.

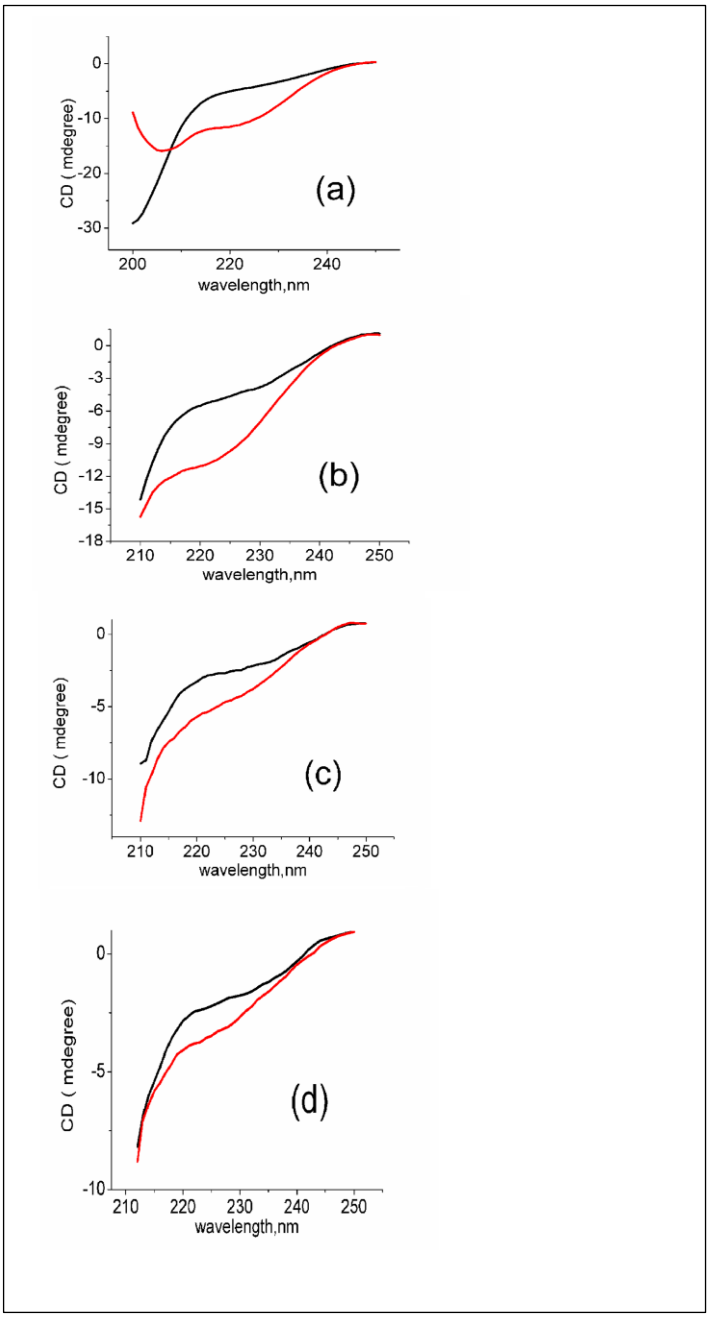

Figure S10: The variation of r_H of BSA-Alexa488 unfolded at pH 2 with sodium perchlorate concentration in the absence (black, the data at the left y axis) and presence of 4M urea (red, the data at right y axis). The data follow the trend identical to that observed with Cytc-TMR.