

## **Supplementary Information**

### **Mixed Macromolecular Crowding: A Protein and Solvent Perspective**

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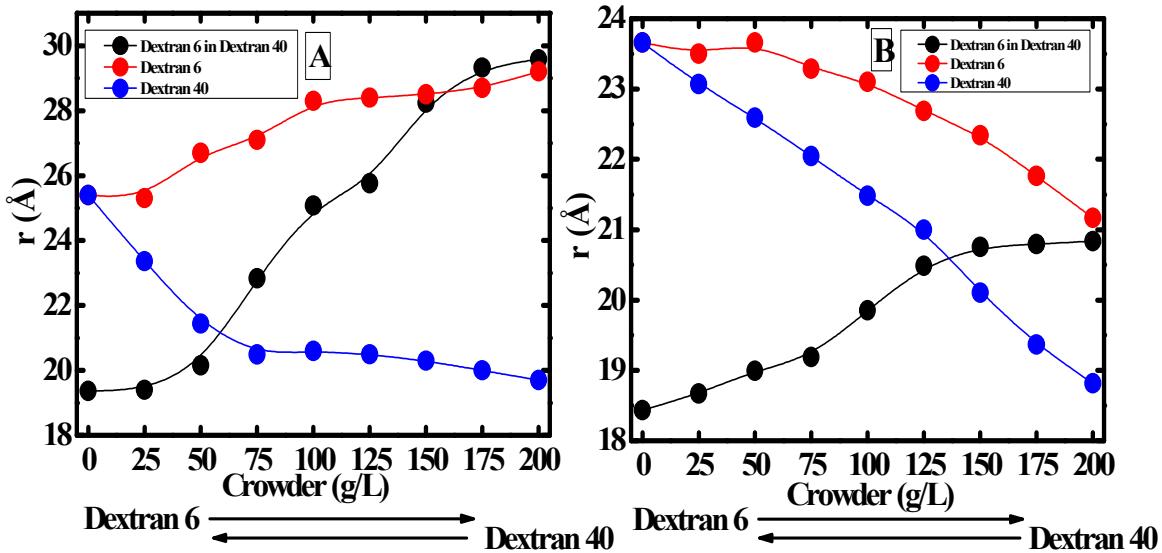
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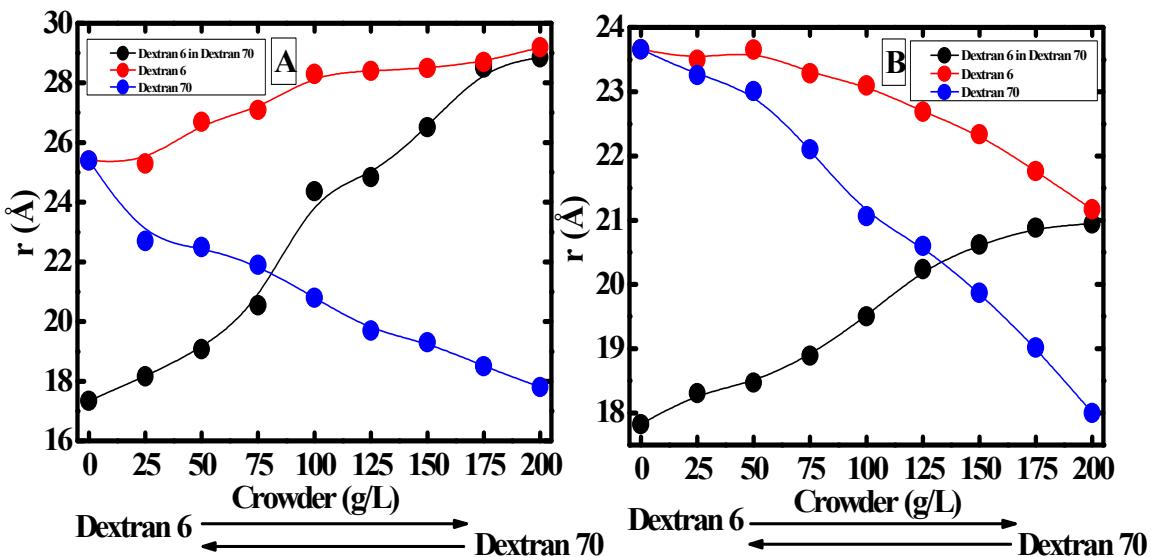
<sup>#</sup>These authors are equal contributors to the manuscript.

## Supplementary Figures

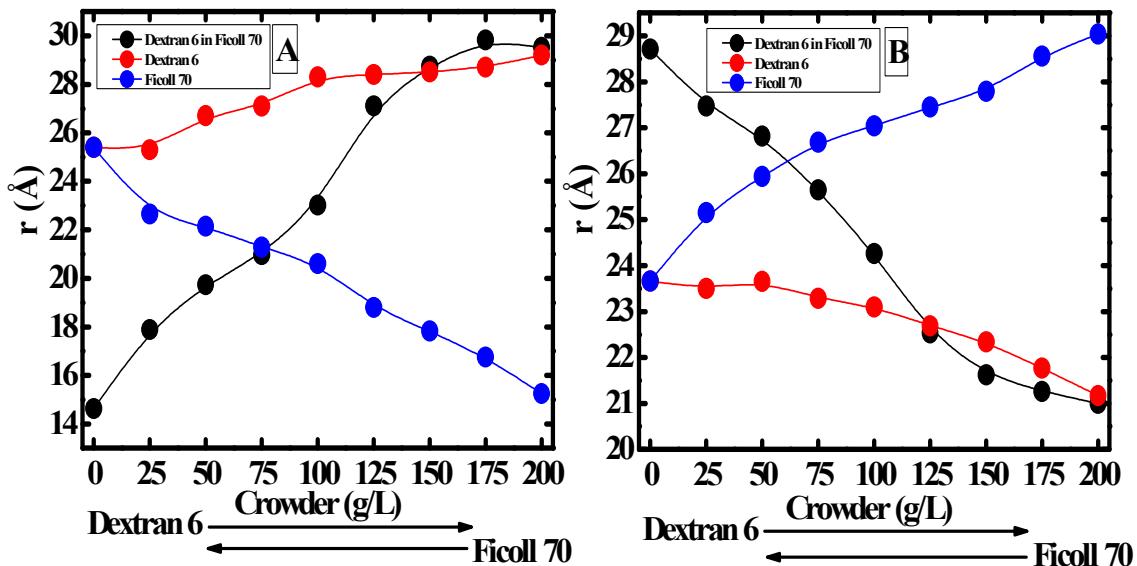
**Figure S1:** Variation of interdomain distances for domain I-II (A) and II-III (B) with increasing Dextran 6 concentration in Dextran 40 (g/L), Dextran 6 and Dextran 40 only.



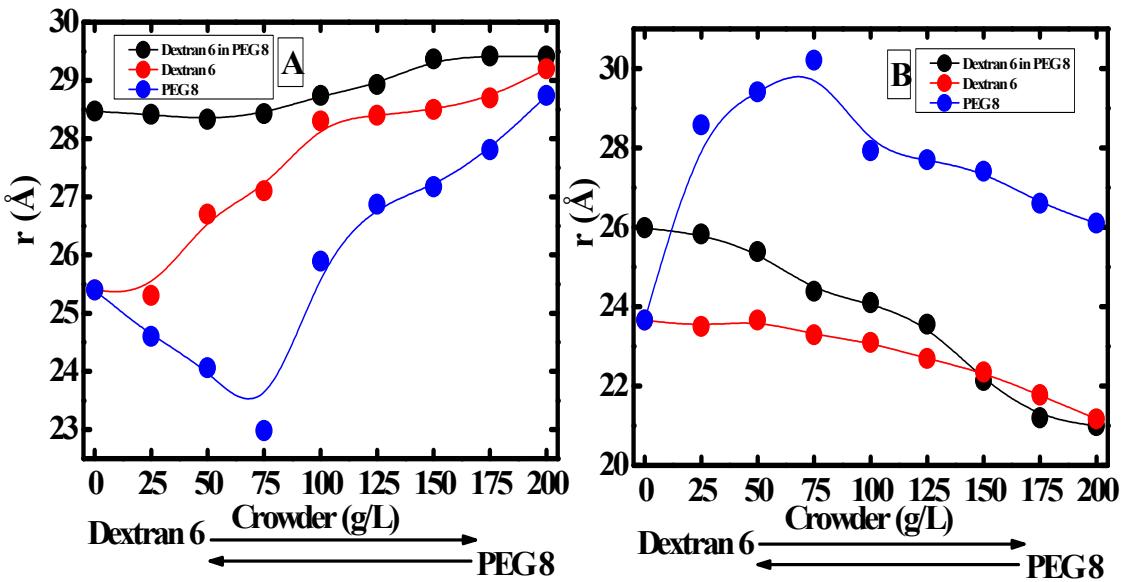
**Figure S2:** Variation of interdomain distances for domain I-II (A) and II-III (B) with increasing Dextran 6 concentration in Dextran 70 (g/L), Dextran 6 and Dextran 70 only



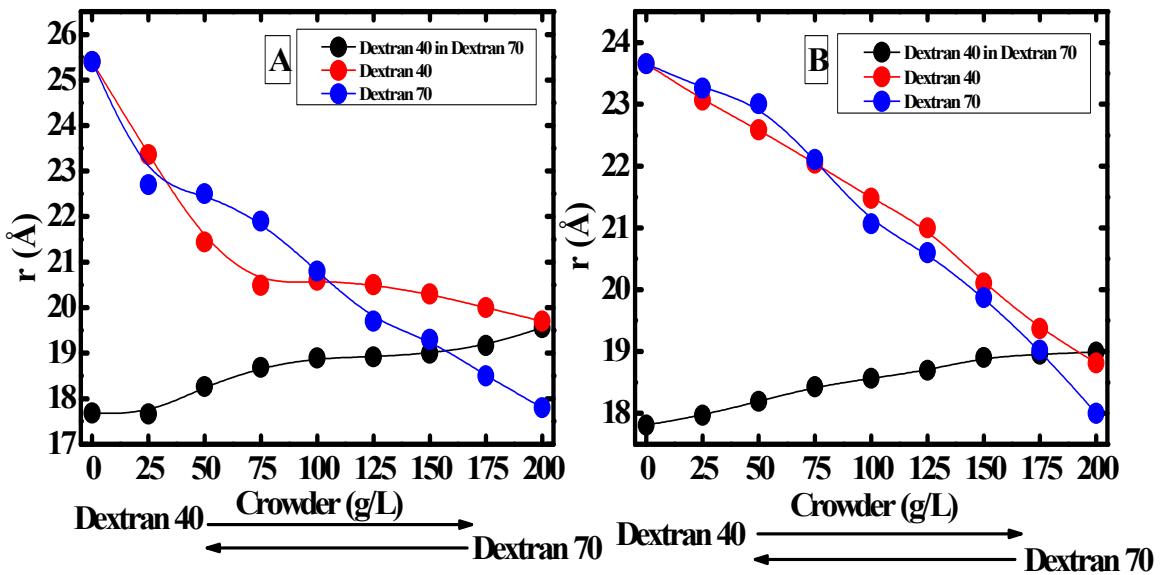
**Figure S3:** Variation of interdomain distances for domain I-II (A) and II-III (B) with increasing Dextran 6 concentration in Ficoll 70 (g/L), Dextran 6 and Ficoll 70 only



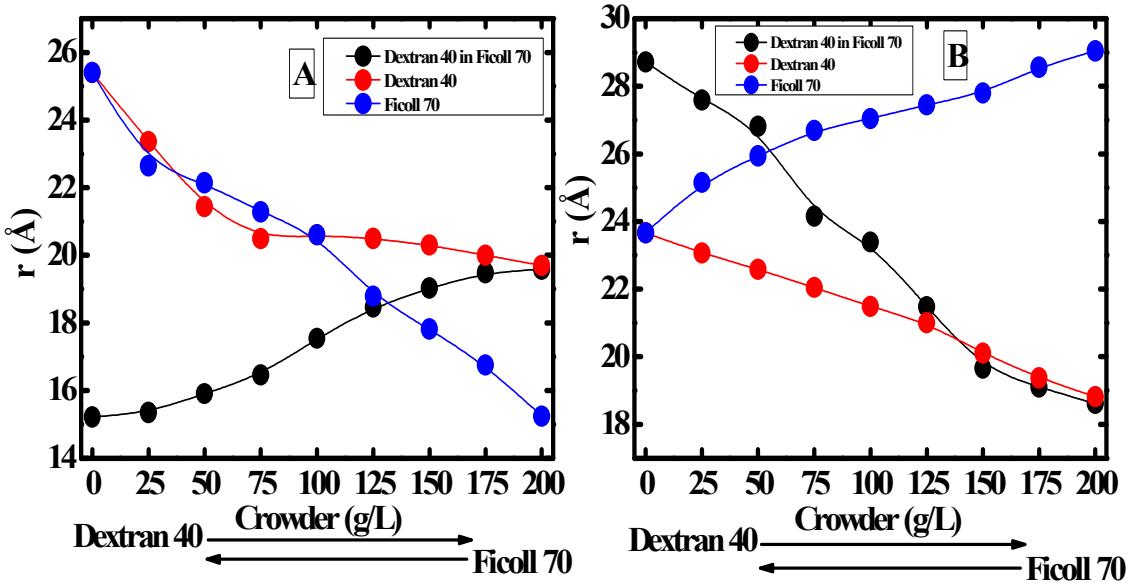
**Figure S4:** Variation of interdomain distances for domain I-II (A) and II-III (B) with increasing Dextran 6 concentration in PEG 8 (g/L), Dextran 6 and PEG 8 only



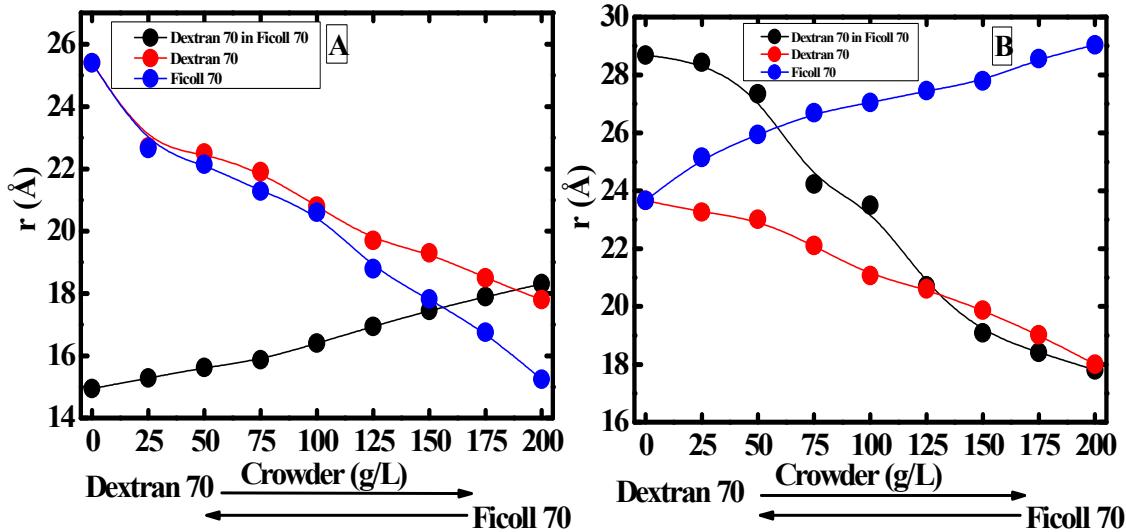
**Figure S5:** Variation of interdomain distances for domain I-II (A) and II-III (B) with increasing Dextran 40 concentration in Dextran 70 (g/L), Dextran 40 and Dextran 70 only



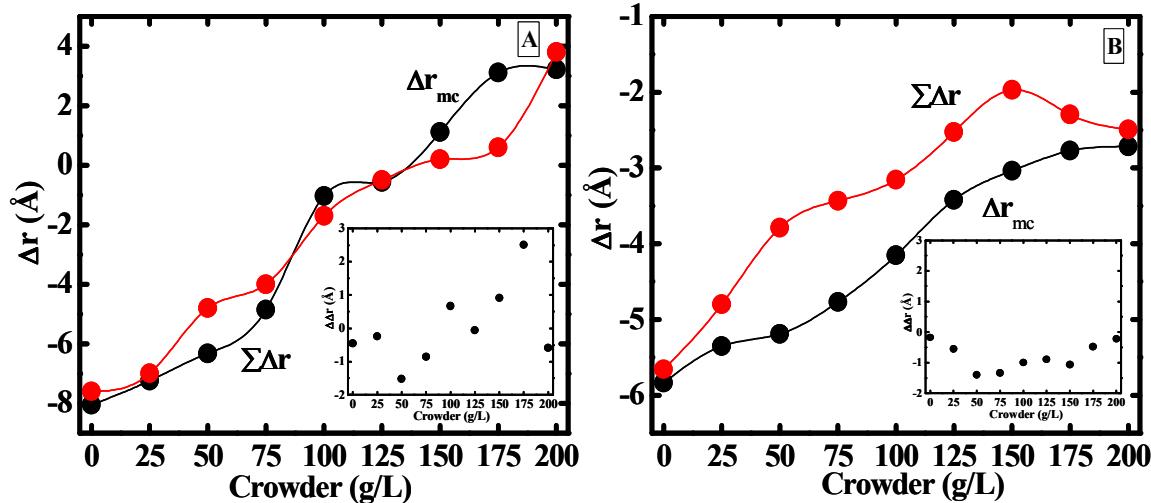
**Figure S6:** Variation of interdomain distances for domain I-II (A) and II-III (B) with increasing Dextran 40 concentration in Ficoll 70 (g/L), Dextran 40 and Ficoll 70 only



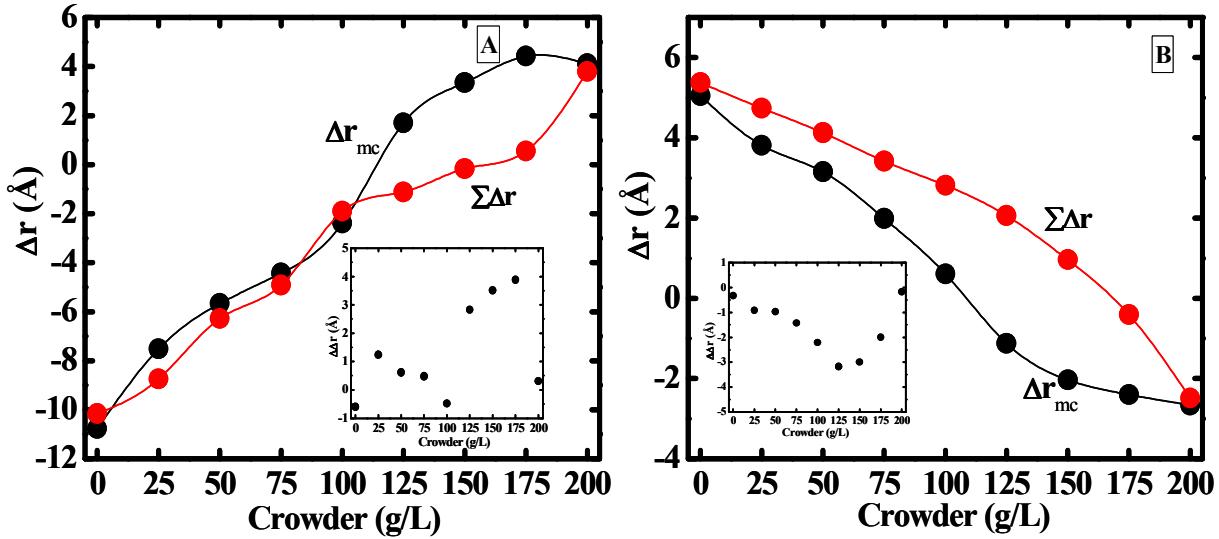
**Figure S7:** Variation of interdomain distances for domain I-II (A) and II-III (B) with increasing Dextran 70 concentration in Ficoll 70 (g/L), Dextran 70 and Ficoll 70 only



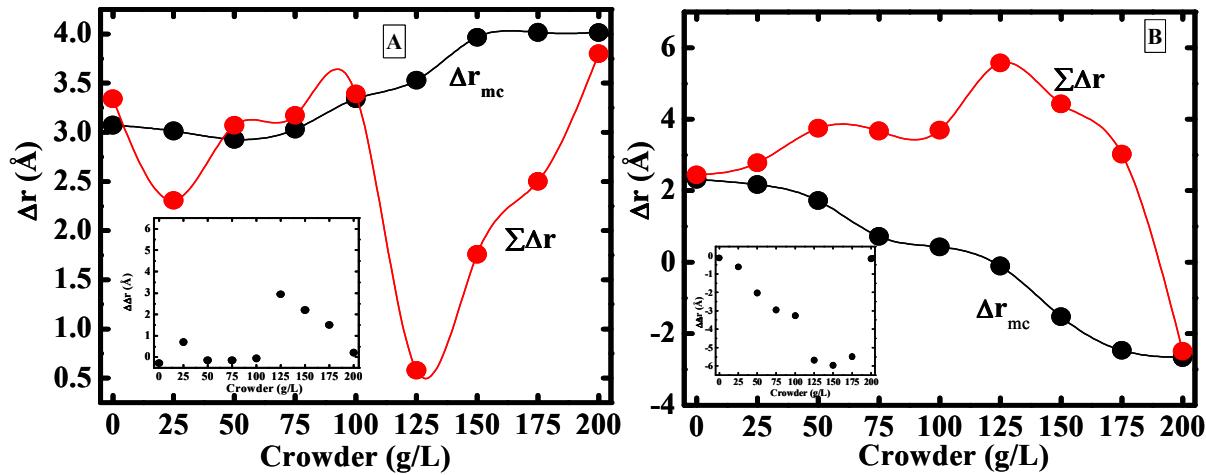
**Figure S8:** The variation of  $\Delta r$  as a function of crowder concentration in mixed macromolecular crowder ( $\Delta r_{mc} = r_{mc} - r_0$ ) (A) and sum of individual crowders ( $\Sigma \Delta r = \Delta r_{C_1(X)} + \Delta r_{C_2(200-X)}$ ) (B), where  $C_1$  and  $C_2$  are Dextran 6 and Dextran 70 respectively. [Inset shows the variation of  $\Delta \Delta r$  ( $\Delta \Delta r = \Delta r_{mc} - \Sigma \Delta r$ )]



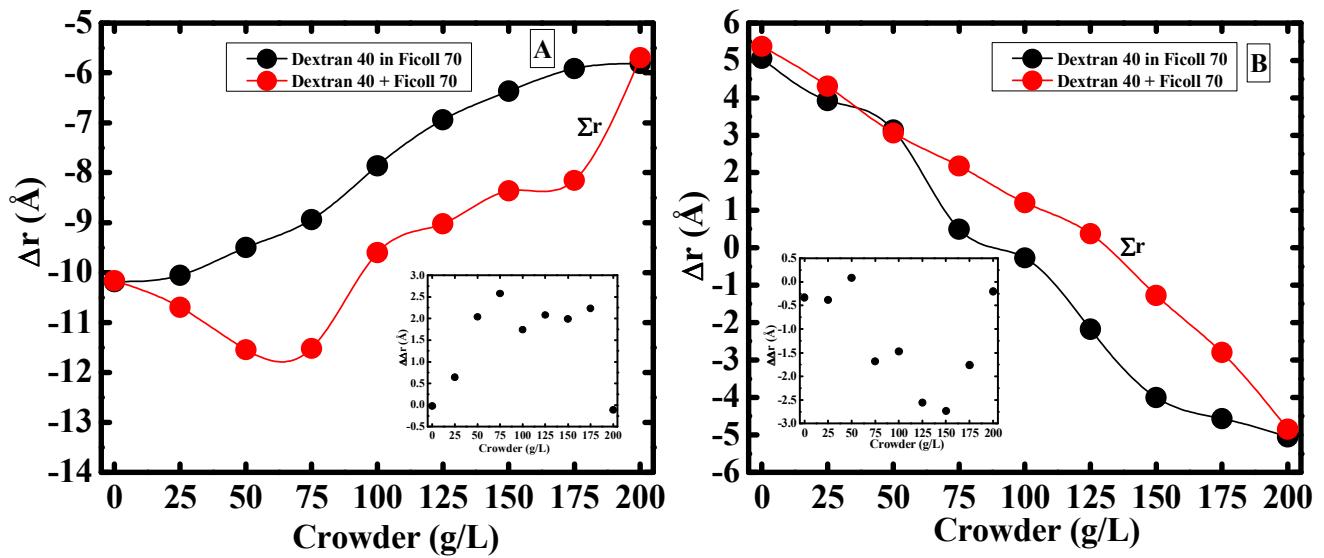
**Figure S9:** The variation of  $\Delta r$  as a function of crowder concentration in mixed macromolecular crowder ( $\Delta r_{mc} = r_{mc} - r_0$ ) (A) and sum of individual crowders ( $\Sigma \Delta r = \Delta r_{C_1(X)} + \Delta r_{C_2(200-X)}$ ) (B), where  $C_1$  and  $C_2$  are Dextran 6 and Ficoll 70 respectively. [Inset shows the variation of  $\Delta\Delta r$  ( $\Delta\Delta r = \Delta r_{mc} - \Sigma \Delta r$ )]



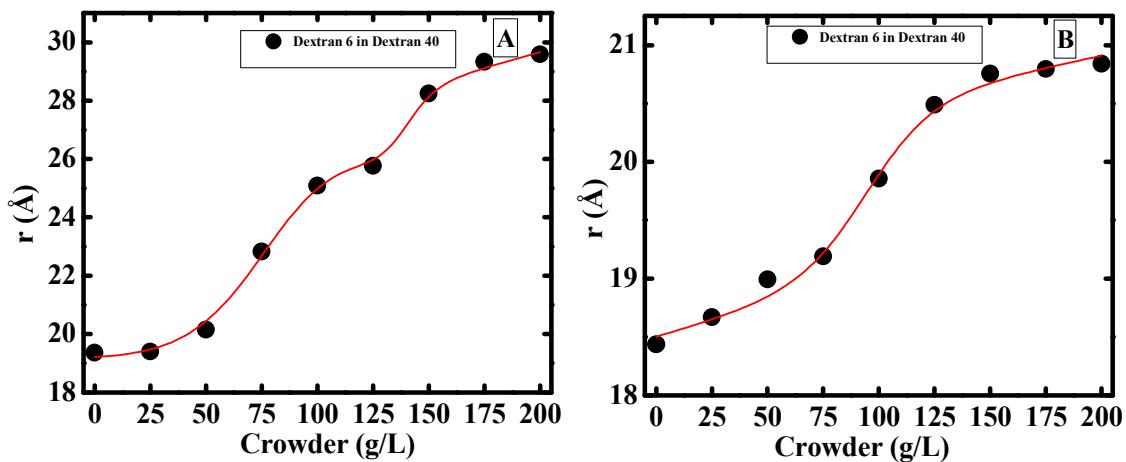
**Figure S10:** The variation of  $\Delta r$  as a function of crowder concentration in mixed macromolecular crowder ( $\Delta r_{mc} = r_{mc} - r_0$ ) (A) and sum of individual crowders ( $\Sigma \Delta r = \Delta r_{C_1(X)} + \Delta r_{C_2(200-X)}$ ) (B), where  $C_1$  and  $C_2$  are Dextran 6 and PEG8 respectively. [Inset shows the variation of  $\Delta\Delta r$  ( $\Delta\Delta r = \Delta r_{mc} - \Sigma \Delta r$ )]



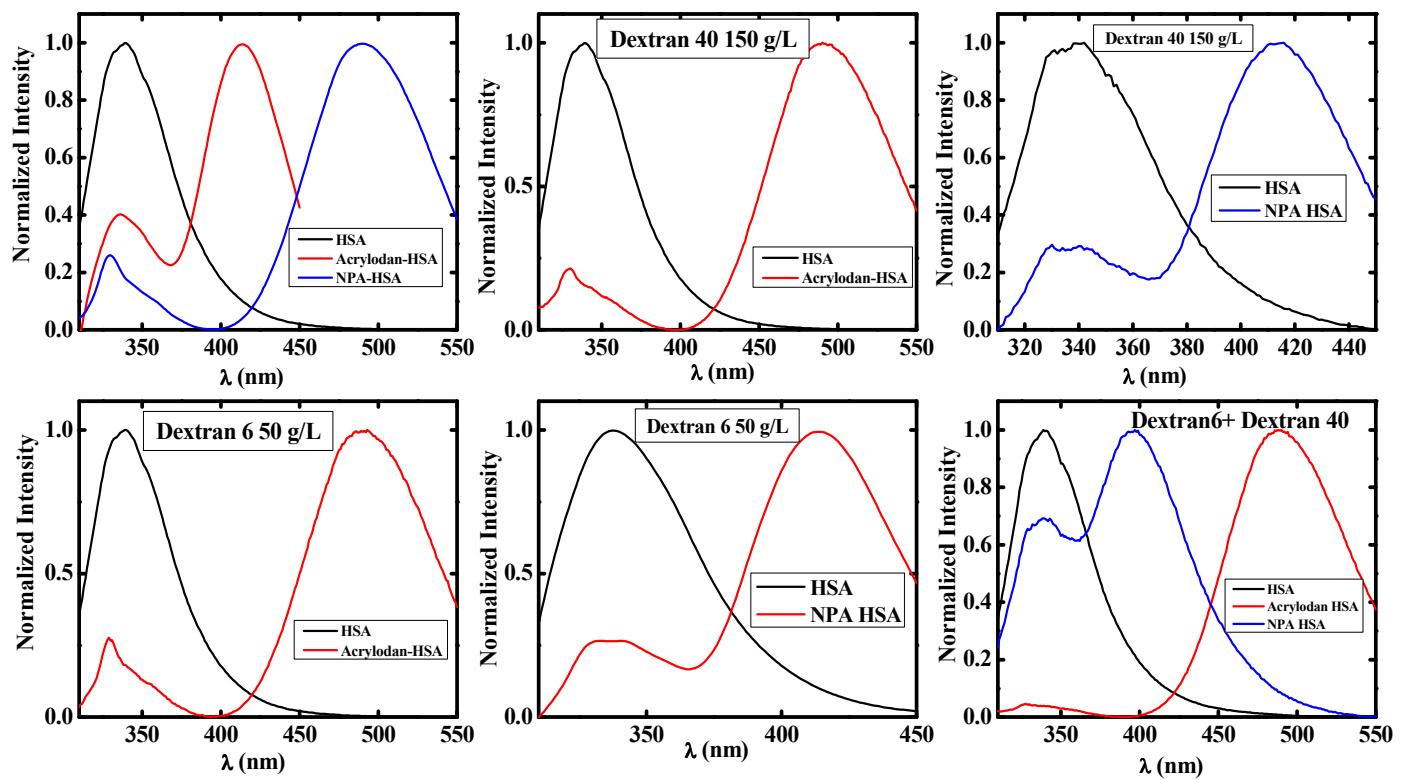
**Figure S11:** The variation of  $\Delta r$  as a function of crowder concentration in mixed macromolecular crowder ( $\Delta r_{mc} = r_{mc} - r_0$ ) (A) and sum of individual crowders ( $\Sigma \Delta r = \Delta r_{C_1(X)} + \Delta r_{C_2(200-X)}$ ) (B), where  $C_1$  and  $C_2$  are Dextran 40 and Ficoll 70 respectively. [Inset shows the variation of  $\Delta \Delta r$  ( $\Delta \Delta r = \Delta r_{mc} - \Sigma \Delta r$ )]



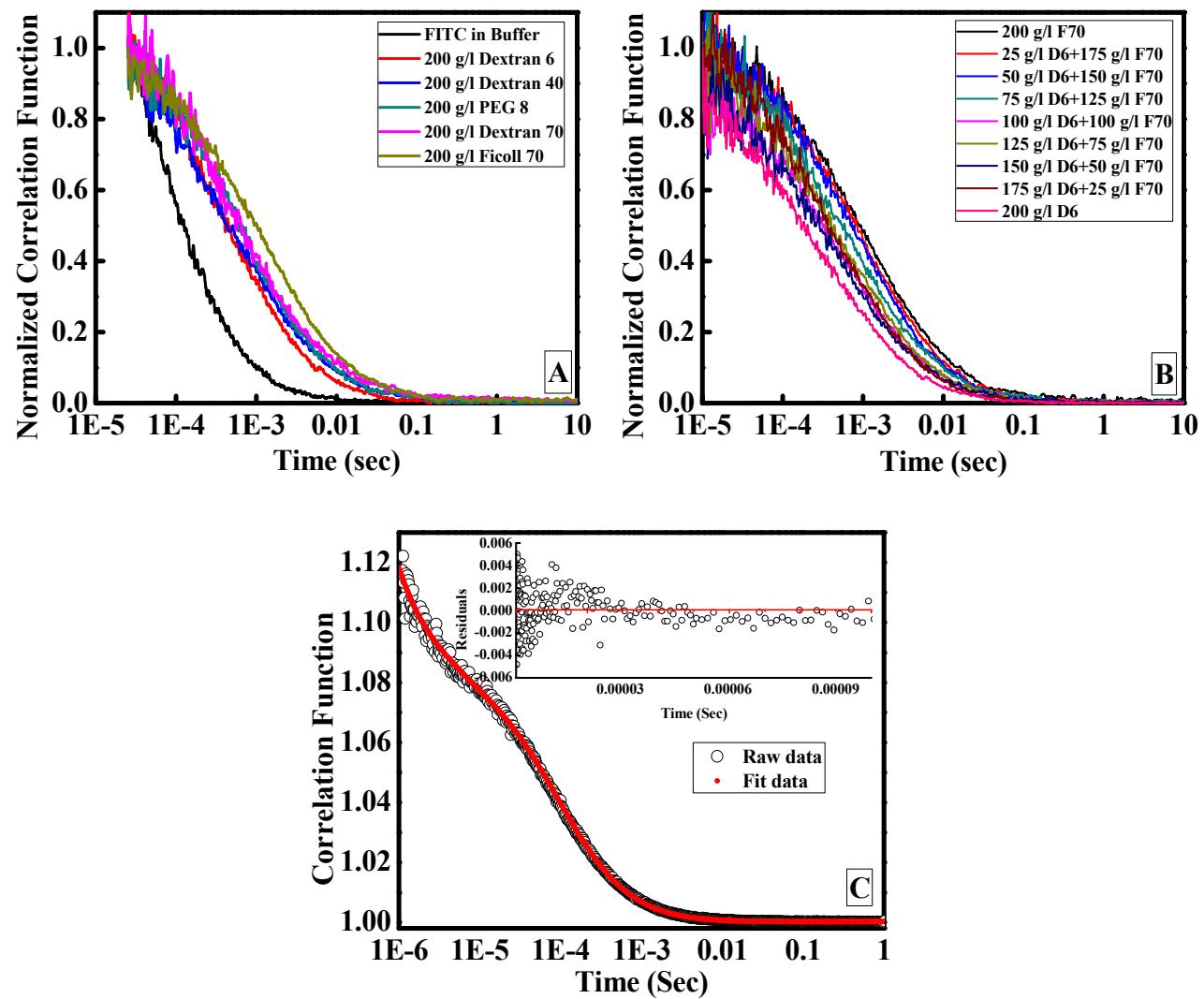
**Figure S12:** Representative three-state and two-state thermodynamic fits of domain movement in Ac-HSA (A) and NPA-HSA (B) in the ‘Dextran6+Dextran 40’ mixture.



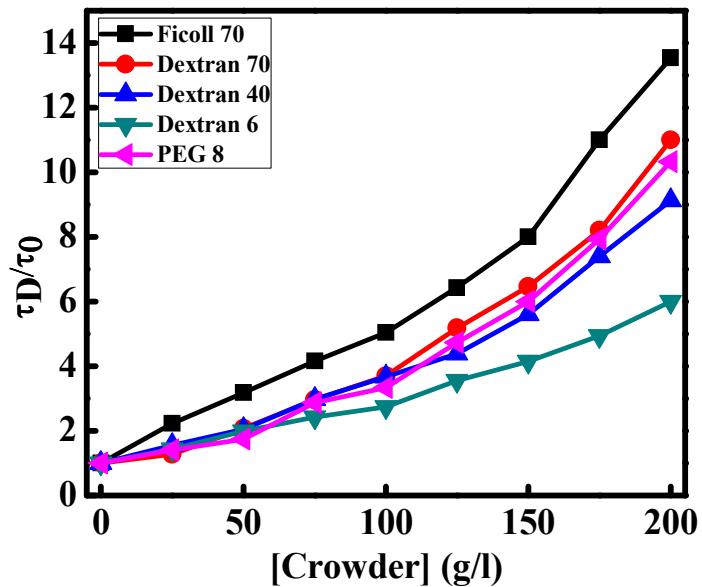
**Figure S13:** Representative plots of fluorescence intensity of HSA, Acrylodan HSA (domain I) and NPA HSA (domain III) in absence of any crowder, 150 g/L Dextran 40, Dextran 6 and binary mixture of 50 g/L Dextran 6+150 g/L Dextran 40 (as mentioned in legends).



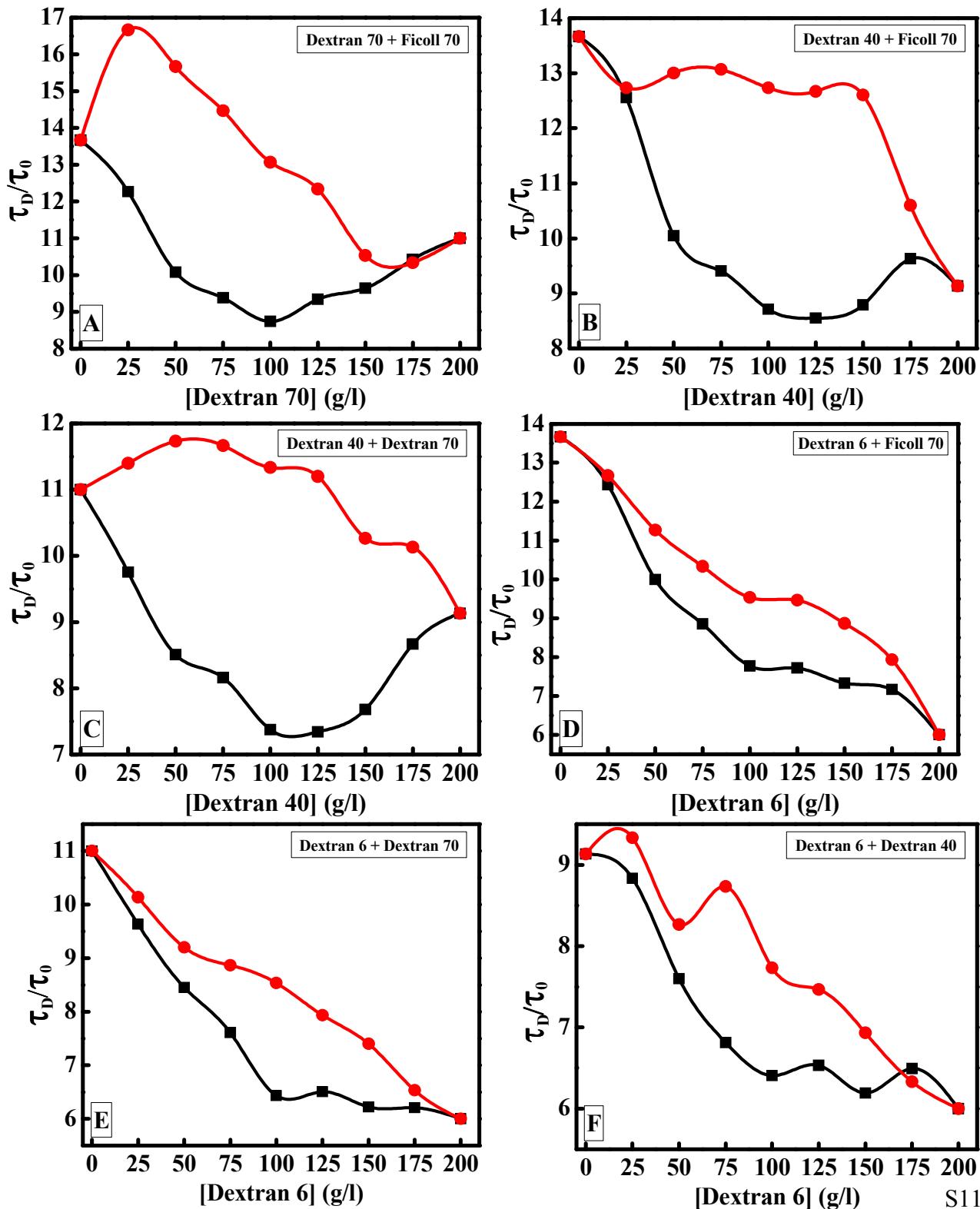
**Figure S14:** Normalized autocorrelation curves of FITC in presence of (A) 200 g/l of various individual macromolecular crowding agents and (B) ‘Dextran 6+Ficoll 70’ mixture with varying concentration of these two crowders. (C) A representative FCS trace showing the fit and the associated residuals.

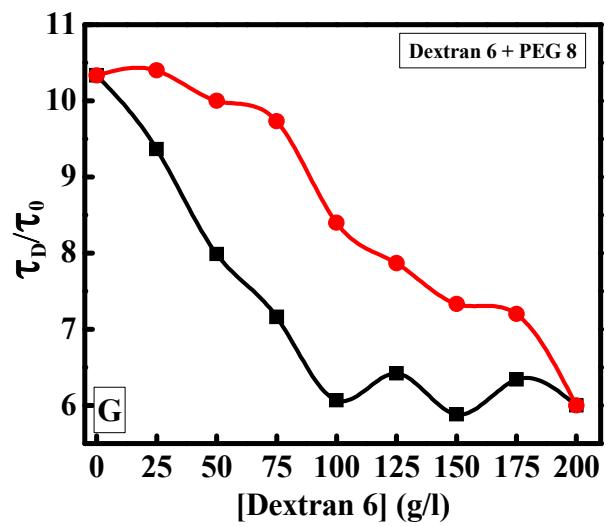


**Figure S15:** Plot of  $\tau_D/\tau_0$  as a function of the crowder concentration. (where  $\tau_D$  is the diffusion time of FITC in presence of crowding agents and  $\tau_0$  is the diffusion time of FITC in simple buffer.)



**Figure S16:** Plot of  $\tau_D/\tau_0$  against crowder concentration for (A) Dextran 70 and Ficoll 70, (B) Dextran 40 and Ficoll 70, (C) Dextran 40 and Dextran 70, (D) Dextran 6 and Ficoll 70, (E) Dextran 6 and Dextran 70, (F) Dextran 6 and Dextran 40 and (G) Dextran 6 and PEG 8 mixture. The red and black line represents the  $\tau_D/\tau_0$  of mixed macromolecular crowding agents and sum of the individual crowding agents respectively.





**Figure S17:** Plot of  $\tau_D/\tau_0$  against crowder concentration for (A) Dextran 70 and Ficoll 70, (B) Dextran 40 and Ficoll 70, (C) Dextran 40 and Dextran 70, (D) Dextran 6 and Ficoll 70, (E) Dextran 6 and Dextran 70, (F) Dextran 6 and Dextran 40 and (G) Dextran 6 and PEG 8 mixture. The red and black line represents the  $\tau_D/\tau_0$  of mixed macromolecular crowding agents and sum of the individual crowding agents respectively.

