Electronic Supplementary Material (ESI) for Chemical Communications

SUPPORTING INFORMATION

A simple strategy for charge selective biopolymer sensing

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Experimental

Materials and methods: CTAB, SDS, BSA, and PS and SCMC were procured from Sigma-Aldrich chemicals co. CS and pyrene was purchased from Fluka. Procured polysaccharides SCMC and CS were in highly pure state (99 %). Water was obtained from a Milli-Q purification system. Required amount of protein was mixed in MQ water to keep concentration 0.5 % weight for all experiments. All sensing experiments pH was kept constant and maintained at 3 with 0.001 N HCl in the medium. pH has been changed only in pH selectivity control experimentation.

Fluorescence Measurements. Fluorescence studies were performed to examine the micro-environment of tryptophan (present in protein molecule) in protein-surfactant system in presence and absence of polysaccharide. All fluorescence studies were performed using a Shimadzu 5301PC spectrophotometer at room temperature. The excitation wavelength was set at 280 nm (for tryptophan and tyrosine residue in protein) and emission spectra were measured from 300 to 600 nm. Slit widths for both (excitation and emission) were set at 10 nm and 10 nm for BSA and pepsin respectively.

ζ-potential Measurements. All ζ-potential measurements were performed in ζ-potential analyzer (Zeta Plus) from Brookhaven Instruments Corporation (BIC) equipped with 25 mW He-Ne, 677 nm laser

source. All samples were prepared in MIlli-Q water and sonicated for 30 minutes before using. The ζ-potential is calculated from the electrophoretic mobility of the charged particles. Measurements were carried out at room temperature and three duplicates were taken for each sample and then averaged.

DLS Measurements. The hydrodynamic sizes of protein-surfactant suspensions in Milli-Q water with and without polysaccharide were measured. DLS measurements were performed on a Brookhaven Instrument (BI 2000) equipped with an autoorrelator (BI-9K) and goniometer (BI-200SM) with BIHV photo multiplier tube. An argon ion laser (LEXEL 95-2) operating at 488 nm was used as a light source. The beam was focused onto the sample cell (standard cylindrical 4.5 ml cuvettes) through a temperature-controlled chamber (the temperature was controlled within 23 ± 0.1 ^{0}C). All experiments were done at a correlation time of 2 min. The measurements were carried out at 90^o. The available software records the autocorrelation of the intensity trace during the experiment. Once the autocorrelation data have been generated, the averaged translational diffusion coefficient of the protein samples is derived mathematically from the fitting parameter, the average decays rate, and scattering vector at a given angle of 90^o. The hydrodynamic radius, R_H, is computed based on the Stokes-Einstein equation. Three measurements were taken for each system and then the average hydrodynamic radius was obtained.

Turbidimetric Measurements. The turbidimetric measurements were performed using Perkin-Elmer Lambda 35 UV/vis spectrophotometer. A wavelength of 400 nm in the visible regime was selected to examine the formation of protein-surfactant complex in presence and absence of polysaccharide. All solutions were prepared in deionized water and mixed well before measurement. Experiments were carried out at room temperature and measurements were taken in transmittance (%T) to maximize the precision. Measurements were carried out on two or three sample solutions prepared at different times from the same materials (replicates).

Binding Curve Generation

The nature of “BSA-CTAB” and “PS-SDS” interaction can be well explained in terms of binding isotherm. Figure S-I shows the binding curve for the “BSA-CTAB” and “PS-SDS” interaction. In the binding curve,

fractions (α) of a BSA or PS molecule bound by CTAB and SDS respectively have been plotted as a function of total surfactant (bound + free) concentration. If a protein molecule have n_0 number of binding sites and at a certain stage surfactant molecules bind to n number of such sites, then the fraction of a protein molecule bound to surfactant is $\alpha = n/n_0$. Thus in the absence of CTAB or SDS, $\alpha = 0$ and $\alpha = 1$, when "BSA-CTAB" and "PS-SDS" complexation has been completed and no further surfactant can bind to protein chain. The binding characteristics in Figure S-I show different regions and are matches with the reported observation, where α has been estimated from the intensity of protein fluorescence as $\alpha = (F_{obs} - F_{free}) / (F_{min} - F_{free})$ where F_{obs} is protein fluorescence intensity at any surfactant concentration, F_{min} is minimum protein fluorescence intensity at a particular surfactant concentration after which addition of more surfactant will not further decrease the fluorescence intensity i.e. saturation binding condition and F_{free} is protein fluorescence in absence of surfactant.

At the beginning binding of some CTAB and SDS molecules occur in high energy sites of BSA and PS protein respectively. The slow rising part after this phase is termed as 'cooperative interaction' region; where protein 'unfolding' starts due to massive cooperative binding between surfactant and protein. This unfolding of the protein molecule promotes formation of micelle-like aggregates by wrapping around them (region A& B, Scheme 1). This is known as '*pearl necklace*' model of surfactant-protein complex. Beyond of this region a plateau is observed indicating no more binding of surfactant and protein i.e. called 'saturation' region. For BSA saturation binding with CTAB occurs at ~5 mM concentration and for PS saturation binding with SDS occurs at ~10 mM concentration. When CTAB or SDS (quencher) interacts with BSA or PS respectively, protein fluorescence may change depending on the impact of such interaction on the protein conformation. On the assumption that there are (n) substantive binding sites for quencher (Q) on protein (B), the quenching reaction can be shown as follows:

$$nQ + B \leftrightarrow Q_nB \qquad (1)$$

The binding constant (K_A) can be calculated as

$$K_A = [Q_nB]/[Q]^n[B] \qquad (2)$$

Where [Q] and [B] are the quencher (surfactant) and protein concentration, respectively, $[Q_nB]$ is the concentration of fluorophore-quencher complex, and $[B_0]$ gives the total protein concentration:

$$[Q_nB] = [B_0] - [B] \quad (3)$$

$$K_A = [B_0] - [B]/[Q]^n[B] \quad (4)$$

The fluorescence intensity is proportional to the protein concentration as described:

$$[B]/[B_0] \propto F/F_0 \quad (5)$$

Results from fluorescence measurements can be used to estimate the binding constant of the protein-surfactant assembly. From eq 4,

$$\ln[(F_0 - F)/F]) = \ln K_A + n \ln[Q] \quad (6)$$

Value of binding constant (K_A) and n for "BSA-CTAB" and "PS-SDS" binary assembly system (at pH~3) is 0.2 X 10^3 (M^{-1}) and 0.15 X 10^3 (M^{-1}), respectively.

To confirm the surfactant induced protein unfolding, we have performed DLS measurement of protein in presence and absence of surfactant. At, pH~3, addition of CTAB to BSA and SDS to PS, both leads to increase in hydrodynamic radius of protein chain. This result suggests potential opening up or unfolding of protein chain due to addition of similarly charged surfactant.

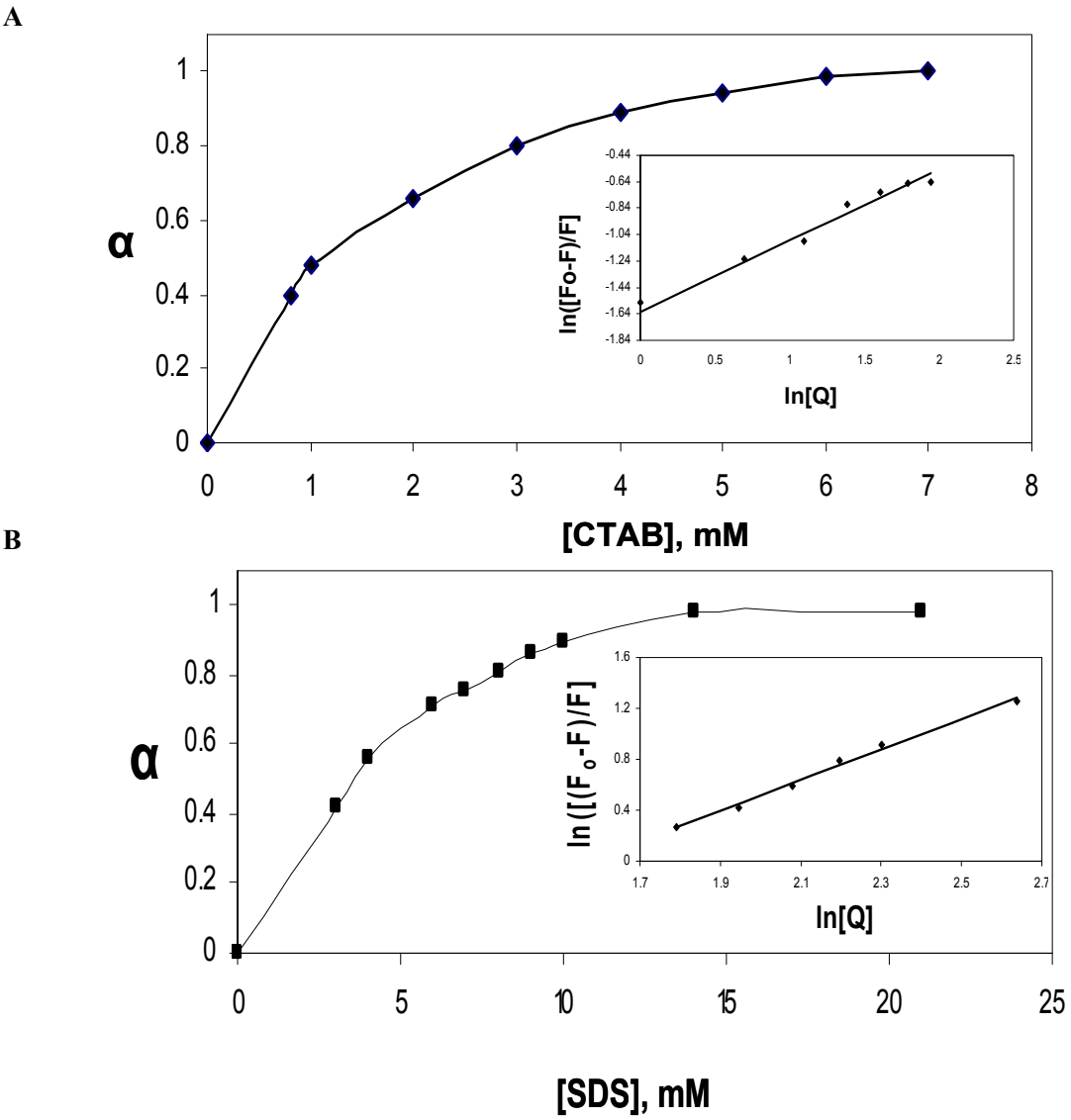

Figure S-1. Binding curve (α vs [surfactant]) showing interaction between A) BSA and CTAB and B) PS and SDS; protein used 0.5%, where α = fraction of a protein molecule bound by similarly charged surfactant at pH 3; Inset: Plot of ln $(F_0\text{-}F)/F$ as a function of ln [quencher] for calculation of binding constant (K_A).

DLS, turbidity, and ζ-potential study of "protein-surfactant-polysaccharide" ternary system. We have performed DLS, turbidity and ζ-potential measurement for protein, surfactant, "Protein-surfactant" binary

system, and "Protein-surfactant-polysaccharide" ternary system, individually at pH~3, to investigate the existence of "Protein-polysaccharide" complex in the "Protein-surfactant-polysaccharide" ternary system.

BSA solution was positively charged at pH 3 with ζ-potential ranging +15 mV, whereas PS solution was negatively charged with ζ-potential -14 mV. The ζ-potential of "BSA-CTAB" and "PS-SDS" system was more cationic and more anionic ranging +35 mV and -60 mV, respectively. This result indicates the binding of cationic CTAB to the small anionic patches on BSA chain, resulting increase in overall +ve charge of the "BSA-CTAB" complex, and similarly, binding of anionic SDS to the small cationic patches on PS chain, resulting in overall -ve charge increment of the "PS-SDS" complex. The effect of SCMC addition decreased the ζ-potential value approximately 30 mV for "BSA-CTAB" system and effect of CS addition increased the ζ-potential value approximately 50 mV for "PS-SDS" system, indicating a new molecular complex formation, which could be "Protein-polysaccharide" or "polysaccharide-surfactant" aggregation in the ternary assembly.

In a set of control experiments, addition of SCMC independently into BSA and CTAB causes increase in turbidity and particle sizes. Turbidity and particle size of "BSA-SCMC" is much higher than that of "CTAB-SCMC", which indicates "BSA-SCMC" complex is much larger in size compared to "SCMC-CTAB". When SCMC has been introduced in "BSA-CTAB" binary system, it causes increase in both turbidity and particle size. The trend of size distributions and turbidity of the "BSA-CTAB-SCMC" ternary system is similar to that for "BSA-SCMC" binary system. Presumably, this complex was formed between positively charged groups on the protein (e.g. $–NH_3^+$) and negatively charged groups on the SCMC (e.g. $–COO^-$). When the pH is low, there would be an increasingly large number of positively charged groups on the protein surface which could act as binding sites for the negatively charged groups on SCMC. This would explain why complexation was observed at pH values below the *p*I of the BSA. Thus in the ternary system due to the formation of more compact, oppositely charged Protein-polysaccharide complex, protein would prefer a folded conformation resulting in fluorescence recovery in the presence of CTAB surfactant.

Similarly, addition of CS into SDS, PS and "PS-SDS" solution shows similar trend as we have observed in SCMC addition in to CTAB, BSA and "BSA-CTAB" system. Thus in the ternary system due the formation of more compact, oppositely charged protein-polysaccharide complex, protein would prefer a folded conformation

resulting in fluorescence recovery. Presumably, this complex was formed between negatively charged groups on the protein (e.g. $–COO^-$-) and positively charged groups on the CS (e.g. $–NH_4^+$). When the pH is low, there would be an increasingly large number of negatively charged groups on the protein surface which could act as binding sites for the positively charged groups on CS. This would explain why complexation was observed at pH values above the *p*I of PS (where protein had a net negative charge and CS had a net positive charge).

I. Binding constant values and number of bound quencher on protein-surfactant binary assembly

Protein-Surfactant system	K_A (M^{-1})
BSA-CTAB	0.20×10^3
PS-SDS	0.15×10^3

II. Surfactant selectivity:

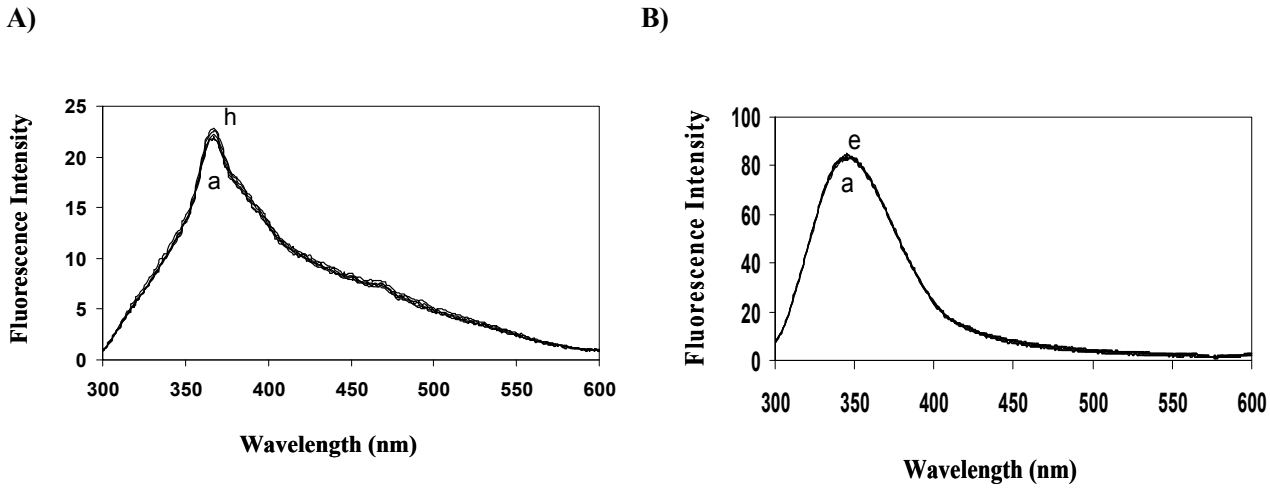

Figure S-II. A) Fluorescence intensity pattern of 'BSA-SDS' ([SDS] 10 mM) system in presence of SCMC [from 0 μM (a) to 2.0 μM SCMC (h)], B) Fluorescence intensity pattern of 'PS-CTAB' ([CTAB] 5mM) system in presence of CS [from 0 μM (a) to 0.20 μM CS (e)],

III. Polysaccharide selectivity:

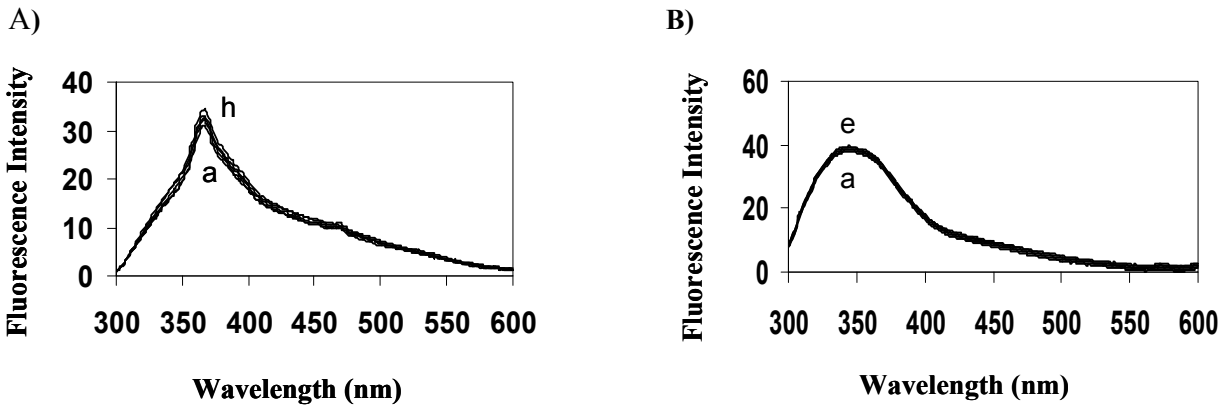

Figure S-III. A) Fluorescence intensity pattern of 'BSA-CTAB' ([CTAB] 5 mM) system in presence of CS [from 0 μM (a) to 0.20 μM CS (h)], B) Fluorescence intensity pattern of 'PS-SDS' ([SDS] 10 mM) system in presence of SCMC [from 0 μM (a) to 0.56 μM SCMC (e)].

IV. ζ-potential study of "protein-surfactant-polysaccharide" ternary system:

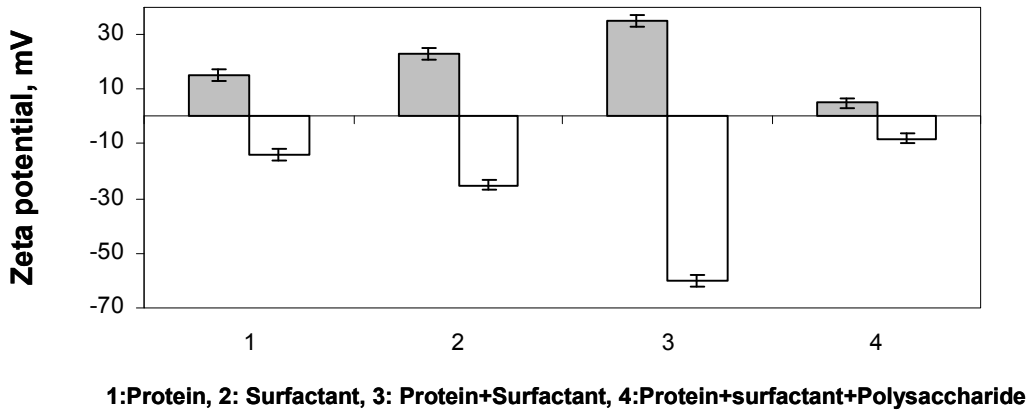

Figure S-IV. Variation of ζ-potential for BSA (0.5%), CTAB (5 mM) and SCMC (0.5 μM) system (■) and in PS (0.5%), SDS (10 mM) and CS (0.20 μM) system ( ) at pH 3.

V. Turbidity study of "protein-surfactant-polysaccharide" ternary system:

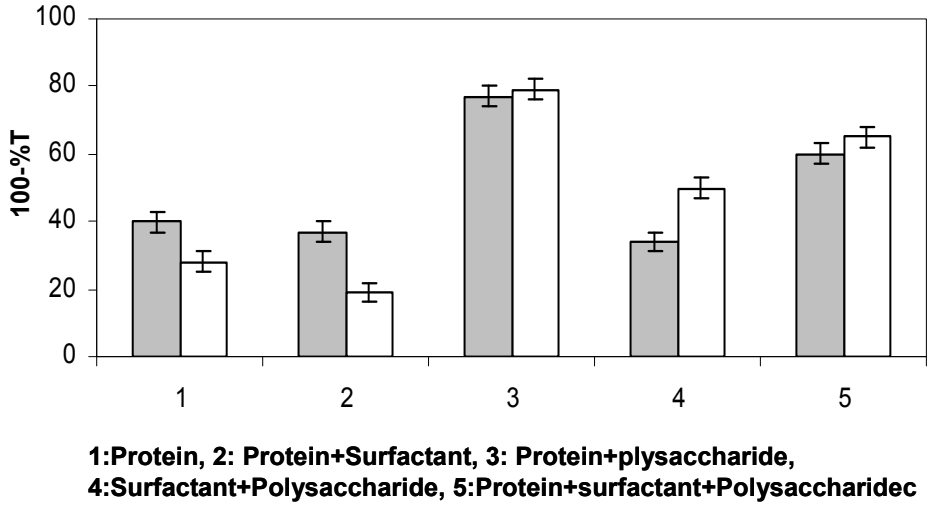

Figure S-V. Variation of turbidity for BSA (0.5%), CTAB (5 mM) and SCMC (0.5 µM) system (■) and in PS (0.5%), SDS (10 mM) and CS (0.20 µM) system (□t pH 3

VI. Turbidity study of "protein-surfactant-polysaccharide" ternary system:

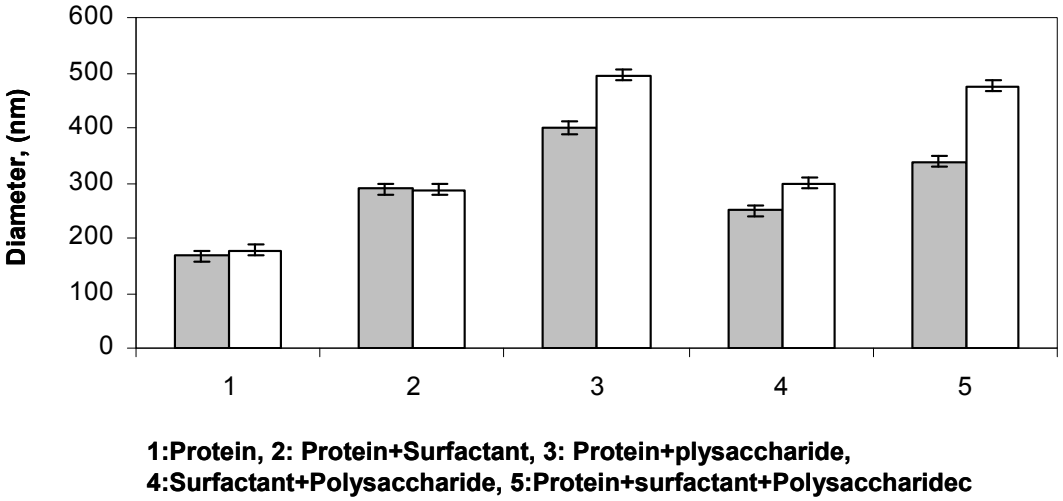

Figure S-VI. Variation of particle diameter in BSA (0.5%), CTAB (5 mM) and SCMC (0.5 μM) system (■) and in PS (0.5%), SDS (10 mM) and CS (0.20 μM) system (□) pH 3.

Pyrene Probe Study of 'protein-surfactant' and 'protein-polysaccharide' binary assembly. Exposure of the protein hydrophobic domains to the solvent, upon unfolding, in the case of "protein-surfactant" interaction and large macromolecular aggregate formation with protein folding in the case of "protein-polysaccharide" can be easily probed using a fluorescence dye, which is highly sensitive towards polarity of the microenvironment. Keeping this in mind, we have been studied the formation of "protein-similarly charged surfactant" assembly and "protein-oppositely charged polysaccharide" aggregation, in the presence of an external hydrophobic probe pyrene. The fluorescence intensity of pyrene (I_1/I_3) is very sensitive to the polarity of the microenvironment. A lower value of I_1/I_3 reflects a more hydrophobic environment and higher value of I_1/I_3 reflects comparably less hydrophobic environment. The I_1/I_3 values of pyrene plotted against $C/[C_{max}]$ where C is the concentration of surfactant or polysaccharide and C_{max} is the maximum concentration of

surfactant or polysaccharide added in to protein solution. Figure S-VII (A) shows pyrene in BSA, where I_1/I_3 value increases with increase in CTAB concentration and decreases with increase in SCMC concentration, indicating pyrene probe is facing more hydrophilic and hydrophobic microenvironment, respectively. Figure S-VII (B) shows pyrene in PS, where I_1/I_3 value increases with increase in SDS concentration, indicating pyrene is facing more polar environment and I_1/I_3 decreases with increase in CS, concentration, indicating pyrene is facing more hydrophobic microenvironment. This observation supports our explanation that addition of similarly charged surfactant to the protein leads to partial unfolding of the protein chain, thus exposure of fluorescent amino acid groups in more hydrophilic solvent, responsible for quenching of protein fluorescence in "Protein-similarly charged" binary assembly. Whereas, addition of oppositely charged polysaccharide to the protein leads to formation of "Protein-polysaccharide" complex, where fluorescent amino acid groups goes into more hydrophobic region, responsible for protein fluorescence enhancement.

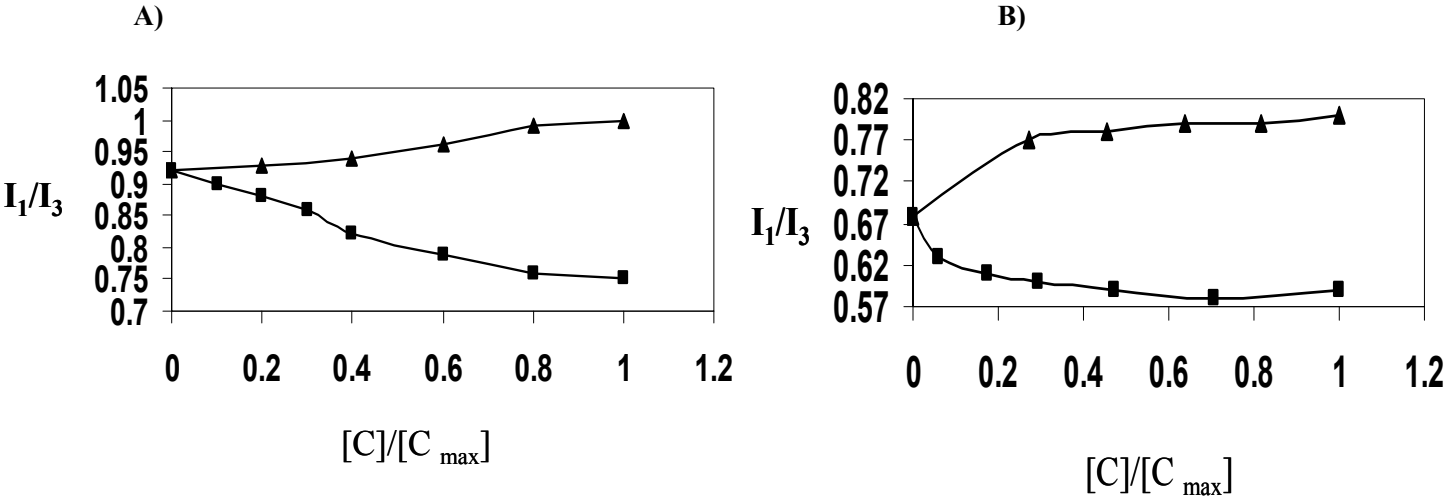

Figure S-VII. I_1/I_3 ratios of pyrene in A) BSA (0.5 %) in the presence of CTAB (▲) and SCMC (■) and B) PS (0.5 %) in the presence of SDS (▲) and CS (■). C is the concentration of surfactant or polysaccharide at any point and C_{max} is the maximum concentration used at pH 3. Here CTAB concentration varied from 0 to 5 mm, SDS concentration varied from 0 to 10 mM, SCMC concentration varied from 0 to 1.0 µM and CS concentration varied from 0 to 0.1 µM.