Supporting Information

Unfolding and Refolding of Protein by Combination of Ionic and Nonionic Surfactants

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Theory of Dynamic Light Scattering

The dynamic light scattering usually measured the temporal fluctuation in scattering light intensity at a specific angle using a monochromatic light. The signal generated by diffusing particles can be analyzed by the normalized intensity autocorrelation function \( g^2(\tau)^{1,2} \)

\[
g^{(2)}(\tau) = \frac{\langle I(t)I(t+\tau) \rangle}{\langle I(t) \rangle^2} \tag{1}
\]

where \( I(t) \) is the scattered light intensity at time \( t \) and \( I(t+\tau) \) the scattered intensity at time \( t \) plus delay time \( \tau \). The normalized intensity correlation function is related to the normalized filed autocorrelation function by the Siegert relation

\[
g^{(2)}(\tau) = 1 + \beta \left| g^{(1)}(\tau) \right|^2 \tag{2}
\]

where \( \beta \) is the spatial coherence factor and depends on the alignment and detection optics.

The field autocorrelation function for a dilute system of monodisperse particles is represented by

\[
g^{(1)}(\tau) = \exp(-\Gamma \tau) \tag{3}
\]

where \( \Gamma \) is the average decay rate of \( g^{(1)}(\tau) \) and extracted from the monomodal fit. The diffusion coefficient \( (D_a) \) and the wave vector \( q \) are related to \( \Gamma \) as \( \Gamma = D_a \omega^2 \)

For a suspension of polydisperse particles, the field autocorrelation function is modified to

\[
g^{(1)}(\tau) = \int_0^\infty G(D_a) \exp(-D_a q \tau) dD_a \tag{4}
\]

where \( G(D_a) \) is the distribution of particles with different diffusion coefficients about the mean value. The mean value of the diffusion coefficient \( (D_m) \) and polydispersity index (PI)
are calculated by using the cumulant analysis method.\(^3\) Including the cumulant analysis, Eq. (4) can be simplified to

\[
g^1(\tau) = \exp \left[ -D_m q^2 \tau + \frac{\mu_e \tau^2}{2} \right]
\]

where PI is calculated from the ratio of variance (\(\mu_2\)) to the square of the mean of the decay rate (\(\Gamma_m = D_m q^2\)). The corresponding effective hydrodynamic size \((d_h)\) is calculated using Stoke-Einstein equation.\(^4\)

**SANS analysis of Form Factor and Structure Factor of pure components**

The ellipsoidal shape is commonly used to define the form factor \(P(q)\) of micelles as it can represent different shapes depending on the axial ratio from spherical to rodlike and disclike. The pure micellar systems have been fitted with core-shell ellipsoidal micelles with its semi axes \(R\) and \(\varepsilon R\) and shell thickness \(t\). Depending on \(\varepsilon\), the shape of particle can be spherical \((\varepsilon = 1)\), prolate ellipsoidal \((\varepsilon > 1)\), oblate ellipsoidal \((\varepsilon < 1)\), rodlike \((\varepsilon > 1)\), and disclike \((\varepsilon < < 1)\).

\(S(q)\), the interparticle structure factor reveals the interactions between the particles. In case of a dilute system, the structure factor is close to the unity \((S(q) \approx 1)\). Particles without having charge can interact with the hard-sphere potential and \(S(q)\) has been calculated using Percus-Yevick approximation.\(^5\) The screened Coulomb potential under rescaled mean spherical approximation is used to calculate \(S(q)\) for charged particles. In our case, the scattering profiles of ionic micelles are fitted with the \(S(q)\) of charged particles interacting through screened Coulomb interaction. The charged particles are assumed to be rigid spheres of equivalent size \(\sigma\) interacting through a potential as given by \(^6\)

\[
u(r) = u_0 \sigma \exp \left[ -\kappa \left( r - \sigma \right) \right] / r, r \sigma
\]

where \(\kappa\) is the Debye- Hückel inverse screening length and \(u_0\) is the contact potential. On the other hand, nonionic micelles of C12E10 and ionic-nonionic mixed micelles are fitted by the hard sphere interaction.

**Scattering Length Density calculation and SANS data analysis of pure components**

The calculated scattering length density of hydrophobic tail for all ionic surfactant is about \(-0.25\times10^{-10}\) cm\(^2\). The scattering length density of hydrophilic part of nonionic surfactant is
0.60\times10^{10} \text{ cm}^{-2}.\textsuperscript{7} The solvent D\textsubscript{2}O has scattering length density of 6.37\times10^{10} \text{ cm}^{-2}. The scattering profile of individual ionic (C10TAB, C12TAB, C14TAB, and C16TAB) and nonionic (C12E10) micelles are shown in Figure S1. Due to the very high CAC (CAC\textsubscript{C10TAB} = 40 \text{ mM}), 1 wt\% C10TAB molecules unable to form micelles and remain as free molecules in the solution.

\textbf{Figure S1.} SANS data of 1 wt \% surfactants (ionic C10TAB, C12TAB, C14TAB, and C16TAB and nonionic C12E10) at pH = 7 in D\textsubscript{2}O.
IFT fitting of SANS profile of 1% BSA in the presence of CnTAB (n=10,12,14, and 16)

Figure S2. IFT fit of SANS profiles of 1% BSA in the presence of 0-80 mM (A) C10TAB, (B) C12TAB, (C) C14TAB, and (D) C16TAB.
|                | BSA   | CnTAB (mM) | C12E10 (mM) | Hydrodynamic Radius (nm) | Remarks |
|----------------|-------|------------|-------------|--------------------------|---------|
| Native BSA     |       |            |             |                          |         |
| 0.0%           | 5     | 0          | 4.3         |                          |         |
| 1.0%           | 5     | 0          | 5.9         | Unfolding                |         |
|                | 10    | 0          | 8.6         | Unfolding                |         |
|                | 20    | 0          | 8.1         | Unfolding                |         |
|                | 40    | 0          | 7.1         | Refolding                |         |
|                | 60    | 0          | 6.0         | Refolding                |         |
| C12TAB         | 20    | 60         | 5.6         | Refolding                |         |
|                | 80    | 5.2        | Refolding   |                          |         |
|                | 100   | 5.0        | Refolding   |                          |         |
|                | 120   | 4.4        | Refolding   | Refolded BSA             |         |
|                | 40    | 0          | 6.8         | Unfolding                |         |
|                | 80    | 0          | 6.3         | Unfolding                |         |
| 1.0%           | 5     | 0          | 9.0         | Unfolding                |         |
|                | 10    | 0          | 7.9         | Unfolding                |         |
|                | 20    | 0          | 7.2         | Unfolding                |         |
|                | 40    | 0          | 6.7         | Refolding                |         |
|                | 60    | 0          | 5.8         | Refolding                |         |
|                | 80    | 0          | 5.4         | Refolding                |         |
| 1.4%           | 140   | 4.4        | Refolding   | Refolded BSA             |         |
|                | 150   | 4.3        | Refolding   | Refolded BSA             |         |
|                | 160   | 4.3        | Refolding   | Refolded BSA             |         |
|                | 40    | 0          | 6.8         | Unfolding                |         |
|                | 80    | 0          | 5.7         | Unfolding                |         |
| 1.0%           | 5     | 0          | 8.1         | Unfolding                |         |
|                | 10    | 0          | 7.9         | Unfolding                |         |
|                | 20    | 0          | 7.2         | Unfolding                |         |
|                | 40    | 0          | 6.5         | Refolding                |         |
|                | 60    | 0          | 6.2         | Refolding                |         |
|                | 80    | 0          | 6.4         | Refolding                |         |
| C14TAB         | 20    | 80         | 5.6         | Refolding                |         |
|                | 100   | 5.0        | Refolding   |                          |         |
|                | 120   | 4.7        | Refolding   |                          |         |
|                | 130   | 4.4        | Refolding   | Refolded BSA             |         |
|                | 140   | 4.2        | Refolding   | Refolded BSA             |         |
|                | 40    | 0          | 6.3         | Unfolding                |         |
|                | 80    | 0          | 5.9         | Unfolding                |         |

**Table S1.** Fitted parameters of DLS data of BSA protein, BSA-CnTAB (n=12,14, and 16) complexes, and BSA-CnTAB-C12E10 (n=12,14, and 16) mixed micelles at different surfactant concentrations.
Unfolding of BSA studied by DLS

Figure S3: DLS data of protein-surfactant complexes of 1 wt% BSA + \( c \) mM CnTAB (A) \( n=10 \) (B) \( n=12 \) and (C) \( n=14 \).
Refolding of BSA protein in the presence of C12E10

Four possible scenarios are considered for the interaction of BSA with mixed surfactant system.

1. It is assumed that the nonionic surfactant neither interacts with protein nor with the ionic surfactants. Therefore, the scattering profile becomes the sum of unfolded BSA induced by ionic surfactants plus the nonionic surfactant micelles. The data fitting revealed that the scenario is not valid for any of our cases (Model 1).

2. It is assumed that the BSA remains in the unfolded state and the unfolding of BSA is driven by the mixed micelles (ionic-nonionic surfactant mixed micelles) formation around the patches of BSA. However, the fitting of SANS profile clearly indicates that this assumption is not valid for any of our cases (Model 2).

3. It is assumed that the ionic surfactant interacts with both the protein and the nonionic surfactant. According to this assumption, the unfolded protein coexists with the mixed micelles. However, the propensity of unfolding could be less for 1 wt% BSA-40 mM CnTAB (n=12, 14, and 16)-C12E10 compared to the reference system (1 wt% BSA-40 mM CnTAB) due to the partial extraction of the ionic surfactant from the cluster. The assumption is valid for 1 wt% BSA-40 mM CnTAB-C12E10 systems (Model 3).

4. It is assumed that the unfolded protein return to its native state and the ionic surfactant molecules comes out from the BSA-surfactant complexes to form mixed micelles with the nonionic surfactant. Therefore, the scattering profiles are considered as a linear combination of the scattering from pure BSA and ionic-nonionic mixed micelles. To adopt this model, the scattering profile of mixed surfactant at the different combination of ionic and nonionic surfactant ratio were measured independently with and without BSA protein. This assumption is partially valid at the intermediate concentrations of C12E10 (cni=5, 10, and 20 mM) and completely match with the scattering profile at higher concentration (cni=40 mM) of C12E10 in the BSA-surfactant complexes. The competition between the electrostatic interaction of BSA and ionic surfactants and the hydrophobic interaction of ionic and nonionic surfactants yielded the complete extraction of ionic micelles from the protein patches. At 40 mM C12E10 concentration, all ionic
micelles come out from the cluster and the protein is refolded back to its native structure (Model 4).

**Figure S4.** The comparison of model scattering with the experimental data of 1 wt % BSA protein with 40 mM C16TAB and (i) 5 mM (ii) 10 mM (iii) 20 mM and (iv) 40 mM C12E10. Model 1: Nonionic micelles coexist of protein–ionic surfactant complexes; Model 2: Mixed micelles driven unfolding of BSA protein; Model 3: Ionic surfactant induced unfolded BSA coexists with the ionic-nonionic mixed micelles; and Model 4: Refolded BSA protein coexists with mixed ionic and nonionic surfactants micelles. The model calculations are done using the experimental data of different individual and mixed components.
Refolding of BSA studied by DLS

Figure S5. DLS data of 1 wt % BSA and 20 mM CnTAB with varying concentration of C12E10 (A) C12TAB and (B) C14TAB. The inset shows the variation of the hydrodynamic radius with the change in the concentration of C12E10.

Figure S6. DLS data of 1 wt % BSA, 40 mM CnTAB (n=12, 14, and 16), 40 mM C12E10 and 40 mM C16TAB-20 mM C12E10 mixed micelles.
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