Electronic Supplementary Information (ESI)

Title:

Assessment of Micro-Polarity Anisotropy as a function of Surfactant Packing in SDS (sodium dodecyl sulphonate)-Hexane Reverse Micelle Systems

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Materials and Sample preparation: The sodium dodecyl sulfate (SDS) reverse micelle (RM) samples having varying ω_0 values were prepared with hexane as solvent. The RM aqueous phase constituted solvent as either H₂O or mixture of H₂O/D₂O (1:1 v/v). SDS surfactants were dissolved in hexane at desired concentrations. Desired amounts of nano-pure H₂O (18.2 m Ω) or H₂O/D₂O solvents were added to hexane. The injection method [1, 2] was used, wherein a known volume of the 'aqueous phase'/ 'enzyme-containing phase' was injected into a known volume of hexane with 10 mM SDS, and as per ω_0 values. The mixtures were magnetically stirred until optically transparent solutions were obtained. HRP enzymes were dissolved in the aqueous phase solvent at desired concentration levels, and were added to hexane to prepare RMs containing HRP. All the chemicals used in this study were of analytical reagent grade, and were obtained from Sigma Aldrich.

ESR data acquisition protocol (determination of a_N and τ_c): A Bruker EMX-EPR was used for the ESR studies. 16-DSA (16-Doxyl stearic acid) was used as a nitroxide radial (N-^O) probe. The probe was dissolved in chloroform to obtain a concentration of 5×10^{-2} M, and subsequently added to the RM samples at 2×10^{-4} M concentration levels. 16-DSA at such low concentration levels negligibly alters the MPA of RMs [3]. The ESR spectra were recorded at room temperature. The Hyperfine splitting constant ^{an} provides the interaction energy between the electron spin and characterizes a magnetic nucleus. The rotational coefficient ^c provides a measure of the rigidity/packing of the surfactant layer (self-assembled surfactant structure of RM). The hyperfine splitting ⁿ value was determined as:

$$a_N = 2.35 \chi a_N^0 - 0.084 \ mT$$
 ... (1)

Where the isotropic hyperfine coupling constant a_N for the spin label in the an environment is given by:

$$a_N = 1/3 (A_{\parallel} + 2A_{\perp}) \dots (2) [4]$$

where A_{\parallel} is identified with the outer maximum hyperfine splitting, and A_{\perp} is obtained from the inner minimum hyperfine splitting. The a_N values are sensitive to the polarity of the environment of the spin labels since increases in a_N values reflect the increase in the polarity of the aqueous phase. The rotation correlation time c, a measure of the surfactant density/packing, was determined as:

$$\tau_c = 6.5 \times 10^{-10} \Delta H \left[\left(\frac{h_0}{h_{+1}} \right)^{0.5} - 1 \right] \dots (3) [4]$$

Where, h_0 , h_{+1} and h_{-1} are the peak-to-peak heights of the 0, +1 and -1 transitions; ΔH represents the line width of the central line in field units.

ATR-FTIR studies: The ATR-FTIR spectroscopic studies of the inter-molecular water micro-structures in the RM was carried out with a Perkin-Elmer Spectrum 100 ATR-IR spectrometer. The RM samples were placed on a ZnSe trough, and 10 total internal reflections were allowed. The ATR-IR spectra were taken at the regular intervals of 3-4 minutes, within the range of 650-4000 cm⁻¹ and at 4cm⁻¹ resolution. In order to obtain information on the microstructural features of water in RM the OH- and OD- stretching vibration line shapes were analysed in the ranges of 3700-

3100 cm⁻¹ and 2800-2200 cm⁻¹, respectively. HRP conformations were analysed as per the changes in the peak positions and reshaping of the Amide I bands in the IR spectra.

Cryo SEM and AUC measurement

The RMs size and shape were determined using Cryo SEM and Analytical Ultra centrifuge (AUC) [5]. A sample was pipetted into a 200 μ m thick hat and frozen by high pressure freezing on a Wohlwend HPF Compact 01 high pressure freezer, then stored in liquid nitrogen. For imaging, the hat was loaded onto a shuttle and loaded into a Quorum cryostage (Quorum Technologies In., East Sussex England) mounted on an FEI Helios Nanolab 650 dual beam FIB/SEM. The sample was kept at -175 °C at all times, and transferred into the microscope. For AUC studies, the densities of the solutions were measured using an Anton Paar density meter. Sedimentation velocity tests were performed using a Beckman Coulter Optima XL-I analytical ultracentrifuge equipped with both absorbance and interference optical detectors.

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MPA contribution of HRP in RM



Fig. S3. Micro-polarity contributions (as $\langle a_N \rangle / \langle \tau_c \rangle$) owing to presence of HRP enzymes in RMs, and as a function of ω_0 .

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Amide I peak of HRP in H₂O/D₂O(-----), and in H₂O/D₂O of RMs (-----) of ω_0 = 20



Fig. S4. ATR-FTIR spectra of HRP in H_2O/D_2O (red spectrum) and in aqueous phase of H_2O/D_2O mixture of RMs (blue spectrum). The red spectrum enumerates the native conformation (α -helix, β -sheets, turns/coils) of HRP. Significant changes in the conformation of the HRP (blue spectrum) in RM could be seen. These conformational changes suggest transformation of α -helix to β -sheets-like structures.