Supporting Information

New Strategy to Prepare Giant Vesicles from Surface Active Ionic Liquids (SAILs): A Study of Protein Dynamics in Crowded Environment Using Fluorescence Correlation Spectroscopic Technique

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**Figure S1**: Schematic presentation of the FLIM and FCS detection
Figure S2. Fluorescence intensity fluctuation traces of FCS signal (alexa fluor-488 tagged BSA in buffer)
1. Synthesis and characterization of SAILs: ([CTA][AOT], [BHD][AOT], [C_{16}mim][AOT], [C_{12}mim][AOT], and [C_{8}mim][AOT])

The SAILs were prepared using anion exchange procedures similar to those reported in the literature.\(^1\) Equimolar (0.05 M) amounts of sodium bis(2-ethylhexyl)sulfosuccinate (NaAOT) salt and CTAB/BHDC/ C_{16}mimCl/ C_{12}mimCl/ C_{8}mimCl were dissolved in a mixture of methylene chloride and water (2:1 v/v) and stirred for 12 h at room temperature. The methylene chloride layer was washed several times with water, and the product was obtained from the organic lower layer and dried by removal of solvent by rotavapour. Further high vacuum pump was used for 60 hours to remove traces of water. The obtained SAILs [CTA][AOT], [BHD][AOT], [C_{16}mim][AOT], [C_{12}mim][AOT], and [C_{8}mim][AOT] were characterized by \(^1\)H, \(^{13}\)C, and DEPT NMR studies. All NMR measurements were carried out with a Bruker 400 MHz NMR spectrometer using C_{6}D_{6} as chemical shift reference for mode locking. We have indicated the numbering against different peaks obtained in \(^1\)H, \(^{13}\)C, and DEPT NMR spectra.
Figure S3: $^1$H, $^{13}$C, and DEPT (135°) NMR spectra of [C$_8$ mim][AOT].
Figure S4: $^1$H, $^{13}$C, and DEPT (135°) NMR spectra of [C$_{12}$mim][AOT].
Figure S5: $^1$H, $^{13}$C, and DEPT (135$^\circ$) NMR spectra of [C$_{16}$mim][AOT].
Figure S6. Fluorescence intensity images of [CTA][AOT] vesicles (a); (b) is the fluorescence lifetime images of the same vesicles after fitting with a biexponential function. The legend in ‘(b)’ shows the lifetime of the vesicles in the different regions. FLIM data format 256×256 pixels, 256 time channels.

Figure S7. Fluorescence intensity images of [BHD][AOT] vesicles (a); (b) is the fluorescence lifetime images of the same vesicles after fitting with a biexponential function. The legend in ‘(b)’ shows the lifetime of the vesicles in the different regions. FLIM data format 256×256 pixels, 256 time channels.
Figure S8. Fluorescence intensity images of \([\text{C}_{16}\text{MIM}][\text{AOT}]\) vesicles (a); (b) is the fluorescence lifetime images of the same vesicles after fitting with a biexponential function. The legend in ‘(b)’ shows the lifetime of the vesicles in the different regions. FLIM data format 256×256 pixels, 256 time channels.
**Figure S9.** Fluorescence intensity images of [C<sub>12</sub>MIM][AOT] vesicles (a); (b) is the fluorescence lifetime images of the same vesicles after fitting with a biexponential function. The legend in ‘(b)’ shows the lifetime of the vesicles in the different regions. FLIM data format 256×256 pixels, 256 time channels.

**Figure S10.** Fluorescence intensity images of [C<sub>8</sub>MIM][AOT] vesicles (a); (b) is the fluorescence lifetime images of the same vesicles after fitting with a biexponential function. The legend in ‘(b)’ shows the lifetime of the vesicles in the different regions. FLIM data format 256×256 pixels, 256 time channels.
**Figure S11.** TEM images of giant vesicles formed by different SAILs; (a) [CTA][AOT], (b) [BHD][AOT], (c) [C$_{16}$mim][AOT], (d) [C$_{12}$mim][AOT], and (e) [C$_{8}$mim][AOT]

**Figure S12.** Fitted correlation curve measures for R6G in water along with the residual.