

**Biamphiphilic Ionic Liquids Based Aqueous Microemulsions as an Efficient Catalytic  
Medium for Cytochrome c**

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**Supporting Information**

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## Annexure S1

### Synthetic procedure for the preparation of Biamphiphilic ionic liquids (BAILs)

1-hexyl-3-methylimidazolium dioctylsulfosuccinate [C<sub>6</sub>mim][AOT], its ester-, [C<sub>6</sub>Emim][AOT] and amide-, [C<sub>6</sub>Amim][AOT], -functionalized counterparts have been synthesized by following the anion exchange method as reported by Rao et al.<sup>1</sup> Equimolar amount of sodium dioctyl sulfosuccinate salt and [C<sub>6</sub>mim][Cl] were dissolved in dichloromethane and the reaction mixture was stirred at room temperature for 12 hours. Dichloromethane layer was washed with water for at least five times and then the organic layer is separated. Then product [C<sub>6</sub>mim][AOT] is separated from the organic layer by using rotavapor.

For synthesizing the functionalized BAIL, firstly SAILs with ester, [C<sub>6</sub>Emim][Cl] and amide, [C<sub>6</sub>Amim][Cl], counterparts were synthesized by following the procedure reported by Singh et al.<sup>2</sup> Intermediates required for the synthesis of functionalized SAILs i.e. hexyl-2-chloroacetate and 2-chloro-*N*-hexylacetamide were synthesized in similar manner as reported earlier.<sup>3,4</sup> Equimolar amount of these intermediates and 1-methylimidazole was stirred for 48 hours. Resulting product was washed with diethyl ether for at least five times to yield [C<sub>6</sub>Emim][Cl] and [C<sub>6</sub>Amim][Cl], respectively. To synthesize [C<sub>6</sub>Emim][AOT] and [C<sub>6</sub>Amim][AOT], equimolar amount of [C<sub>6</sub>Emim][Cl]/[C<sub>6</sub>Amim][Cl] and sodium dioctyl sulfosuccinate salt were dissolved in dichloromethane and stirred for 12 hours at room temperature. The organic layer was washed with water for at least five times and the organic layer was separated using separatory funnel. Final product was obtained by rotavapor to remove the dichloromethane. Resulting BAILs were further characterized using NMR spectroscopy. <sup>1</sup>H NMR spectra was recorded on Brüker Ascend 500 spectrometer (AVANCE III HD console) in CDCl<sub>3</sub> as solvent.

[C<sub>6</sub>mim][AOT] (BAIL-1): <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>, δ-ppm),

[AOT]: 0.88 (m, 6H, -O-CH<sub>2</sub>-(CH<sub>2</sub>)<sub>6</sub>-CH<sub>3</sub>), 1.35 (m, 20H, -O-CH<sub>2</sub>-CH<sub>2</sub>-(CH<sub>2</sub>)<sub>5</sub>-CH<sub>3</sub>), 1.55 (m, 2H, -O-CO-CH<sub>2</sub>-CH-SO<sub>3</sub>-CO-O-CH<sub>2</sub>-(CH<sub>2</sub>)<sub>5</sub>-CH<sub>3</sub>), 1.63 (m, 2H, -CH<sub>2</sub>-CO-O-CH<sub>2</sub>-(CH<sub>2</sub>)<sub>5</sub>-CH<sub>3</sub>), 3.24 (m, 2H, -O-CO-CH<sub>2</sub>-CH-SO<sub>3</sub>-CO-O-CH<sub>2</sub>-(CH<sub>2</sub>)<sub>5</sub>-CH<sub>3</sub>), 4.00 (m, 4H, -O-CH<sub>2</sub>-(CH<sub>2</sub>)<sub>6</sub>-CH<sub>3</sub>), 4.19 (m, 1H, -O-CO-CH<sub>2</sub>-CH-SO<sub>3</sub>-CO-O-)

[C<sub>6</sub>mim]: 0.88 (m, 3H, -N<sup>+</sup>-CH<sub>2</sub>-(CH<sub>2</sub>)<sub>4</sub>-CH<sub>3</sub>), 1.35 (m, 6H, -N<sup>+</sup>-CH<sub>2</sub>-CH<sub>2</sub>-(CH<sub>2</sub>)<sub>3</sub>-CH<sub>3</sub>), 1.88 (m, 2H, -N<sup>+</sup>-CH<sub>2</sub>-CH<sub>2</sub>-(CH<sub>2</sub>)<sub>3</sub>-CH<sub>3</sub>), 4.01 (s, 1H, -N-CH<sub>3</sub>), 4.26 (t, 2H, -N<sup>+</sup>-CH<sub>2</sub>-CH<sub>2</sub>-(CH<sub>2</sub>)<sub>3</sub>-CH<sub>3</sub>), 7.18 (t, 1H, -N<sup>+</sup>-CH-CH-N-), 7.23 (t, 1H, -N<sup>+</sup>-CH-CH-N-), 9.76 (s, 1H, -N<sup>+</sup>-CH-N-).

[C<sub>6</sub>Emim][AOT] (BAIL-2): <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>, δ-ppm)

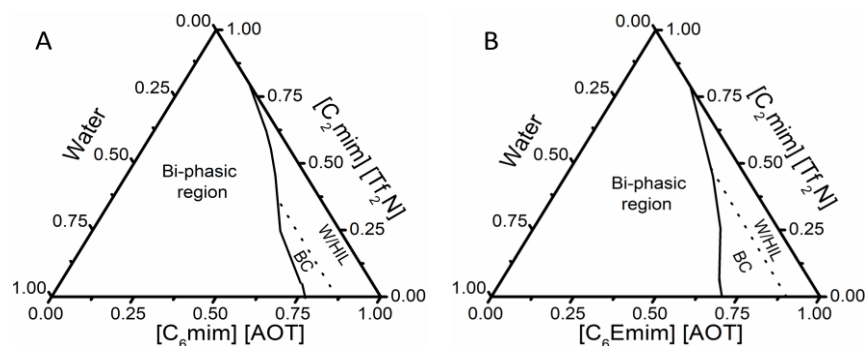
[AOT]<sup>-</sup>: 0.88 (m, 6H, -O-CH<sub>2</sub>-(CH<sub>2</sub>)<sub>6</sub>-CH<sub>3</sub>), 1.34 (m, 20H, -O-CH<sub>2</sub>-CH<sub>2</sub>-(CH<sub>2</sub>)<sub>5</sub>-CH<sub>3</sub>), 1.54 (m, 2H, -O-CO-CH<sub>2</sub>-CH-SO<sub>3</sub>-CO-O-CH<sub>2</sub>-(CH<sub>2</sub>)<sub>5</sub>-CH<sub>3</sub>), 1.63 (m, 2H, -CH<sub>2</sub>-CO-O-CH<sub>2</sub>-(CH<sub>2</sub>)<sub>5</sub>-CH<sub>3</sub>), 3.11 (m, 2H, -O-CO-CH<sub>2</sub>-CH-SO<sub>3</sub>-CO-O-CH<sub>2</sub>-(CH<sub>2</sub>)<sub>5</sub>-CH<sub>3</sub>), 3.95 (m, 4H, -O-CH<sub>2</sub>-(CH<sub>2</sub>)<sub>6</sub>-CH<sub>3</sub>), 4.09 (m, 1H, -O-CO-CH<sub>2</sub>-CH-SO<sub>3</sub>-CO-O-)

[C<sub>6</sub>Emim]<sup>+</sup>: 0.88 (m, 3H, -O-CH<sub>2</sub>-(CH<sub>2</sub>)<sub>4</sub>-CH<sub>3</sub>), 1.34 (m, 6H, -O-CH<sub>2</sub>-CH<sub>2</sub>-(CH<sub>2</sub>)<sub>3</sub>-CH<sub>3</sub>), 2.60 (m, 2H, -O-CH<sub>2</sub>-CH<sub>2</sub>-(CH<sub>2</sub>)<sub>3</sub>-CH<sub>3</sub>), 3.98 (s, 1H, -N-CH<sub>3</sub>), 4.19 (t, 2H, -O-CH<sub>2</sub>-CH<sub>2</sub>-(CH<sub>2</sub>)<sub>3</sub>-CH<sub>3</sub>), 5.20 (s, 2H, -N<sup>+</sup>-CH<sub>2</sub>-CO-), 7.42 (t, 1H, -N<sup>+</sup>-CH-CH-N-), 7.47 (t, 1H, -N<sup>+</sup>-CH-CH-N-), 9.76 (s, 1H, -N<sup>+</sup>-CH-N-).

[C<sub>6</sub>Amim][AOT] (BAIL-3): <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>, δ-ppm)

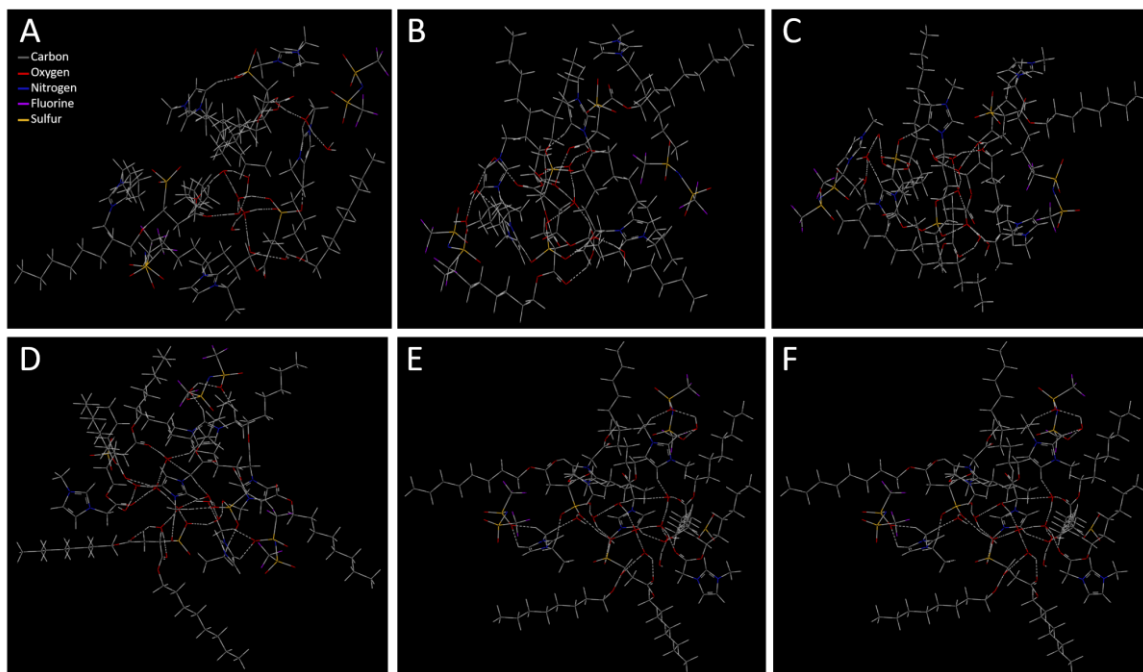
[AOT]<sup>-</sup>: 0.85 (m, 6H, -O-CH<sub>2</sub>-(CH<sub>2</sub>)<sub>6</sub>-CH<sub>3</sub>), 1.32 (m, 20H, -O-CH<sub>2</sub>-CH<sub>2</sub>-(CH<sub>2</sub>)<sub>5</sub>-CH<sub>3</sub>), 1.51 (m, 2H, -O-CO-CH<sub>2</sub>-CH-SO<sub>3</sub>-CO-O-CH<sub>2</sub>-(CH<sub>2</sub>)<sub>5</sub>-CH<sub>3</sub>), 1.62 (m, 2H, -CH<sub>2</sub>-CO-O-CH<sub>2</sub>-(CH<sub>2</sub>)<sub>5</sub>-CH<sub>3</sub>), 3.11 (m, 2H, -O-CO-CH<sub>2</sub>-CH-SO<sub>3</sub>-CO-O-CH<sub>2</sub>-(CH<sub>2</sub>)<sub>5</sub>-CH<sub>3</sub>), 3.97 (m, 4H, -O-CH<sub>2</sub>-(CH<sub>2</sub>)<sub>6</sub>-CH<sub>3</sub>), 4.12 (m, 1H, -O-CO-CH<sub>2</sub>-CH-SO<sub>3</sub>-CO-O-)

[C<sub>6</sub>Amim]<sup>+</sup>: 0.85 (m, 3H, -O-CH<sub>2</sub>-(CH<sub>2</sub>)<sub>4</sub>-CH<sub>3</sub>), 1.32 (m, 6H, -O-CH<sub>2</sub>-CH<sub>2</sub>-(CH<sub>2</sub>)<sub>3</sub>-CH<sub>3</sub>), 2.96 (m, 2H, -O-CH<sub>2</sub>-CH<sub>2</sub>-(CH<sub>2</sub>)<sub>3</sub>-CH<sub>3</sub>), 3.94 (s, 1H, -N-CH<sub>3</sub>), 4.12 (t, 2H, -O-CH<sub>2</sub>-CH<sub>2</sub>-(CH<sub>2</sub>)<sub>3</sub>-CH<sub>3</sub>), 5.03 (s, 2H, -N<sup>+</sup>-CH<sub>2</sub>-CO-), 7.24 (t, 1H, -N<sup>+</sup>-CH-CH-N-), 7.57 (t, 1H, -N<sup>+</sup>-CH-CH-N-), 8.12 (t, 1H, -CO-NH-CH<sub>2</sub>-), 9.21 (s, 1H, -N<sup>+</sup>-CH-N-).

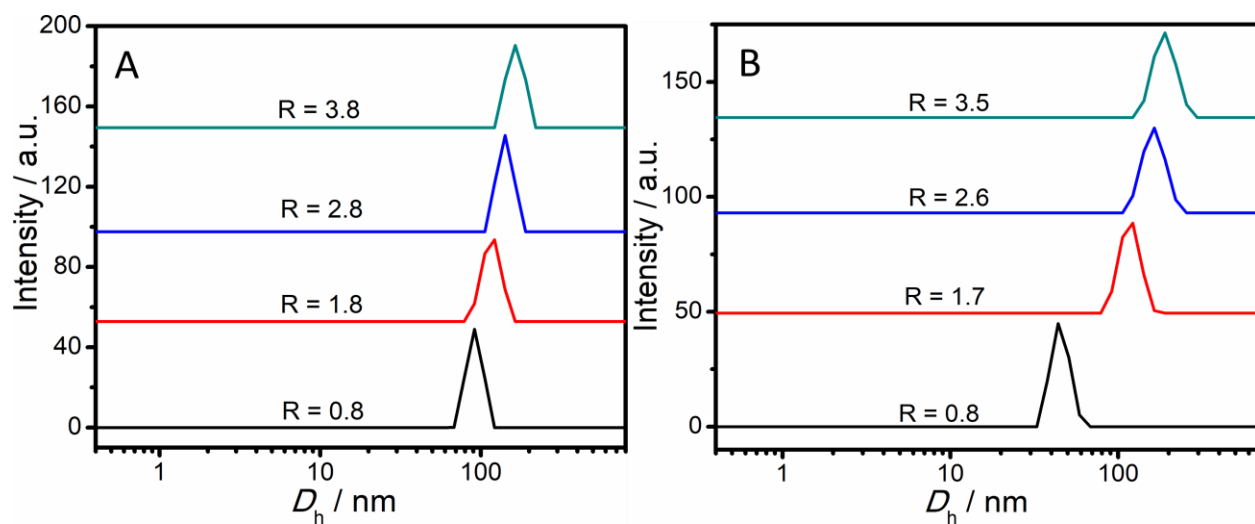


**Figure S1.** Phase diagram of (A)  $\mu$ E-1 and (B)  $\mu$ E-2 showing different regions of microemulsions.

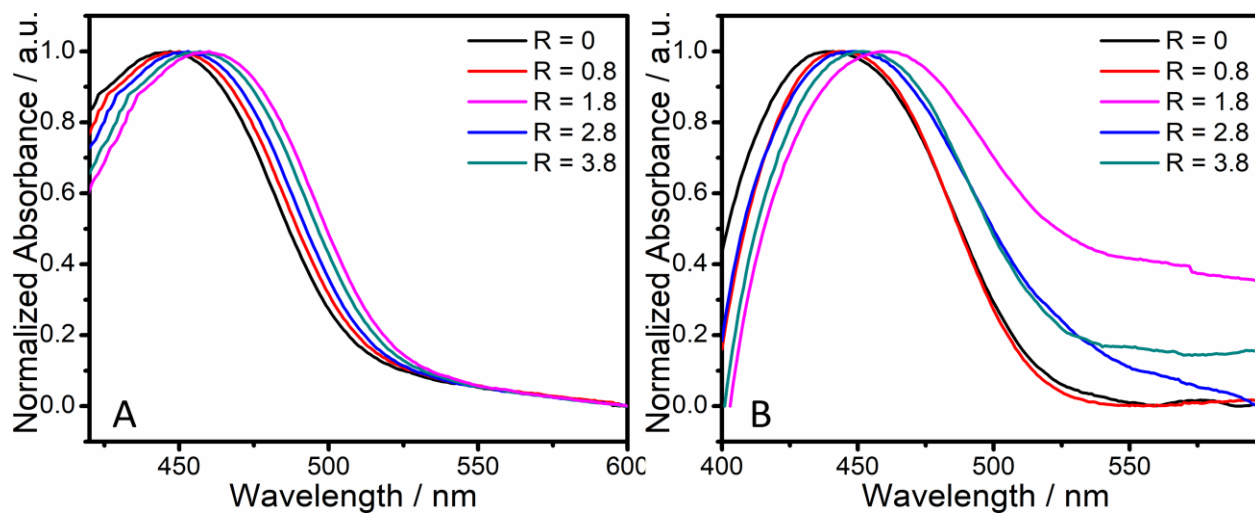
To optimize the geometric configuration of different  $\mu$ Es, DFT (Density Functional Theory) calculations have been carried out using Gaussian 09 software package.<sup>5</sup> In this work, B3LYP hybrid functional along with 6-311+G(d, p) basis set was used by taking the molar composition of different components on which the formation of  $\mu$ E occurs. The Figure S2 shows that the outcome of DFT is in good agreement with the experimental results. Figure S2 reveals the presence of *H*-bonding interactions between BAIL and water in the  $\mu$ Es. Moreover, in case of  $\mu$ E-2, dipole-dipole interactions are also observed along with *H*-bonding interactions as shown in Figure D-F. Also, we have calculated dipole moments (Table S1) of different ions constituting the  $\mu$ E systems using same computation method.



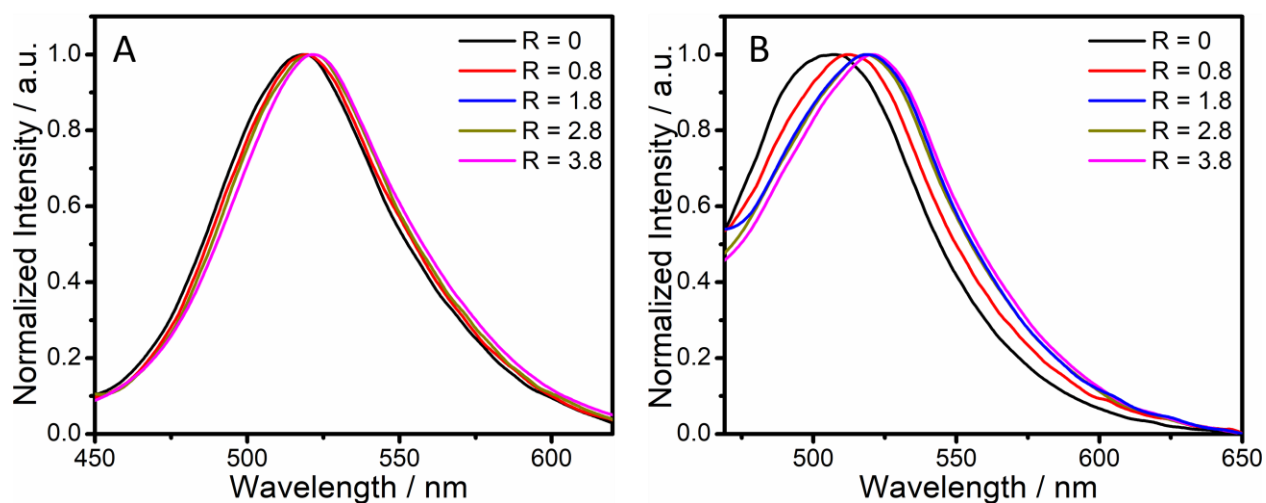
**Figure S2.** Images showing formation of (A-C)  $\mu$ E-1 and (D-F)  $\mu$ E-2.



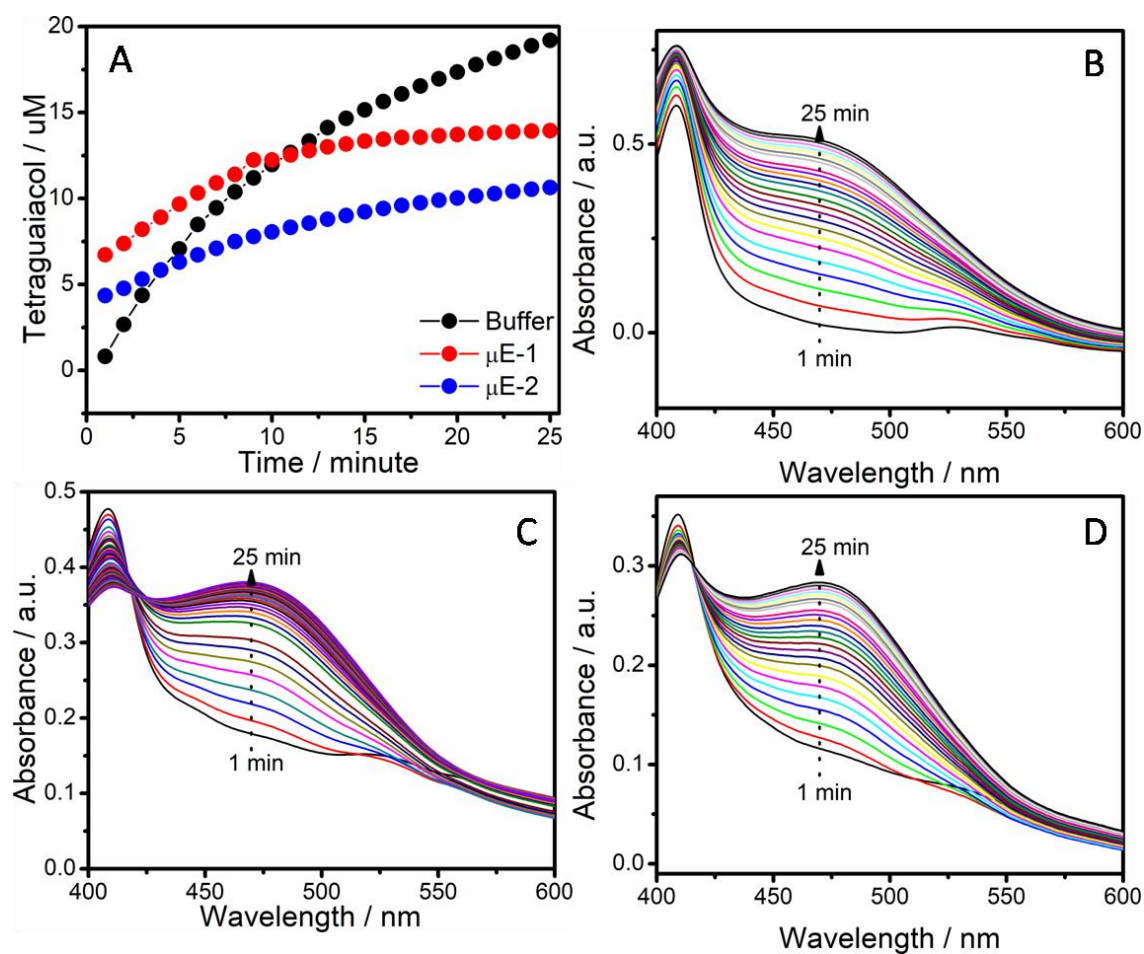
**Figure S3.** Intensity weighted size distribution of (A)  $\mu\text{E-1}$ ; (B)  $\mu\text{E-2}$  as a function of  $R$ .



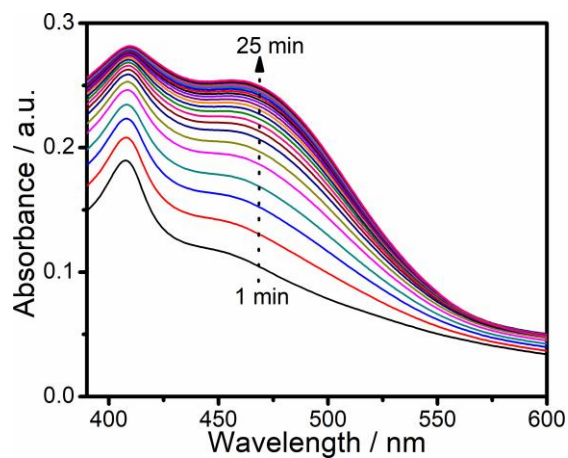
**Figure S4.** Absorption spectra of MO in (A)  $\mu\text{E-1}$ ; (B)  $\mu\text{E-2}$  as a function of  $R$ .



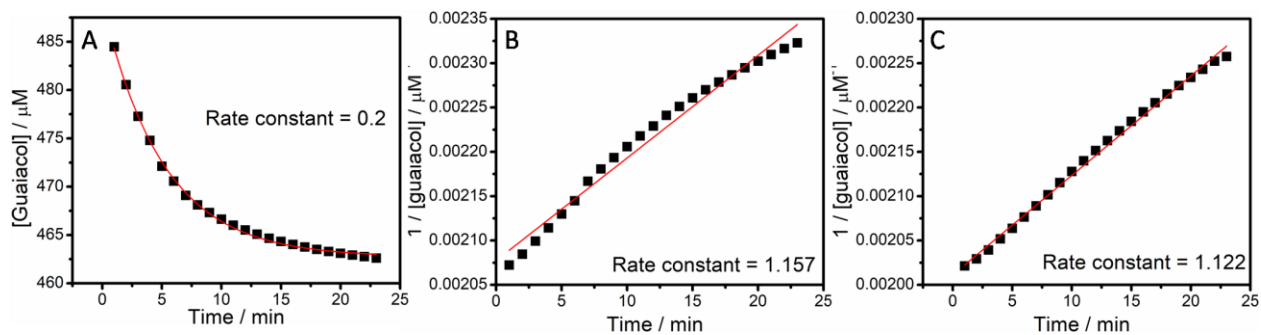
**Figure S5.** Emission spectra of C-153 in (A)  $\mu$ E-1; (B)  $\mu$ E-2 as a function of R.



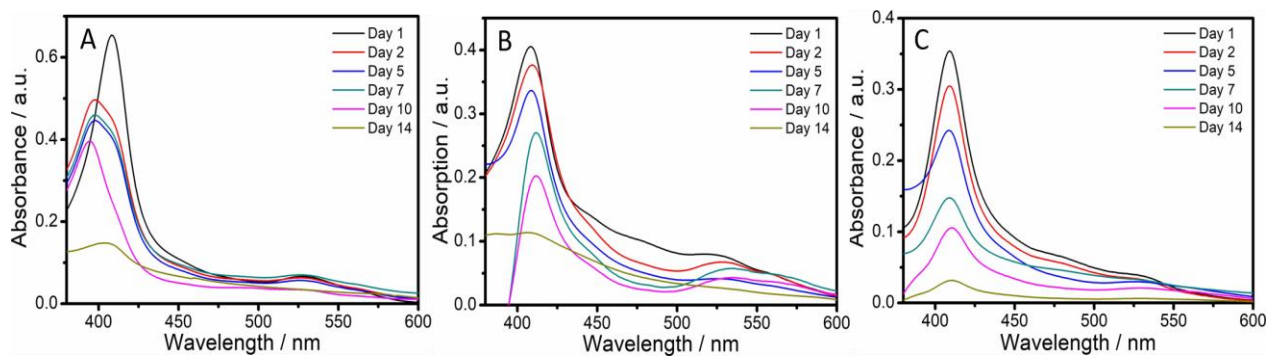
**Figure S6.** (A) Catalytic activity of Cyt C in buffer,  $\mu$ E-1 and  $\mu$ E-2: Absorption spectra of tetraguaiacol at room temperature for 25 minutes in (B) buffer; (C)  $\mu$ E-1; (D)  $\mu$ E-2.



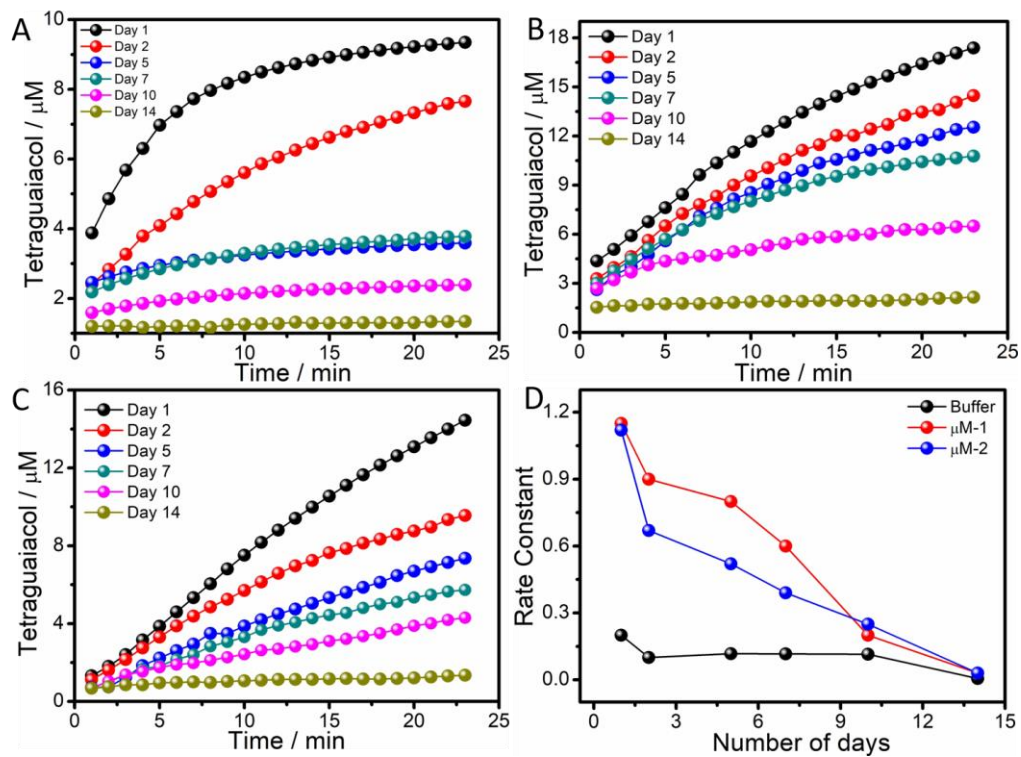
**Figure S7.** Absorption spectra of tetraguaiacol at room temperature in buffer-ethanol mixture.



**Figure S8.** Kinetics of (A) buffer; (B)  $\mu$ E-1 and (C)  $\mu$ E-2.



**Figure S9.** Absorption spectra of Cyt-c in (A) Buffer; (B)  $\mu$ E-1 and (C)  $\mu$ E-2 with time.



**Figure S10.** (A) Catalytic activity of Cyt-C in (A) Buffer, (B)  $\mu$ E-1 and (C)  $\mu$ E-2 with time; (D) Rate constant of Cyt-c in buffer and  $\mu$ Es as a function of days.

**Table S1.** Dipole moments of ionic liquid ions

Ions	Dipole moment ( $\mu / D$ )
[AOT] <sup>-</sup>	16.7
[C <sub>6</sub> mim] <sup>+</sup>	10.6
[C <sub>6</sub> Emim] <sup>+</sup>	14.18
[C <sub>2</sub> mim] <sup>+</sup>	1.65
[Tf <sub>2</sub> N] <sup>-</sup>	4.32



## References

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