Supplementary Material (ESI) for Green Chemistry

Supporting Information for: Water-in-Ionic Liquid Microemulsions as a New Medium for Enzymatic Reactions

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Materials: IL [C_8mim][Tf_2N] (1-octyl-3-methyl imidazolium bis(trifluromethyl sulfonyl) amide) was synthesized and stored as described elsewhere.[1,2] The purity of IL was verified by elementary analysis, showing that analytically calculated values and experimental values were the same. The reagents for the synthesis of IL, the surfactant AOT, and the substrate *p*-nitrophenyl butyrate (*p*-NPB) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO) and used as received. All the enzymes were commercial preparations, i.e., lipase PS from Amano Enzyme (Nagoya, Japan); α-chymotrypsin from Sigma; horse radish peroxidase (HRP) from Wako; and *Candida antarctica* lipase B (CALB) from Novoenzyme. Enhanced green fluorescence protein (EGFP) was obtained as reported previously.[3]

Spectrophotometric study of substrate and product: *Fig. S-1* shows absorption spectra for the substrate, *p*-nitrophenyl butyrate (*p*-NPB) and for the product, *p*-nitrophenol *(p*-NP) in AOT/water/1-hexanol/IL systems at W_0 (molar ratio of water to AOT) 4. The reaction product, *p*-NP is partially in the form of the *p*-nitrophenolate anion (p*K*≈ 7.0), which absorbs light at 410 nm.[4] These absorption spectra indicate that the interference from the substrate with a *p*-NP absorbance at 410 nm is negligible. The same trend was observed during a spectrophotometric study of the substrate and the product in AOT/water/isooctane microemulsions (data not shown). Accordingly, the reactions were followed recording the absorbance at 410 nm versus time.

Determination of the molar extinction coefficient (ε) of *p*-nitrophenol in AOT/water/1-hexanol/IL systems: For determining **ε,** the standard curve (absorbance vs. concentration of *p*-NP) has been prepared (Fig.S-2). For this purpose, we dissolved *p*-NP in the IL at various concentrations and measured the absorbance of each solution using a UV-Vis spectrophotometer (Jasco V-570) at 410 nm. The molar extinction coefficient of *p*-NP in water-in-IL microemulsions was determined using the prepared standard curve and found to be 1080 M^{-1} cm^{-1} at the given conditions. The molar extinction coefficient of *p*-NP in AOT/water/isooctane microemulsions (at $W_0 = 15$) was also determined to be 3500 M^{-1} cm^{-1} at 410 nm. The initial reaction rate was calculated based on these coefficients in individuals systems.

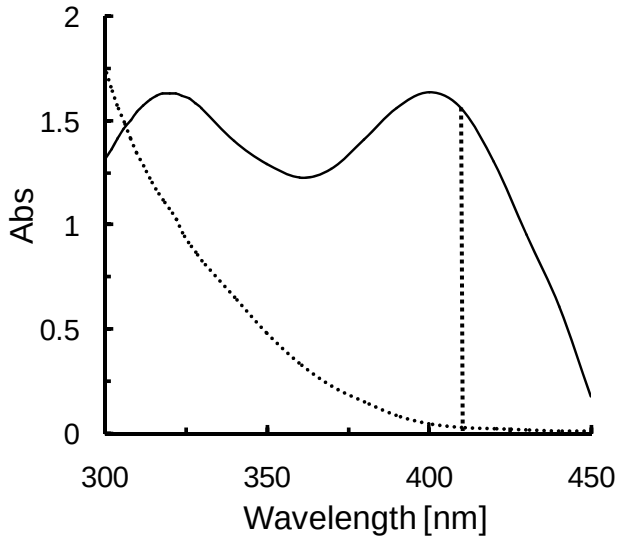

Fig.S-1 UV-Vis spectra of *p*-NPB (dotted line) and *p*-NP (solid line).

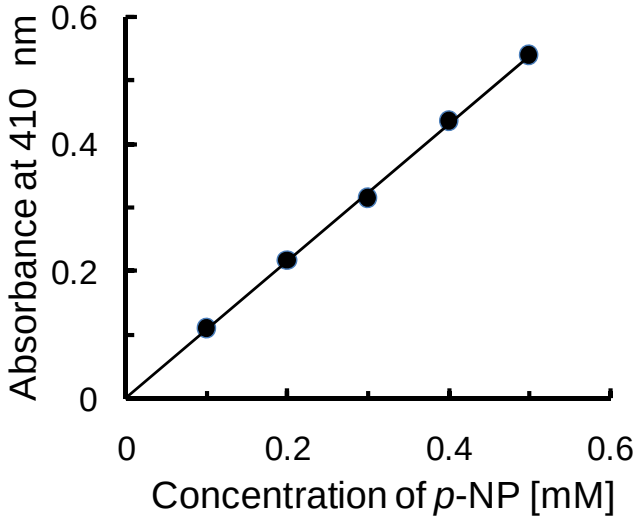

Fig.S-2 Calibration curve for the absorbance of *p*-NP in AOT/water/1-hexanol/IL microemulsional system at W_0 =4.

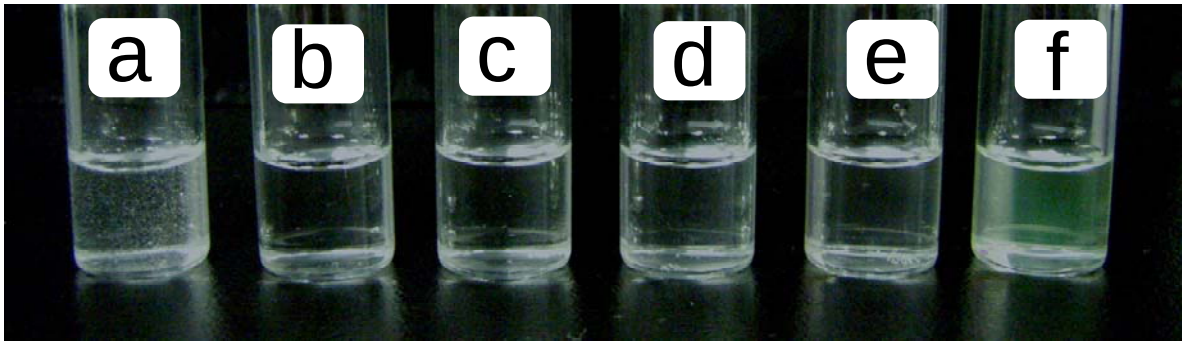

Fig.S-3 Photograph showing the solubility of enzymes(1mg/ml based on the water pool volume of the system) at 25ºC: (a) PS lipase in IL [C_8mim][Tf_2N] and (b-f) enzymes in AOT/IL/1-hexanol/water microemulsions with W_0 = 4; (b) PS lipase, (c) *Candida antarctica* lipase B (CALB) (d) α-chymotrypsin (e) *Horse radish* peroxidase (HRP) and (f) Enhanced Green fluorescent protein (EGFP).

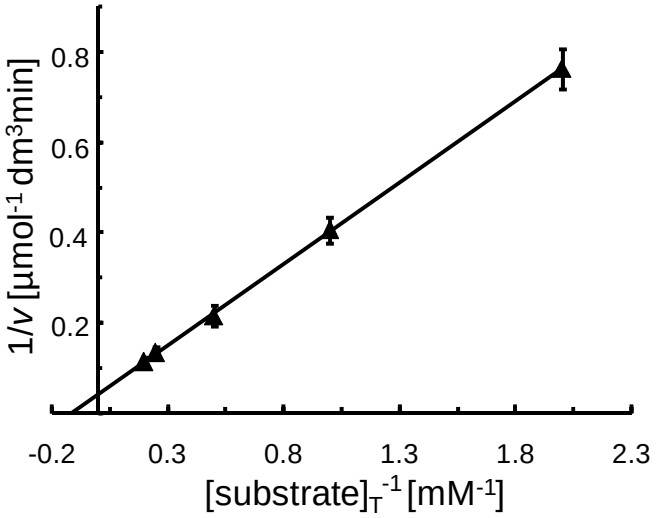

Fig.S-4 A typical Lineweaver-Burk plot for lipase-catalyzed hydrolysis of *p*-NPB in AOT/water/ IL microemulsion. Experimental conditions were [AOT] = 0.15 M, [lipase] =0.64 μM, W_0= 4 and buffer pH=8.0.

References

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2. J. G. Huddleston, A. E. Visser, W. M. Reichert, H. D. Willauer, G. A. Broker and R. D. Rogers, *Green Chem*., 2001, **3**, 156.
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4. R.C. Weast, 1980, *in Handbook of Chemistry and Physics 61st edition*, CRC Press, Boca Raton.