Supporting Information

Characterization of Ionic Liquid Aqueous Two-Phase Systems: Phase Separation Behaviors and the Hydrophobicity Index between the Two Phases

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Supplementary Method for Hydrophobicity Factor (*HF*) of Aqueous Two-Phase Systems (ATPS)

A systematic approach to the characterization and quantitative determination of surface properties of biomolecules has previously been reported by using an aqueous two-phase system (ATPS).[S1] Previous researchers mainly focused on the use of functional polymers in the ATPS, such as charged ligands (tetra-methyl-acetate-PEG or PEG-sulfonate),[S2, S3] hydrophobic ligands (palmitate- PEG,[S4, S5] and biospecific ligands (antibodies-PEG).[S6] If such ligands are not attached to the polymer, the partition coefficient of biomolecules in an ATPS has been found empirically to depend upon several factors, which act independently. The partitioning coefficients of biomolecules, such as amino acids, peptides, proteins (enzymes), liposomes and cells, may therefore be expressed as follows:[S7, S8]

$$\ln K = K_{electorostatic} + K_{hydrophobic} + K_{salt} + K_{lignd} + \ldots \quad (1)$$

where $K_{electorostatic}$, $K_{\mathrm{hydrophobic}}$, K_{salt}, and K_{lignd} represent the contribution to the partitioning of the biomolecules by electrostatic, hydrophobic, salt and ligand effects, respectively. From consideration of these effects, the surface of biomolecules can be systematically characterized. It is then considered that the ATPS method can also be applied to the characterization of the surface properties of bacterial cells, which are highly organized biomolecular assemblies.

Tjerneld has found that the pH dependence of the partition coefficient of cells in an ATPS containing some types of salt showed a common cross point at pI (cross partition method).[S7] At the pI and low ionic strength, the values of ln $K_{electorostatic}$ and lnK_{salt} can be ignored, and the following relationship can then be obtained,

$$\ln K = K_{hydrophobic} \quad (2)$$

Nozaki and Tanford evaluated the hydrophobicities of several amino acids in water/ethanol and water/dioxane systems.[S9] In our previous study, a relationship between the Nozaki-Tanford values and the partition coefficients of amino acids was elaborated,[S1] and the following equation on the definition of hydrophobicity factor (*HF*) values can then be obtained:

$$\ln K = HF \times (RH + B) \quad (3)$$

where *RH* is the relative hydrophobicity based on the Nozaki-Tanford value and *B* is the normalization constant defined as the ratio of the partition coefficient and the hydrophobicity of glycine, $\ln K_{Gly}/\Delta G_{Gly}$. The hydrophobicity differences between the two phases in an ATPS can be described as *HF*, as for example shown in **Fig. S1** and the surface net hydrophobicity of protein (*HFS*) from the slope of Eq. (4) using ATPS.

$$\ln K = HFS \times HF \quad (4)$$

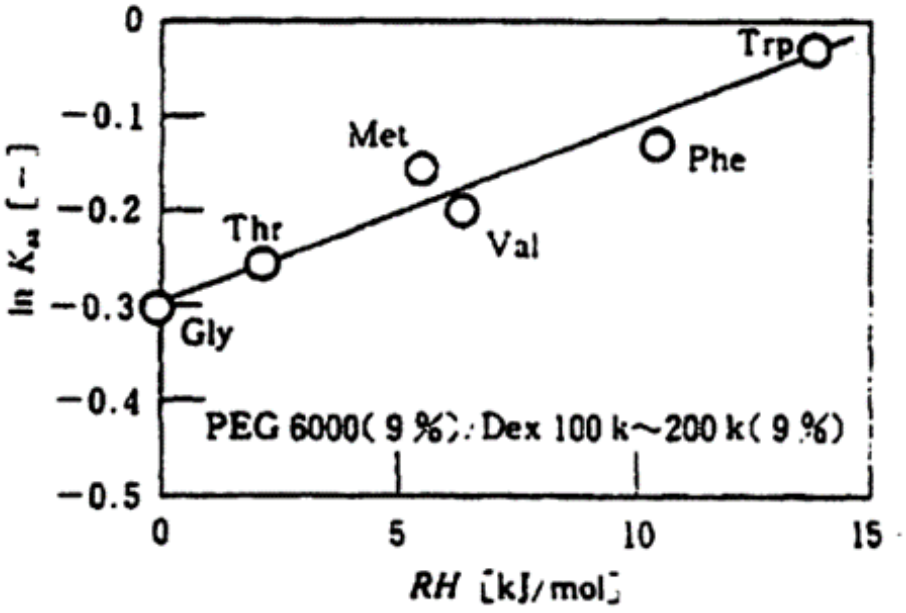

Figure S1. Relationship between the partition coefficients of amino acids and their hydrophobicities determined by Nozaki and Tanford.[S9]

The above equation can provide quantitative data based on the surface properties of a variety of biomolecules such as amino acids, peptides, proteins (enzymes), liposomes and bacterial cells. As an analogy, the hydrophobicity of other types of aqueous two-phase systems such as reverse micellar systems (RVMS)[S10] and liposome membrane systems (LMS)[S11] can also be evaluated based on the partition behavior of amino acids between two phases. **Figure S2** shows the *HF* values of some aqueous systems such as RVMS and LMS, which are evaluated based on the relationship between the

partitioning behaviors of amino acids and their hydrophobicity (**Fig. S2a**).[S12] As shown in **Fig. S2b**, the ATPS was in general found to provide the wide spectra of HF values from 0.005-0.35mol/kJ, depending on the type and concentration of phase forming components such as poly (ethylene glycol), dextran, phosphate salts and the random copolymer of PO and EO (BREOX).[S13] The *HF* values for the RVMS were higher than that usually found for ATPSs and they varied with the water contents and types of detergents. The LMS indicated lower *HF* value and, especially in the cease of the POPC liposome, the *HF* value was nearly equal to that of PEC6 (9%) /dextran (9%) two phase system, which can be classified as an ATPS with a higher *HF* value in PEG/dextran aqueous polymer two phase system. It was this found that the *HF* values for various types of aqueous two phase systems could be characterized on the same scale based on the Nozaki-Tanford value and the partitioning behaviors of large target biomolecules in the systems could be controlled by the *HF* values.

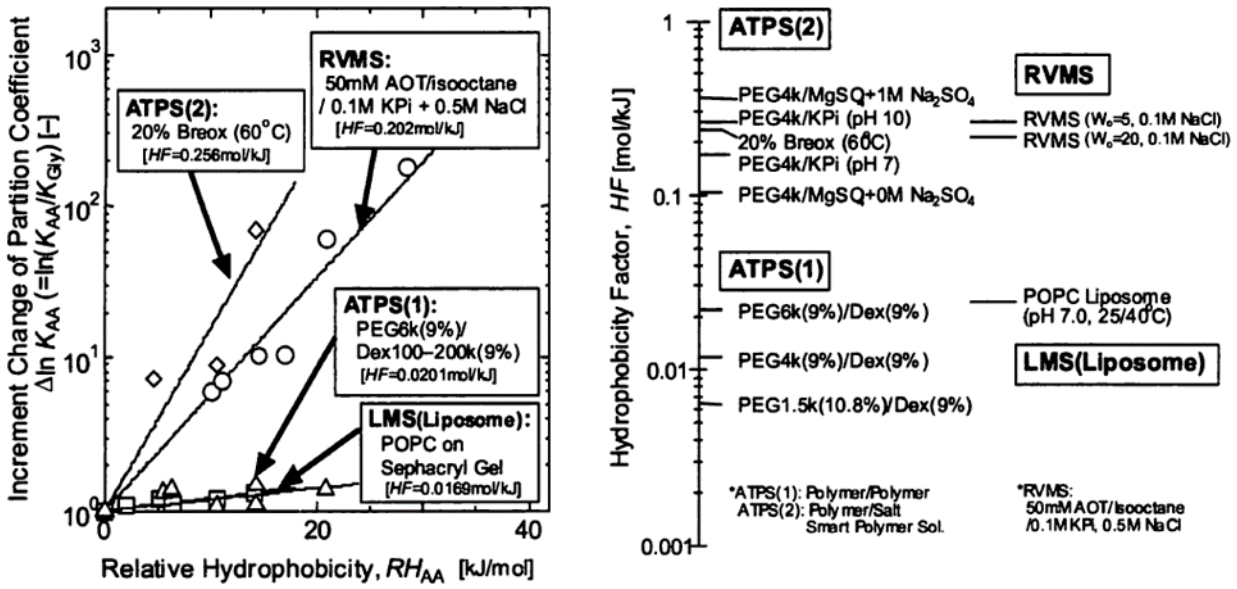

Figure S2. *HF* value of various aqueous two-phase systems.[S12] (a) Relationship between the partition coefficients of amino acid and their hydrophobicities determined by Nozaki and Tanford in various aqueous two-phase systems such as ATPS, RVMS, and LMS. (b) *HF* ladder for these systems.

Evaluation of HFS value of cellulase using conventional ATPS.

The *HFS* value of the cellulase from *Trichoderma viride* (Yakult Pharmaceutical Industry (Tokyo, Japan)) was determined based on the partitioning behaviors in PEG/Dex system (**Fig. S3**). The *HFS* value is −222.1 kJ/mol, indicating that cellulase has hydrophilic surface as well as bovine serum albumin (ca. -215 kJ/mol[S12]). Considering the *HF* values of IL-ATPS (0.13-0.41 mol/kJ), it is assumed that most of cellulase could be distributed into bottom phase.

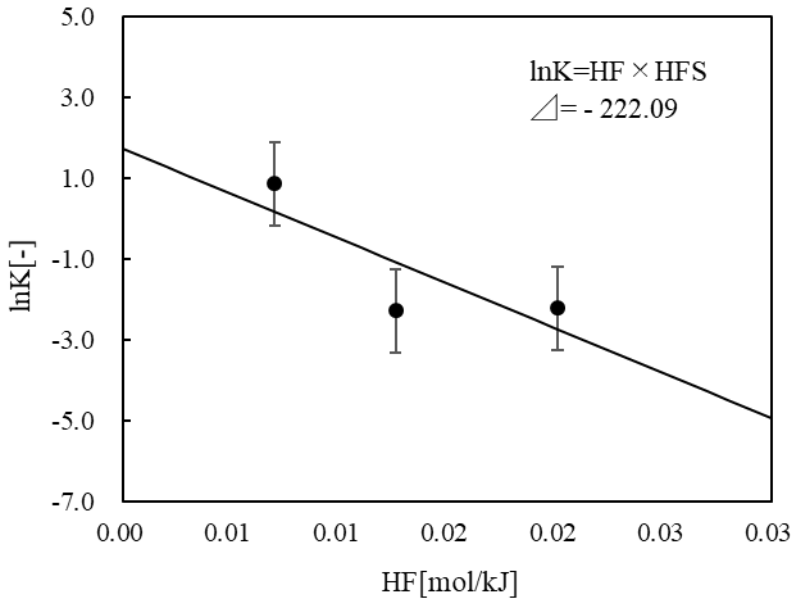

Figure S3. Partitioning behaviors of cellulase in PEG/Dex ATPS. The amount of cellulase in top and bottom phases were determined by fluorescamine method. Experiments were carried out at 25℃.

Nomenclature

HF = hydrophobic index [mol/kJ]

K = partitioning coefficient [-]

K_{aa} = partitioning coefficient of amino acid [-]

RH = relative hydrophobicity [kJ/mol]

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