Supporting Information

Active-site motions and polarity enhance catalytic turnover of hydrated subtilisin dissolved in organic solvents

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Enzyme Structure in Tetrahydrofuran

Active-site structure was assessed through the steady state ^1H-^{19}F Nuclear Overhauser Effect (NOE) enhancement factor. The NOE in THF was -0.73 at a_w=0, indicative of a folded but not tightly packed active-site, and increased sharply at between a_w = 0.1-0.3 (Figure S1A). This evinces a loss of proton contact around the fluorine nucleus and is typical of an unstructured environment.[1] Interestingly, at the hydration level where the enzyme exhibited maximum catalytic activity, a_w=0.2, an NOE of -0.6 was recorded, suggesting that the active-site was at least partially unfolded. The NOE in isooctane at a_w=0 was -0.82, close to the aqueous enzyme NOE of -0.85, and did not change at full hydration.

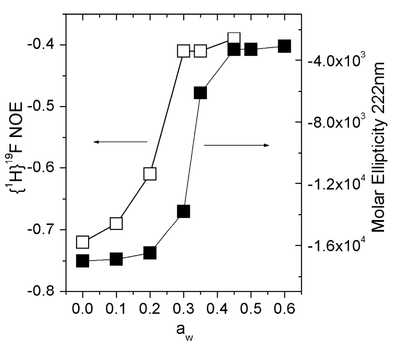

Figure S1. Global and active-site stability of 4FBS-subtilisin in tetrahydrofuran. (A) ^1H-^{19}F NOE (□) and molar ellipticity (■) were calculated as described in *Experimental.*

Empirical Relation Between ^{19}F Chemical Shift and Solvent Dielectric Constant

Values for the chemical shift were taken at infinite dilution for acetone, isooctane, and 1-propanol.

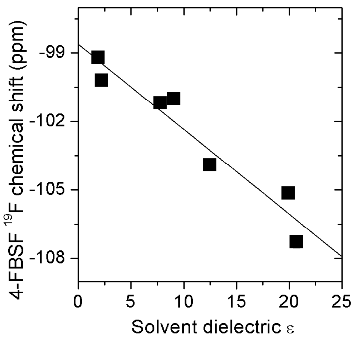

Figure S2. Calibration of ^{19}F chemical shift with solvent dielectric constant. The aromatic ^{19}F resonance of 4FBSF (10-500 μM) is referenced to $CFCl_3$ at 376MHz. Solvents used were 1-PrOH (ε=20.1), acetone (20.6), THF (7.8), methylene chloride (8.9), t-butanol (12.5), hexane (1.9), isooctane (2.0)

References

(1) Kairi, M.; Gerig, J. T. *Mag Res Chem* **1990**, *28*, 47-55.