Supporting Information

Substrate catalysis enhances single enzyme diffusion

Hari S. Muddana[1], Samudra Sengupta[2], Thomas E. Mallouk[2], Ayusman Sen[2], Peter J. Butler[1*]*

[1]Department of Bioengineering, The Pennsylvania State University, University Park, Pennsylvania 16802

[2]Department of Chemistry, The Pennsylvania State University, University Park, Pennsylvania 16802

Email: pbutler@psu.edu, asen@psu.edu

I. Experimental Methods

A. Fluorescence tagging of urease enzyme and urea inhibition studies

Jack bean urease (type C-3) was purchased from Sigma-Aldrich, Inc. and tagged with thiol-reactive dyes, Dylight 549 (ex/em: 549/568; Thermo Fisher Scientific, Inc.) for single molecule diffusion measurements, or 5-(and-6) chloromethyl SNARF-1 acetate (ex/em:550/640nm; Invitrogen, Inc.), for time-resolved single-molecule fluorescence lifetime measurements. Reaction of fluorescence probe with urease was carried out in 150 mM phosphate buffer (pH 7.2) under room temperature conditions overnight. Enzyme-probe complex was further purified using membrane dialysis to reduce free dye concentration. Inhibition experiments were conducted using pyrocatechol at 1 mM concentration. Kot *et al*. have shown that pyrocatechol acts as a time- and concentration-dependent irreversible inactivator of

urease.[1] Urease was incubated with the inhibitor at room temperature for at least two hours prior to conducting diffusion and lifetime measurements. At higher concentrations (10 mM and above), it was found that pyrocatechol denatured the protein significantly, as evidenced by the significantly lower diffusion coefficients.

B. Time-correlated single-photon counting (TCSPC)

Single-molecule diffusion and fluorescence lifetime measurements were performed using a custom-built time-correlated single photon counting (TCSPC) setup developed in our lab.[2] The optical setup for TCSPC was based on an Olympus IX71 inverted microscope. A water-cooled 80 MHz, 5.4 ps, 75 mW, pulsed solid-state laser (λ = 532 nm; High-Q laser, Hohenems, Austria) was used as the excitation light source. Laser light was cleaned by appropriate excitation filter and expanded to slightly underfill the back aperture of the objective to ensure a Gaussian illumination profile. The laser power as measured at the back aperture of the objective was adjusted to 50 μW to avoid photobleaching and triplet state formation. Excitation light from the laser was reflected by a suitable dichroic mirror and focused in the sample using a 60x water immersion objective (UPLAPO60XW, Olympus). Fluorescence emission from the sample was passed through the dichroic mirror, a set of high quality emission filters (580 nm-640 nm), and a polarizer fixed at the magic angle, before focusing onto a 50 μm/0.22NA optical fiber that served as the confocal pinhole. The optical fiber was coupled to a GaAsP photomultiplier tube (H7422-40P, Hamamatsu, Tokyo, Japan) with a peak quantum efficiency of 40% at 580 nm wavelength. Photon arrival times were recorded using the SPC-630 TCSPC module (Becker and Hickl, Berlin, Germany).

Fluctuations in fluorescence intensity arising from diffusion of molecules into and out of the diffraction-limited confocal observation volume were aucorrelated and fit by a multi-component 3D diffusion model (equation 1) to determine the diffusion coefficient of individual species,[3-5]

$$G(\tau) = \sum_{i=1}^{n} \frac{1}{N_i} \left[1 + \left(\frac{\tau}{\tau_D^i}\right)\right]^{-1} \left[1 + \left(\frac{1}{w}\right)^2 \left(\frac{\tau}{\tau_D^i}\right)\right]^{-\frac{1}{2}} \text{ with } \tau_D^i = \frac{r^2}{4D_i} \quad (1)$$

where N_i is the average number of diffusing fluorophores of i^{th} species in the confocal observation volume, τ is the autocorrelation time, w is the structure factor, and $\tau_D{}^i$ is the characteristic diffusion time of the fluorophores of i^{th} species with diffusion coefficient D_i crossing a circular area with radius r. Autocorrelation curves were fit using Levenberg-Marquardt nonlinear least-squares regression algorithm with the aid of Origin software (Originlab, Northampton, Massachusetts). Radius of the observation volume determined using the known diffusion coefficient of Rhodamine 6G (D = 2.8*10^{-6} cm^2/sec in water) was ~400 nm and the structure factor was between 4 and 6 for all experiments. Assuming that the diffusing fluorophore behaves as a hard sphere, hydrodynamic radius (R_h) of the fluorophore was calculated from the diffusion coefficient using Stokes-Einstein relationship given by [6],

$$R_h = \frac{K_B T}{6\pi\eta D} \quad (2)$$

where K_B is Boltzmann's constant, T is absolute temperature, and η is solvent viscosity.

C. Fluorescence lifetime of SNARF-1

Fluorescence lifetime was measured using a pulsed-laser excitation source. Fluorescence decay curves were extracted from histograms of photon arrival times relative to the laser pulse time and fit to a bi-exponential decay model [5]. Fluorescence emission from the sample was detected at the magic angle (54.7^0) to avoid the effects of rotational diffusion on fluorescence lifetime. Curve fitting was done using Fluofit software (PicoQuant, GmbH, Berlin, Germany) by a process of iterative reconvolution given by [2],

$$I(t) = \int_{-\infty}^{t} IRF(t') \sum_{i=1}^{n} A_i e^{-\left(\frac{t-t'}{\tau_i}\right)} dt' \quad (3)$$

where $I(t)$ is the experimental decay function, $IRF(t)$ is the instrument response function, A_i is the amplitude of i^{th} lifetime, τ_i is the i^{th} lifetime, and n is the number of exponents. The instrument response function (IRF) was collected from a sample of dilute scattering solution, prior to the experiment. The full width at half maximum (FWHM) of the IRF was calculated to be ~330 ps. The quality of the fitted curves was evaluated based on χ^2 values and the autocorrelation of residual curves. Fitting the decay curves to a decay model with more than two exponentials did not improve the goodness-of-fit significantly. The amplitude-weighted average lifetimes $\langle\tau\rangle$ were calculated by [5],

$$\langle\tau\rangle = \frac{\sum_{i=1}^{n} A_i \tau_i}{\sum_{i=1}^{n} A_i} \tag{4}$$

D. Brownian dynamics simulation methodology

Brownian dynamics simulations were performed using the position Langevin dynamics, that is, inertial effects are assumed to be negligible. Position Langevin dynamics equation is as follows,

$$\frac{d\vec{r}}{d\vec{t}} = \frac{\mathbf{1}}{\gamma} F(r) + G \tag{5}$$

where γ is the friction coefficient, F is the force acting on the particle, and G is the Gaussian noise process with $\langle G_i(t).G_j(t+\Delta t)\rangle = 2.\Delta t.\delta_{ij}.K_B.\frac{T}{\gamma}$. The above Langevin dynamics equation was numerically integrated using the following equation,

$$r(t+\Delta t) = r(t) + \frac{\Delta t}{\gamma} F(t) + \sqrt{2K_B T \frac{\Delta t}{\gamma}}\, r^G \tag{6}$$

where γ is the Stokes-Einstein drag coefficient for a spherical particle, K_B is the Boltzmann's constant, r^G is the Gaussian distributed noise with mean = 0 and standard deviation = 1, and Δt is the integration

time step (10 ns). Total simulation time was 100 ms.

II. Supplementary Results

A. Local Temperature

The local rise in the temperature due to enzymatic urea hydrolysis reaction was estimated. The heat liberated (***q***) by a single enzyme (urease) molecule from the catalytic hydrolysis of urea will be,

$$q = nk_{cat}\Delta H/N_A \tag{7}$$

where, n is number of catalytic sites per enzyme molecule (6 catalytic sites per urease molecule), k_{cat} is enzyme (urease) turnover number (2.3×10^4 s^{-1}) , ΔH is the enthalpy change in the reaction (-59,580 J mol^{-1}), and N_A is Avogadro's number (6.023×10^{23} molecules mol^{-1}). The heat transfer (***q***) from the enzyme (urease) to the surrounding solvent is

$$q = 4\pi\kappa R\Delta T \tag{8}$$

where, κ is thermal conductivity of water (0.58 W $m^{-1}K^{-1}$), R is radius of the enzyme (7×10^{-9} m), and ΔT is change in temperature in K. Comparing (7) and (8), we calculate ΔT to be 2.6×10^{-7} K.

B. Electrolytic friction coefficient

The complete protein sequence of Canavalia ensiformis urease was obtained from the GenBank database (AccessID: AF468788). The charge of the urease enzyme at different pH values was estimated using the Protein Calculator v3.3 available at http://www.scripps.edu/~cdputnam/protcalc.html, as shown in Table S.1. The estimated isoelectric point was at pH 5.75, which is in reasonable agreement with the available experimental value of 5.2.[7] Note that the charge of urease decreases from -19.3 at pH 7.0 to -28.4 at pH 8.0.

pH	Charge
4.0	63.7
4.5	39.2
5.0	17.1
5.5	4.3
6.0	-3.9
6.5	-12.0
7.0	-19.3
7.5	-24.2
8.0	-28.4
8.5	-33.6
9.0	-40.5

Table S.1. Estimated charge of urease enzyme at different pH values.

The decrease in electrolyte friction coefficient due to the increase in ionic strength and the decrease in enzyme charge were estimated as described in Noyola et al.[8] The diffusion coefficient of a charged Brownian particle in an electrolyte is given by the following equation,

$$D = \frac{K_B T}{\xi^h + \xi^e}, \qquad \xi^h = 6\pi\eta R, \quad \xi^e = \left[\frac{Q^2}{12\varepsilon D^0 \kappa R^2}\right]\left[1 - e^{-2\kappa R}(1 + 2\kappa R)\right] \tag{9}$$

where, ξ^h and ξ^e are the hydrodynamic and electrolyte friction coefficients, respectively, η is viscosity, R is the radius of the molecule, Q is the charge, D^0 is the diffusion coefficient of ions (2 x 10^{-5} cm^2/s), κ^{-1} is the Debye length, K_B is the Boltzmann constant, and T is temperature. Upon hydrolysis, each urea molecule forms two ammonium ions and one bicarbonate ion, as a dihydrogen phosphate ion in the buffer is deprotonated; that is, complete hydrolysis of one molar of urea results in the net production of three moles of ions according to equation (10).

$$H_2NCONH_2 + 2\ H_2O + H_2PO_4^- \rightarrow 2\ NH_4^+ + HCO_3^- + HPO_4^{2-} \tag{10}$$

The ionic strength (I) of the buffer used in all the experiments was 150 mM. Based on equation 9, the

predicted diffusion coefficient of urease in buffer (pH = 7.2; I = 150 mM) was 3.11 x 10^{-7} cm^2/s, whereas at the maximum urea concentration (pH = ~8.0 and I = 3.15 M) it was 3.25 x 10^{-7} cm^2/s. That is, a maximum of 4.5% change in diffusion coefficient is expected due to changes in pH and ionic strength without contributions from diffusiophoresis.

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