Brownian dynamics assessment of enhanced diffusion exhibited by ‘fluctuating-dumbbell enzymes’
Supplementary Information

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S1. SUBSTRATE DEPENDENT DIFFUSION COEFFICIENT

Following the arguments from ref. 1, we consider an enzyme in three states: open (o), close (c), and close with a substrate attached to its active site (cs). Denoting by E_o and E_c an enzyme in the open and close state, respectively, and by SE_{cs} a substrate-enzyme complex, one can assume the following simple kinetics

$$\mathrm{E}_c \underset{\mathrm{k}_o}{\overset{\mathrm{k}_c}{\rightleftharpoons}} \mathrm{E}_o\,, \tag{S1}$$

$$\mathrm{E}_o + \mathrm{S} \underset{\mathrm{d}}{\overset{\mathrm{a}}{\rightleftharpoons}} \mathrm{S}\,\mathrm{E}_{cs} \xrightarrow{\mathrm{k}} \mathrm{P} + \mathrm{E}_o, \tag{S2}$$

where k_c and k_o are the close-to-open and open-to-closed ‘reaction’ rate constants, respectively, a and d are the association and dissociation rate constants, respectively, and k is the turnover number. The last step in eqn (S2) is the irreversible step of a catalytic reaction, which may or may not occur, depending on whether S is a substrate or a competitive inhibitor. Note that here one accounts for the possibility that the configuration of the enzyme in the complex with the substrate S may differ from the one in the c (without substrate) state.

Assuming that the times taken by the $E_o \rightleftharpoons E_c$ or $E_o \rightleftharpoons SE_{cs}$ transitions are negligibly small (as compared to the life time of the corresponding states), one can write the (long-time) diffusion coefficient of such a model enzyme

$$D = D_o p_o + D_c p_c + D_{cs} p_{cs}, \tag{S3}$$

where p_α is the probability that the enzyme is in the state $\alpha = \{o, c, cs\}$ and D_α is the corresponding diffusion coefficient. If one further assumes that the shapes of the enzyme in the states c and cs are similar, by writing $D_{cs} = D_c + \Delta D_{cs}^{\text{fluct}}$ one identifies $\Delta D_{cs}^{\text{fluct}}$ as the contribution to the diffusion coefficient solely due to the reduction of internal (conformational) fluctuations by binding a substrate. Since the number of enzymes is conserved, it follows that the steady-state probabilities of finding an enzyme in each of the three states are

$$p_o(\rho_{\text{S}}) = \frac{K_M p^{(0)}}{K_M + \rho_{\text{S}}\, p^{(0)}}, \tag{S4a}$$

$$p_{cs}(\rho_{\text{S}}) = \frac{\rho_{\text{S}}\, p^{(0)}}{K_M + \rho_{\text{S}}\, p^{(0)}}, \tag{S4b}$$

and

$$p_c(\rho_{\text{S}}) = 1 - p_o(\rho_{\text{S}}) - p_{cs}(\rho_{\text{S}}) = \frac{K_M(1 - p^{(0)})}{K_M + \rho_{\text{S}}\, p^{(0)}}, \tag{S4c}$$

where ρ_{S} is the number density of a substrate, $K_M = (d + k)/a$ is the Michaelis-Menten constant, and

$$p^{(0)} = p_o(\rho_{\text{S}} = 0) = k_c/(k_o + k_c) \tag{S5}$$

is the probability that an enzyme is in the open state in the absence of substrates. Note that eqn (S4) capture the limiting behaviors $p_o(\rho_{\text{S}} \to \infty) \sim K_M/\rho_{\text{S}} \to 0$ and $p_{cs}(\rho_{\text{S}} = 0) = 0$, as expected. By combining eqn (S3) and (S4), and denoting by $\mathcal{D}$ the diffusion coefficient of the enzyme in the absence of substrate,

$$\mathcal{D} = D(\rho_{\text{S}} = 0) = D_o p^{(0)} + D_c(1 - p^{(0)}), \tag{S6}$$

we have obtained for the relative change $\delta D(\rho_{\text{S}})$ in the diffusion coefficient, as a function of substrate concentration,

$$\delta D(\rho_{\text{S}}) = \frac{D(\rho_{\text{S}}) - \mathcal{D}}{\mathcal{D}} = \frac{\rho_{\text{S}}\, \delta D_{\text{max}}}{\rho_{\text{S}} + K_M/p^{(0)}}, \tag{S7a}$$

where $\delta D_{\text{max}} = \delta D(\rho_{\text{S}} = \infty)$ denotes the maximum relative enhancement of the diffusion coefficient. The latter can be written as a sum of contributions arising solely from the shape (size) changes and from the suppression of the internal fluctuations, respectively [1, 2]:

$$\delta D_{\text{max}} = \frac{D_c - \mathcal{D}}{\mathcal{D}} + \frac{\Delta D_{cs}^{\text{fluct}}}{\mathcal{D}} = \delta D^{\text{size}} + \delta D^{\text{fluct}}\,, \tag{S7b}$$

with $\delta D^{\text{size}} \geq 0$ since $D_c \geq D_o$.

The probability $p^{(0)}$ can be determined from experiments at low and high (saturated) substrate densities ρ_{S} as follows. From measurements of the diffusion constant $D(\rho_{\text{S}})$ in the saturation regime, one obtains $\delta D_{\max}$. At low densities, $\rho_{\text{S}} \ll K_M/p^{(0)}$, one has

$$\delta D \approx \frac{\delta D_{\max} p^{(0)}}{K_M} \rho_{\text{S}} \,, \tag{S8}$$

and thus $p^{(0)}$ can be unambiguously extracted from the slope of δD at low substrate/inhibitor densities.

S2. FLUCTUATING DUMBBELL MODEL OF ENZYMES

We consider a system of two beads (enzyme subunits) with the bead-bead interaction potential given by eqn (6) in the main text. This potential models the open and closed states of enzymes, allowing the jumps between these states. In order to test the additivity of diffusion constant for such enzymes (eqn (S3) and eqn (1) of the main text), we considered additionally two systems with the single-well interaction potentials U_c and U_o, corresponding to the closed and open states. For U_c we took

$$U_c(l) = \frac{16\kappa}{(l_o - l_c)^4}(l_c - l)^2 g_c(l), \tag{S9}$$

where

$$g_c(l) = \begin{cases} (l_o - l)^2, & \text{if} \quad l < l_c \\ (l - 2l_c + l_o)^2, & \text{if} \quad l > l_c \end{cases}, \tag{S10}$$

and similarly for U_o

$$U_o(\ell) = \frac{16\kappa}{(\ell_o - \ell_c)^4}(\ell_o - l)^2 g_o(l), \tag{S11}$$

where

$$g_o(\ell) = \begin{cases} (\ell_c - \ell)^2, & \text{if} \quad \ell > \ell_o \\ (\ell - 2\ell_o + \ell_c)^2, & \text{if} \quad \ell < \ell_o \end{cases}. \tag{S12}$$

It is easy to see that functions $g_\alpha(\ell)$ are continuous with continuous first derivatives, thus producing continuous forces.

A. Brownian dynamics simulations

To perform Brownian dynamics (BD) simulations, we have used a customized version of the simulation package BD_BOX [3, 4]. The customization was to implement the interaction potentials defined by eqn (5) and (6) of the main text, and eqn (S11).

Brownian dynamics trajectories have been generated by using the second order Iniesta-de la Torre algorithm [4, 5]; within this algorithm, the position of ith bead at time t is

$$\boldsymbol{r}_i = \boldsymbol{r}_i^0 + \frac{1}{2}\frac{\Delta t}{k_B T}\sum_{j=1}^{N}\left(\boldsymbol{D}_{ij}^0 \boldsymbol{F}_j^0 + \boldsymbol{D}'_{ij}\boldsymbol{F}'_j\right) + \boldsymbol{R}_i, \tag{S13}$$

where N is the number of beads (two in our case), $\Delta t = t - t_0 > 0$ is the time step, $\boldsymbol{r}_i^0$ is the position of the ith bead at time t_0, k_B is the Boltzmann constant and T temperature. The (position-dependent) diffusion matrix $\boldsymbol{D}_{ij}^0$ (see below) and the force $\boldsymbol{F}_j^0$ acting on the jth bead are evaluated at time t_0, while $\boldsymbol{D}'_{ij}$ and $\boldsymbol{F}'_j$ are evaluated for beads in a configuration with the positions at an intermediate corrector step [5].

The $3N$ vector of random forces, $\hat{\boldsymbol{R}} = \{\boldsymbol{R}_i\}$, is given by $\hat{\boldsymbol{R}} = \hat{\boldsymbol{B}}\hat{\boldsymbol{X}}$, where $\hat{\boldsymbol{X}}$ is a random Gaussian vector, and the matrix $\hat{\boldsymbol{B}} = \{\boldsymbol{B}_{ij}\}$ is a 'square root' of the diffusion tensor, *i.e.*,

$$\hat{\boldsymbol{D}} = \hat{\boldsymbol{B}}\hat{\boldsymbol{B}}^T \tag{S14}$$

where $\hat{\boldsymbol{D}} = \{\boldsymbol{D}_{ij}\}$. For the Iniesta-de la Torre algorithm, the diffusion matrix used in eqn (S14) is $(\hat{\boldsymbol{D}}^0 + \hat{\boldsymbol{D}}')/2$, so that the random forces satisfy

$$\langle \boldsymbol{R}_i \rangle = 0, \quad \langle \boldsymbol{R}_i \boldsymbol{R}_j^T \rangle = \Delta t \left(\boldsymbol{D}_{ij}^0 + \boldsymbol{D}'_{ij} \right). \tag{S15}$$

We used Cholesky decomposition to calculate $\hat{\boldsymbol{B}}$, as implemented in BD_BOX. In all simulations, the time step was 0.1 ps.

To account for hydrodynamic interactions, we employed the generalized Rotne-Prager-Yamakawa tensor [6, 7], which reads (a_i is the bead's hydrodynamic radius, r_{ij} the center-to-center separation between the i'th and j'th beads, η is viscosity and $\boldsymbol{I}$ is a 3×3 unit matrix):

$$\boldsymbol{D}_{ii} = \frac{k_B T}{6\pi\eta a_i} \boldsymbol{I}; \tag{S16a}$$

$$\boldsymbol{D}_{ij}(r_{ij}) = \frac{k_B T}{8\pi\eta r_{ij}} \left[\left(1 + \frac{a_i^2 + a_j^2}{3r_{ij}^2} \right) \boldsymbol{I} + \left(1 - \frac{a_i^2 + a_j^2}{r_{ij}^2} \right) \frac{\boldsymbol{r}_{ij}\boldsymbol{r}_{ij}^T}{r_{ij}^2} \right] \tag{S16b}$$

for $r_{ij} > a_i + a_j$;

$$\boldsymbol{D}_{ij}(r_{ij}) = \frac{k_B T}{8\pi\eta r_{ij}} \left[\frac{16r_{ij}^3(a_j + a_j) - [(a_i - a_j)^2 + 3r_{ij}^2]^2}{32r_{ij}^3} \boldsymbol{I} + \frac{3[(a_i - a_j)^2 - r_{ij}^2]^2}{32r_{ij}^3} \frac{\boldsymbol{r}_{ij}\boldsymbol{r}_{ij}^T}{r_{ij}^2} \right] \tag{S16c}$$

for $a_{ij}^M - a_{ij}^m < r_{ij} < a_i + a_j$, where a_{ij}^M is the largest and a_{ij}^m the smallest of a_i and a_j; and

$$\boldsymbol{D}_{ij} = \frac{k_B T}{6\pi\eta a_{ij}^M} \boldsymbol{I}, \tag{S16d}$$

for $r_{ij} < a_{ij}^M - a_{ij}^m$.

It is necessary to stress that the diffusion matrix, eqn (S16), takes into account only two-body far-field hydrodynamic interactions (formally extended to overlapping distances between the beads). Accounting for the long-range many-body and for the near-field forces may alter the simulation results quantitatively. Although we do not expect the main conclusions of this work to be altered (particularly regarding the effect of the dumbbell fluctuations), it would nevertheless be interesting to study such effects explicitly (which can be done, for instance, by using the HYDROLIB library [8, 9]). We note that our simulations are already the next order approximation to the results of Refs. [1, 2], where only the contribution from the Oseen tensor was taken into account. However, our simulations demonstrate a good agreement with these (theoretical) results, and it is reasonable to expect that even higher order terms in the diffusion matrix will not change the results significantly.

In order to single out the effects related to the suppression of fluctuations and hydrodynamic interactions, we neglected all interactions, except of the bead-bead binding potentials U, as defined by eqn (5) and (6) of the main text, and eqn (S11); the force acting on a bead is then $\boldsymbol{F} = -\nabla U$. In a few cases, we also added the hard core repulsion between the beads. However, no significant differences with the case of no steric interactions have been observed that could have changed the conclusions of this work.

The box size was $1000\text{Å} \times 1000\text{Å} \times 1000\text{Å}$ and periodic boundary conditions were applied in all three directions. In order to account for the long-range character of the hydrodynamic interactions, we used the Ewald summation as proposed by Smith *et al.* [10] and implemented in BD_BOX [3, 4]. It is known that periodic boundary conditions lead to a correction in the diffusion coefficient, inversely proportional to the box length [11, 12]. For our setup, we estimated this correction to be about 6.2% to 3.5%, depending on the parameters. However, here we are primarily interested in the *difference* between the diffusion coefficients at different spring constants, in which case this correction simply cancels out; in other words, it will merely shift the curves in Fig. 1e of the main text, Fig. S2e and Fig. S1e, by a few percent.

For all dumbbell enzymes, the starting configurations (*i.e.*, the separations between the beads) were chosen randomly according to the Boltzmann distribution defined by the bead-bead interaction potentials (eqn (5) and (6) of the main text, and eqn (S11)). We performed 4000 to 6000 simulations (each of a duration of 1 μs), depending on the parameters, to gather good statistics.

B. Open and close state probabilities

For the interaction potential $U(\ell)$, the probability of an enzyme to be in the open state in the absence of the substrate ($\rho_\mathrm{S} = 0$) is

$$p_o(0) = \frac{\int_0^{\ell_m} \ell^2 \mathrm{e}^{-U(\ell)/k_BT} d\ell}{\int_0^{\infty} \ell^2 \mathrm{e}^{-U(\ell)/k_BT} d\ell}\,, \tag{S17}$$

and, accordingly, $p_c(0) = 1 - p_o(0)$. Here $\ell_m = (\ell_c + \ell_o)/2$ is the mid-point between the two minima, which coincides with the location at which $U(\ell)$ attends a local maximum.

For $\kappa = 6.8\,k_BT$, $\ell_o = 2.5$ nm, and $\ell_c = 1.5$ nm, eqn (S17) renders $p_c(0) \approx 73\%$, which agrees well with the results of our Brownian dynamics simulations.

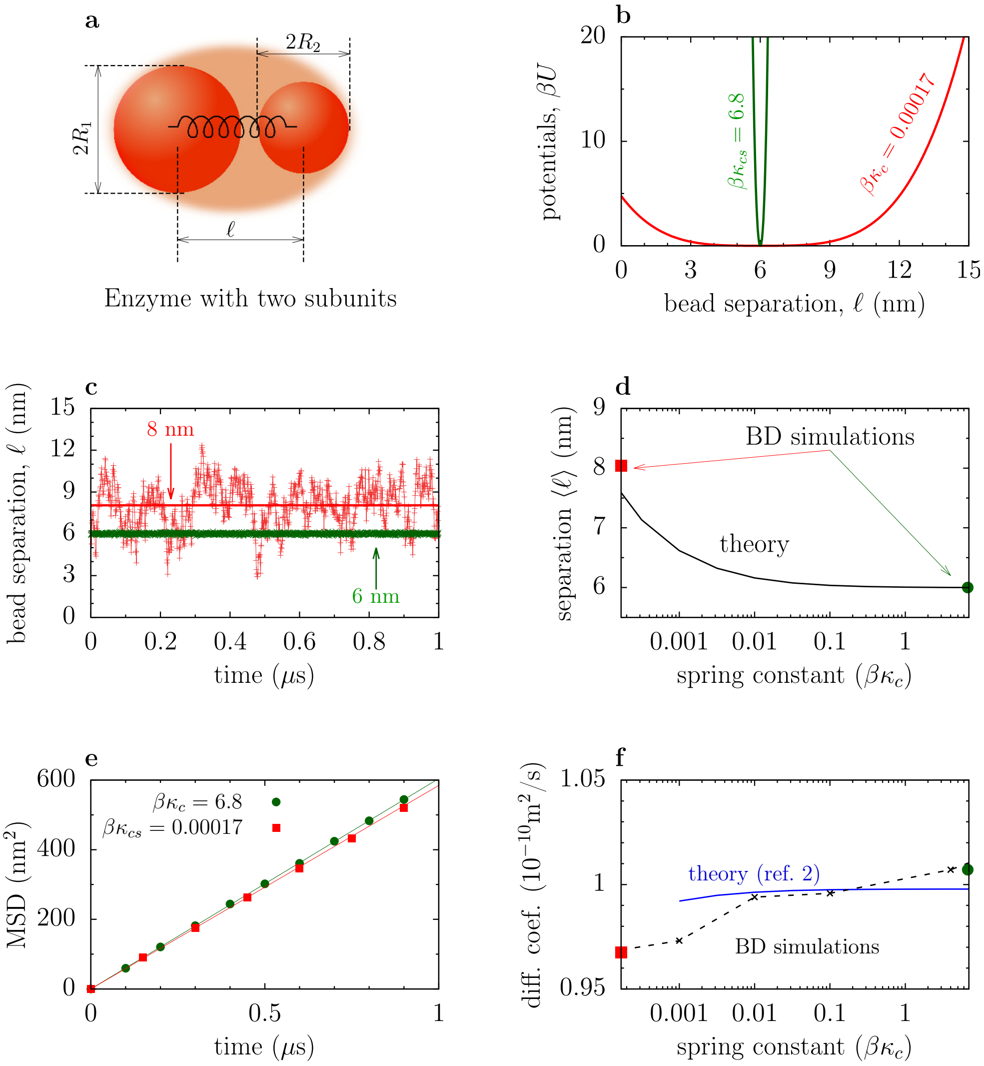

FIG. S1. **Effect of stiffness of the interaction potential on the diffusion of flexible dumbbell enzymes.** The parameters and the presentation is the same as in Fig. 1 of the main text, but without the hard core repulsion between the enzyme subunits. Here and in Fig. S2, the second term in the parantheses in eqn (S18) is not defined (due to the soft potential, which allows $\ell \to 0$) and thus the diffusion coefficent was approximated by the $\langle m_0 \rangle$ term only.

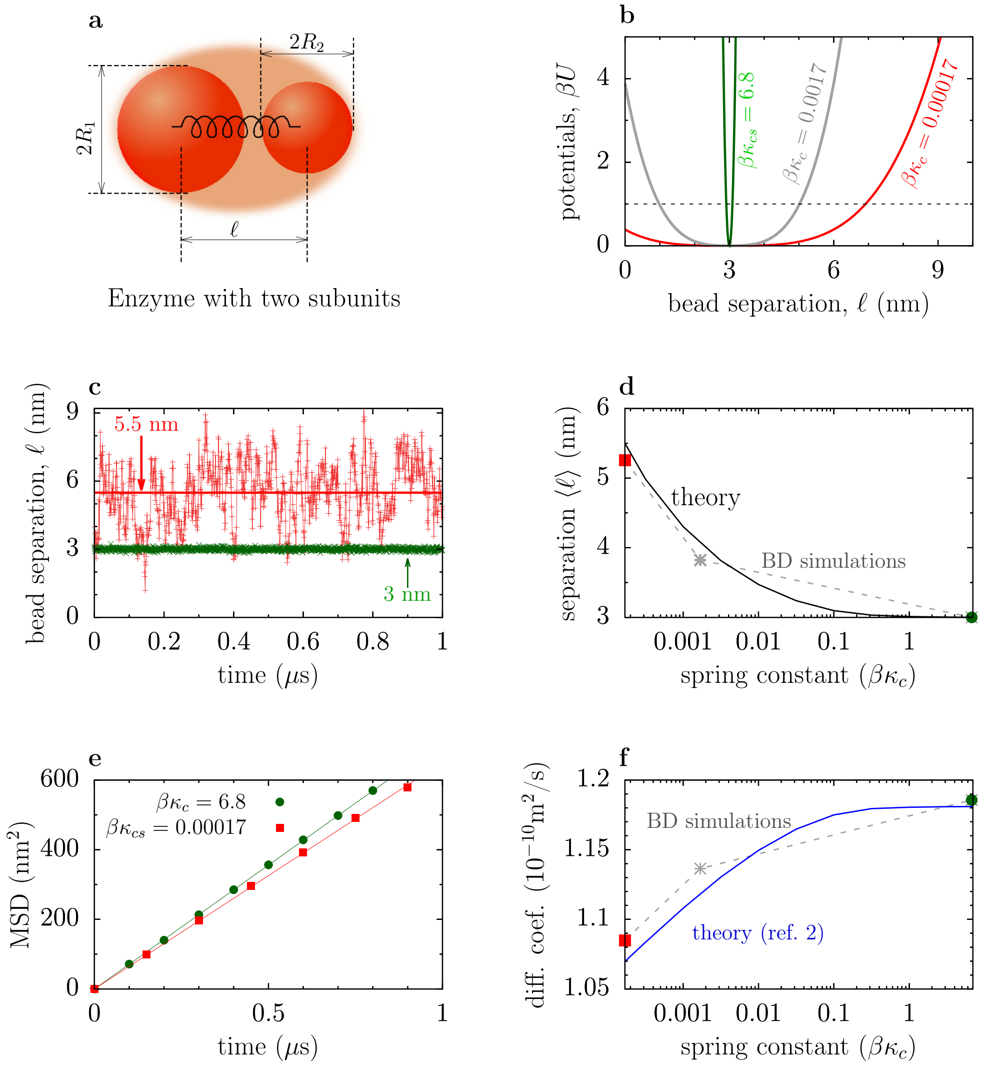

FIG. S2. **Effect of stiffness of the interaction potential on the diffusion of flexible dumbbell enzymes.** The presentation is the same as in Fig. 1 of the main text but for smaller averaged separations between the enzyme subunits. The following parameters have been used: The radii $R_1 = 1.5$ nm and $R_2 = 1$ nm, as in Fig. 1 of the main text, and the parameters of the interaction potential were $\ell_o = 3$ nm and $\ell_c = 2$ nm. The subunits were allowed to overlap (no hard core repulsion). Here and in Fig. S1, the second term in the parantheses in eqn (S18) is not defined (due to the soft potential, which allows $\ell \to 0$) and thus the diffusion coefficient was approximated by the $\langle m_0 \rangle$ term only.

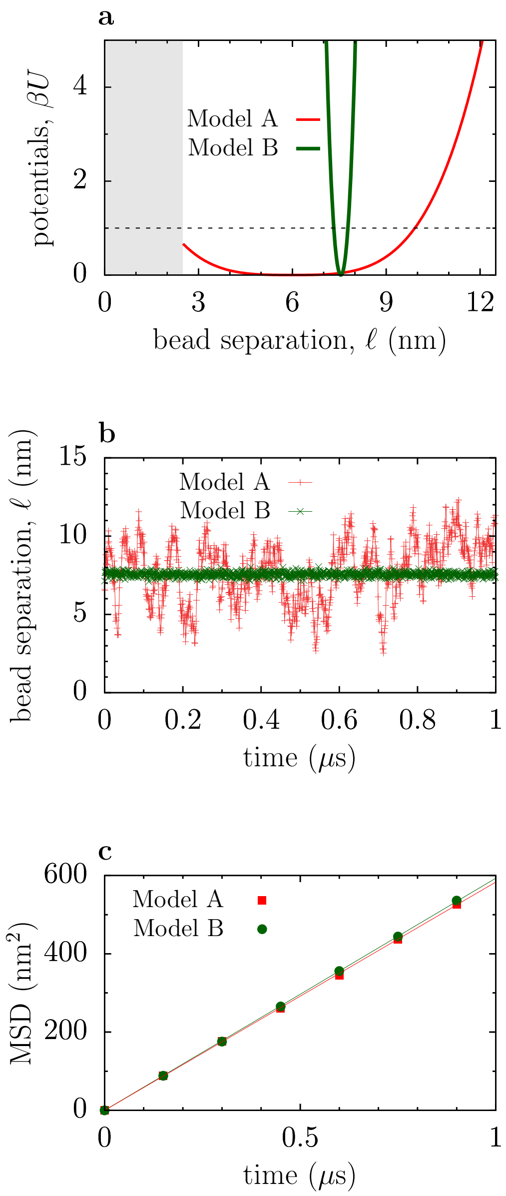

FIG. S3. **Effect of fluctuations on the diffusion of flexible dumbbell enzymes.** The following parameters have been used: The bead radii $R_1 = 1.5$ nm and $R_2 = 1$ nm, and parameter $\ell_c = 5$ nm, as in Fig. 1 of the main text; the spring constant $\kappa = 0.00017 k_B T$ and parameter $\ell_o = 6$ nm (model A), and $\kappa = 6.8 k_B T$ and $\ell_o = 7.55$ nm (model B). The parameters were chosen such that the average bead-bead separations in the two models are comparable, $\langle \ell \rangle_{\rm A} \approx 7.55$ nm and $\langle \ell \rangle_{\rm B} \approx 7.56$ nm, implying that the main contribution to the difference in the diffusion coefficients comes from fluctuations. (a) Interaction potentials for models A and B. (b) Examples of bead-bead separation from a *single* simulation, demonstrating that in both models the beads fluctuate around the same average separation and that the fluctuations in model A are much stronger than in model B. (c) Mean square displacements (MSDs) for models A and B. The MSDs are averages over 4000 independent simulaitons. The extracted diffusion coefficients are $D_{\rm A} \approx 0.972 \times 10^{-10}$ m^2/s and $D_{\rm B} \approx 0.989 \times 10^{-10}$ m^2/s, which differ by approximately 1.7%.

S3. THEORETICAL ESTIMATE OF THE DIFFUSION CONSTANT

In order to estimate theoretically the diffusion coefficient of the flexible dumbbell enzyme, we employ the results derived in ref. 1. In particular, the following expression, which accounts for the hydrodynamic interactions between the two spherical beads in the Oseen limit (far-field, large separation between beads, i.e., $\ell \gg R_1 + R_2$) ,

$$D_{eff} = \frac{1}{4\beta}\left(\langle m_0\rangle - \frac{2}{3}\frac{\langle \gamma_0/\ell\rangle^2}{\langle w_0/\ell^2\rangle}\right) , \tag{S18}$$

is used; here $\beta := 1/(k_B T)$ (with k_B the Boltzmann constant and T the temperature),

$$m_0 := \frac{1}{6\pi\eta}\left(\frac{1}{R_1} + \frac{1}{R_2}\right) + \frac{1}{4\pi\eta\ell} , \tag{S19a}$$

$$\gamma_0 := \frac{1}{6\pi\eta}\left(\frac{1}{R_1} - \frac{1}{R_2}\right) , \tag{S19b}$$

$$w_0 := \frac{1}{6\pi\eta}\left(\frac{1}{R_1} + \frac{1}{R_2}\right) - \frac{1}{4\pi\eta\ell} , \tag{S19c}$$

where

$$\langle O\rangle := \frac{\int\limits_0^\infty d\ell\, \ell^2 O \exp[-\beta U(\ell)]}{\int\limits_0^\infty d\ell\, \ell^2 \exp[-\beta U(\ell)]} ,$$

and $U(\ell)$ denotes the potential between the two beads (see Eqs. (4), (S6) - (S8) in ref. 1).

In addition to the potential $U_c(\ell)$, eqn (5) in the main text, supplemented by the hard core repulsion, i.e., $\ell > R_1 + R_2$ (results shown in Fig. 1 in the main text) we have also used a harmonic potential (with the same location, ℓ_0, of the minimum)

$$U_h(\ell) := K_h(\ell/\ell_0 - 1)^2 , \tag{S20}$$

which was employed as an example in ref. 1, also combined with the hard core repulsion, i.e., $\ell > R_1 + R_2$. The following values of the geometric parameters have been chosen: $R_1 = 1.5$ nm, $R_2 = 1$ nm, $\ell_0 = 6$ nm and $\ell_c = 5$ nm, and the viscosity is set to the value 10^{-3} Pa s, i.e., the viscosity of water. The ranges for the "spring constants" κ and K_h are set by noting that $\Delta = 16\beta\kappa[(2 - \ell_c/\ell_0)/(1 - \ell_c/\ell_0)]^2 \approx 784\beta\kappa$ and $\Delta = \beta K_h$, respectively, represent the energy (in units of the thermal energy) needed to stretch the corresponding dumbbell to twice its equilibrium length ℓ_0. Accordingly, κ and K_h are varied such that the "stretching energy" Δ varies from fractions of $k_B T$ (a very loose spring) to thousands of $k_B T$ (a very stiff spring).

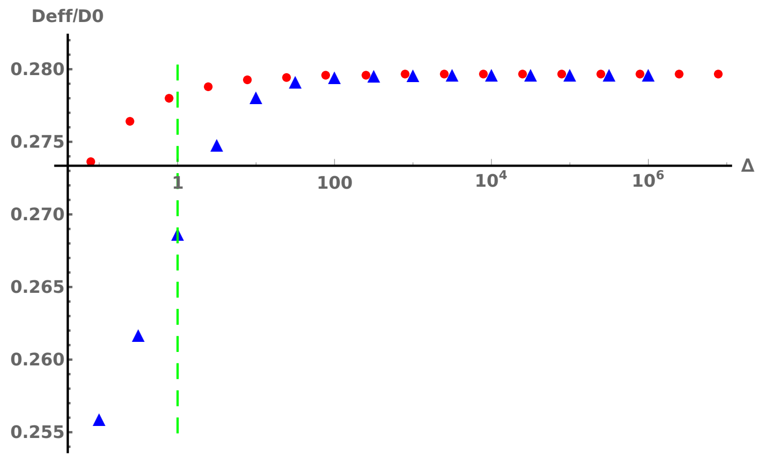

FIG. S4. **Theoretical estimates for the effective diffusion constant.** Diffusion constant D_{eff} is shown in units of $D_0 = (k_B T)/(6\pi\eta R) \approx 3.6 \times 10^{-10}$ m^2/s (where $1/R = 1/R_1 + 1/R_2$, and R_1 and R_2 are the bead radii) and presented as a function of the 'stretching energy' Δ (see the text). The results correspond to hard-core interaction between the beads and a confining potential given by eqn (5) of the main text (red circles) and by eqn (S20) (blue triangles), respectively. The green dashed line marks the value $\Delta = 1$ of the 'stretching energy'.

The diffusion constants D_{eff}, in units of $D_0 := (k_B T)/(6\pi\eta R) \simeq 3.6 \times 10^{-10}$ m^2/s (where $1/R := 1/R_1 + 1/R_2$), corresponding to the two potentials are compared in Fig. S4. It can be seen that in both cases for values $\Delta_{c,cs} > 1$, which are both physically rational (in that the thermal fluctuations of the extension of the dumbbell enzyme should not become unreasonably large) as well as a necessary condition for the theoretical calculations leading to eqn (S18) (separation of time scales between the relaxation of the vibrational modes and the rotational diffusion, see ref. 1), the increase in the diffusion coefficient upon a switch of the spring constant from the c state to the cs one is expected to be well below 3 - 4% (which is the increase if the c state is taken as the extreme case $\Delta = 1$).

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