Supporting Information

Elber et al. 10.1073/pnas.0909636107

SI Text

Correlated Motions Between Secondary Structure Elements. Coupling between switch 1 and the relay helix is shown in Fig. S1. An S shape plot suggests that the relay helix moves first, then SW1 takes the (sequential) lead and finally the relay helix completes its' own motion. This S shaped motion may be related to the seesaw model of Fischer et al. (ref. 12). The specific sequential mechanism observed at room temperature trajectories (within the Milestoning approximation) supports the picture of a direct and mechanical path which is preserved even with significant thermal noise (Fig. S1).

Another correlated motion worth examining is of switch 2 (internal RMSD) and the relay helix. Similarly to the P-loop (Fig. 4*A*), switch 2 shows early relaxation to its final state (Fig. S2).

Connection Between Milestoning and Nonequilibrium Theory. Eq. **S1**

$$P_s(t) = \int_0^t Q_s(t')\left[1 - \sum_{s'} \int_0^{t-t'} K_{ss'}(\tau)d\tau\right]dt'$$

$$Q_s(t) = P_s(0)\delta(t^+) + \sum_{s'} \int_0^t Q_{s'}(t')K_{s's}(t-t')dt', \qquad \textbf{[S1]}$$

where $P_s(t)$ is the probability that the last Milestone the system crossed is s. In our example of a reaction coordinate the system is found between Milestones $s \pm 1$. The density $Q_s(t)$ is the probability that Milestone s is passed exactly at time t. The input for the above equation is the calculated (from trajectories) $K_{ss'}(t)$ and the initial distribution $P_s(0)$. The above equation is formally equivalent to the Generalized Master Equation

$$\frac{dP_s(t)}{dt} = \int_0^t \Gamma_{ss'}(t')P_{s'}(t-t')dt', \qquad \textbf{[S2]}$$

where the Laplace transform of the memory kernel $\Gamma_{ss'}(t)$ is directly related to the Laplace transform of the kernel $K_{ss'}(t)$, $\Gamma_{ss'} = \frac{uK_{ss'}(u)}{1-K_{ss'}(u)}$ (the Laplace transform of a function $G(t)$ is: $(G(u) = \int_0^\infty \exp(-ut)G(t)dt)$). Initial conditions are sampled from a stationary distribution at s. Instead of solving [equation 1] directly it is possible to calculate moments in time. The first moment is the Mean First Passage Time (MFPT). The inverse of the MFPT is the usual rate constant for exponential kinetics (refs. 21, 23, 24)

$$\langle\tau\rangle = \mathbf{1}^t \int_0^\infty \tau \cdot \mathbf{K}(\tau)d\tau \cdot \left[\mathbf{I} - \int_{\mathbf{0}}^\infty \mathbf{K}(\tau)d\tau\right]^{-1}\mathbf{P}(0). \qquad \textbf{[S3]}$$

An alternative view of Milestoning with exact limit is discussed in ref. 24.

Computing the Kernel. We estimate the elements of the kernel ($K_{s,s+1}(\tau)$ or $K_{s,s-1}(\tau)$) in two steps. We (*i*) initiate trajectories at Milestone s and (*ii*) propagate the trajectories in time until they terminate at Milestones $s \pm 1$ (Fig. 1).

Initial conditions. The preparation of the initial conditions at Milestone s is approximate. The initial conditions are sampled from the canonical ensemble constrained to be at s ($p(X|s) \propto \exp(-\beta U(X|s))$ $\beta = 1/k_BT$ where k_B is the Boltzmann constant and T the absolute temperature). In principle a complete trajectory from reactants to products should be computed; instead we assume loss of memory between Milestones (and stationary distribution in s). In ref. 24 this approximation was discussed in the context of overdamped Langevin dynamics. A crrection for the present study (the curvature of the reaction coordinate) was examined numerically and found to be small (a few percent).

Trajectories between Milestones. Let the number of structures sampled at Milestone s be L. Each of the L coordinates is a starting point of a trajectory that is no longer constrained to s. The trajectories are terminated when they reach the hyperplane $s+1$ (or $s-1$) and their arrival times, τ_{sl} $l = 1, \ldots, L$, are recorded. Binning the termination events provides estimates of $K_{s+1,s}(\tau)$ (or $K_{s-1,s}(\tau)$). While possible (ref. 21) the direct calculation of the Kernel is statistically demanding. It is easier to compute the zero and the first moments of the kernel that are sufficient to compute the MFPT Eq. **S3**. For the zero moment we have $\int_0^\infty K_{s+1,s}(\tau)d\tau$; $(1/L)\lim_{\tau\to\infty}\sum_{l=1}^L G(X(\tau), s+1)$ where $\lim_{\tau\to\infty} G(X(\tau), y)$ is equal one if $X \in y$ and zero otherwise. The expression for $\bar{\tau}_s$ (the average termination times of Milestoning trajectories initiated at s) is $\bar{\tau}_s = \int_0^\infty \tau \sum_{s'=s+1,s-1} K_{s's}(\tau).d\tau \cong (1/L)\sum_{l=1}^L \tau_{sl}$. The zero moment and $\bar{\tau}_s$ of all Milestones are sufficient to determine the overall MFPT.

Numerical Implementation of the Reaction Path Calculations (Unnumbered Main Text Equation $F[X(l)] = \int_{X_R}^{X_P}\sqrt{\nabla U^t \nabla U}dq$). The computed reaction path is a function of the subset of coordinates $\mathbf{q}$. For myosin $\mathbf{q}$ includes all the C_α coordinates. For numerical computations the path is discretized to have S structures $\{X_i\}_{i=1}^S$ and the functional becomes $F \approx \sum_s \sqrt{\nabla U^t(X_s) \cdot \nabla U(X_s)} \cdot \Delta q_{s,s+1}$ where $\Delta q_{s,s+1} = \sqrt{(\mathbf{q}_s - \mathbf{q}_{s+1})^2}$. The functional is minimized subject to path and Eckart constraints (refs. 25, 26). The outputs of these calculations are the Milestones or hyperplanes perpendicular to the reaction coordinate. (Milestones) are defined with two vectors. Each Milestone s is determined by a vector $\mathbf{q}_s$ in the plane and by a unit vector $\hat{e}_s$ perpendicular to hyperplane s $\hat{e}_s \approx (X_{s+1} - X_{s-1})/|X_{s+1} - X_{s-1}|$.

Milestoning and Molecular Dynamics Simulations. All water molecules (TIP3P or SPC/E, we repeated the calculation with the two water models) are kept rigid with a matrix variant (ref. 31) of the SHAKE algorithm (ref. 32). Long range electrostatic is calculated with Particle Mesh Ewald (ref. 33), and the time step was 1 femtosecond using the Velocity Verlet algorithm. Configurations of the first 40 picoseconds are ignored and structures are saved each 0.2 ps in the rest of the simulation (40 ps). Note that the sampling is not to cover the complete 3N-1 configuration space but rather to explore the neighborhood of the reaction coordinate. Milestoning evaluates a proposed reaction coordinate (and a reaction mechanism). The total number of structures sampled in each Milestone is 200.

Computational Efficiency. The calculations of the transition kernel $K_{ss'}(\tau)$ are the bottleneck of he simulations. Nevertheless, they are considerably cheaper than straightforward Molecular Dynamics (MD) simulations and in some cases exponentially faster (in the number of Milestones). Different speed up factors were discussed elsewhere (refs. 20, 21). Here we consider one

case that provides exponential speed up. Consider the passage over a barrier of height B. In a usual MD simulation the probability of reaching the top of the barrier would be proportional to $\exp(-\beta B)$ and the time to reach the top will scale like $\exp(+\beta B)$. The use of M milestones along the barrier split the process to independent calculations each with a barrier to cross of order of. The transition time between two Milestones is therefore proportional to $\exp(\beta B/M)\ B/M$, which is proportional to the time required to estimate $K_{ss'}(\tau)$ (depending on the number of trajectories that are used to generate the sample). The total time of the transitions for the M milestones $M\exp(\beta B/M)$. Hence the speed up in this case is exponential in the number of Milestones.

The concrete application to Myosin is discussed below. The length of a typical myosin trajectory between Milestones is in picoseconds. A few Milestones have $\bar{\tau}_s$ of tens of picoseconds. Overestimating the average local transition times at 10 picoseconds, the total computational time (on a single core to generate a single "connected" trajectory from reactants to protducts) is estimated as $10\ \text{ps} \times 200$ trajectories $\times 240$ milestones $+ 80\ \text{ps} \times 240$ sampling $= 499,200$ ps.

This is less than a half of a microsecond. Of course the trajectories are trivially run in parallel, which significantly shortens the clock time. We have employed in the calculations about 1,000 cores; each core simulates trajectories for about a nanosecond. A nanosecond is a factor of million shorter than the time scale of simulating directly a single event of recovery stroke.

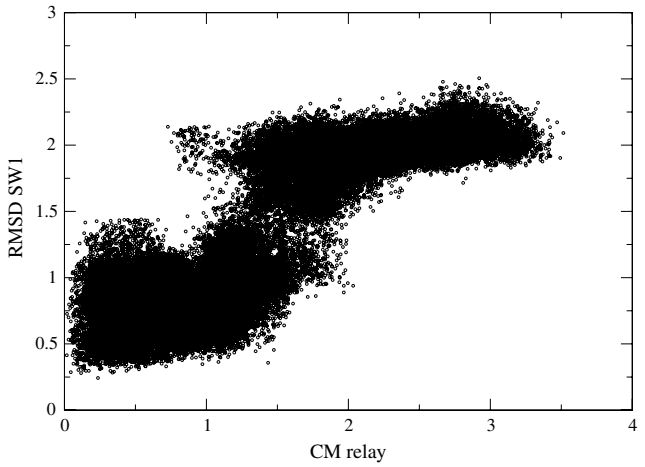

Fig. S1. Correlation of the relay helix center of mass motion and switch 1 internal RMSD.

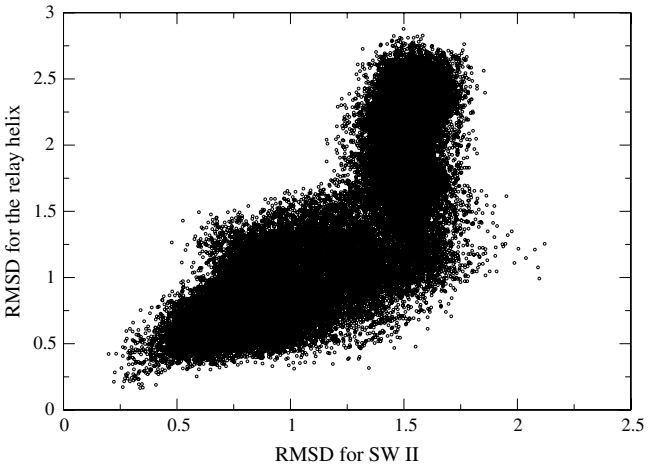

Fig. S2. Correlation of the relay helix and switch 2.