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Motor-like Properties of Nonmotor Enzymes

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ABSTRACT Molecular motors are thought to generate force and directional motion via nonequilibrium switching between energy surfaces. Because all enzymes can undergo such switching, we hypothesized that the ability to generate rotary motion and torque is not unique to highly adapted biological motor proteins but is instead a common feature of enzymes. We used molecular dynamics simulations to compute energy surfaces for hundreds of torsions in three enzymes—adenosine kinase, protein kinase A, and HIV-1 protease—and used these energy surfaces within a kinetic model that accounts for intersurface switching and intrasurface probability flows. When substrate is out of equilibrium with product, we find computed torsion rotation rates up ~140 cycles s^{-1}, with stall torques up to ~2 kcal mol^{-1} $cycle^{-1}$, and power outputs up to ~50 kcal mol^{-1} s^{-1}. We argue that these enzymes are instances of a general phenomenon of directional probability flows on asymmetric energy surfaces for systems out of equilibrium. Thus, we conjecture that cyclic probability fluxes, corresponding to rotations of torsions and higher-order collective variables, exist in any chiral molecule driven between states in a nonequilibrium manner; we call this the "Asymmetry-Directionality" conjecture. This is expected to apply as well to synthetic chiral molecules switched in a nonequilibrium manner between energy surfaces by light, redox chemistry, or catalysis.

INTRODUCTION

A biological molecular motor is an enzyme that transduces chemical energy to mechanical motion. This motion must have a specific direction to fulfill the motor's functional role. For example, a corkscrew-shaped flagellum must rotate in the appropriate sense to propel the organism. The ability to generate such directional motion appears to be a complex molecular property, perhaps unique to highly adapted motor proteins.

A system at equilibrium is guaranteed not to undergo net directional motion, due to the principle of microscopic reversibility. When the system is thrown out of equilibrium, there is the possibility of directional motion, but this requires a mechanism to capture and transduce the available chemical energy. Molecular motors work by using the chemical energy to drive stochastic switching between conformational energy surfaces in a manner that leads to directional motion (1–8). Nonmotor enzymes also switch between at least two conformational free energy surfaces as they bind and release substrate. It is thus of interest to ask whether enzymes not normally thought of as motor proteins have motor-like properties when operating away from chemical equilibrium.

This article provides theoretical reasoning in support of this view, and then uses modeling to test the hypothesis that all periodic coordinates of any enzyme catalyzing an out-of-equilibrium reaction must undergo some degree of directional rotation. To uncover these rotations and begin to assess their magnitudes, we used molecular simulations and kinetic analysis to analyze the rotational dynamics of bond torsions induced by nonequilibrium, stochastic, state-switching in three, diverse, nonmotor enzymes: adenosine kinase (ADK), protein kinase A (PKA), and HIV-1 protease (HIVP).

Theoretical overview: asymmetric systems out of equilibrium

Consider a periodic molecular coordinate, θ, such as a bond torsion or a higher-order collective variable, in an enzyme undergoing a catalytic cycle of substrate-binding, catalysis, and product release. When binding of substrate switches the effective energy surface from that of the apo state, $\mu_0(\theta)$, to that of the bound state, $\mu_1(\theta)$, probability flows along θ toward a new equilibrium distribution corresponding to $\mu_1(\theta)$ (Fig. 1). Such a flow of probability can be modeled with the Fokker-Planck equation, for example (4). Because enzymes are chiral, neither energy landscape, $\mu_0(\theta)$ and $\mu_1(\theta)$, can possess mirror symmetry; i.e., neither can be an exactly even function around any value of θ. Thus, there will be more probability flow in one direction than

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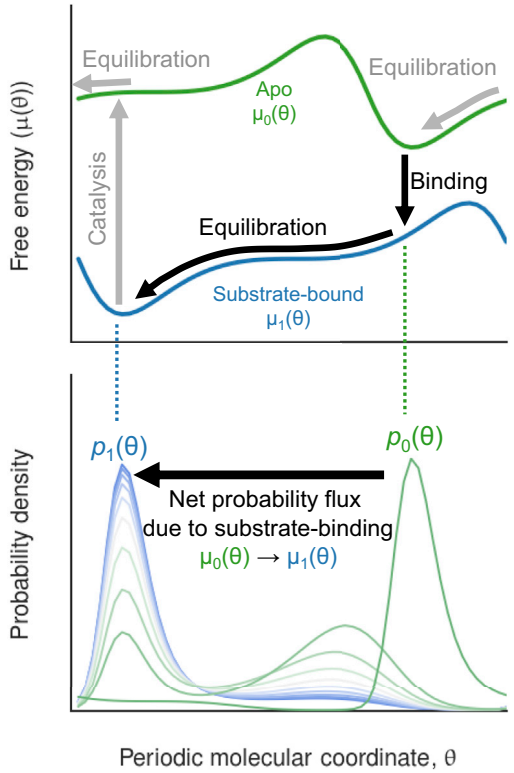

FIGURE 1 Illustrative apo and substrate-bound free energy surfaces of an enzyme (*solid green* and *blue lines*, respectively) and the flow of probability along coordinate θ, after the effective potential changes due to substrate binding (*green* to *blue* probability distribution curves).

the other, due to the asymmetry of the starting probability distribution and of the patterns of barriers and troughs along the bound free energy surface. After the substrate is catalytically removed, this process repeats itself, with a probability distribution that has approached equilibrium on the substrate-bound surface diffusing asymmetrically under the potential of the apo free energy surface, $\mu_0(\theta)$. Unless there is an infinitely high energy barrier at the same location on both surfaces, there is no basis to expect that the net flows will cancel identically for a chiral molecule, and the resulting asymmetric net flow of probability during this two-step process constitutes directional motion. Note that any such motion will be superimposed on a background of rapid, nondirectional stochastic fluctuations in θ.

MATERIALS AND METHODS

The one-dimensional free energy surfaces of protein main- and side-chain torsions, discretized into bins, were obtained from equilibrium molecular dynamics simulations of the enzymes in their apo and substrate-bound states (Supporting Material). These data, coupled with literature values for the enzyme kinetic parameters (Table S1), enabled us to define first-order rate constants for transitions along and between the free energy surfaces (Fig. S1). The resulting set of rate equations was solved for the nonequilibrium steady-state probability distribution, for a given concentration of substrate, and this, in turn, was used to compute the probability flux on each surface. The net flux, J, in units of torsional rotational cycles per second, is an indication of directional rotation; e.g., a positive value implies clockwise rotation of the torsion.

The three nonmotor enzymes studied have distinctive characteristics: ADK, with 214 residues and a relatively high $k_{cat} \sim 300\ s^{-1}$ (9,10), undergoes extensive conformational change on binding substrate, with two domains reorienting to form a compact conformation (11,12); PKA, with 350 residues and $k_{cat} \sim 140\ s^{-1}$ (13), acts as a "dynamic switch," with long-range allosteric interactions and domain rearrangement upon ligand binding (14); and HIVP, with 200 residues and lower $k_{cat} \sim 10\ s^{-1}$ (15–17), contains two flexible flaps that lose mobility in the substrate-bound state (18,19) (Fig. S2).

RESULTS

For all three enzymes, we find that multiple side-chain and main-chain torsion angles undergo directional rotations, as indicated by nonzero probability flux, when excess substrate is present. Thus, at high substrate concentration, ~40 torsions in ADK and PKA rotate faster than 10 cycles s^{-1}, and ~140 rotate faster than 1 cycle s^{-1} (Fig. 2 *a*). Directional rotation is also observed for HIVP, although the rates are lower (Fig. 2 *b*, *red*). The lower rates largely reflect the lower k_{cat} value of HIVP, relative to PKA and ADK. Thus, artificially assigning $k_{cat} = 200\ s^{-1}$ to HIVP leads to substantial increases in the number of torsions with fluxes of at least 10 cycles s^{-1} and at least 1 cycle s^{-1} (Fig. 2 *b*, *orange*; and Fig. S4). The tendency toward lower fluxes in HIVP may also reflect the smaller scale of its conformational changes (Fig. S2): a small conformational change may lead to more similar energy surfaces in the two states, and hence less opportunity to generate rotational flux by the mechanisms discussed below. For all three enzymes, there are main-chain torsions with substantial directional flux, but directional flux is more prevalent among side-chain torsions than main-chain torsions; see Fig. 3 and Fig. S3.

Although the maximum rotation rates are different for ADK, PKA and HIVP (180, 70, and 5.6 cycles s^{-1}, respectively), the maximum numbers of rotations per catalytic step are similar, at 0.5–0.6 cycles per catalytic turnover (Fig. 4). (Assuming $k_{cat} = 200\ s^{-1}$ for HIVP yields a maximum rate of 112 cycles s^{-1}, which corresponds to 0.6 cycles per catalytic turnover.) This ratio is akin to a 2:1 gearing of catalysis to torsional rotation. Figs. S4 and S5 provide further details regarding the relationships between catalytic rate and torsional flux for additional torsions. The angles with highest directional flux are localized near the substrate binding pocket or mobile regions (Fig. S2, *right two columns*).

We furthermore evaluated power output and performance under load by tilting the energy surfaces to generate a torque, τ, opposite to the directional flux, which modifies the intrasurface bin-to-bin rate constants (Supporting Material). The power output is the product of load torque and flux: $P = \tau J$. Both the maximum power and the stall torque, τ_{stall}, defined as the torque that brings the directional flux to zero, were found by scanning across values of applied torque. The results indicate that torsions in these enzymes can do work

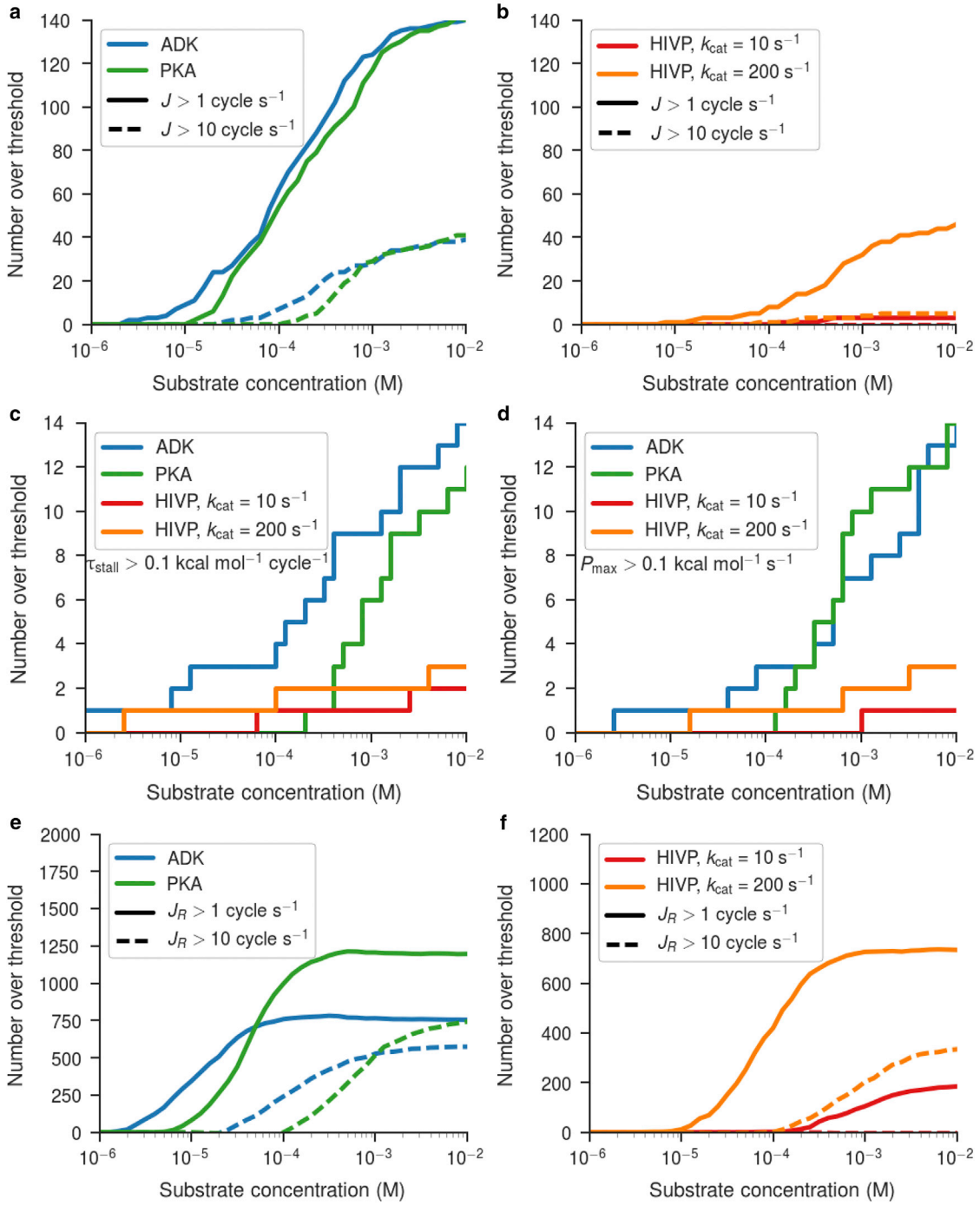

FIGURE 2 The number of torsions above various thresholds of directional flux magnitude, reciprocating flux magnitude, stall torque, and maximum power, as a function of substrate concentration. (*a* and *b*) The number of torsions with directional flux >1 (*solid*) or 10 (*dotted*) cycles s^{-1} in ADK, PKA, and HIVP is shown. (*c* and *d*) The number of angles with (*c*) maximal stall force >0.1 kcal/(mol•cycle) and (*d*) power >0.1 kcal/(mol•s) is shown. (*e* and *f*) The number of torsions with reciprocating flux >1 (*solid*) or 10 (*dotted*) cycles s^{-1} and, at the same time, directional flux <1 cycle s^{-1} is shown.

against small mechanical loads and thus generate power (Fig. 2, *c* and *d*). In particular, at high substrate concentrations, torsions in ADK and PKA are predicted to generate stall torques up to 2.4 and 1.6 kcal mol^{-1} $cycle^{-1}$, respectively, and maximum power outputs per torsion of 70 and 28 kcal mol^{-1} s^{-1}.

The mechanism by which high rates of directional flux are generated is illustrated by the torsion of ADK Thr-175 (Fig. 5 *a*). This angle has a two-peaked probability distribution in both the bound and apo states, but the peak near $+\pi/2$ is favored in the apo state, whereas that near $-\pi/2$ is favored in the bound state (Fig. 5, *b* and *c*).

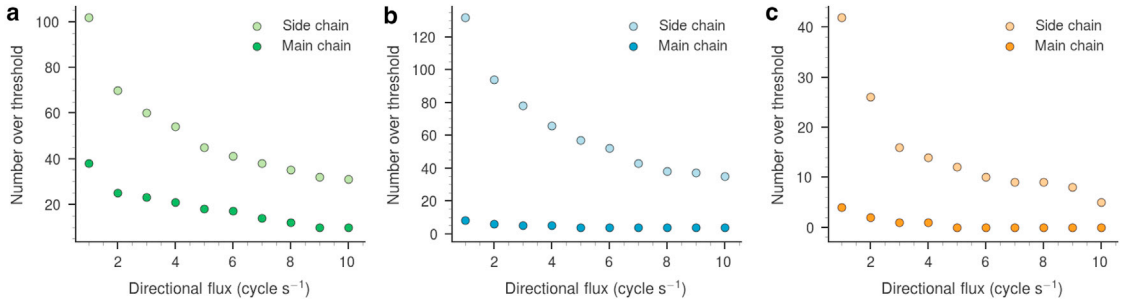

FIGURE 3 The number of main-chain (ϕ and ψ) and side-chain (χ) torsions above various thresholds of directional flux magnitude in each enzyme. Results are for (*a*) PKA, (*b*) ADK, and (*c*) HIVP (assuming $k_{cat} = 200\ s^{-1}$; see main text) at a substrate concentration of 10^{-2} M.

In the presence of substrate, the bound-state energy minimum near $-\pi/2$ is highly occupied (Fig. 5, *b* and *c*). Catalytic breakdown of substrate pumps the system to the secondary energy minimum of the apo state at $-\pi/2$ (Fig. 5 *c*; *arrow 1*). Probability then flows primarily to the left on the apo surface, because this is the lowest-barrier path to the apo state's global energy minimum near $+\pi/2$ (*arrow 2*; this flux goes through the periodic boundary at $\theta = -\pi \equiv +\pi$). Probability pooled in the global energy minimum of the apo state near $+\pi/2$ then flows primarily to the bound state, by binding substrate and landing in the secondary energy minimum of the bound state (*arrow 3*). It then flows back to the global minimum of the bound state via the lowest-barrier path, which is again leftward (*arrow 4*). The net effect is a leftward flux of up to -140 cycles s^{-1}. Fig. 5 *d* shows the steady-state flux on each surface: leftward flux predominates overall, but occurs on the apo surface between $-\pi/2$ and $+\pi/2$, and on the bound surface elsewhere, with crossovers between surfaces at the energy minima.

This process parallels fluctuating potential mechanisms previously invoked to explain highly evolved molecular motors (3,4,7,8,20–23). Intuitively, high flux can be generated when each energy surface (apo and bound) has a main energy barrier and a main energy well, and the two surfaces are offset, so that probability flow in a given direction on each surface bypasses the barrier on the other surface, as schematized in Fig. S6, *a* and *b*. Also, as noted above, generation of net flux requires asymmetric energy surfaces; i.e., energy surfaces that are not even functions around any value of θ. Accordingly, symmetrizing the energy surfaces of a high-flux torsion abolishes directional flux, as shown in Fig. S7.

There are also many torsions whose net directional flux is small (<1 cycle s^{-1}), and whose dynamics are instead dominated by reciprocating motion, in which probability flows in one direction in the bound state and in the other direction in the apo state, within some angular range. These reciprocating probability fluxes correspond to driven, oarlike motion of the atoms controlled by the torsion. The enzymes ADK and PKA, respectively, are predicted to have ~1250 and ~750 torsions with reciprocating motions at rates of at least 1 cycle s^{-1} (Fig. 2, *e* and *f*), and minimal directional flux. The maximal reciprocating fluxes are greater than the maximal directional fluxes, and, for ADK and PKA, approach the catalytic rates (Fig. S5). The mechanism of reciprocating motion is illustrated by χ_2 of Asn-138 in ADK (Fig. 5 *e*), which has a net flux that is nonzero but well below 1 cycle s^{-1}, yet has reciprocating fluxes of up to 130 cycles s^{-1} (Fig. 5, *e–h*). Reciprocating flux occurs when the main energy wells on the two surfaces are in different locations, but little probability flow on each surface bypasses the main barrier on the other surface, as schematized in Fig. S6, *c* and *d*. Thus, if the main barriers in the

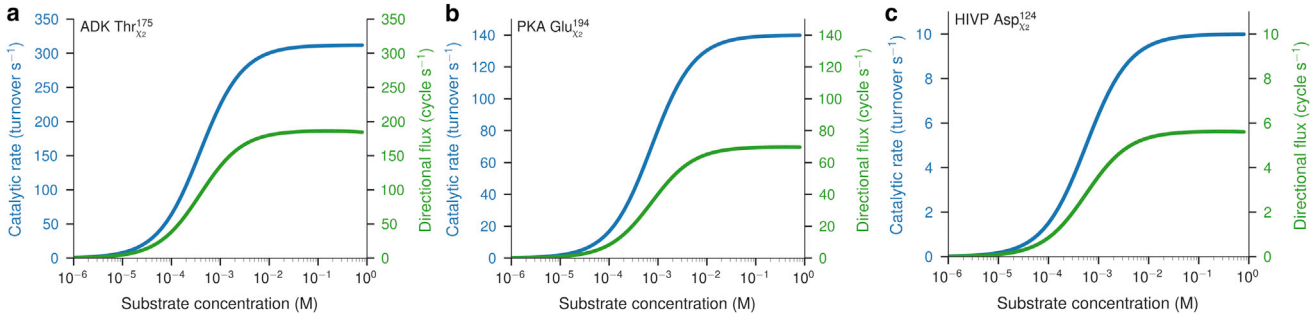

FIGURE 4 Dependence of catalytic rates and of the magnitude of directional flux on substrate concentration, for torsion angles in each enzyme. (*a*) The χ_2 angle of Thr-175 in ADK reaches a high level of directional flux. (*b*) The χ_2 angle of Glu-194 in PKA reaches a moderate level of directional flux. (*c*) Although the total amount of flux in the χ_2 angle of Asp-124 in HIVP is low, the ratio of directional flux to the enzyme velocity is similar to that in ADK and PKA.

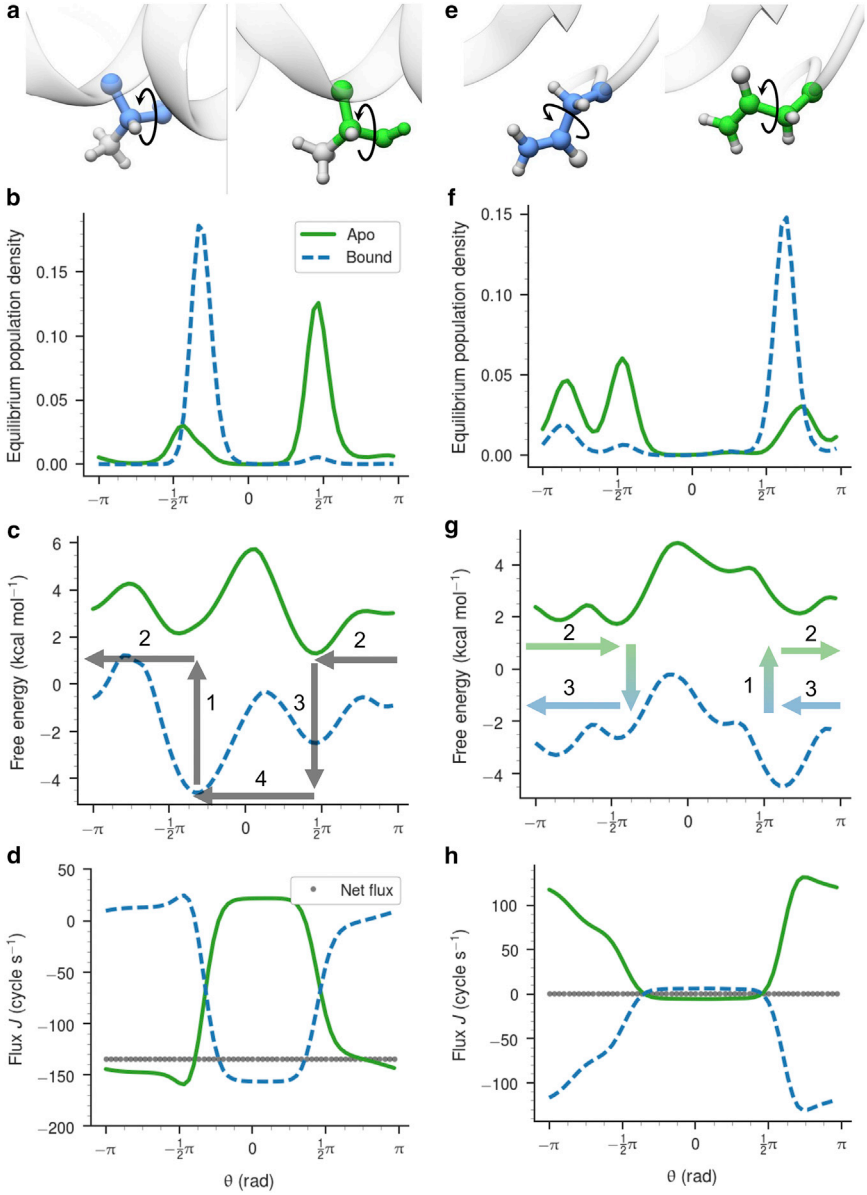

FIGURE 5 Protein torsion angles show directional and reciprocating motion. (*a*) ADK Thr-175 in its crystallographic conformations for the apo (*green*) and bound (*blue*) forms (see Supporting Material for PDB accessions) with the χ_2 angle denoted. The coloring is the same for (*a*)–(*d*). (*b*) Equilibrium population densities of this angle from molecular dynamics simulations are shown (Supporting Material). (*c*) Shown are free energy surfaces of this angle (Supporting Material) derived from the population densities in (*b*). Arrows indicate the direction of probability flux along, and between, the two surfaces. (*d*) The probability flux drawn separately for each surface and as a sum (*gray points*), indicating large directional and reciprocating fluxes. (*e–h*) Same as (*a–d*) for ADK Asn-138. In all cases the substrate concentration is 10^{-3} M.

two states are coincident, movement will be dominated by reciprocating motion. It is worth noting that such reciprocating motion could generate net directional motion, if there were an additional mechanism that decoupled the load from the torsion for motion in either the bound or apo state. This would require correlated motion along at least one additional degree of freedom.

DISCUSSION

We have provided reasoning and evidence that directional rotation that can work against a load is not limited to highly adapted biological motor proteins. Instead, any enzyme operating out of equilibrium should generate directional conformational fluxes. The magnitudes of these motions will depend on a number of factors, including the levels and locations of the barriers and energy wells on the energy surfaces associated with different states of the system, and the chemical driving forces and kinetic constants. We also observe high reciprocating flux in torsions with energy surfaces that are not well tuned to generate net directional motion.

This modeling approach uses atomistic simulations to obtain effective energy surfaces, and then embeds these in a kinetic model. This efficient technique is broadly applicable. For example, it could be used to study not only proteins but also synthetic molecular motors. It can also be extended to higher-dimensional distribution functions, and hence multidimensional energy surfaces, which will come into play when considering larger-scale cooperative

motions. Such motions could lead, for example, to state-dependent coupling of a load to a driven, reciprocating degree of freedom (see above). Note, however, that substantially more conformational sampling would be necessary to populate the multidimensional histograms arising in this application.

The basic mechanism by which directional motion is generated here is not limited to enzymes. We conjecture that the same statistical mechanical considerations will cause any system that undergoes stochastic, nonequilibrium switching between asymmetric energy surfaces to generate directional, cyclic motion. We term this idea the "Asymmetry-Directionality" (AD) conjecture. The AD conjecture applies to many molecular systems other than enzymes, such as ones where states are switched by absorption of light, changes in electrical potential, or changes in solution conditions. Thus, the occurrence of directional cycles of conformational change in molecular systems out of equilibrium should be regarded as the rule, rather than the exception.

The same reasoning applies not only to one-dimensional torsional motions, but also to protein motions associated with large-scale conformational change, because the associated multidimensional energy surfaces have asymmetric potentials, due to chirality. Such motions might help explain the observation that the translational diffusion coefficients of some nonmotor enzymes rise with substrate concentration and hence with enzyme velocity (24,25). The generality of the AD conjecture also means that enzymes from earliest evolutionary time would have had the ability to generate directional motion, and thus would have had a good starting point from which to embark on an evolutionary path to today's highly adapted motor proteins.

SUPPORTING MATERIAL

Supporting Materials and Methods, nine figures, and two tables are available at http://www.biophysj.org/biophysj/supplemental/S0006-3495(18)30211-X.

AUTHOR CONTRIBUTIONS

M.K.G. conceived and designed the project. D.R.S. implemented the model and performed the simulations. M.K.G. and D.R.S. analyzed the data and wrote the manuscript.

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