Manubot Rootstock: Manuscript Title

Response to reviewers for “Motor-Like Properties of Non-Motor Enzymes” by Slochower and Gilson, PNAS 2017-11059

We thank the reviewers for their considered responses of our manuscript “Motor-Like Properties of Non-Motor Enzymes”. We believe the reviewers have highlighted some important issues with the phrasing of our conclusions. However, we also believe some of the reviewers’ comments can be addressed quickly and succinctly.

Reviewer 1

Major criticisms

> First, using the same model, the authors should explain carefully how the enzymes without significant conformational change exhibit enhanced diffusivity upon catalysis?

We would like to clarify that the goal of the manuscript was not to explain the enhanced diffusivity of enzymes upon catalysis. Rather, we focus on revealing and describing the dynamics of chiral molecules placed out of equilibrium, sampling two potential energy surfaces. This model has been discussed before (!!!) but the emergence of directional flux has not been discussed.
Our conclusions state that the “the hydrodynamic coupling […] might help explain why some enzymes diffuse faster when catalytically active.” We will modify the text of our conclusion to focus more on …

> Likewise, for the enzymes with significant conformational changes that have very low kcat, what will the model predict?

In the main text, we describe the case of HIV protease with $k_{\text{cat}} \sim 1\,\text{s}^{-1}$, compared to the relatively high values of catalysis for ADK and PKA.

Directional flux is limited by this low level of catalysis and we show that increasing the catalytic rate increases the level of flux.

Figure 1 shows the results for HIV protease with both high and low levels of catalysis. Figure S4 specifically addresses the effect of k_{cat} (shown below). Furthermore, we have studied the effect of lowering the catlytic rate for ADK, but we did not include this plot in the manuscript. Shown below, it is clear that flux deceases as K_{cat} decreases.

> Finally, the authors should carefully explain the role of change in torsional landscape and kcat (specifically in some simplistic models and fluctuating landscapes) in producing directed motion and then show the link of either landscape and kcat for the motor proteins, thus showing the evolutionary link.

We have looked at simplistic models for the landscapes (flat, monotonically increasing, sawtooth waves) and concluded that landscapes that show the largest amount of flux

We have looked at the effect of symmetrizing landscapes as "controls."

Minor criticisms

> The choice of force-field and simulation time can alter the sampled states of torsional angles? It needs to be explored and explained in detail that there is no bias due to force-field or simulation length.

This is a good point The choice of force field will have an effect. However, since our goal was to highlight htis motion The torsional population histograms converge rapidly during simulation time. [Figure]

> The choice of bin size (why was it chosen as 60 per conformation?) Does different bin size alter the results?

Bin size was chosen arbitrarily, but the bin size and number of bins does not alter the results. [Figure]

> The choice of D is not clear, what happens in different D values? Finally, transition in adjacent states, I think detailed balance should be taken into account.

D was chosen to replicate free diffusion in the absence of any energy barriers. That is, *D* controls how rapidly the landscape is sampled. We calculated this using the diffusion of butane in free … (as described in the SI). Further, the choice of *D* is saturating.

Reviewer 2

Major criticisms

> Why did they choose this model? In what way is it better than more straightforward free energy and rate calculations used in atomistic simulations?

Separate simulations of apo and bound state protiens

> The procedure for determining the chemical potential and rate coefficients seems a bit convoluted and not as straightforward as it can be.

We mixed experimental data