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Pickin' Up Good Vibrations

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Although conformational change has long been recognized as critical to protein function, whether the same goes for equilibrium dynamical fluctuations has been the subject of myriad squabbles. There are those who rigidly deny any dynamical effects, those who claim fluctuations drive functional conformational change, and those who claim to have snared exquisitely evolved function-channeling vibrations (1–4).

Thermal fluctuations must influence function at least insofar as ligand or substrate binding formally leads to a change in the vibrational density of states (frequency distribution) with a corresponding effect on the partition function and thus binding thermodynamics. Indeed, dating back to the 1980s in a series of calculations on different systems, this effect was shown to favor binding (5–7), and this was finally confirmed by density of states measurements on the binding of methotrexate to dihydrofolate reductase using inelastic neutron scattering (8).

More contentious is whether proteins have evolved equilibrium (or nonequilibrium) motions to promote activity, such as the reactive steps in enzyme catalysis. Whereas several molecular dynamics simulation studies have been interpreted as suggesting this, others have not (1,3). Of particular importance here is which motions one is looking at. Local rearrangements, as detectable by nuclear magnetic resonance, are fundamentally different beasts to global vibrations. A role in function for the latter, though, is an appealing idea as they are collective in character. However, individual collective vibrations have proven fiendishly difficult to detect experimentally.

A fillip toward escaping this impasse would be an experimental technique capable of detecting collective, long-range vibrations in proteins. Inelastic neutron scattering can do this only with difficulty, and typical density-of-states measurements have been on isotropic powder samples. The article by Niessen et al. (9) takes a noteworthy step in the right direction. Global vibrations are low frequency relative to internal vibrations in small molecules, vibrating on the picosecond timescale or longer. Niessen et al. (9) use a novel approach, anisotropic terahertz microscopy, which directly probes optically active protein vibrations. In this technique, the terahertz light absorbance of an aligned molecular sample (protein crystals in this case) is measured as a function of the direction of polarization of the light relative to the orientation of the sample.

The authors find a dramatic change in the anisotropic terahertz microscopy signal, and thus the vibrational directionality, with inhibitor binding to lysozyme. Directly interpreting the results requires computer simulation, and normal mode calculations found that whereas in the apo enzyme a clamping motion around the binding site was found, for the holo form a twisting motion around the binding site was calculated.

Challenges, of course, remain. Calculating the observed spectra requires a knowledge of the dipole derivatives of the modes and this requires both accurate electrostatic and dynamics modeling. Overcoming the need to align the proteins would also be a major advance. Also, experiments would be useful in complementing some calculations that the authors report, comparing wild-type lysozyme with a higher catalytic rate double-deletion mutant, which suggests a reorientation of motions. Moreover, large-amplitude, collective motions in proteins are a quagmire in themselves (10). How, for example, can one detect harmonic motions that are overdamped, i.e., which follow a parabolic potential of mean force but move stochastically? Low-frequency motions, by virtue of their large amplitudes, are susceptible to solvent damping. Nonetheless, the results in Niessen et al. (9) are clearly a technical advance in a promising direction. The dream of experimentally discovering large-scale vibrations intimately intertwined with protein function may be one step closer.

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REFERENCES

1. Kamerlin, S. C. L., and A. Warshel. 2010. At the dawn of the 21st century: is dynamics the missing link for understanding enzyme catalysis? *Proteins.* 78:1339–1375.
2. Henzler-Wildman, K. A., M. Lei, …, D. Kern. 2007. A hierarchy of timescales in protein dynamics is linked to enzyme catalysis. *Nature.* 450:913–916.
3. Agarwal, P. K., S. R. Billeter, …, S. Hammes-Schiffer. 2002. Network of coupled promoting motions in enzyme catalysis. *Proc. Natl. Acad. Sci. USA.* 99:2794–2799.
4. Dunn, R. V., V. Réat, …, R. M. Daniel. 2000. Enzyme activity and dynamics: xylanase activity in the absence of fast anharmonic dynamics. *Biochem. J.* 346:355–358.
5. Tidor, B., and M. Karplus. 1994. The contribution of vibrational entropy to molecular association. The dimerization of insulin. *J. Mol. Biol.* 238:405–414.
6. Fischer, S., and C. S. Verma. 1999. Binding of buried structural water increases the flexibility of proteins. *Proc. Natl. Acad. Sci. USA.* 96:9613–9615.
7. Fischer, S., J. C. Smith, and C. S. Verma. 2001. Dissecting the vibrational entropy change on protein/ligand binding: burial of a water molecule in bovine pancreatic trypsin inhibitor. *J. Phys. Chem. B.* 105:8050–8055.
8. Balog, E., T. Becker, …, J. C. Smith. 2004. Direct determination of vibrational density of states change on ligand binding to a protein. *Phys. Rev. Lett.* 93:028103.
9. Niessen, K. A., M. Xu, …, A. G. Markelz. 2017. Moving in the right direction: protein vibrational steering toward function. *Biophys. J.* 112:933–942.
10. Hong, L., D. C. Glass, …, J. C. Smith. 2013. Elastic and conformational softness of a globular protein. *Phys. Rev. Lett.* 110:028104.