doi:10.1038/nature14079

BIOPHYSICS

Enzymes surf the heat wave

Molecular diffusion of some enzymes is enhanced when they catalyse reactions, but the reason for this was obscure. Dissipation of heat generated by catalysis through the protein is now thought to propel the molecules.

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Ever since 1894, when Emil Fischer proposed that enzyme substrates fit perfectly into active sites like a key in a lock[1], biochemists have been fascinated by the catalytic action of enzymes. For more than a century, the focus has been almost entirely on the details and principles that govern the interconversion of reactant and product molecules. The fate of the accompanying heat of enzymatic reactions has been largely ignored. It is from this perspective that Riedel *et al.*[2], in a paper published on *Nature*'s website today, investigate the origin of the anomalous diffusion of several enzymes, which they ascribe to an acoustic wave generated by the heat released during catalysis.

Previous studies indicated that the hydrolysis of urea by urease[3] and the conversion of hydrogen peroxide to molecular oxygen and water by catalase[4] enhanced the molecular diffusion of these enzymes. These observations for urease were interpreted as self-phoretic effects — that is, they were thought to be generated by the release of charged products from the enzyme's surface[5]. Riedel and colleagues examined an alternative hypothesis: that dissipation of the heat of the catalysed reaction is responsible.

Using a technique known as single-molecule fluorescence correlation spectroscopy, the authors demonstrated that the diffusion of four enzymes — including urease and catalase — correlates with the rates of the reactions catalysed by those enzymes, and therefore with the heat produced. Several crucial control experiments eliminated various other potential causes, such as local solvent-heating effects or simple binding of a ligand to the enzyme. The researchers used the enzyme triose phosphate isomerase as a negative control because it has little heat of reaction, and observed no anomalous diffusion in this case.

Riedel *et al.* report that, for each enzyme, the fractional increase in diffusivity of the macromolecule is proportional to the velocity of the reaction (and so to the heat generated). There is no common proportionality, however, suggesting that the microscopic details of each protein are key. To simulate the proposed effect, the authors heated the catalytic centre of catalase using a short laser pulse and observed qualitatively the same anomalous diffusion seen during catalysed reactions. It therefore seems that the flow of heat through the protein molecule does indeed give rise to the peculiar diffusion of these enzymes.

But how does the heat of reaction enable the enzyme molecule to move? Here, it must be noted that proteins exist in a world in which Brownian motion is governed by viscous forces, rather than by inertia[6,7]. Coasting is not an option — continuous force generation is required. And not all types of motion can cause translational diffusion[6].

To explain their observations, Riedel and colleagues develop a simple model whereby the heat generated from each catalytic cycle is transmitted through the enzyme as a pressure wave (Fig. 1). In this model, the active site must be asymmetrically placed — that is, not at the enzyme's centre of mass. The pressure wave creates differential stress at the enzyme–solvent interface, which in turn propels the enzyme. The authors call this a 'chemoacoustic' effect. The model itself is barren of microscopic details and assumes that the enzyme, solvent water and their interface are individually homogeneous. This simple view nevertheless gives reasonable estimates of the force generated at the enzyme–water interface from a modest fluctuation in volume created by the thermal activation of a protein's motional modes.

Over the past few decades it has become abundantly clear that proteins (including enzymes) are dynamic entities rich in motion over a vast range of timescales. The picosecond-to-nanosecond time frame relevant to Riedel and co-workers' findings is no exception. Sophisticated molecular-dynamics simulations[8] suggest that transmission of energy through a protein can be remarkably fast — on the order of 5 ångströms per picosecond (1 picosecond is 10^{-12} seconds) — and non-uniformly distributed. The complexity of the internal motion of protein molecules has also been exemplified using nuclear magnetic resonance spectroscopy[9]. This complexity is particularly apparent in the context of applied pressure[10], which is highly relevant to the present study. But perhaps most importantly, proteins have elements (often termed

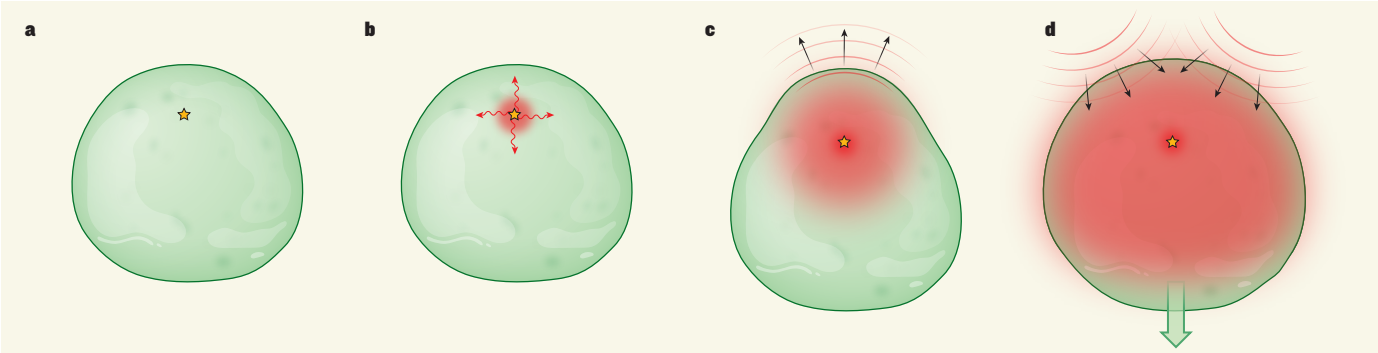

Figure 1 | The chemoacoustic model of anomalous enzyme diffusion. Riedel *et al.*[2] report that dissipation of heat generated during enzymatic reactions increases the diffusion of certain enzyme molecules. **a**–**c**, They suggest that heat released at the active site (yellow star) during a catalytic event generates a radial deformation wave that causes the enzyme to rapidly expand; orange areas indicate passage of the wave. This causes acoustic waves (black arrows) in the surrounding solvent. **d**, If reflected back on the protein, the waves cause the molecule to move (green arrow). Because enzyme molecules also undergo rotational Brownian motion (not shown), this mechanism of locomotion will not generate overall motion in a particular direction.

'foldons') that cooperatively fold to adopt a particular substructure and dictate many of the kinetic and thermodynamic properties of protein molecules[11]. Given all this complexity, the precise mechanism by which pressure waves move through protein molecules remains uncertain.

What happens when the transient expansion of an enzyme arrives at the enzyme–water interface is also more complicated than the situation in Riedel and colleagues' simple model. Theory[12,13], simulation[14] and experiment[15] suggest that the interaction of a protein surface with surrounding water molecules is context-dependent and variable. Furthermore, evidence[14,16] seems to suggest that proteins can induce long-range ordering of water, beyond the traditional 'hydration layer' of water molecules that immediately surrounds a dissolved protein molecule. Clearly there is much to do to fully understand how heat generated by enzyme catalysis is dissipated and how this can result in a locomotive protein molecule.

Finally, the most intriguing question of all is whether the anomalous diffusion of protein molecules through the chemoacoustic effect is a product of evolution or simply an accidental unselected result of heat flow in proteins. It seems reasonable to think that enzymes might have evolved the capacity to seek 'greener pastures' of substrates. But this view is more complicated than it might at first look[6,7], and any selective advantage may ultimately be quite obscure. ■

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