CellPress

Cragg, G.M., and Newman, D.J. (2013). Biochim. Biophys. Acta *1830*, 3670–3695.

Cronan, J.E. (2014). Biochem. J. *460*, 157–163.

Crosby, J., and Crump, M.P. (2012). Nat. Prod. Rep. *29*, 1111–1137.

Lambalot, R.H., and Walsh, C.T. (1995). J. Biol. Chem. *270*, 24658–24661.

Lambalot, R.H., Gehring, A.M., Flugel, R.S., Zuber, P., LaCelle, M., Marahiel, M.A., Reid, R., Khosla, C., and Walsh, C.T. (1996). Chem. Biol. *3*, 923–936.

Nguyen, C., Haushalter, R.W., Lee, D.J., Markwick, P.R.L., Bruegger, J., Caldara-Festin, G., Finzel, K., Jackson, D.R., Ishikawa, F., O'Dowd, B., et al. (2014). Nature *505*, 427–431.

Ray, T.K., and Cronan, J.E., Jr. (1976). Proc. Natl. Acad. Sci. USA *73*, 4374–4378.

Enzyme Dynamics and Engineering: One Step at a Time

Nobuhiko Tokuriki[1] and Colin J. Jackson[2,*]
[1]Michael Smith Laboratories, University of British Columbia, Vancouver, BC V6T 1Z4, Canada
[2]Research School of Chemistry, Australian National University, Canberra, ACT, 2601, Australia
*Correspondence: colin.jackson@anu.edu.au
http://dx.doi.org/10.1016/j.chembiol.2014.10.003

Although protein dynamics are accepted as being essential for enzyme function, their effects are not fully understood. In this issue of *Chemistry and Biology*, Gobeil and coworkers describe how engineered changes in the millisecond motions of a mutant TEM-1 β-lactamase do not significantly affect substrate turnover. This mutational robustness has implications for protein engineering and design strategies.

Protein dynamics, excluding protein translation and trafficking dynamics, generally refers to conformational changes in protein structure that occur over a broad range of timescales. Although the process of protein folding, essential for the function of a large proportion of all proteins, drastically minimizes the conformational freedom and dynamics of a polypeptide relative to the unfolded state, it has long been appreciated that some conformational change must be involved in protein function. The predominance of protein crystallography in structural biology over the last 50 years yielded a vast amount of structural data, yet crystal structures have traditionally been reported as a single ground state structure. In recent years there has been a natural progression to the study of protein dynamics; as our ability to solve protein structures and simulate their dynamics in silico has developed, more time has been invested in studying their movement, particularly through new nuclear magnetic resonance (NMR)-based methods (Mittermaier and Kay, 2006). The field has seen a number of landmark papers in recent years (for a recent review, see Ma and Nussinov, 2010), and there is a level of appreciation for the role of dynamics in protein function and evolution (Tokuriki and Tawfik, 2009). Accordingly, there is increasing interest in incorporating dynamic effects into protein engineering and design experiments.

Like any rapidly developing field, there are areas of consensus and contention. If enzymes are considered, several impressive works have demonstrated the importance of dynamics in the catalytic cycles of enzymes (Boehr et al., 2006) and their evolutionary conservation among members of some protein families (Gagné et al., 2012), implying that protein dynamics are under evolutionary selection and affect the fitness of proteins. The steady-state turnover number (k_{cat}), which is the most commonly measured kinetic rate, is only the rate-limiting step of a number of microscopic rate constants that, together, comprise the full catalytic cycle. While the role of dynamics in allowing proteins to transit between different conformations suited for various steps in the cycle is widely acknowledged, there are different views regarding the role of dynamics in catalysis: the increase in the rate of "chemical steps" in a catalytic cycle (Kamerlin and Warshel, 2010; Klinman and Kohen, 2014).

In this issue of *Chemistry and Biology*, Gobeil et al. (2014) have investigated millisecond protein motions in two related extant β-lactamases and an engineered chimeric protein produced through recombination. This work makes a significant contribution to the discussion of the role of millisecond dynamics in protein function by providing an in-depth analysis of the effects of engineering on both dynamics and catalytic activity. Interestingly, although the chimera exhibits almost identical substrate turnover rates to the wild-type β-lactamases with a range of substrates, the millisecond dynamics of the chimera are substantially different from those of the extant enzymes; i.e., in this case, there seems to be little correlation between substrate turnover and millisecond dynamics. This work complements and contrasts other NMR studies that showed close correlation between millisecond motions and turnover rates (Eisenmesser et al., 2005) as well as engineering studies that showed turnover rates for enzymes are highly sensitive to changes in dynamics when conformational change is rate limiting (Jackson et al., 2009). Specifically, this study shows that the effects of changes in dynamics on substrate turnover are particular to the enzyme. It is important to consider that, in the case of the TEM-1 β-lactamase, a chemical step

 ©2014 Elsevier Ltd All rights reserved

in the catalytic cycle (lactam ring opening for cephalosporins or enzyme deacylation for penicillins) is rate limiting (Saves et al., 1995). Thus, it appears that alterations to millisecond dynamics and the steps in the catalytic cycle that they contribute to, such as loop motions, might not significantly affect the turnover rate, provided that they are not so deleterious that conformational change becomes rate limiting.

What does this mean for protein engineering and design? It will largely depend on the aim of the engineering experiment. In most cases, protein engineering and design experiments seek to improve an activity that is catalyzed very poorly, such as a low-level promiscuous activity in an enzyme with a different primary function. In these cases, the chemical step is often rate limiting because the active site is unlikely to be optimal for catalyzing the new activity. Thus, the results of Gobeil et al. (2014) should be encouraging to protein engineers and designers in that it appears many enzymes might be quite tolerant to changes in millisecond dynamics, at least until the energy barrier to the chemical step is substantially reduced (Figure 1). In contrast, if the aim is to improve an enzyme that is already efficient in the chemical step, or an enzyme in which dramatic conformational change is required in substrate recognition, dynamics will need to be a primary consideration.

There are, of course, some caveats to this discussion of the role of dynamics and engineering, largely because the term "dynamics" itself is so broad. For instance, this work specifically analyzes a subset of motions on the millisecond timescale. Thus, the flexibility or level of preorganization of side chains in active sites, which can affect transition state stabilization in chemical reactions (Warshel et al., 2006), will still be relevant. Likewise, changes to the conformational landscape of a protein, particularly increased conformational sampling of a new or minor conformation that is better suited to catalyze the reaction, will be catalytically beneficial. Indeed, the tolerance to changes in dynamics observed in this work could increase the likelihood of new conformations being sampled, which has been proposed to promote evolvability (Boehr et al., 2009). This work highlights the mutational robustness of naturally evolved enzymes and reinforces the importance of understanding the catalytic cycles of proteins, particularly the nature of the rate-determining step, because of the impact this will have on our engineering and design strategies.

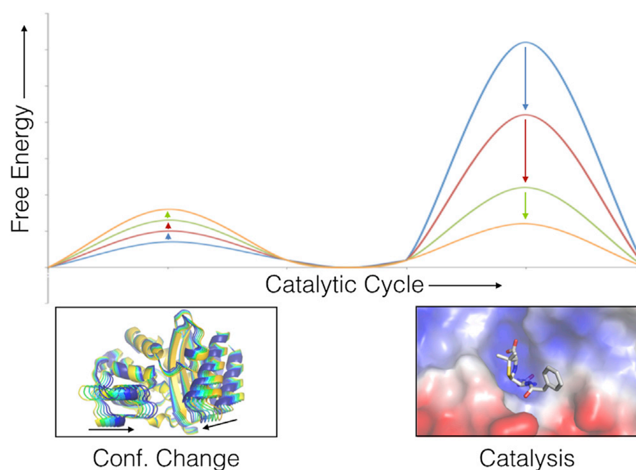

Figure 1. A Simplified Free Energy Diagram for a Protein Engineering Experiment in which Catalysis Is Rate Limiting
A typical scenario at the start of a protein engineering experiment in which the chemical step (catalysis) has a substantially higher barrier than a barrier to a conformational change that precedes it (blue). Mutations (red and green) that reduce the barrier for the chemical step will be beneficial, even though they might increase the barrier for conformational change, until the energy barrier for catalysis begins to approach that of conformational change (orange).

REFERENCES

Boehr, D.D., McElheny, D., Dyson, H.J., and Wright, P.E. (2006). Science *313*, 1638–1642.

Boehr, D.D., Nussinov, R., and Wright, P.E. (2009). Nat. Chem. Biol. *5*, 789–796.

Eisenmesser, E.Z., Millet, O., Labeikovsky, W., Korzhnev, D.M., Wolf-Watz, M., Bosco, D.A., Skalicky, J.J., Kay, L.E., and Kern, D. (2005). Nature *438*, 117–121.

Gagné, D., Charest, L.A., Morin, S., Kovrigin, E.L., and Doucet, N. (2012). J. Biol. Chem. *287*, 44289–44300.

Gobeil, S.M.C., Clouthier, C.M., Park, J., Gagné, D., Berghuis, A.M., Doucet, N., and Pelletier, J.N. (2014). Chem. Biol. *21*, this issue, 1330–1340.

Jackson, C.J., Foo, J.L., Tokuriki, N., Afriat, L., Carr, P.D., Kim, H.K., Schenk, G., Tawfik, D.S., and Ollis, D.L. (2009). Proc. Natl. Acad. Sci. USA *106*, 21631–21636.

Kamerlin, S.C., and Warshel, A. (2010). Proteins *78*, 1339–1375.

Klinman, J.P., and Kohen, A. (2014). J. Biol. Chem. *10*, 565515.

Ma, B., and Nussinov, R. (2010). Curr. Opin. Chem. Biol. *14*, 652–659.

Mittermaier, A., and Kay, L.E. (2006). Science *312*, 224–228.

Saves, I., Burlet-Schiltz, O., Maveyraud, L., Samama, J.P., Promé, J.C., and Masson, J.M. (1995). Biochemistry *34*, 11660–11667.

Tokuriki, N., and Tawfik, D.S. (2009). Science *324*, 203–207.

Warshel, A., Sharma, P.K., Kato, M., Xiang, Y., Liu, H., and Olsson, M.H. (2006). Chem. Rev. *106*, 3210–3235.

 ©2014 Elsevier Ltd All rights reserved