ENZYME ENGINEERING

## Just a jump to the left

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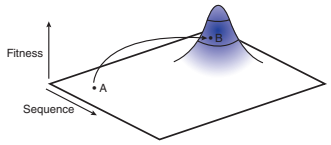
ANDERS SANDSTRÖM




Enzymes have been used by humans to carry out chemical transformations on small-molecule substrates for hundreds of years—for example, for brewing beer or making cheese. However, the high substrate specificity of many enzymes makes it challenging to generate new 'biocatalysts' that efficiently carry out chemical transformations on non-native substrates. Sandström *et al.* now show that it is possible to generate a very small library of mutant lipases and still find a highly active variant that is able to perform an enantioselective reaction on a larger non-native substrate. The authors selected nine amino acids in the substrate-binding pocket for simultaneous mutation, making a library that only contained 1,024 variants. The most active variant identified had mutations at five of the targeted positions. An analysis of single and double mutants suggested that the native enzyme lay in a relatively 'flat' or inactive region of the protein fitness landscape, and the authors believe that 'walking' (via directed evolution) from that position would not have yielded a highly enantioselective mutant lipase for the new substrate. However, that several mutations were simultaneously incorporated meant that the authors had 'hopped' to a different, more active region of the protein fitness landscape. This approach may facilitate the discovery of new biocatalysts for other enantioselective chemical transformations. *JMF*

METHODS

## Zebrafish click

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AMERICAN CHEMICAL SOCIETY

Changes in protein expression in the vertebrate nervous system are regulated by chemical stimuli as well as by changes in behavioral states, and this process is important for various neurological processes, including long-term memory. However, newly synthesized proteins, particularly low-abundance ones, have been difficult to identify. Recently, bio-orthogonal metabolic labeling techniques such as BONCAT or FUNCAT have been developed in which tagged proteins are isolated or visualized, respectively. Hinz *et al.* for the first time use these techniques in an intact organism, the larval zebrafish. The authors showed that the 'clickable' azidohomoalanine tag—compatible with both techniques—was metabolically incorporated with high specificity into a broad range of newly synthesized proteins, including those found in structures deep within the organism, such as those that make up the nervous system. They concluded that BONCAT could be used to detect newly synthesized proteins by MS after affinity enrichment, whereas FUNCAT could be used to identify regions of nascent protein synthesis as well as metabolically active cells and cell groups. The authors then used these methods to show that stimulation with a GABA receptor antagonist causes a general increase in protein synthesis throughout the proteome. These and related techniques paired with chemical stimuli or changes in behavioral state can be used to identify proteome adjustments in distinct regions of the nervous system. *MB*

ALLOSTERY

## A systematic search

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Identifying small-molecule effectors that affect enzyme activity often relies on functional enzyme assays as a readout, but this strategy can overlook metabolites that indirectly alter enzyme activity. To search for these species, Orsak *et al.* report a new method, mass spectrometry integrated with equilibrium dialysis for the discovery of allostery systematically (MIDAS), that detects protein-mediated accumulation of small molecules. In particular, the authors tested a panel of 138 endogenous metabolites against 5 protein targets, including NAD kinase and L-glutamate dehydrogenase type III (GDH), and identified 16 known and 13 unknown interactions. Unknown interactions were further investigated using traditional assays, identifying competitive, noncompetitive and uncompetitive inhibitors as well as activators and those with no obvious effect on function. For example, glucose-6-phosphate's role as an activator of NAD kinase was identified, along with several new inhibitors; the authors speculate that these molecules might work in combination to switch the cell from a low-energy state, in which the reducing equivalents generated by the enzyme are not needed, to a high-energy state, supportive of biogenesis and growth. Similarly, the authors' discovery that *trans*-aconitate functions as an inhibitor of GDH suggests a new point of regulation within the citric acid cycle. Although the full biological significance of these interactions requires further study, this method should provide important starting points for understanding cellular metabolism. *CG*

PROTEIN-PROTEIN INTERACTIONS

## In the TANK

*Cell* **147**, 1340–1354 (2011)

Tankyrase (TNKS) is a poly(ADP-ribose)polymerase that transfers ADP-ribose onto protein substrates, regulating their stability and function. Although mutation of the TNKS-interaction site in the substrate 3BP2 underlies the human disease cherubism, little is known about the molecular determinants for substrate recognition. To address this gap, Guettler *et al.* evaluated the five substrate-binding ankyrin repeat clusters (ARCs) in TNKS2 to confirm at least four substrate binding sites. They solved the X-ray crystal structure of ARC4 with or without 3BP2 substrate peptide and used these structures to predict substrate-bound structures for the remaining ARCs. Four groups of substrate-contacting residues, including two regions that the authors termed an 'arginine cradle' and an 'aromatic glycine switch', were validated with mutagenesis and in multiple protein-protein interaction assays. X-ray crystal structures of ARC4 with peptides from additional protein substrates confirmed the generality of the configuration observed for ARC4-3BP2. Using a peptide library containing substrates that vary at positions in the recognition motif, the authors use fluorescence polarization assays to confirm a requirement for arginine and glycine residues at positions 1 and 6, respectively, and use a position-specific scoring matrix to predict new substrates. Coimmunoprecipitations confirmed 13 new TNKS2 interactors as well as the dependence of these protein-protein interactions on the recognition motif. Together, these data provide the first molecular-level view of tankyrase-substrate interaction and predict additional substrates for these enzymes. *AD*