Recent work (*9*) precisely implements an extended version of this scheme, using a longer NW and a gate voltage to tune the number of conducting channels to two channels in the N-region. This establishes two quasiparticle doublets and allows excitation of spin-flip transitions between the different doublets. However, the intra-doublet spin-flip qubit transition was beyond reach.

The groundbreaking result is that Hays *et al*. (*1*) have been able to demonstrate that the device (*9*) can be operated as a spin qubit, ASQ, in a strong-coupling cQED setup, creating Rabi oscillation with 52 ns spin-echo coherence time and detecting it with single-shot readout. This establishes the ASQ as an interesting alternative but also heralds the need for advanced materials science.

For the ASQ to compete in a decisive manner, the coherence time must approach seconds. This criterion requires the NW N-region to be fabricated with semiconductor material that does not create unwanted coupling to nuclear spins, while conserving the Andreev interface properties and spin-orbit coupling. Another challenge is to control and extend the quasiparticle trapping times into the subsecond domain. The bottom line is that there are no shortcuts to qubit paradigm shifts because basic physics and materials science will determine the progress of quantum computing. ■

"...looking for alternative qubits holds the promise of a breakthrough, if it does not end up becoming a wild goose chase."

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10.1126/science.abk0929

BIOCHEMISTRY

The inner workings of an enzyme

A high-throughput mutation screen dissects the mechanistic basis of enzyme activity

By **Zachary T. Baumer** *and* **Timothy A. Whitehead**

Predictive understanding for how a particular amino acid sequence encodes enzymatic function is a grand challenge in molecular biology, with profound impacts in fields ranging from industrial biotechnology, computational protein design, and agriculture to predictive identification of disease mutations (*1*) and medicinal chemistry. Innovative methods for high-throughput and quantitative measurements of different aspects of enzymatic function are needed to achieve this goal. On page 411 of this issue, Markin *et al*. (*2*) describe a laboratory-on-a-chip platform called High-Throughput Microfluidic Enzyme Kinetics (HT-MEK) as a step in this direction. The technique allows high-fidelity in vitro biochemical and biophysical characterization of more than 1000 mutants of the model enzyme PafA (phosphate-irrepressible alkaline phosphatase of Flavobacterium). HT-MEK identifies partially overlapping yet distinct networks of amino acids that undergird individual reaction steps of PafA, illuminating the mechanistic basis of catalysis for this enzyme.

The ability to read and write DNA cheaply has enabled high-throughput, massively parallel experiments, broadly known as deep mutational scanning (*3*), which can be used to probe how a given enzyme sequence determines function. Typically, many different mutants of an enzyme are passed through some screen or genetic selection, changing the underlying frequency of mutants in that population. Next, the frequency change for each library variant is quantified and converted to a score. A key advantage of this method is scale: Tens of thousands of mutations can be evaluated in a single experiment. However, for enzymes there is usually, although not always (*4*), a narrow dynamic range of approximately fivefold in activity. This is sometimes advantageous in that small changes in activity can substantially enhance cellular fitness, which is useful for understanding steps in molecular evolution. Additionally, robust identification of small improvements in enzymes are particularly valuable for engineering, in which many mutations conferring small improvements can be combined (*5*, *6*). However, deciphering enzyme mechanisms requires activity measurements that span orders of magnitude. Another challenge in deep mutational scanning is that a given mutation may affect several catalytic or biophysical properties that are flattened to a single score. For example, a low score may be the result of protein folding, activity effects, or a combination of both. Thus, there are numerous challenges in interpretating these datasets that cloud a fuller understanding of mechanistic enzymology.

HT-MEK is a conceptually different approach than deep mutational scanning (see the figure). HT-MEK involves the construction of a reaction chamber around an immobilized DNA sequence that encodes an enzyme variant fused to a fluorescent protein. An in vitro reaction produces the enzyme, which is then assayed against different reactants. The fluorescent protein fusion allows quantification of the amount of enzyme in the reaction chamber, enabling determination of Michaelis-Menten kinetic parameters over a large dynamic range spanning several orders of magnitude. Segregation of enzymes into individual chambers affords measurement of enzyme activity as a function of environmental conditions, temperatures, inhibitors, and substrates. Integration of these experiments yields mechanistic dissection of the enzyme.

Alkaline phosphatases have been extensively studied for decades; what more was revealed by Markin *et al*.? The key data generated from HT-MEK—the mutational effects of individual PafA reaction steps for nearly 1000 mutants—allows precise delineation of networks of residues identified by a particular kinetic characteristic. In many cases, these networks extend large distances, from the active site to positions on the enzyme surface more than 20 Å away. Some of the findings have precedence in the literature, such as elucidating that the majority of residues with large catalytic effects are more than 10 Å beyond the active site (*7*). Yet the ability to separate the effects of protein folding from individual catalytic effects offers unparalleled clarity into these allosteric communication channels. HT-MEK thus offers a tantalizing possibility for the unbiased identification of allosteric networks in

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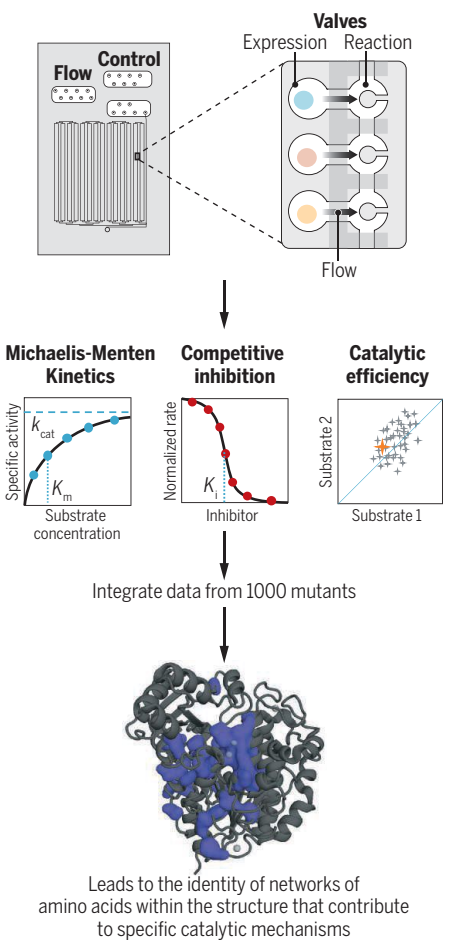
other enzymes, with applications in medicinal chemistry and synthetic biology. A new finding of Markin *et al.* is that mutations to PafA are more likely to result in tighter binding than weaker binding of the competitive inhibitor phosphate. This is unusual because mutations on average are expected to decrease binding. It suggests that strong evolutionary mechanisms in enzymes ensure rapid and efficient turnover (catalytic rate) without excessive end-product inhibition.

Although there are limitations in the demonstrations of HT-MEK, there are clear ways to improve generalizing the method and its throughput. HT-MEK requires product formation to be linked to fluorescence. Markin *et al.* used both a non-natural fluorescent substrate and a fluorescent biosensor (*8*) that detects the phosphate product. The biosensor can be used directly for many diverse enzyme classes that produce inorganic phosphate. Probing other enzyme classes will require innovative methods for high-affinity biosensor design in which the product is linked to fluorescence at a response rate faster than that of the internal enzyme kinetics. Also, PafA is a fast enzyme with a catalytic efficiency approximately 10-fold higher than an average enzyme (*9*). Evaluating less efficient enzymes, such as those involved in secondary metabolism, may result in a diminished dynamic range and sensitivity for assay measurements. In addition, Markin *et al.* used a clever experimental design to disentangle protein misfolding from protein activity by performing activity measurements at different temperatures and choosing mutations unlikely to result in global unfolding. PafA is more stable than typical enzymes, and whether this experimental design can be generalized is an open question. Also, the throughput tested by Markin *et al.* was a little more than 1000 mutants, or less than 10% of the throughput of deep mutational scanning. Several technologies can be envisioned to overcome this throughput limit, including parallelization of devices, advances in on-chip oligonucleotide synthesis of entire synthetic genes, and scale down of the reaction chambers.

Beyond exploring the limits of mechanistic enzymology, there are several near-term applications, such as the de novo design of enzymes (*10*). This approach has succeeded in generating active sites of enzymes with atomic resolution, but turnover rates are generally poor without substantial directed evolution; something is missing in the design concept. Teasing apart the mechanistic basis of activity and inactivity for hundreds or thousands of enzyme designs could help identify potentially missing factors. ■

Enzyme dissection at scale

The High-Throughput Microfluidic Enzyme Kinetics (HT-MEK) approach allows the simultaneous characterization of more than 1000 single-residue mutants of an enzyme. Each chamber expresses a single mutant that is flowed into an enzyme reaction compartment. Kinetic characterization [such as apparent unimolecular rate constant (k_{cat}), Michaelis constant (K_m), inhibition constant (K_i), and substrate specificity] of each mutant is integrated and mapped back onto the structure of the enzyme, revealing networks of residues involved in different catalytic and functional effects.



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ACKNOWLEDGMENTS

The authors are supported by the National Science Foundation (NSF) (Chemical, Bioengineering, Environmental and Transport Systems award 2030221 to T.A.W.; NSF Graduate Research Fellowship Program to Z.T.B.) and National Institutes of Health, National Institute of General Medical Sciences under award R21GM129559-01 to T.A.W.

10.1126/science.abj8346

SPECTROSCOPY

Proximity and single-molecule energetics

Scanning probes measure how nearby oxygen molecules affect triplet lifetimes of pentacene

By **Linfei Li** *and* **Nan Jiang**

Probing single molecules in their nanoenvironment can reveal site-specific phenomena that would be obscured by ensemble-averaging experiments on macroscopic populations of molecules. Particularly in the past decade, major technological breakthroughs in scanning probe microscopy (SPM) have led to unprecedented spatial resolution and versatility and enabled the interrogation of molecular conformation, bond order, molecular orbitals, charge states, spins, phonons, and intermolecular interactions. On page 452 of this issue, Peng *et al.* (*1*) use SPM to directly measure the triplet lifetime of an individual pentacene molecule and demonstrate its dependence on interactions with nearby oxygen molecules with atomic precision. In addition to allowing the local tuning and probing of spin-spin interactions between molecules, this study represents a notable advance in the single-molecule regime and provides insights into many macroscopic behaviors and related applications in catalysis, energy-conversion materials, or biological systems.

Single-molecule studies have benefited from the high resolution achieved with well-defined functionalized probes, especially with carbon monoxide–terminated atomic force microscopy (AFM) tips (*2*). The versatility and applicability of AFM have also been enhanced by biasing the tip with gate voltages and supporting molecules on insulating substrates. In this configuration, the conductive AFM tip serves as an atomically controlled charge injector with single-charge sensitivity. Such electrical addressing of electronic states of single molecules (*3*) allows for the study

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Science, 373 (6553), • DOI: 10.1126/science.abj8346