Published in final edited form as:

Nat Chem Biol. 2015 July ; 11(7): 438–441. doi:10.1038/nchembio.1844.

Quo Vadis, Enzymology?

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Abstract

Enzymology has been a vital link between chemistry and biology in the second half of the 20th century. A range of emerging scientific challenges is presenting the field with exciting opportunities to continue thriving in the future.

Although the study of enzymes dates back to the late 19th century, enzymology, or the study of the structure, function, mechanism & regulation of biological catalysts, emerged as a *bona fide* research field in the post-World War II era. In particular, in the 1960s, the physical organic chemist's insights into catalysis merged with the art of *in vitro* enzyme reconstitution, spurring landmark insights into diverse biological processes ranging from nutrition, energy metabolism and the biosynthesis of the building blocks of life to defining themes of molecular biology including DNA replication and protein translation. In turn, this mechanistic knowledge was extensively harnessed for societal benefit through medicine and agriculture.

Like many mature disciplines, the luster of the name has faded over time. Whereas most contemporary research at the chemistry-biology interface involves enzymes, one does not frequently run into young investigators who introduce themselves as enzymologists. Perhaps it simply reflects the fact that those who study biological catalysts today are more cognizant of the broader implications of their investigations. Or perhaps the gradual decrease in self-identified enzymologists is a consequence of Arthur Kornberg's admonition nearly three decades ago that "biochemistry has ... failed to fill the gulf between chemistry and biology" [1]. Regardless of the reason, my perspective below is motivated by a deep admiration for the timeless aspects of enzymology as well as a strong interest in ensuring its vibrancy.

Why do we need enzymologists?

There are at least three very good reasons why neither chemistry nor biology can afford to overlook enzymology as a discipline.

First, the toolbox for studying biological catalysts has undergone a profound revolution within the first decade of this century. If recombinant DNA expanded the scope of enzymology in the late 20th century, whole-genome sequencing and cheap DNA synthesis have blown the field wide open. What took an entire doctoral dissertation as recently as the nineties, can be achieved today in a matter of weeks. Meanwhile, modern synchrotrons have made protein structures readily accessible to the non-specialist. Advances in computing have brought us within striking distance of simulating complete catalytic cycles. And perhaps most remarkably, single molecule spectroscopy has turned on its head the most basic paradigm of observing enzyme function in an ensemble-averaged manner. All of these advanced tools, and many others, have greatly empowered the core strength of an enzymologist, which is to develop and test mechanistic hypotheses explaining the chemical logic underlying a biological process involving a catalyst. This strength has served chemistry, biology and society very well, and must be nurtured.

Second, the range of problems that need an enzymologist's expertise has also expanded immensely. Many grand challenges in biology and bioengineering depend upon mechanistic insights into biological catalysis. I will have more to say about this in the next section. What is particularly interesting is that "old" fields that depended upon such insights are undergoing rejuvenation. As an example (one that is particularly close to my own heart), metabolism is in vogue again. In part, this is because the \$1,000 human genome reminds us almost daily that, while bad genes may underlie most diseases, metabolic circuits still remain the most fertile therapeutic targets. At the same time, the avalanche of sequenced microbial genomes has revealed that the full scope of metabolic chemistry is no longer appreciably captured in the IUBMB-Nicholson Metabolic Pathways chart (<http://www.iubmb-nicholson.org/chart.html>).

Third, the study of biological catalysis remains one of the most rigorous training opportunities at the interface between chemistry and biology. At a time when data-driven discovery science has become a dominant paradigm, there is considerable pedagogical merit

to formulating and testing mechanistic hypotheses that, on one hand, involve chemical bond formation/cleavage while, on the other, have direct biological relevance. From a technical standpoint, the time-honored approach to interrogating enzyme action through *in vitro* reconstitution remains an essential skill in mainstream biology, as attested by the discovery of catalytic RNA, protein ubiquitination, and vesicle trafficking mechanisms.

With this backdrop, what are the emerging challenges that confront future generations of enzymologists (regardless of what they call themselves)? In no particular order, I cite as examples six broad challenges in enzymology that most readers would recognize as having considerable potential for impact.

Enzyme function annotation

It has long been appreciated that assigning function to enzymes based on sequence alone is difficult. While enzymology will remain a predominantly experimental science in the foreseeable future, one cannot avoid a sense of helplessness when one considers the huge (and growing) deficit in functionally annotated sequences. By now, there are approximately 100 million non-redundant protein sequence entries in GenBank, but a reliably curated protein database such as SwissProt contains fewer than one million entries. This is a quintessential “big data” problem, where the rate at which data is generated continues to outpace the rate at which it is curated. It is unlikely that more resource-intensive curation alone can solve the problem. As the proverbial saying goes, this may be a situation where the most desirable approach would involve user-friendly tools that teach a novice how to fish instead of serving fish. Such tools would ideally capture the essence of the enzymologist’s judgment in layers of increasing sophistication, depending on the user’s actual needs. Community-based efforts such as the Enzyme Function Initiative (<http://enzymefunction.org/>) are making significant headway in this regard.

Understanding biological machines

Notwithstanding a few elegant recent examples to the contrary [2,3], our understanding of energetic coupling in catalytic biological machines is still in its infancy. As early as the late 1970’s, William Jencks formulated general principles by which such systems transform chemical energy into other forms of energy (e.g., active transport, muscle contraction (Fig. 1), ribosomal protein translation, channeling of metabolic intermediates over long distances) [4]. However, for most naturally occurring catalytic machines, our ability to describe at a chemical level how these principles actually operate is lacking. In part this is because the sheer size and conformational flexibility of biological machines impedes structural analysis. The inability to connect small yet significant structural changes to small yet significant energetic differences also contributes to this challenge. More generally, the development of new theories to explain “action at a distance” through an extended network of non-covalent interactions could have a huge impact on this class of problems, because many of these biological machines will continue to elude full-blown computational simulation for the foreseeable future.

RNA enzymology

Although most of this article has implicitly equated enzymology with protein chemistry, biological catalysts are no longer confined to polypeptides. More and more naturally occurring RNA enzymes are being discovered [5]. Meanwhile, non-coding RNAs have possibly become the largest family of cryptic molecules encoded within the human genome. Some of them can be expected to have physiologically relevant catalytic functions [6]. If so, then they may also be therapeutic targets. The example of the bacterial ribosome highlights the potential for drugs that target RNA. However, for such potential to be realized, enzymologists must strengthen their conceptual and technical frameworks for interrogating catalytic RNA. Ribozyme enzymology may also be a particularly attractive venue for testing Richard Feynman's famous adage, "What I cannot create, I do not understand", because of their potential as a platform for *de novo* engineering of allosterically regulated catalysts in synthetic biology applications.

Enzymology of secondary metabolism

The discovery that secondary metabolism is genetically clustered in microorganisms has added a new dimension to enzymology. Initially, the field of natural products chemistry was the beneficiary, as biosynthetic gene clusters revealed unimaginable catalytic strategies in rapid succession. While the trend continues, it leaves in its wake an embarrassment of unprecedented chemical mechanisms (especially in cases of certain metalloenzyme-catalyzed reactions) that have only been scratched on the surface (Fig. 2a) [7–10]. A related challenge, albeit one at a different scale, pertains to understanding nature's myriad strategies for complex molecule construction. Given the exquisite ability of metabolism to operate in a one-pot environment, such problems are most holistically approached through *in vitro* reconstitution of entire biosynthetic pathways [11,12]. More recently, the study of secondary metabolism has also been extended to commensal microorganisms. Although their catalytic genius may not be as awesome as their free-living cousins in soil or in oceans, their ability to coordinate complex, multi-step chemical transformations has profound physiological or pathophysiological consequences for human health.

Post-translational modifications

Post-translational modifications (PTMs) represent an incredibly rich palette for genetic code expansion (Fig. 2b), but their function and regulation (and, in a few instances, even chemistry) is poorly understood. With a few notable exceptions (e.g., GFP fluorophore maturation), PTMs require enzymatic catalysis. What started off as an area of enzymology dominated by ubiquitin- and glycosyl-transfer reactions has spawned a dizzying biochemical lexicon. Each new PTM brings with it an entourage of catalytic isoforms and protein-protein interactions. Nearly 10% of the coding capacity of the human genome appears to be involved in PTMs [13]. The challenge is to develop an integrative and quantitative picture of their chemical as well as biological logic. Because of their immense potential as chemical signatures of human health, at a minimum such knowledge can be expected to transform the practice of precision medicine in the future.

Enzymology in vivo

Although enzymologists invariably analyze biological catalysts in ~55 M water, the biological function of some enzymes is inadequately captured in aqueous buffer (Fig. 3). In such cases, large quantitative (and sometimes qualitative) gaps are evident between *in vitro* and *in vivo* observations. We lack general strategies to understand the contributions of quintessentially cellular features such as anisotropic membranes, gel-like environments, or weak (but specific) macromolecular interactions to biological catalysis. This may be a case where the first step to solving a problem is simply to acknowledge its existence [14]. Remarkably, the earliest examples of *in vivo* analysis of kinetic parameters of enzymes go back several decades [15]. More recently, conceptually straightforward but technically challenging approaches to simulate *in vivo* conditions in test tubes have been gaining attention [16]. Not surprisingly, the awesome power of molecular biology is starting to be harnessed to probe the function of certain enzymes directly *in vivo* [17]. As super-resolution microscopy of cells becomes less invasive, one can look forward to a day when the Kornberg paradigm for purifying an enzyme before studying it may be no more than a “practice problem” offered to future students of enzymology before they take on the real challenge.

An evolving discipline

As Darwin might have predicted, the pressure to have a broader impact on science and society is motivating enzymologists to evolve. Whether this is achieved through brand makeover or by embracing (or even coining) new labels, the study of biological catalysts will continue to flourish. Therefore, with utmost respect to Darwin, I offer three concluding thoughts about the future of the field.

First, those who study biological catalysts will increasingly need to justify their pursuits based on more than chemical novelty. This is not to take anything away from the importance of advancing the frontiers of chemistry *per se*. However, if the experience of a previous generation of physical organic chemists is a harbinger, then relating enzyme chemistry to biology will be the key to this discipline’s future. In articulating how enzymology will inform biology (or biotechnology), it must be recognized that the biologist (or the engineer) will be the ultimate judge of impact. This consideration is perhaps most useful at the time of problem selection.

Second, enzymologists must embrace messy systems. Messy biocatalysts are elaborately regulated multicomponent systems, often with weakly interacting parts. Understanding their properties frequently demands multi-scale analysis, both with respect to length and time. Not only are messy biocatalysts more challenging to reconstitute; *in vitro* models do not capture key qualitative and/or quantitative features of the actual biological system. An expanding toolkit for *in vivo* enzymology could be particularly powerful for comparison purposes, as would an emerging theoretical base that is better suited to the pragmatic constraints of experimentally interrogating such complex systems.

Finally, it behooves me to point out that, although the data explosion in biology runs against the enzymologist’s penchant for mechanistic depth, this trend is here to stay and must

therefore be acknowledged. If so, then, like the proverbial lighthouse, the future of enzymology could depend upon how skilled its practitioners become in saving other life scientists from drowning in their own data.

Acknowledgments

I am grateful to Chris Walsh for numerous discussions on the subject and for his feedback on an early draft of this article. Research on enzymology in the author's laboratory has been generously supported by the National Institutes of Health (R01 GM087934 and R01 DK063158).

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Figure 1.

Energy coupling in catalytic biological machines. More than 30 years ago, Jencks proposed a simple but powerful theoretical framework to explain how free energy of a reaction catalyzed by a biological machine is coupled to the work it performs. In the above example, the energy of ATP hydrolysis is coupled to motion of myosin on an actin filament. In order for this to occur, as Jencks pointed out, myosin must undergo a change in specificity as a result of ATP hydrolysis (step B) to enable it to move directionally on the actin filament.

(a)

(b)

Figure 2.

(a) Two examples of remarkable enzyme-catalyzed chemical transformations involved in secondary metabolism. (Left) Conversion of L-tyrosine into p-hydroxyphenylacetaldehyde oxime by CYP79 from the sorghum plant (18); and (Right) EncM-catalyzed conversion of a highly reactive protein-bound polyketide intermediate into desmethyl-5-deoxyenterocin (19). (b) Examples of post-translational modifications observed in natural proteins.

Figure 3.
Brownian dynamics simulation of the cytoplasmic environment (20).