OPEN ACCESS
Freely Available Online

JOURNAL OF ENZYMES
ISSN NO: 2690-4829

Editorial

DOI: 10.14302/issn.2690-4829.jen-18-2010

Enzymology in its Second Century

Phillip Brumm[1,*]

[1]C5-6 Technologies LLC

Enzymology has a long and illustrious history, dating back to the seminal work by Sumner on crystallization of urease and demonstration that enzymes were proteins (as reviewed in [1]) .

Isolating and purifying individual enzymes, followed by determining the enzyme's properties, has been a mainstay of enzymology for the past 90 years. This type of work is still valuable and remains the backbone of enzymology.

I purified my first enzyme as a college freshman in 1973. At that time, there was no choice but to use native sources of the enzyme. A limited number of chromatography media types were available, and all suffered from slow flow and poor resolution. Complete protein sequences were practically unknown, and temperature and pH optima, and K_m and V_{max} values on defined small-molecule substrates were the major defining properties that defined the enzyme. Over the past 45 years, enzymology has seen quantum leaps in technology:

- Edman degradation made determining protein sequences a tedious, but routine practice, opening up enzymology to asking questions about sequence versus function [2].
- Determining the sequence of enzymes by DNA sequencing [3].
- Cloning of genes to eliminate the need for native sources [4].
- Improved promoters for high level expression combined with enzyme tags and affinity chromatography [5].
- Complete chemical synthesis of genes coding for enzymes
- High throughput enzyme crystal structure determination [6].

The result of these technology leaps is a generally faster, easier route to pure, single enzymes. What is needed, as we approach the start of the second

Corresponding Author: Phillip Brumm, C5-6 Technologies LLC 5627 Old Oak Drive Fitchburg, WI 53711 USA. Email: pbrumm@c56technologies.com

Received: Feb 27, 2018 **Accepted:** Feb 27, 2018 **Published:** Mar 01, 2018

century of enzymology, is a new set of challenges for enzymology. Some of these challenges include:

- Natural product production by assembling biosynthetic pathways *in vitro*.
- Degradation of complex natural substrates by single enzymes, mixtures of enzymes, and enzyme complexes such as cellulosomes [7].
- Non-traditional enzymes including single-turnover enzymes such as Cas9 [8].
- Enzymology of microbial immune systems [9].
- Role of glycosylation [10] and other post-translational modifications on enzyme activity.
- Functional characterization of "hypothetical proteins" identified in genomic and metagenomic sequencing [11].

There are many routes forward for enzymology in this dawning new century. Enzymologists should boldly explore new techniques and new collaborations to continue advancing the field.

References

1. Simoni RD, Hill RH, Vaughan M. Urease, the first crystalline enzyme and the proof that enzymes are proteins: the work of James B. Sumner. J Biol Chem. 2002;277(35):23e.
2. Heinrikson RL. Application of automated sequence analysis to the understanding of protein structure and function. Ann Clin Lab Sci. 1978;8(4):295-301.
3. Smith M. The first complete nucleotide sequencing of an organism's DNA. Am Sci. 1979;67(1):57-67.
4. Ehrlich SD, Bursztyn-Pettegrew H, Stroynowski I, Lederberg J. Expression of the thymidylate synthetase gene of the Bacillus subtilis bacteriophage Phi-3-T in Escherichia coli. Proc Natl Acad Sci U S A. 1976;73(11):4145-9.
5. Kroll DJ, Abdel-Malek Abdel-Hafiz H, Marcell T, Simpson S, Chen CY, Gutierrez-Hartmann A, et al. A multifunctional prokaryotic protein expression system: overproduction, affinity purification, and selective detection. DNA Cell Biol. 1993;12(5): 441-53.
6. Walter TS, Diprose JM, Mayo CJ, Siebold C, Pickford MG, Carter L, et al. A procedure for setting up high-throughput nanolitre crystallization experiments. Crystallization workflow for initial screening, automated storage, imaging and optimization. Acta Crystallogr D Biol Crystallogr. 2005;61(Pt 6):651-7.
7. Brumm PJ. Bacterial genomes: what they teach us about cellulose degradation. Biofuels. 2013;4(6): 69-81.
8. Jinek M, Jiang F, Taylor DW, Sternberg SH, Kaya E, Ma E, et al. Structures of Cas9 endonucleases reveal RNA-mediated conformational activation. Science. 2014;343(6176):1247997.
9. Doron S, Melamed S, Ofir G, Leavitt A, Lopatina A, Keren M, et al. Systematic discovery of antiphage defense systems in the microbial pangenome. Science. 2018.
10. Nothaft H, Szymanski CM. Bacterial protein N-glycosylation: new perspectives and applications. J Biol Chem. 2013;288(10):6912-20.
11. Doyle S. Fungal proteomics: from identification to function. FEMS Microbiol Lett. 2011;321(1):1-9.