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Enzymology in its Second Century

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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

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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.

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