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ACS chemical biology

Ralph F. Hirschmann, 1922–2009. A Perspective

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Ralph F. Hirschmann, an organic chemist who bridged the chemistry–biology interface, died June 20, 2009 at his home in Pennsylvania. He was 87 years old. Recent graduate students reading this article might recall Ralph F. Hirschmann, Professor of Chemistry at the University of Pennsylvania, who was a major innovator in the field of peptide mimetics from 1987 to the present. Senior faculty might also know that Ralph Hirschmann was Senior Vice President for Research at Merck during the company's most productive drug discovery era. Members of the general scientific community might remember that Ralph Hirschmann was awarded the National Medal of Science in 2000 by President of the United States Bill Clinton. But in its July 18 obituary for Ralph Hirschmann, the *New York Times* recalled him as "a leader of a team of biochemists that for the first time synthesized an enzyme, one of the master chemicals of life" and reprinted a photograph that was published in January 17, 1969 on the front page of that paper, titled "An Enzyme is Synthesized for the First Time". The total synthesis of an enzyme certainly was a landmark achievement in organic chemistry and biochemistry. What is generally forgotten is that it was also an early example of what has now become known as chemical biology.

Ralph Hirschmann led the team of Merck chemists (which included Dr. Robert G. Denkewalter, Dr. Daniel F. Veber, and Dr. Frederick W. Holly) who accomplished the total synthesis of the enzyme, Ribonuclease-S (RNase-S) in 1969. At the same time, Bruce Merrifield and Bernd Gutte accomplished the synthesis of RNase-A, a closely related enzyme, and the two groups reported their results at the same press conference. This achievement was considered significant enough to be front page news in the *New York Times* as well as *Chemical & Engineering News*, February 10, 1969.

Ralph F. Hirschmann and the author at a 2005 ACS meeting. Image courtesy of Garland R. Marshall.

Merrifield and Gutte accomplished their synthesis by using the solid-phase synthetic method invented by Merrifield in 1963, for which he received the 1984 Noble Prize in Chemistry. In contrast, the Merck team used a more classical organic approach of assembling segments in solution and coupling them in multiple steps to assemble the fully protected protein. The success of their approach was dependent on the team's invention of several novel protecting groups and coupling reagents to effect an efficient overall yield and on very careful process research to optimize reaction conditions. The concurrent achievement of these syntheses by different routes instantly corroborated both results, and the importance of their

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Published online September 18, 2009

10.1021/cb900208u CCC: $40.75

discoveries had a significant impact on the emerging fields of bio-organic chemistry and chemical biology. Looking back, it is difficult to remember just how risky this research was in the late 1960s.

Biologically active peptides that contained 20−30 amino acids had been prepared about 10 years earlier, but larger peptides and proteins represented a much bigger challenge. Peptides of that size tend to aggregate and to be insoluble and were very difficult to characterize with the analytical methods available. For these reasons, the Merck group chose to utilize a fragment condensation strategy that affords pure, more readily characterized intermediates but suffers for the difficulty of the penultimate step, the coupling of two 5 kD segments in solution. Even when this was achieved, the next step necessitated removal of many side chain protecting groups that were known to undergo intramolecular rearrangements under certain conditions. Finally, after the two previous obstacles had been overcome, the protein had to be folded from a mixture of ill-defined conformations into the biologically active conformation of the catalytically active enzyme.

There was evidence from the work of Christian Anfinsen (first reported in 1957−58) that strongly suggested that the amino acid sequence of a protein contained all the information needed for the protein to fold correctly into the biologically active form, the so-called "thermodynamic hypothesis". However, Anfinsen's hypothesis was based on a series of studies in which native proteins were denatured in aqueous media containing urea and other solutes that break up intramolecular hydrogen bonds; the so-called denatured protein was then refolded slowly as the solutes were removed by dialysis. Anfinsen's prior work had established that several proteins treated in this fashion did refold as expected. These experiments appeared to confirm the thermodynamic hypothesis, but only if no portion of the denatured protein existed in its native catalytic form that could "seed" or facilitate refolding. But did Anfinsen really know that there was "no residual conformation" to seed the correct refolding? In the 1960s there were only limited ways to detect denaturation: loss of catalytic (or biological) activity or loss of spectroscopic signals (CD/ORD) characteristic of native conformations. Anfinsen himself was aware that trace amounts of native conformations might not be detectable by these methods and spoke of this in some of his lectures. And knowing what we know in the modern era about prions, which in trace amounts are thought to catalyze conformational transitions in native proteins, or of recent NMR studies that reveal the presence of non-random, residual structure between amino acid side chains in supposedly totally denatured protein, Anfinsen was wise to recognize the limits to which his hypothesis had been tested prior to the work of Hirschmann and of Merrifield.

The definitive experiment to prove that the amino acid sequence in a protein determines its 3D conformation was to synthesize a fully protected protein precursor, remove the protecting groups, and let the unprotected amino acid sequence fold up. Since the fully protected protein was assembled from fragments (in Hirschmann's work), the deprotected sequence had never existed in the native conformation and therefore had no "memory" (residual clusters) to initiate folding *de novo* along a particular pathway. The successful synthesis of catalytically active RNase-A by Merrifield and RNase-S by Hirschmann *et al.* removed the last ambiguity in Anfinsen's thermodynamic hypothesis, for which he received the 1972 Nobel Prize in Chemistry. These two total syntheses of enzymes are an early example of experimental chemical biology.

How did a chemist trained in synthetic chemistry and hired to do process research in industry end up bridging the chemistry biology interface? Organic chemists trained in the 1950s rarely strayed into biology and for good reason: organic chemistry was in the midst of a renaissance and entering a remarkably creative era. D. H. R. Barton's discoveries of conformational analysis, announced in 1950 and which led to his 1969 Nobel Prize in Chemistry, stimulated and guided all sorts of studies to characterize the conformational properties of organic molecules. Studies of reactive chemical intermediates illuminated fundamental organic reaction mechanisms, and these in turn led to logical invention of new reagents and methods to prepare increasingly complex natural products. The commercialization of radioactive isotopes, notably ^{14}C and ^{3}H, enabled biosynthetic mechanisms to be elucidated, another early important contribution of chemistry to chemical biology.

Hirschmann came of scientific age during this era. He received his Ph.D. in chemistry from the University of Wisconsin in 1950, where he worked on the synthesis of steroids, and was then hired to do process research at Merck, again working primarily on steroids. But pharmaceutical companies bring together both chemists and biologists in their search for new drugs. A chemist, who must be an expert in organic synthesis, works together with biologists, who must be experts in some therapeutic discipline. Sometimes chemists want to understand both fields, and Hirschmann was one who showed a penchant for solving problems at the chemistry−biology interface. A notable achievement during this early phase of his career was his use of steroidal *N*-acetylglucosamides to direct anti-inflammatory agents to their site of action, thus demonstrating both the "prodrug" concept and the targeted drug release concept long before these terms entered our scientific vocabulary. From 1970 to 1987, Hirschmann focused more and more on research administration, culminating as Senior Vice President of Basic Research from 1979−1984 and Senior Vice President of Chemistry from 1984−1987. Under his leadership, Merck scientists developed the antiparasitic Ivermectin used for the treat-

ment of river blindness, the statins Mevacor and Zocor for reducing cholesterol in patients at risk of cardiovascular disease, the β-lactam-family broad-spectrum antibiotic Primaxin, the synthetic androgen Proscar used for the treatment of enlarged prostate and prostate cancer, and the ACE inhibitor Vasotec for treating hypertension.

In 1987, Hirschmann became the first Research Professor in Chemistry at the University of Pennsylvania. Later he was named the Rao Makineni Professor of Bio-Organic chemistry and in 2002 this became the Hirschmann-Makineni Professorship in Chemistry. During this phase of his career, he invented novel ways to synthesize and design nonpeptide mimics called "peptidomimetics". In collaboration with Professor Amos Smith, he developed a series of β-D-glucose scaffolds that mimic β-turns. Initially targeted to the SRIF receptor subtype 4, a G-protein-mediated signal transducing receptor, closely similar analogues of these compounds inhibited other G-protein coupled receptors for substance P and b2-adrenergic receptors. This work had major impact on the growth of the peptidomimetic research and also augmented the rationale for preparing closely related organic structures by combinatorial chemical synthesis.

Ralph Hirschmann, who was recognized with an Honorary Doctor of Science Degree in 1996 from the University of Wisconsin-Madison, remains one of the University of Wisconsin's most distinguished graduates. Beginning as an organic chemist trained in the synthesis of steroids, he matured into a world-class scientist who made important contributions to peptide chemistry, protein chemistry, medicinal chemistry, bio-organic chemistry, and early chemical biology. He accomplished this through highly productive collaborations with chemists, biologists, clinicians, biophysical chemists, and pharmacologists, both while at Merck and throughout his academic career. His integrity was impeccable and the loyalty of his associates admirable. That he could recruit these scientists to his team and retain their respect, admiration, and friendship throughout his life may well be his greatest legacy and an accomplishment for all to emulate.