Guest Editorial: Chemical Protein Synthesis

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Chemical Protein Synthesis

Proteins are numerous and diverse; they are the workhorse molecules of the cellular world, carrying out almost all of the biochemistry that goes on in cells. Chemistry has a unique role to play in our efforts to understand the principles that govern the architecture and consequent functions of protein molecules. Because of the introduction of modern chemical ligation methods, the application of chemistry to protein science has made great strides in recent years. Chemical ligation represented a radical shift in the way chemists approach the synthesis of the large polypeptide chains found in protein molecules. The underlying principle — chemoselective covalent condensation of unprotected peptide segments, to give the full-length polypeptide chain of a target protein molecule — is at once simple yet powerful.

Of all the chemistries that have been introduced based on the chemical ligation principle, the most effective is native chemical ligation, the thioester-mediated, amide-forming covalent condensation of unprotected peptide segments at a Xaa-Cys ligation site. Native chemical ligation is equally applicable to total chemical protein synthesis from a set of synthetic peptide segments, or to semi-synthesis using a recombinantly expressed polypeptide having a C-terminal alpha-thioester or an N-terminal Cys. Recent extensions of native chemical ligation include the use of "one pot" synthesis to facilitate the preparation of long polypeptide chains from multiple peptide segments, and the fully convergent synthesis of target polypeptides from four synthetic peptide building blocks. In addition, effective methods for extending native chemical ligation to a wider range of Xaa-Yaa ligation sites have been developed. The key conceptual breakthrough was made by Yan and Dawson, who introduced ligation at Xaa-Ala sites by native chemical ligation at Xaa-Cys followed by facile desulfurization. Variations on this theme, and other enhancements of native chemical ligation, provide the researcher with a set of effective tools that enable synthetic access to a wide range of target protein molecules.

Once synthetic access has been established, the researcher has complete atom-by-atom control over the covalent structure of those parts of the protein molecule made by chemical means. This provides for great versatility in the incorporation of, for example, fluorescent, NMR, or spin labels at specific sites within a protein molecule, in order to use advanced physical techniques to study conformational aspects of protein function. Chemical protein synthesis also enables the researcher to use an unlimited range of non-coded amino acid building blocks, and uniquely enables the synthesis of mirror-image protein molecules — made entirely from D-amino acids and the achiral amino acid glycine — which are useful in racemic crystallography and in drug discovery. Many of these capabilities are so recent that investigators have only just begun to explore the myriad utilities of chemical protein synthesis.

The articles in this special issue of the Israel Journal of Chemistry represent a "snapshot" of topics from the current state of the art of research involving chemical protein synthesis. Seitz describes the preparation and application of DNA–peptide molecular hybrids. Macmillan reports on the generation of peptide-thioesters, for use in native chemical ligation, from peptides, by N-to-S acyl shift under special conditions; this is one approach to overcoming a challenge faced by all researchers who use native chemical ligation in protein science: how to generate the (poly)peptide thioester building blocks. Dawson reviews the application of desulfurization in conjunction with native chemical ligation, and its extension to selective deselenization. Muir describes the applications of protein trans-splicing using split-inteins, to carry out on-demand generation of active protein species in vivo.

Correct folding of chemically synthesized polypeptide chains to give the unique tertiary structure of the functional protein molecules is an essential practical consideration for the total chemical synthesis of proteins. In particular, there are numerous small disulfide-containing proteins with potent and specific biological activities, including secretory proteins and venom-derived proteins, and effective methods for folding these proteins with concomitant formation of native disulfides are important. Here, Hilvert reports on the use of selenocysteine in high-efficiency folding of disulfide-containing proteins.

Other contributions to this special issue describe the application of chemical protein synthesis to challenging and important research areas that include: the synthesis of ubiquitinated protein constructs of defined structure (Brik); the use of mirror-image proteins to developed D-peptide therapeutic lead molecules (Lu); the study of "cyclotides" — proteins with circular polypeptide chains (Craik); and single molecule studies of enzyme activity (Kent).

Glycoprotein structure and function, and integral membrane proteins, are two areas of research that are the subject of great current interest. The application of chemical

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synthesis to glycoproteins is represented by contributions from the Danishefsky group and from the Kajihara group. These articles describe distinct and complimentary approaches to this important topic. Integral membrane proteins are abundant in nature, and play key roles in a variety of important biology. Here, two contributions (Becker; Liu) describe the state of the art and prospects for the application of chemical protein synthesis to integral membrane proteins.

Proteins are the essential "natural products" of the 21st century, and as such are fitting targets of research by the world's best chemists. Despite the breadth of topics covered in this special issue on Chemical Protein Synthesis, there are an equal number of applications of chemical synthesis to the study of proteins that are not represented, but that can be found in the current literature. Chemical protein synthesis has virtually unlimited applicability to, for example, the elucidation of novel structures and functions for the plethora of predicted protein molecules, known only as open reading frames in genome sequence data, and for the manufacture of protein therapeutics by total chemical synthesis, to name just two important applications. Despite the efficacy of current methods for the total synthesis of proteins, there is plenty of room for exciting innovations in synthetic chemistry, and for new applications of current synthetic capabilities to elucidate the molecular basis of protein functions. As the research community continues to apply synthetic chemistry to protein molecules, we can be assured that new chemistries will be forthcoming. The 21st century will be a Golden Age of chemical protein science.

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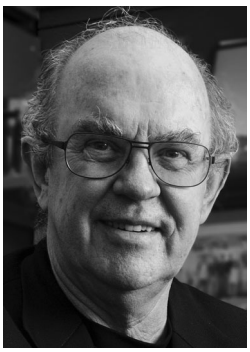

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