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“Use of Modern Chemical Protein Synthesis and Advanced Fluorescent Assay Techniques to Experimentally Validate the Functional Annotation of Microbial Genomes”

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Summary

The objective of this research program was to prototype methods for the chemical synthesis of predicted protein molecules in annotated microbial genomes. High throughput chemical methods were to be used to make large numbers of predicted proteins and protein domains, based on microbial genome sequences. Microscale chemical synthesis methods for the parallel preparation of peptide-thioester building blocks were developed; these peptide segments are used for the parallel chemical synthesis of proteins and protein domains. Ultimately, it is envisaged that these synthetic molecules would be ‘printed’ in spatially addressable arrays. The unique ability of total synthesis to precision label protein molecules with dyes and with chemical or biochemical ‘tags’ can be used to facilitate novel assay technologies adapted from state-of-the art single molecule fluorescence detection techniques. In the future, in conjunction with modern laboratory automation this integrated set of techniques will enable high throughput experimental validation of the functional annotation of microbial genomes.

Results

During the course of this research program we developed parallel, automated high-throughput chemical synthesis of peptide-thioesters and we prepared by total chemical synthesis a variety of protein molecules in order to prototype our novel synthetic methods. Work in the total period of the research program has resulted in 15 published papers. Each paper contains the following acknowledgment: “This research was supported by the Office of Science (BER), U.S. Department of Energy, Grant No. DE-FG02-07ER64501.” These papers are listed below under ‘Papers and Products’.

Papers and Products

Published papers are:

1. Synthesis of photo-active analogs of a cystine knot trypsin inhibitor protein. Thomas Durek, Junliang Zhang, Chuan He, Stephen B.H. Kent, *Organic Letters*, **9**, 5497-500 (2007).
2. Mirror image forms of snow flea antifreeze protein prepared by total chemical synthesis have identical antifreeze activities. Brad L. Pentelute, Zachary P. Gates, Jennifer Dashnau, Jane M. Vanderkooi, Stephen B. H. Kent, *J Am Chem Soc*, **130**, 9702-9707 (2008).

3. X-ray structure of snow flea antifreeze protein determined by racemic crystallization of synthetic protein enantiomers. Brad L. Pentelute, Zachary P. Gates, Valentina Tereshko, , Jennifer Dashnau, Jane M. Vanderkooi, Anthony A. Kossiakoff, Stephen B. H. Kent, *J Am Chem Soc*, **130**, 9695-9701 (2008).
4. Crystal structure of chemically synthesized HIV-1 protease and a ketomethylene isostere inhibitor based on the p2/NC cleavage site. V. Yu. Torbeev, K. Mandal, V. A. Terechko, S. B. H. Kent, *Bioorganic & Medicinal Chemistry Letters*, **18**, 4554-7 (2008).
5. Comparative properties of insulin-like growth factor 1 (IGF-1) and [Gly7D-Ala]IGF-1 prepared by total chemical synthesis. Youhei Sohma, Brad L. Pentelute, Jonathan Whittaker, Qin-xin Hua, Linda J. Whittaker, Michael A. Weiss, and Stephen B. H. Kent, *Angewandte Chemie Int Ed Engl*, **47**, 1102-1106 (2008).
6. Dynamics of 'flap' structures in three HIV-1 protease/inhibitor complexes probed by total chemical synthesis and pulse-EPR spectroscopy. Vladimir Yu. Torbeev, H. Raghuraman, Kalyaneswar Mandal, Sanjib Senapati, Eduardo Perozo, Stephen B. H. Kent, *J Am Chem Soc*, **131**, 884-5 (2009).
7. X-ray structure of native scorpion toxin BmBKTx1 by racemic protein crystallization using direct methods. Kalyaneswar Mandal, Brad L. Pentelute, Valentina Tereshko, Anthony A. Kossiakoff, Stephen B. H. Kent, *J Am Chem Soc*, **131**, 1362-3 (2009).
8. Total chemical synthesis and racemic protein crystallography used to determine the X-ray structure of plectasin by direct methods. Kalyaneswar Mandal, Brad L. Pentelute, Valentina Tereshko, Anthony A. Kossiakoff, Stephen B. H. Kent, *Protein Science*, **18**, 1146-1154 (2009).
9. Chemical protein synthesis: inventing new chemistries to reveal how proteins work (Merrifield Award address). Stephen B.H. Kent, in *"Proceedings of the 21st American Peptide Symposium"*, M. Lebl, ed., Prompt Scientific Publishing, San Diego, CA, 2009, pp.3-8.
10. Synthesis and comparative properties of two amide-generating resin linkers for use in solid-phase peptide synthesis. Fang-Kun Deng, Kalyaneswar Mandal, Sam Luisier, Stephen B. H. Kent, *J. Peptide Science*, **16**, 545-550 (2010).
11. Determination of the X-ray structure of the snake venom protein omwaprin by total chemical synthesis and racemic protein crystallography. James R. Banigan, Kalyaneswar Mandal, Michael R. Sawaya, Vilasak Thammavongsa, Antoni P. A. Hendrickx, Olaf Schneewind, Todd O. Yeates, Stephen B. H. Kent, *Protein Science*, **9**, 1840-1849 (2010).
12. Total chemical synthesis of biologically active vascular endothelial growth factor. Kalyaneswar Mandal, Stephen B.H. Kent, *Angewandte Chem Int Ed*, **50**, 8029–

8033, (2011).

13. On the role of protein conformational dynamics in the chemical mechanism of HIV-1 protease catalysis. Vladimir Yu. Torbeev, H. Raghuraman, Donald Hamelberg, Marco Tonelli, William M. Westler, Eduardo Perozo & Stephen B. H. Kent, *Proc Natl Acad Sci USA*, **108**, 20982-20987 (2011).
14. Convergent chemical synthesis of [Lysine^{24,38,83}]human erythropoietin. Suhuai Liu, Brad L. Pentelute, Stephen B. H. Kent, *Angewandte Chem*, **51**, 993-999 (2012).
15. Design, total chemical synthesis, and X-ray structure of a protein having a novel polypeptide chain topology. Kalyaneswar Mandal, Brad L. Pentelute, Duhee Bang, Zachary P. Gates, Vladimir Yu. Torbeev, Stephen B. H. Kent, *Angewandte Chem Int Ed*, **51**, 1481-1486 (2012).