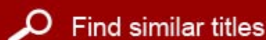
This PDF is available from The National Academies Press at http://www.nap.edu/catalog.php?record_id=18354

Directed Evolution for Development and Production of Bioactive Agents: A Meeting Summary

ISBN
978-0-309-28626-8

27 pages
8 1/2 x 11
PAPERBACK (2013)

Daniel Talmage and Dionna Ali, Rapporteurs; Committee on Science and Technology for Defense Warning; Division on Engineering and Physical Sciences; National Research Council



Visit the National Academies Press online and register for...

- Instant access to free PDF downloads of titles from the
  - NATIONAL ACADEMY OF SCIENCES
  - NATIONAL ACADEMY OF ENGINEERING
  - INSTITUTE OF MEDICINE
  - NATIONAL RESEARCH COUNCIL
- 10% off print titles
- Custom notification of new releases in your field of interest
- Special offers and discounts

Distribution, posting, or copying of this PDF is strictly prohibited without written permission of the National Academies Press.
Unless otherwise indicated, all materials in this PDF are copyrighted by the National Academy of Sciences.
Request reprint permission for this book

Copyright © National Academy of Sciences. All rights reserved.

THE NATIONAL ACADEMIES
Advisers to the Nation on Science, Engineering, and Medicine

Directed Evolution for Development and Production of Bioactive Agents

A Meeting Summary

Daniel Talmage and Dionna Ali
Rapporteurs

Committee on Science and Technology for Defense Warning

Division on Engineering and Physical Sciences

NATIONAL RESEARCH COUNCIL
OF THE NATIONAL ACADEMIES

THE NATIONAL ACADEMIES PRESS
Washington, D.C.
www.nap.edu

Copyright © National Academy of Sciences. All rights reserved.

THE NATIONAL ACADEMIES PRESS 500 FIFTH STREET, NW Washington, DC 20001

NOTICE: The project that is the subject of this report was approved by the Governing Board of the National Research Council, whose members are drawn from the councils of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine.

This study was supported by Contract HHM402-10-D-0036 between the Defense Intelligence Agency and the National Academy of Sciences. Any views or observations expressed in this publication are those of the author(s) and do not necessarily reflect the views of the organizations or agencies that provided support for the project.

International Standard Book Number-13: 978-0-309-28626-8
International Standard Book Number-10: 0-309-28626-3

Limited copies of this report are available from the Division on Engineering and Physical Sciences, National Research Council, 500 Fifth Street, NW, Washington, DC 20001; (202) 334-2400.

Copyright 2013 by the National Academy of Sciences. All rights reserved.

Printed in the United States of America

Copyright © National Academy of Sciences. All rights reserved.

THE NATIONAL ACADEMIES

Advisers to the Nation on Science, Engineering, and Medicine

The **National Academy of Sciences** is a private, nonprofit, self-perpetuating society of distinguished scholars engaged in scientific and engineering research, dedicated to the furtherance of science and technology and to their use for the general welfare. Upon the authority of the charter granted to it by the Congress in 1863, the Academy has a mandate that requires it to advise the federal government on scientific and technical matters. Dr. Ralph J. Cicerone is president of the National Academy of Sciences.

The **National Academy of Engineering** was established in 1964, under the charter of the National Academy of Sciences, as a parallel organization of outstanding engineers. It is autonomous in its administration and in the selection of its members, sharing with the National Academy of Sciences the responsibility for advising the federal government. The National Academy of Engineering also sponsors engineering programs aimed at meeting national needs, encourages education and research, and recognizes the superior achievements of engineers. Dr. Charles M. Vest is president of the National Academy of Engineering.

The **Institute of Medicine** was established in 1970 by the National Academy of Sciences to secure the services of eminent members of appropriate professions in the examination of policy matters pertaining to the health of the public. The Institute acts under the responsibility given to the National Academy of Sciences by its congressional charter to be an adviser to the federal government and, upon its own initiative, to identify issues of medical care, research, and education. Dr. Harvey V. Fineberg is president of the Institute of Medicine.

The **National Research Council** was organized by the National Academy of Sciences in 1916 to associate the broad community of science and technology with the Academy's purposes of furthering knowledge and advising the federal government. Functioning in accordance with general policies determined by the Academy, the Council has become the principal operating agency of both the National Academy of Sciences and the National Academy of Engineering in providing services to the government, the public, and the scientific and engineering communities. The Council is administered jointly by both Academies and the Institute of Medicine. Dr. Ralph J. Cicerone and Dr. Charles M. Vest are chair and vice chair, respectively, of the National Research Council

www.national-academies.org

Copyright © National Academy of Sciences. All rights reserved.

Copyright © National Academy of Sciences. All rights reserved.

COMMITTEE ON SCIENCE AND TECHNOLOGY FOR DEFENSE WARNING

BRENDAN B. GODFREY, *Chair*, University of Maryland
THOMAS E. ROMESSER, *Vice Chair,* Northrop Grumman Aerospace Systems (retired)
ILHAN A. AKSAY, Princeton University
ANN N. CAMPBELL, Sandia National Laboratories
JOHN F. CASHEN, Aerospace and Defense Consultant
EDWARD M. GREITZER, Massachusetts Institute of Technology
ELLIOTT D. KIEFF, Harvard University
FRANCIS R. LANDOLF, Core Consulting, LLC
PATRICK LINCOLN, SRI International
DARRELL D.E. LONG, University of California, Santa Cruz
DAVID F. McQUEENEY, IBM Research
RANDOLPH L. MOSES, Ohio State University
MONICA OLVERA DE LA CRUZ, Northwestern University
MIKHAIL SHAPIRO, University of California, Berkeley
ELIAS TOWE, Carnegie Mellon University

Staff

TERRY JAGGERS, Lead DEPS Board Director
DANIEL E.J. TALMAGE, JR., Study Director
DIONNA ALI, Senior Program Assistant
CHRIS JONES, Financial Associate

Copyright © National Academy of Sciences. All rights reserved.

Acknowledgment of Reviewers

This report has been reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise, in accordance with procedures approved by the National Research Council's (NRC's) Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making its published report as sound as possible and to ensure that the report meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. We wish to thank the following individuals for their review of this report:

Brendan B. Godfrey, University of Maryland,
Diane E. Griffin (NAS/IOM), Johns Hopkins Bloomberg School of Public Health,
J. Jerome Holton, The Tauri Group, and
Mikhail G. Shapiro, University of California at Berkeley.

Although the reviewers listed above have provided many constructive comments and suggestions, they were not asked to endorse the views of individual participants, nor did they see the final draft of the report before its release. The review of this report was overseen by Gerald F. Joyce (NAS), The Scripps Research Institute. Appointed by the NRC, he was responsible for making certain that an independent examination of this report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this report rests entirely with the authors and the institution.

Copyright © National Academy of Sciences. All rights reserved.

Contents

1 MEETING SUMMARY 1
Objectives and Scope, 1
Presentations, 1
Protein Switches and the Genetic Code, 2
Directed Evolution of New Viruses for Gene Therapy, 3
Expanding the Synthetic Capabilities of Yeast, 5
One Handful of Soil: 10,000,000,000 Microbes, 100,000 Different Species, 6
Digital Chemistry: The Fabrication and Application of Ordered Molecular Arrays, 7
Cell-Penetrating Mini-Proteins, 9
Discussion, 10
Final Comments, 11

APPENDIXES

A Workshop Presentations and Participants 15
B Biographies 17

Copyright © National Academy of Sciences. All rights reserved.

Copyright © National Academy of Sciences. All rights reserved.

1
Meeting Summary

In 2012, the Defense Intelligence Agency (DIA) approached the National Research Council and asked that an ad hoc committee be formed to develop a list of workshop topics to explore the impact of emerging science and technology. One topic that came out of that list was directed evolution for development and production of bioactive agents. This workshop was held on February 21-22, 2013.

OBJECTIVES AND SCOPE

The objectives for the workshop were to explore the potential use of directed evolution[1] for military science and technology. The statement of task for the meeting is given in Box 1-1. Understanding the current research in this area, and the potential opportunities for U.S. adversaries to use this research, might allow the DIA to advise U.S. policy makers in an appropriate and timely manner. The workshop featured invited presentations and discussions that aimed to:

- Inform the U.S. intelligence community of the current status of directed evolution technology and related research, and
- Discuss possible approaches involving directed evolution that might be used by an adversary to develop toxic biological agents that could pose a threat to the United States or its allies, and how they could be identified.

Members of the Committee on Science and Technology for Defense Warning planned the agenda for the workshop (Appendix A), selected the presenters (see Appendix B for biographies), and helped moderate discussions in which meeting participants probed issues of national security related to directed evolution in an effort to gain an understanding of potential vulnerabilities. Experts were invited from the areas of directed evolution, biosynthesis, detection, and biological agents. This report summarizes the views expressed by individual meeting participants and was constrained by the meeting agenda; the views described are not necessarily those of all meeting participants, the committee, or the National Research Council. This meeting was not intended to provide a comprehensive review of the state of directed evolution. The rapporteurs are responsible for the overall quality and accuracy of the report as a record of what transpired at the meeting,

PRESENTATIONS

Dr. Mikhail Shapiro, committee member, started the meeting by describing the committee's origin and how the meeting had been devised. He also thanked the presenters for sending in read-aheads that complemented their presentations. He then requested that the participants introduce themselves.

[1]Directed evolution mimics natural selection on an accelerated timescale to create novel organisms, proteins, or nucleic acids, with desirable properties not found in nature. For more information see the National Research Council report, *The Scinece and Applications of Synthetic and Systems Biology: Workshop Summary*, The National Academies Press, Washington, D.C., 2011, available at http://www.nap.edu/catalog.php?record_id=13239.

Copyright © National Academy of Sciences. All rights reserved.

Box 1-1

Statement of Task

An ad hoc committee will plan and conduct a two-day review of selected national security implications of recent advances in Directed Evolution for Development and Production of Bioactive Agents. The meeting will feature invited presentations and panelists and include discussions on directed evolution, research, and application that could create a technological surprise to our Nation's security and require US Defense Department intelligence monitoring and advance warning to allow national leaders to take action. The committee will plan the agenda, select and invite speakers and discussants, and moderate the discussions.

Dr. Shapiro also suggested that participants think about how an adversary could use the technology discussed during the meeting and noted that there would be time for open discussion. After the introductions were completed Dr. Shapiro introduced the first presenter.

Protein Switches and the Genetic Code

Marc Ostermeier, Professor and Vice Chair, Chemical and Biological Engineering
Johns Hopkins University

Dr. Marc Ostermeier opened his presentation by discussing protein switches. He mentioned that activity at one site in a protein is regulated by changes at a distal site in the same protein (e.g., binding of ligand at a distal site). Communication between the two sites occurs through conformational changes, and these proteins can be described as switches and can be seen as transducers of information. He then showed an example of building protein switches by domain fusion.[2] On the basis of this work Dr. Ostermeier was able to start building a library of gene fusions using random circular permutation and random insertion; his group's researchers have been able to identify additional switches in the library by using positive selection and screening for β–lactamase activity using a colorimetric assay with and without the desired allosteric ligand, maltose. He then discussed the sugar-activated β–lactamases and how well their binding proteins work. Dr. Ostermeier explained that switches are specific and reversible; switches are modular; switches create new phenotypes; and switches can have emergent properties. He then discussed how cell phenotype can be exploited to create new switches/binders and how they can be used in therapeutics.[3] He described examples in which cancer cells were selectively destroyed by protein switches sensitive to hypoxia.[4] His team found that the therapeutic potential of cellular environment-dependent switches is linked to the following properties: switches are inherently specific; they avoid a requirement for targeted delivery; the target and therapeutic mechanism are not inextricably linked; and they offer a route to the therapeutic exploitation of undruggable targets.[5]

Dr. Ostermeier then discussed the genetic code, suggesting that it is not randomly connected and indicating that there are different theories to account for nature's assignment of 20 amino acids to 64 three-base codons. He stated that the genetic code minimizes the deleterious effects of mutations and that directed evolution is constrained by the genetic code. For example, for TEM-1, which has 287 amino acids, of the 18,081 different hypothetical codon substitutions only 2,583 are accessible for evolution as

---

[2]M. Ostermeier, "Protein Switches and the Genetic Code," presentation at the directed evolution for development and production of bioactive agents meeting, February 21, 2013.

[3]G. Guntas, T.J. Mansell, J.R. Kim, and M. Ostermeier, Directed evolution of protein switches and their application to the creation of ligand-binding proteins, *Proceedings of the National Academy of Sciences U.S.A.* 102:11224, 2005.

[4]C.M. Wright, R.C. Wright, J.R. Eshleman, and M. Ostermeier, A protein therapeutic modality founded on molecular regulation, *Proceedings of the National Academy of Sciences U.S.A.* 108:16206, 2011.

[5]Ibid.

Copyright © National Academy of Sciences. All rights reserved.

Directed Evolution for Development and Production of Bioactive Agents: A Meeting Summary

single-based substitutions, of which 1,722 result in amino acid mutations. Dr. Ostermeier used comprehensive codon mutagenesis (CCM) to form a library of mutated TEM-1 alleles and devised an experiment to test the fitness of all codon substitutions in parallel for screening survival. He found that adaptive mutations are enriched among evolutionarily accessible substitutions. He said that this work will be presented in a future paper by Fimberg and Ostermeier that shows the distribution of these fitness effects.[6] He finished his talk by discussing theories on the genetic code's origin from work by Koonin and Novozhilov,[7] whose paper described the following possibilities:

- Frozen accident—too hard to change once it was established;
- Stereochemical theory—inherent interactions between amino acids and nucleotide triplets;
- Coevolution theory—amino acid biosynthetic pathways and the genetic code coevolved; and
- Adaptive theory—the genetic code evolved under selective pressure to minimize the deleterious effects of mutations and mistranslations.

Dr. Ostermeier suggested that there should be a fifth theory:

- Evolvability theory—the genetic code evolved to make adaptive mutations more likely.

After the presentation participants were given a short time to ask questions. Most dealt with specific technical questions regarding protein switches, and Dr. Ostermeier clarified as needed what he had presented.

Directed Evolution of New Viruses for Gene Therapy

David Schaffer, Professor of Chemical and Biomolecular Engineering, Bioengineering, and Neuroscience
University of California, Berkeley

Dr. Schaffer opened his presentation by noting that 100 years ago life expectancy was 47 years and the leading cause of death was tuberculosis. Today life expectancy has improved to 77 years, but with that improvement new long-term chronic diseases have been discovered, including Alzheimer's, retinitis pigmentosa, multiple sclerosis, diabetes, and cancer, to name a few. He pointed out that these diseases have driven up heath care costs and that they are expected to continue to increase.[8] Dr. Schaffer reminded the audience that gene therapy is the introduction of genetic material into an individual's cells for therapeutic benefit. It has had recent success in clinical trials in some areas, including Leber's congenital amaurosis, hemophilia, and x-linked adrenoleukodystrophy, but the challenge remains that despite the potential for cures, many targets still are beyond the reach of current gene delivery technology.[9] Dr. Schaffer then showed examples of research with adeno-associated virus (AAV) and how this approach to gene therapy using the viral vector has had success with Leber's congenital amaurosis. He then discussed the engineering challenges of gene therapy using AAV, murine retrovirus, lentivirus, and adenovirus—viruses that did not evolve in nature to be used in therapy for humans. He discussed the different criteria

[6]M. Ostermeier, "Protein Switches and the Genetic Code," presentation at the directed evolution for development and production of bioactive agents meeting, February 21, 2013.

[7]E.V. Koonin and A.S. Novozhilov, Origin and evolution of the genetic code: The universal enigma, *IUBMB Life* 61(2):99-111, 2009.

[8]Centers for Medicare and Medicaid Services, Office of the Actuary, "National Health Expenditure Accounts-Projected, Table 1: National Health Expenditures; Aggregate and Per Capita Amounts, Percent Distribution, and Average Annual Percent Growth, by Source of Funds: Calendar Years 2003-2018," undated.

[9]D. Schaffer, "Directed Evolution of New Viruses for Gene Therapy," presentation to the directed evolution for development and production of bioactive agents meeting, February 21, 2013.

Copyright © National Academy of Sciences. All rights reserved.

for success in nature versus medicine and how directed evolution can be a valuable approach to re-evolve viruses for novel objectives. He outlined the challenges, safety measures needed, and barriers to gene transfer. To address these issues his research group constructed a novel library involving sequencing of 50 variants of infectious viruses. They took three libraries—error-prone PCR (simple diversification of cap), a shuffled library (chimeric cap sequences), and a "loop swap" library (diversifying surface regions of AAV2)—and put them together. He showed an example of the evolution of immune system-evading AAV, and another evolved variant that yielded high-level gene expression in human airway epithelia.[10] There were two modifications in the viral sequence, a point mutation in the region that binds to the cellular receptor, and a change near the N-terminus in the phospholipase domain. Either modification alone did not improve infectivity, but both together resulted in a 50-fold increase in infectivity.[11] This is a result that only an evolutionary approach would have revealed to the researcher. Dr. Schaffer then showed examples of how viruses evolved for better transport through layers of cells in the retina that allowed improvement of retinal degeneration in humans, mice, and nonhuman primates.

Dr. Schaffer then talked about weaponizable viruses, and how directed evolution could potentially be used to enhance the process, or develop new "strategies." He discussed ways to improve spread/ infectivity, including altering viral tropism, and pointed the meeting participants to articles in *Science* and *Nature* that reported mutations in H5N1 "avian flu" that enabled transmission between mammals.[12,13] He also discussed how one could use directed evolution of HIV to achieve replication in pig-tailed macaques.[14] Next he showed how cytopathicity and immune interactions could be tuned to enhance virulence. He concluded that evolution works best when biological function has a toehold. Dr. Schaffer concluded his presentation with the following statements:[15]

- Viruses are potent gene delivery vehicles, and directed evolution could be harnessed to optimize them as human therapeutics.
- Directed evolution is a powerful method for creating "designer" viruses, even when mechanistic knowledge is limited.
- We have used this approach to alter receptor-binding specificity, increase efficiency, target virus, enhance immune evasion, and improve safety of viral integration
- This approach could conceivably be utilized to enhance the pathogenicity of viral or bacterial agents.

A short question-and-answer period was held after the presentation. The key questions from meeting participants discussed accessibility and the size of the commercial market, and how companies are starting to target orphan diseases based on small patient markets. These companies feel that the cost from start to finish in technological protein production is cheaper for rare diseases. One participant voiced that the lower cost was especially true in the regulatory arena.

---

[10]K.J.D.A. Excoffon, J.T. Koerber, D.D. Dickey, M. Murtha, S. Keshavjee, B.K. Kaspar, J. Zabner, and D.V. Schaffer, Directed evolution of adeno-associated virus to an infectious respiratory virus, *Proceedings of the National Academy of Sciences U.S.A.* 106(10):3865-3870, 2009.

[11]Ibid.

[12]S. Herfst, E.J.A. Schrauwen, M. Linster, S. Chutinimitkul, E. de Wit, V.J. Munster, E.M. Sorrell, T.M. Bestebroer, D.F. Burke, D.J. Smith, G.F. Rimmelzwaan, A.D.M.E. Osterhaus, and R.A.M. Fouchier, Airborne transmission of Influenza A/H5N1 virus between ferrets, *Science* 336(6088):1534-1541, 2012.

[13]M. Imai, T. Watanabe, M. Hatta, S.C. Das, M. Ozawa, K. Shinya, G. Zhong, A. Hanson, H. Katsura, S. Watanabe, C. Li, E. Kawakami, S. Yamada, M. Kiso, Y. Suzuki, E.A. Maher, G. Neumann, and Y. Kawaoka, Experimental adaptation of an influenza H5 HA confers respiratory droplet transmission to a reassortant H5 HA/H1N1 virus in ferrets, *Nature* 486:420-428, 2012.

[14]K. Pekrun, R. Shibata, T. Igarashi, M. Reed, L. Sheppard, P.A. Patten, W.P. Stemmer, M.A. Martin, and N.W. Soong, Evolution of a human immunodeficiency virus type 1 variant with enhanced replication in pig-tailed macaque cells by DNA shuffling, *Journal of Virology* 76(6):2924-2935, 2002.

[15]D. Schaffer, "Directed Evolution of New Viruses for Gene Therapy," presentation to the directed evolution for development and production of bioactive agents meeting, February 21, 2013.

Copyright © National Academy of Sciences. All rights reserved.

Expanding the Synthetic Capabilities of Yeast

Virginia W. Cornish, Helena Rubinstein Professor, Departments of Chemistry and Systems Biology
Columbia University

Dr. Cornish opened her presentation by stating that nobody makes cells as well as nature itself and that humans will harness them by mimicking evolution. Her research first focused on a chemical complementation system in yeast linking chemical-protein binding to gene expression. Specifically, she used methotrexate and dexamethasone anchors to enable a selection of enzymes capable of cleaving linker that connects these anchors.[16] The proof of principle was the use of cephem hydrolysis by a cephalosporinase.[17] The two challenges were the number of rounds needed to get to an enzyme library size of 10^8, and the levels of enzyme range that have to be established. She noted that cellulose engineering is improved for synthetic biology with these new reporter genes. Dr. Cornish described using a heritable recombination system to exploit mutagenesis in yeast that shows some potential to leverage sexual reproduction mechanisms to increase the size and diversity of mutant libraries. The process uses the yeast's natural homologous recombination capabilities. For example, by introducing a particular nuclease gene in *trans,* Dr. Cornish enabled recombination from multiple plasmids co-localized in a yeast cell. This allows libraries of yeast to be continually crossed with each other. Two types of recombination are being worked on in her lab: mutagenesis by transformation[18] and heritable recombination mutagenesis.[19] After selecting for the best combined beneficial mutations, Dr. Cornish used endonuclease-induced recombination to get a very high recombination efficiency, and her group quantified the efficiency of the endonuclease-induced recombination to get target optimal rates for sporulation.[20] After crossing beneficial mutations, Dr. Cornish found that the evolution of hisA could be achieved in a very straightforward way and results could be obtained rapidly without the extra mutation.[21]

Dr. Cornish is now doing pathway engineering for terpenoid biosynthesis through reiterative recombination: mutagenesis.[22] Two genes can be used in this process, which it is simple and easy to do. She noted that the process makes a large number of recombinants and is an "everyman" tool for pathway construction (8 rounds, 21 items) in yeast chromosomes. This tool is highly efficient for terpenoid production and live cell imaging. She stated that there is an engineering opportunity for a yeast biosensor with development of translational machinery. Dr. Cornish concluded by stating that synthetic chemistry is moving into new and exciting areas. A participant asked what the limits are for creating multienzyme systems and systems that are more complex. Dr. Cornish responded that she would like to look at multienzyme systems but that library size remains a big problem. She suggested that there is a greater impact from simultaneously mutating one or more genes as opposed to synthesizing an entire genome.

---

[16] H. Lin, W. Abida, R.T. Sauer, and V.W. Cornish, Dexamethasone-methotrexate: An efficient chemical inducer of protein dimerization in vivo, *Journal of the American Chemical Society* 122:4247, 2000.

[17] K. Baker, C. Bleczinski, H. Lin, G. Salazar-Jimenez, D. Sengupta, S. Krane, and V.W. Cornish, Chemical complementation: A reaction-independent genetic assay for enzyme catalysis, *Proceedings of National Academy of Sciences U.S.A.* 99:16537-16542, 2002.

[18] N. Pirakitikulr, N. Ostrov, P. Peralta-Yahya, and V.W. Cornish, PCRless library mutagenesis via oligonucleotide recombination in yeast, *Protein Science* 19(12):2336-2346, 2010.

[19] D.W. Romanini, P. Peralta-Yahya, V. Mondol, and V.W. Cornish, A heritable recombination system for synthetic Darwinian evolution in yeast, *ACS Synthetic Biology* 1(12):602-609, 2012.

[20] In biology, a mode of generation consisting of the interior division of the body into a mass of spores or germs, which are freed upon the rupture of the body wall; also, the process and act of spore formation; from the website Wordnik, available at http://www.wordnik.com/words/sporation.

[21] D.W. Romanini, P. Peralta-Yahya, V. Mondol, and V.W. Cornish, A heritable recombination system for synthetic Darwinian evolution in yeast, *ACS Synthetic Biology* 1(12):602-609, 2012.

[22] L. Wingler and V. Cornish, Reiterative recombination for the in vivo assembly of libraries of multigene pathways, *Proceedings of the National Academy of Sciences U.S.A.* 108(35):15135-14140, 2011.

Copyright © National Academy of Sciences. All rights reserved.

Meeting participants continued to ask Dr. Cornish questions that dealt mostly with limitations in library size and volume.

One Handful of Soil: 10,000,000,000 Microbes, 100,000 Different Species

Michael Fischbach, Assistant Professor, Department of Bioengineering and Therapeutic Sciences University of California, San Francisco

Dr. Fischbach started his presentation by stating that there are more microorganisms in a handful of soil than there are humans on Earth. Researchers understand genes in the laboratory but are still trying to understand them in the real world. Many antibiotics in the past few years came from these microorganisms, including 70 percent of new antibiotics, 60 percent of new anticancer drugs, and 50 percent of new immunosuppressants.[23] He discussed the metabolic cost of one natural product, daptomycin, and how it is produced by thousands of proteins made from ribosomes. He went on to discuss the roles of natural products and hypothesized that natural products mediate interspecies interactions. These strange and mindboggling interactions are what drive microorganisms' evolution. Raising the question of the natural roles of natural products Dr. Fischbach postulated that natural products mediate interactions between species with genes as the missing link. These individual genes are found in clusters from which researchers can develop drugs. Researchers need to know what each part does to make this complex cluster. Dr. Fischbach then stated that computational insight into these parts before lab work helps to find the clusters by generating a strong hypothesis. Algorithms can be developed for searching through bacterial products and looking at the different gene clusters. This creates an opportunity to look at various groups in a cluster. He noted that using a 90,000-liter-scale vessel to grow erythromycin showed many novel natural products in a drug-producing bacterium that researchers already knew very well.[24] Researchers found that the algorithms pointed them to many other potential drugs from *Saccharopolyspora erythraea* where erythromycin was found.

Dr. Fischbach next related that looking at human-associated bacteria and using automated gene cluster identification along a chromosome yielded probabilities and identified 20,000 potential gene clusters. The most interesting bacteria finds were in the gut, skin, and mouth. He stated that using the most interesting discoveries for the wet lab instead of special soils or seaweed increases the speed of discovery. Dr. Fischbach then showed examples of natural products from symbionts including patellamide C[25,26] and podophyllotoxin.[27,28] He discussed thiopeptide antibiotics and how the human body is already doing an end run to make antibiotics. Researchers are now able to look at gene clusters and better understand what these new drugs are doing.[29] He pointed out that engineering biosynthesis is challenging. When directed evolution produces a new molecule, it then has to be investigated.[30] Half of

---

[23] D.J. Newman, G.M. Cragg, and K.M. Snade, Natural products as sources of new drugs over the period 1981-2002, *Journal of Natural Products* 66:1022-1037, 2003.

[24] M. Oliynyk, M. Samborskyy, J.B. Lester, T. Mironenko, N. Scott, S. Dickens, S.F. Haydock, and P.F. Leadlay, Complete genome sequence of the erythromycin-producing bacterium *Saccharopolyspora erythraea* NRRL23338, *Nature Biotechnology* 24:447-453, 2007.

[25] J. Piel, Metabolites from symbiotic bacteria, *Natural Product Report* 26:338-362, 2009.

[26] D.-C. Oh, M. Poulsen, C.R. Currie, and J. Clardy, Dentigerumycin: A bacterial mediator of an ant-fungus symbiosis, *Nature Chemical Biology* 5:391-393, 2009.

[27] M.S. Donia, B.J. Hathaway, S. Sudek, M.G. Haygood, M.J. Rosovitz, J. Ravel, and E.W. Schmidt, Natural combinatorial peptide libraries in cyanobacterial symbionts of marine ascidians, *Nature Chemical Biology* 2:729-735, 2006.

[28] A.L. Eyberger, R. Dondapati, and J.R. Porter, Endophyte fungal isolates from *Podophyllum peltatum* produce podophyllotoxin, *Journal of Natural Products* 69:1121-1124, 2006.

[29] L.C. Wieland Brown, M.G. Acker, J. Clardy, C.T. Walsh, and M.A Fischbach, Thirteen posttranslational modifications convert a 14-residue peptide into the antibiotic thiocillin, *Proceedings of the National Academy of Sciences U.S.A.* 106:2549-2553, 2009.

[30] MA. Fischbach, J.R. Lai, E.D. Roche, C.T. Walsh, and D.R. Liu, Directed evolution can rapidly improve the activity of chimeric assembly-line enzymes, *Proceedings of the National Academy of Sciences U.S.A.* 104(29):11951-11956, 2007.

Copyright © National Academy of Sciences. All rights reserved.

the time spent in his lab studying evolution in bacteria is devoted to understanding why these clusters are better at evolving. Nature finds ways to make new molecules very efficiently, and gene clusters have subclusters that specify biosynthesis. He went on to discuss the changes to the gene roster using a subclusters process and showed two examples, clorobiocin and landomycin.[31] He stated that evolutionary gene clusters are complex and have many interactions. One example is that nature has many different ways to bind iron. It has mutually interoperable domains that allow these bindings. Dr. Fischbach has found that the domains look more like each other, and so do the gene clusters. He suggested that these interoperable domains are interesting areas for research in the future. He also discussed how natural product toxins will be another area of investigation, and that saxitoxin can be made very easily because the instructions are in the public domain.[32,33] In the near future, he indicated, tetrodotoxin and maitotoxin would be areas that would be investigated.

After Dr. Fischbach's presentation there was a discussion plus questions about the importance of cell biology and biochemistry. Dr. Fischbach pointed out that even with the algorithms, there is still a need for biochemistry, and discovery is still "slow going." One mystery he mentioned was understanding why bacteria in nature still make antibiotics. Dr. Shapiro asked Dr. Fischbach what he considered the most important role directed evolution plays in the work on biosynthetic gene clusters. Dr. Fischbach commented that one of the most important aspects of this field, relevant for the present discussion, is the heterologous expression of gene clusters in common laboratory hosts, such as *E. coli*. Attempts to obtain such expression often do not work on the first try, and it is necessary to "massage" the gene cluster to fit well into its new host. This can be done by making random changes to the cluster and screening for variants that work well within the new context—directed evolution.

Digital Chemistry: The Fabrication and Application of Ordered Molecular Arrays

Neal Woodbury, Co-Director of the Center for Innovations in Medicine,
Professor of Chemistry and Biochemistry
Arizona State University

Dr. Woodbury started his presentation by discussing how he builds large ordered libraries of molecules using ordered molecular arrays. There are two platforms that his research team uses, one that employs fabricated printed arrays of 10,000 peptides, and one that uses in situ synthesized molecular arrays. The fabrication approach allows for standard fabrication instruments and has features at the 8-micron level. The process allows for ~100,000,000 peptides per wafer. The process is high density, has high information content, and is high volume and low in cost. The in situ approach has 330,000 peptides per array, allows for nearly random sequences, and has a feature density of about 660,000 peptides/cm^2. Each slide has 24 arrays and 312 arrays per wafer. Each array can be individually addressed using commercially available biological assay equipment. Dr. Woodbury showed an example of stepwise coupling yields and found that these levels are very high. A typical peptide is a few amino acids long, but some have shown side products under differing conditions. Research on quantitative analysis is ongoing.

[31] P.M. Flatt and T. Mahmud, Biosynthesis of aminocyclitol-aminoglycoside antibiotics and related compounds, *Natural Product Report* 24:358-392, 2007.

[32] R. Kellman, T.K. Mihali, Y.J. Jeon, R. Pickford, F. Pomati, and B.A. Neilan, Bio-synthetic intermediate analysis and functional homology reveal a saxitoxin gene cluster in cyanobacteria, *Applied and Environmental Microbiology* 74(13):4044-4053, 2008.

[33] F.R. Sidell, E.T. Takafuji, and D.R. Franz, eds., *Medical Aspects of Chemical and Biological Warfare*, Textbook of Military Medicine Series, Office of the Surgeon General, Department of the Army, Washington, D.C., 1997, Chapter 30.

Copyright © National Academy of Sciences. All rights reserved.

Dr. Woodbury went on to discuss immunosignaturing,[34] currently a key research area in his lab.[35,36] The process is very simple, requiring only microliters of blood. His research staff dilutes the sample for preparation, and the process uses only one chip with the same array to look at all diseases (chronic and infectious). Some of the benefits include the fact that samples stay stable on dry filter paper for weeks and can be mailed to researchers. Dr. Woodbury has been collaborating with Dr. John Galgiani, looking at immunosignaturing of Valley Fever. Valley Fever is caused by *Coccidioides immitis*, a category C pathogen, prevalent in the Sonoran Desert and other parts of Arizona. Their research using immunosignaturing has been very promising, with 100 percent specificity and sensitivity. The goal of the research is health monitoring for various diseases. His lab is also collaborating with researchers doing work on classification of breast cancer and brain tumors[37] using immunosignaturing. This process has also shown promise not only for detecting brain cancer, but also for distinguishing the type and stage of cancer. Dr. Woodbury is now researching the process for simultaneous detection of five cancers on one chip by visualization using support vector machine classification. While most of the research discussed was completed using the printed array process, Dr. Woodbury did discuss with meeting participants some initial results from the in situ synthesized arrays. The initial research has shown that using the 330,000-peptide arrays allows for better separation of seven infections researched simultaneously. Some of the infections being categorized include those due to dengue virus (category A), West Nile virus, and hepatitis B virus. He noted that the peptide sequence analysis has identified NS1, a nonstructural viral protein that is secreted from infected cells and is one of the most active dengue virus antigens. The two predominant epitopes in this protein both have substantial identity with some of the strongest binding peptides. This same analysis identified peptides associated with known antigens for influenza and type 1 diabetes.

Dr. Woodbury then discussed combining molecular recognition elements and molecular engineering. One area that his lab is working on is synthetic antibodies (synbodies).[38,39] The idea is to rapidly produce these antibodies, while keeping them small (<6000 molecular weight), chemically pure, and not restricted to aqueous solutions or physiological conditions. One synbody specifically captures AKT1 from cell lysate and is selective for AKT1 versus AKT2 (92 percent identity) or AKT3 (87 percent identity). Examples of other targets include Gal-80, influenza, and *Staphylococcus aureus*. His team has also identified binders that were linked on the synbody scaffold. Dr. Woodbury also talked about nanostructured enzyme systems.[40,41,42] He showed how his team is organizing a multienzyme system with controlled spacing. They have also used a flexible arm to facilitate a multienzyme reaction and have regulated a nanostructured enzyme system by using a DNA nanotweezer to turn on and off an NADH-dependent dehydrogenase. Next he showed the use of nM binding in synbodies to assemble modules in

[34]In the patent, Compound Arrays for Sample Profiling, by Stephen Johnson and Phillip Stafford, *immunosignaturing* is defined as a medical diagnostic test that uses arrays of random-sequence peptides to associate antibodies in a blood sample with a disease.

[35]K.F. Sykes, J.B. Legutki, and P. Stafford, Immunosignaturing: A critical review, *Trends in Biotechnology* 31(1):45-51, 2013.

[36]J.B. Legutki, D.M. Magee, P. Stafford, and S.A. Johnston, A general method for characterization of humoral immunity induced by a vaccine or infection, *Vaccine* 28(28):4529-4537, 2010.

[37]A.K. Hughes, Z. Cichacz, A. Scheck, S.W. Coons, S.A. Johnston, and P. Stafford, Immunosignaturing can detect products from molecular markers in brain cancer, *PLoS ONE* 7(7):e40201, 2012.

[38]C.W. Diehnelt, M. Shah, N. Gupta, P.E. Belcher, M.P. Greving, P. Stafford, and S.A. Johnston, Discovery of high-affinity protein binding ligands—Backwards, *PLoS ONE* 5(5):e10728, 2010.

[39]B.A. Williams, C.W. Diehnelt, P. Belcher, M. Greving, N.W. Woodbury, S.A. Johnston, and J.C. Chaput, Creating protein affinity reagents by combining peptide ligands on synthetic DNA scaffolds, *Journal of the American Chemical Society* 131(47):17233-17241, 2009.

[40]J. Fu, M. Liu,Y. Liu, N.W. Woodbury, and H. Yan, Interenzyme substrate diffusion for an enzyme cascade organized on spatially addressable DNA nanostructures, *Journal of the American Chemical Society* 134(12):5516-5519, 2012.

[41]J. Fu, J. Reinhold, and N.W. Woodbury, Peptide-modified surfaces for enzyme immobilization, *PLoS ONE* 6(4):e18692, 2011.

[42]J. Fu, K. Cai, S.A. Johnston, and N.W. Woodbury, Exploring peptide space for enzyme modulators, *Journal of the American Chemical Society* 132(18):6419-6424, 2010.

Copyright © National Academy of Sciences. All rights reserved.

DNA nanostructures. The process selects synbodies that stabilize activity and orient optimally. Finally Dr. Woodbury talked about the future use of immunosignaturing as a platform for a host-based biological or chemical threat event monitoring system. He noted such a system would need to have comprehensive detection coupled to standard health care monitoring. The two-phase approach would include comprehensive immunosignaturing of military/government personnel around the country. The second phase would be immunosignaturing in the context of vaccine monitoring of the general public to efficiently monitor chronic diseases and influenza. Dr. Woodbury then answered specific questions regarding his presentation, talking especially about timelines and specificity of libraries and disease recognition.

Cell-Penetrating Mini-Proteins

Gregory L. Verdine, Erving Professor of Chemistry
Harvard University

Dr. Verdine opened his presentation by mentioning that his lab's focus was more on applications of directed evolution. He discussed the importance of drugs and the areas that pharmaceutical companies are targeting to make new drugs. The two classes of drugs most companies focus on are biologics (protein therapeutics) and small molecules, which are limited in their target ranges. Biologics are limited to approximately 10 percent of all human targets that are outside the cell.[43] Small molecules are limited to approximately 10 percent of human proteins with hydrophilic pockets.[44] Dr. Verdine argued a need for entirely new classes of drugs due to the limited human target ranges (approximately 20 percent) of the drugs described above. These new drugs, developed from research involving directed evolution, would have "coded target recognition or access to huge combinatorics from directed evolution; enablement of lead-to-drug conversion by chemical synthesis; uptake into cells (most likely by an endocytic mechanism); and, preferentially, some clinical history."

Dr. Verdine discussed the use of peptides as therapeutic agents and outlined their properties, such as rapid disposal from the body (peptide structure allows for renal clearance in seconds to minutes) and the decomposition of the α-helix. He then described the effects of the removal of α-helix from a protein, which are low binding affinity, rapid degradation, and poor cell permeability. To address some of the problems of the use of peptides for therapeutics, researchers use hydrocarbon-stapled α-helical peptides: "synthetic miniproteins locked into their bioactive α-helical fold through the site-specific introduction of a chemical brace, an all hydrocarbon staple."[45] Dr. Verdine's lab uses the all-hydrocarbon α-helix stapling system, which enhances the peptide's α-helicity, proteolytic stability, and serum half-life.[46,47] His presentation highlighted the system's use of α-methyl groups and a ring closing olefin (ethylene); the synthesis of the system is documented in Kim, Grossman, and Verdine.[48,49]

Dr. Verdine discussed the properties and uses of the stapled peptides SAHB and SAHM1. SAHBs, are stapled peptides that enhance the pharmacological properties of BH3 peptides, which mediate protein interactions essential in the regulation of programmed cell death (apoptosis).[50] He opened the discussion by describing cellular uptake as a property of $SAHB_A$ and then described how SAHB suppresses the

[43]G.L. Verdine and G.J. Hilinski, Stapled peptides for intracellular drug targets, *Methods in Enzymology* 503:3-33, 2012.
[44]Ibid.
[45]Ibid.
[46]Ibid.
[47]Y.-W. Kim, T.N. Grossmann, and G.L Verdine, Synthesis of all-hydrocarbon stapled α-helical peptides by ring-closing olefin metathesis, *Nature Protocols* 6:761-767, 2011.
[48]Ibid.
[49]G.L. Verdine and G.J. Hilinski. Stapled peptides for intracellular drug targets. *Methods in Enzymology* 503: 3-33, 2012.
[50]L.D. Walensky, A.L. Kung, I. Escher, T.J. Malia, S. Barbuto, R.D. Wright, G. Wagner, G.L. Verdine, and S.J. Korsmeyer, Activation of apoptosis in vivo by a hydrocarbon-stapled BH3 helix, *Science* 305(5689):1466-1470, 2004.

Copyright © National Academy of Sciences. All rights reserved.

growth of human leukemia cells inserted in live mice, thus improving their overall survivability.[51,52] Dr. Verdine described how SAHM1 bridges the ICN/CSL interface, thus preventing the assembly of the NOTCH transactivation complex, which is important in the activation of genes that can cause cancers such as leukemia.[53] He then discussed how SAHM1 is used in the suppression of leukemia growth in mice.[54] Dr. Verdine discussed how stapled peptides can be used as dual antagonists of the cancer genes hDM2 and hDMx/4.[55,56,57] He also showed how the discovery of direct-acting β-catenin antagonists would be useful to future research.[58] Next, he discussed how single α-helixes do not work for all targets and how his group is researching other options including stapling *β*-hairpins and active and passive cell penetration. Finally, he discussed PeptiDream codon reassignment and how a tRNA[59] can be loaded synthetically to make new drugs.

DISCUSSION

The participants used the remainder of the workshop to discuss current and future uses of directed evolution, including (1) the potential for and probable use of directed evolution in the design and creation of bioreactive agents by state and non-state actors; (2) scientific, technological, and other advances needed for state and non-state actors to use directed evolution in the creation of potential agents; and (3) the likelihood and consequences of potential actors developing agents.

Asked to give examples in the first category, meeting participants mentioned taking an animal pathogen and evolving it to a human pathogen, the evolution of pathogens to target non-humans, and the expression of a toxin-synthesizing gene cluster by a simple organism such as *E coli*. One participant described a process for introducing diversity into an animal pathogen and the steps it takes to develop a human pathogen. Another participant mentioned that existing literature details methods for evolution of existing pathogens such as avian influenza viruses for transmission effectively to humans.[60,61]

Participants discussed several technological, scientific, and other advances that would make directed evolution more accessible to others, including state and non-state actors. Some of the areas discussed were evolution of existing pathogens, potential technological "game changers," and improvement of screening methods. Several participants noted the availability of existing biological weapons that are easy to weaponize but also highlighted several potential uses of directed evolution. One participant suggested

---

[51] Ibid.

[52] L.D. Walensky, K. Pitter, J. Morash, K. Joon, S. Barbuto, J. Fisher, E. Smith, G.L. Verdine, and S.J. Korsmeyer, A stapled BID BH3 helix directly binds and activates BAX, *Molecular Cell* 24(2):199-210, 2006.

[53] R.E. Moellering, M. Cornejo, T.N. Davis, C. Del Bianco, J.C. Aster, S.C. Blacklow, A.L. Kung, D.G. Gilliland, G.L. Verdine, and J.E. Bradner, Direct inhibition of the NOTCH transcription factor complex, *Nature* 462:182-188, 2009.

[54] Ibid.

[55] S. Baek, P.S. Kutchukian, G.L. Verdine, R. Huber, T.A. Holak, K.W. Lee and G.M. Popowicz, Structure of the stapled p53 peptide bound to Mdm2, *Journal of the American Chemical Society* 134:103-106, 2012.

[56] F. Bernal, A.F. Tyler, S.J. Korsmeyer, L.D. Walensky, and G.L. Verdine, Reactivation of the p53 tumor suppressor pathway by a stapled p53 peptide, *Journal of the American Chemical Society* 129:2456-2457, 2007.

[57] F. Bernal, M. Wade, M. Godes, T.N. Davis, D.G. Whitehead, A.L. Kung, G.M. Wahl, and L.D. Walensky, A stapled p53 helix overcomes HDMX-mediated suppression of p53, *Cancer Cell* 18:411, 2010.

[58] T. Grossman, J. Yeh, B. Bowman, and G. Verdine, Inhibition of oncogenic Wnt signaling through direct targeting of β-catenin, *Proceedings of the National Academy of Sciences U.S.A.* 109:17942-17947, 2012.

[59] *tNRA* is one of a class of RNA molecules that transports amino acids to ribosomes for incorporation into a polypeptide undergoing synthesis; also called *transfer RNA*. From *The American Heritage Medical Dictionary,* Copyright 2007, 2004 by Houghton Mifflin Company.

[60] S. Herfst, E.J.A. Schrauwen, M. Linster, S. Chutinimitkul, E. de Wit, V.J. Munster, E.M. Sorrell, T.M. Bestebroer, D.F. Burke, D.J. Smith, G.F. Rimmelzwaan, A.D.M.E. Osterhaus, and R.A.M. Fouchier, Airborne transmission of Influenza A/H5N1 virus between ferrets, *Science* 336(6088):1534-1541, 2012.

[61] M. Imai, T. Watanabe, M. Hatta, S.C. Das, M. Ozawa, K. Shinya, G. Zhong, A. Hanson, H. Katsura, S. Watanabe, C. Li, E. Kawakami, S. Yamada, M. Kiso, Y. Suzuki, E.A. Maher, G. Neumann, and Y. Kawaoka, Experimental adaptation of an influenza H5 HA confers respiratory droplet transmission to a reassortant H5 HA/H1N1 virus in ferrets, *Nature* 486:420-428, 2012.

Copyright © National Academy of Sciences. All rights reserved.

that there is a lot to gain by using directed evolution to combine two or more existing pathogens to make them more virulent. Another participant suggested as a related example the creation of a new pathogen that would be immune to current vaccines.

In the opening discussion of day two, a participant challenged others to think about how other technologies from other disciplines such as high-throughput structural analysis could interact with directed evolution technology. This commentary prompted discussion about the use of high-throughput screening in directed evolution research as a potential "game changer" and led one participant to note the use of high-throughput phenotype screening especially of mammalian cells. Another participant said that people "had doubted in vivo selection, and how it connects phenotype to survival to do selection" and asked, "If you could remove the limitation of separating the phenotype from the selection, would that dramatically change what we can do?" The discussion on high-throughput screening led to a discussion of existing screening methods, with several participants viewing the right screening approach as one of the biggest challenges to directed evolution. One participant emphasized as a current limitation the lack of simple screening methods that do not require interpretation by experts.

Throughout the meeting, there was discussion of the likelihood of potential actors entering the field of directed evolution and their ability to produce agents. Some participants noted significant barriers to entry into the practice of directed evolution, such as capital investment and attainment of the required knowledge and expertise. A shift toward directed evolution research could allow access to the results to a new class of actors (i.e., state, non-state, adversary, or ally), a participant suggested. Another proposed that "for the short term the concern is less that non-state actors would use directed evolution themselves and more that they would commandeer the product if directed evolution came from the United States. An example of this would be the use of a heterologous host engineered to produce a toxin." The participant suggested that "DNA synthesis and sequencing capabilities" should be monitored especially as "synthetic biology becomes more routine." Another participant suggested that there is a low to medium risk (perhaps more for state actors), with major potential consequences in the next 5-10 years.

FINAL COMMENTS

At the conclusion of the meeting, Dr. Shapiro asked that the participants comment on what they had learned from the meeting and where they expected directed evolution to go in the future. The comments below summarize some salient points.

Mikhail Shapiro—"Directed evolution of viral pathogens and biosynthetic pathogens produced by gene clusters in heterologous systems are fairly recent and are interesting. I particularly want to emphasize the role of directed evolution in making heterologous genes 'fit' in a new host."

Marc Ostermeier—"The idea that struck me was taking some small pathway and putting it into a non-pathogen or even a single gene product that produces a toxin. I was thinking of the scenario of the disgruntled graduate student or the unhappy employee of a biotech company because they have access to many things in the lab. Many basic evolutionary techniques can be performed outside of the lab."

Neal Woodbury—"Fascinating set of discussions. I was amazed how sophisticated the field is and perhaps this is why it is constrained to small numbers. It is amazing that you can do selection in an eye in vivo, and I never thought about it until now. I have not considered these orthogonal techniques and ideas. What are the orthogonal techniques that are simple enough so that anyone can do them?"

Jerome Holton— "This discussion has dispelled the acquired prejudice that bioterrorism with natural organisms is the only easy option. The advances that you all have discussed here make me want to keep up with the field, and they call into question the difficulty or the ease of the engineered pathogen—for example, an altered or new homogeneous bug that has a gene sequence inserted in it so that it produces a toxin of choice. The reason this is of concern is because a bioterrorism event utilizing an engineered pathogen moves into a more likely category on the risk cube that I talked about yesterday. I ask that you carry with you an awareness of what your research can be used for. This is not to dissuade you from going down certain intellectual and experimental paths, but to encourage you to be aware of what that new knowledge can do in nefarious hands."

Copyright © National Academy of Sciences. All rights reserved.

Copyright © National Academy of Sciences. All rights reserved.

Appendixes

Copyright © National Academy of Sciences. All rights reserved.

Copyright © National Academy of Sciences. All rights reserved.

Appendix A
Workshop Presentations and Participants

PRESENTATIONS

Directed Evolution for Development and Production of Bioactive Agents
February 21-22, 2013
Washington, D.C.

Protein Switches and the Genetic Code
Marc Ostermeier, Professor and Vice Chair, Chemical and Biological Engineering
Johns Hopkins University

Directed Evolution of New Viruses for Gene Therapy
David Schaffer, Professor of Chemical and Biomolecular Engineering, Bioengineering, and Neuroscience
University of California, Berkeley

Expanding the Synthetic Capabilities of Yeast
Virginia W. Cornish, Helena Rubinstein Professor, Departments of Chemistry and Systems Biology
Columbia University

One Handful of Soil: 10,000,000,000 Microbes, 100,000 Different Species
Michael Fischbach, Assistant Professor, Department of Bioengineering and Therapeutic Sciences
University of California, San Francisco

Digital Chemistry: The Fabrication and Application of Ordered Molecular Arrays
Neal Woodbury, Co-Director of the Center for Innovations in Medicine, Professor of Chemistry and Biochemistry
Arizona State University

Cell-Penetrating Mini-Proteins
Gregory L. Verdine, Erving Professor of Chemistry
Harvard University

PARTICIPANTS

Committee

Mikhail Shapiro, University of California, Berkeley

Copyright © National Academy of Sciences. All rights reserved.

Speakers

Virginia W. Cornish, Columbia University
Michael Fischbach, University of California, San Francisco
Marc Ostermeier, Johns Hopkins University
David Schaffer, University of California, Berkeley
Gregory L. Verdine, Harvard University
Neal Woodbury, Arizona State University

Staff

Terry Jaggers, Board Director
Daniel Talmage, Study Director
Dionna Ali, Senior Program Assistant

Agencies Represented

Defense Advanced Research Projects Agency
Defense Intelligence Agency

Copyright © National Academy of Sciences. All rights reserved.

Appendix B
Biographies

SPEAKERS

Virginia W. Cornish is the Helena Rubinstein Professor in the Department of Chemistry at Columbia University. She graduated summa cum laude with a B.A. in biochemistry in 1991 from Columbia University, where she did undergraduate research with Professor Ronald Breslow. She earned her Ph.D. in chemistry with Professor Peter Schultz at the University of California, Berkeley, and then was a postdoctoral fellow in the Biology Department at the Massachusetts Institute of Technology (MIT) under the guidance of Professor Robert Sauer. Dr. Cornish joined the faculty of the Chemistry Department at Columbia in 1999, where she carries out research at the interface of chemistry and biology, and was promoted to associate professor with tenure in 2004 and then professor in 2007. Her laboratory brings together modern methods in synthetic chemistry and DNA technology to expand the synthetic capabilities of living cells. Her research has resulted in 59 research publications and several patents and currently is supported by multiple grants from the National Institutes of Health (NIH) and the National Science Foundation (NSF). Dr. Cornish has been recognized for her research by awards including an NSF CAREER Award (2000), a Sloan Foundation Fellowship (2003), the Protein Society Irving Sigal Young Investigator Award (2009), and the American Chemical Society Pfizer Award in Enzyme Chemistry (2009).

Michael Fischbach is an assistant professor in the Department of Bioengineering and Therapeutic Sciences at University of California, San Francisco (UCSF), and a member of the California Institute for Quantitative Biosciences (QB3). Dr. Fischbach is a recipient of the NIH Director's New Innovator Award, a fellowship for science and engineering from the David and Lucille Packard Foundation, a medical research award from the W.M. Keck Foundation, and the Young Investigator Grant for Probiotics Research from the Global Probiotics Council. His laboratory uses a combination of genomics and chemistry to identify and characterize small molecules from microbes, with an emphasis on the human microbiome. Dr. Fischbach received his Ph.D. in chemistry in 2007 from Harvard University, where he studied the role of iron acquisition in bacterial pathogenesis and the biosynthesis of antibiotics. Before coming to UCSF, he spent 2 years as an independent fellow at Massachusetts General Hospital coordinating a collaborative effort based at the Broad Institute to develop genomics-based approaches to the discovery of small molecules from microbes. Dr. Fischbach is a member of the scientific advisory boards of Schiff Nutrition, Second Genome, and Warp Drive Bio, and he is a consultant for Achaogen, Agraquest, and Genentech.

Marc Ostermeier is a professor and vice chair of chemical and biomolecular engineering at Johns Hopkins University. He received a B.S. in chemical engineering from the University of Wisconsin in 1990 and a Ph.D. in chemical engineering from the University of Texas, Austin, in 1996. He was an NIH postdoctoral fellow in the Chemistry Department at Pennsylvania State University before joining the faculty at Johns Hopkins University in 2000. Dr. Ostermeier was

Copyright © National Academy of Sciences. All rights reserved.

promoted to associate professor in 2007 and professor in 2011. His research is in the areas of protein engineering, synthetic biology, protein evolution, and allostery. He is a recipient of the NSF CAREER Award.

David Schaffer is a professor of chemical and biomolecular engineering, bioengineering, and neuroscience at University of California, Berkeley, where he also serves as the director of the Berkeley Stem Cell Center. He graduated from Stanford University with a B.S. degree in chemical engineering in 1993. Afterward, he attended MIT and earned his Ph.D. in chemical engineering in 1998 with Professor Doug Lauffenburger, while minoring in molecular and cell biology. Finally, he conducted a postdoctoral fellowship in the laboratory of Fred Gage at the Salk Institute for Biological Studies in La Jolla, California, before moving to Berkeley in 1999. At Berkeley, Dr. Schaffer applies engineering principles to enhance stem cell and gene therapy approaches for neuroregeneration. This work includes mechanistic investigation of stem cell control, as well as molecular evolution and engineering of viral gene delivery vehicles. He has received an NSF CAREER Award, an Office of Naval Research Young Investigator Award, and a Whitaker Foundation Young Investigator Award, and he was named a Technology Review Top 100 Innovator. He was also awarded the American Chemical Society BIOT Division Young Investigator Award in 2006 and the Biomedical Engineering Society Rita Shaffer Young Investigator Award in 2000, and he was elected to the college of fellows of the American Institute of Medical and Biological Engineering in 2010.

Gregory L. Verdine is the Erving Professor of Chemistry at Harvard University. He is also the director of the Harvard/Dana-Farber Program in cancer chemical biology and executive director of the Chemical Biology Initiative at the Dana-Farber Cancer Institute. In recent years, Dr. Verdine has established himself as one of the pioneers of the emerging discipline known as chemical biology, which seeks to understand the functions of small molecules, their interplay in the cell, and their effect on biological processes. He has studied the processes underlying control of gene expression and preservation of genomic integrity, and his work has shed light on the biochemical and structural basis for enzymatic recognition and repair of mutagenic damage in DNA. He joined the faculty of Harvard University's Department of Chemistry in 1988 and eventually became the Erving Professor of Chemistry in 2002. Dr. Verdine has received numerous awards and honors, including the NSF Presidential Young Investigator Award, the Sloan Fellowship, the Searle Scholar Award, and an Eli Lilly Award in Biological Chemistry.

Neal Woodbury is a professor in the Department of Chemistry and Biochemistry at Arizona State University (ASU). As co-director and chief scientific officer of the Biodesign Institute, Innovations in Medicine, he leads a team that seeks to develop molecular devices and nanoscale hybrid electronics for use in biomedicine, environmental remediation and monitoring, threat detection, and agriculture. His research into the structure/function relationships in photosynthesis led him to realize the awesome potential of harnessing the energy of light to direct chemical reactions. His efforts have been directed at building synthetic systems that can do this: speed up natural evolution. Dr. Woodbury is an advocate of interdisciplinary science as a means of providing researchers greater vision in addressing real-world problems. His published work includes more than 75 articles and studies. He is a member of the NSF Biophysics Panel and the NSF Integrative Graduate Education and Research Traineeship Panel and is an associate editor of *Photochemistry and Photobiology*. He has served as the director of the Photosynthesis Center at ASU and is an active member of the American Chemical Society, the Biophysical Society, and the American Photobiology Society. Dr. Woodbury received his B.S. in biochemistry from the University of California, Davis, and his Ph.D. from the University of Washington.

Copyright © National Academy of Sciences. All rights reserved.

FACILITATOR

Mikhail Shapiro is a neuroscientist, engineer, and technology entrepreneur focused on developing better ways to study the brain's activity and treat neurological and psychiatric disease. Dr. Shapiro has been named as a Miller Research Fellow at the University of California, Berkeley, to develop an independent research program focused on ways to non-invasively sense and manipulate brain activity at the molecular level. He studied neuroscience at Brown University and received his Ph.D. in biological engineering from MIT as a Hertz and Soros Fellow. Working with Alan Jasanoff and Robert Langer, Dr. Shapiro created the first-ever functional magnetic resonance imaging sensors for neurotransmitters. He was also a cofounder of Cyberkinetics Neurotechnology Systems, whose BrainGate technology allowed paralyzed people to control external devices directly with their thoughts. As a venture principal at Third Rock Ventures, an $800 million life sciences venture capital firm, Dr. Shapiro helped launch companies focused on novel treatments for chronic pain, cancer, and other diseases. In 2010 he was recognized by the *MIT Technology Review* as one of the world's top 35 innovators under age 35.

Copyright © National Academy of Sciences. All rights reserved.