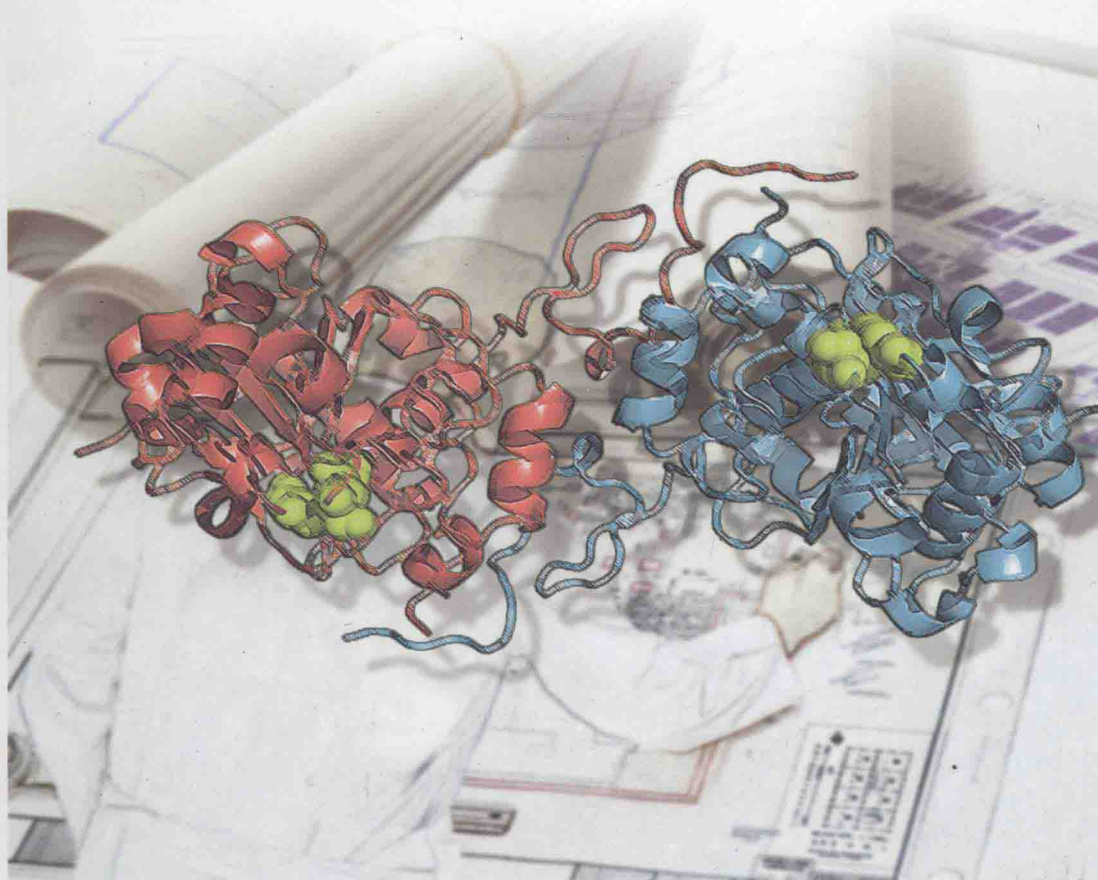


Edited by Stefan Lutz
and Uwe T. Bornscheuer

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Protein Engineering Handbook

Volume 1

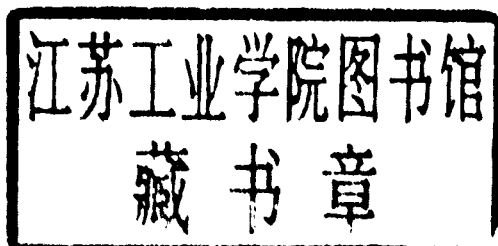


Protein Engineering Handbook

Volume 1

Edited by

Stefan Lutz and Uwe T. Bornscheuer



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The Editors

Prof. Dr. Stefan Lutz

Dept. of Chemistry
Emory University
1515 Dickey Drive
Atlanta GA 30322
USA

Prof. Dr. Uwe T. Bornscheuer

Dept. of Biotechnology and Enzyme Catalysis
Institute of Biochemistry
Greifswald University
Felix-Hausdorff-Str. 4
17487 Greifswald

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**Protein Engineering
Handbook**

Volume 1

*Edited by
Stefan Lutz and
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Further Reading

Cox, M. M., Phillips, G. N. (eds.)

Handbook of Proteins

Structure, Function and Methods. 2 Volume Set

2008

Hardcover

ISBN: 978-0-470-06098-8

Miller, L. W. (eds.)

Probes and Tags to Study Biomolecular Function

2008

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ISBN: 978-3-527-31566-6

Lengauer, T. (ed.)

Bioinformatics – From Genomes to Therapies

2007

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Chemical Biology

From Small Molecules to Systems Biology and Drug Design

2007

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Aehle, W. (ed.)

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Production and Applications

2007

Hardcover

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Meyers, R. A. (ed.)

Proteins

From Analytics to Structural Genomics

2006

Hardcover

ISBN: 978-3-527-31608-3

Preface

Protein engineering is pursued by scientists from many different disciplines. Chemists, biochemists, biologists, and engineers alike are engaged in tailoring enzymes. As diverse as their intellectual background is their motivation to do so, varying from a desire to understand the fundamentals of biocatalysis such as the intimate relationship of structure, dynamics and function to questions of evolution, from a need to adjust enzyme properties for industrial processes to the challenge of generating novel proteins for therapeutic and biomedical applications. To meet their objectives, researchers are using highly creative and innovative approaches to introduce beneficial changes to enzymes, focusing on – among other properties – greater activity, altered substrate specificity, improved enantioselectivity, and increased stability.

As a field of research, protein engineering has made significant contributions towards a better understanding of the physical and chemical properties of proteins. In return, it has benefited from advances in traditional areas of biochemistry and biophysics. Insights into the role of protein structure from x-ray crystallography and NMR spectroscopy experiments have been rapidly growing and, together with clever mechanistic studies by enzymologists, have greatly contributed towards a better rationale for function. Separately, the emerging appreciation for protein dynamics, as well as the implementation of single-molecule studies has given us an intimate look at the performance of not just bulk catalyst but individual molecules as they move along the reaction coordinate. Paralleling advances in our understanding of the fundamentals, the last two decades have brought three paradigm shifts on the technological side of protein engineering. Starting with the introduction of the polymerase chain reaction and recombinant gene technology, progress in the field has empowered researchers to manipulate amino acid sequences in a relatively straightforward fashion and obtain vast quantities of selected polypeptides in heterologous expression systems. Next, the recreation of protein evolution processes in the laboratory, using random mutagenesis and *in vitro* recombination techniques, has opened up exciting and powerful new opportunities for protein engineers in all disciplines. Lastly, the recent development of predictive computer algorithms has added an important new tool, complementing experimental approaches by guiding the design and, in some cases, allowing for complete *de novo* construction of enzymes.

Capturing these exciting developments, Volume 1 of this book series focuses on fundamental aspects of protein engineering. While the opening chapter by Edmondson defines some of the terminology related to the characterization of engineered enzymes and the comparison to its natural parents, the contributions of Kazlauskas, Hollfelder and Miller concentrate on the active site, exploring enantioselectivity and substrate promiscuity. An often neglected yet critical aspect of protein engineering is folding of the polypeptide chain. While Fersht highlights the application of protein engineering for the studies of the folding process, DeLisa recapitulates some of the strategies to identify properly folded proteins. Along the same line, protein dynamics is another largely overlooked aspect of protein engineering. The contribution by Romesberg introduces a series of spectrophotometric techniques to capture protein motion while the article by Pelletier summarizes recent findings by NMR spectroscopy and x-ray crystallography. In one example for putting protein folding and dynamics data to work in the context of enzyme engineering, Sagermann presents a simple yet elegant method to explore and exploit conformational changes for creating functional protein switches.

Giving thought to the observation that not all proteins are equally suitable for laboratory evolution, Höcker provides a more practical perspective on the selection of protein frameworks as starting points for enzyme engineering. New activity and promising templates for engineering can also be found in the vastness of the metagenome. Many new opportunities in this emerging research area are discussed in the chapters by Ferrer and Eck. Separately, the contributions by Wood and Horswill review the utilization of intein sequences as protein engineering tools. Finally, the application of computational methods to guide protein engineering and *de novo* design is examined in the section by Saven.

In Volume 2, mutagenesis and shuffling strategies for generating libraries are described in the contributions by Reetz, Jäger and Tawfik while computational and experimental tools for chimeragenesis are reviewed in the chapters by Arnold and Lutz. Less conventional but highly useful, Ostermeier discusses the impact of circular permutation on the structure and function of proteins.

As library generation represents only half the challenge in directed evolution, effective methods for searching the often substantial library diversity are necessary. Such screening or selection protocols are commonly performed *in vivo* or with the help of display systems as reported by Hilvert, Withers, Soumillion and Wittrup. The combination of such systems with spectroscopic assays offers a highly versatile screening strategy as outlined by Reymond and Coco. Alternatively, Ueda and Tawfik describe elegant *in vitro* strategies for library analysis. More product-oriented, Zhao and Bornscheuer discuss the application of protein engineering towards altering substrate and cofactor specificity, as well as enantioselectivity in individual enzymes. These strategies are not limited to single-enzyme systems. The chapters by Khosla, Steinbüchel, and Schmidt-Dannert demonstrate their application towards the manipulation of entire pathways. Similarly, protein engineering also offers new opportunities for tailoring biomaterials as described in the contributions of Taguchi, Conticello and Kaplan.

In summary, this book series attempts to capture some of the diverse interests and approaches in protein engineering, reflecting the many different disciplines and individual motivations and objectives in this area. We hope that it offers solutions to existing protein engineering problems and inspires new ideas to tackle the challenges in the field. In today's fast-moving world, it is unrealistic to expect an all-inclusive, up-to-date collection of knowledge and methods in any printed media. The current research literature is a more appropriate source for the latest hypotheses and technology. Aiming for scientists new to the field, we instead emphasize a review of the basics in the field, as well as introduce selected new and promising strategies for protein engineering. We hope that this will provide readers with a comprehensive overview of this highly interdisciplinary research topic. For the experienced protein engineer, the book series might offer some new inspiration as well.

A book project such as this would never succeed without the wonderful support of many individuals that inspired, encouraged, and assisted in its assembly. In addition to thanking all of the authors for their efforts, we would like to acknowledge our colleagues and students at Emory University and the University of Greifswald for their advice in managing such a project, as well as their willingness to review and proof-read the pages that make up the two volumes. Finally, our special thanks also extend to the people at Wiley Publisher, namely Dr. Frank Weinreich and Dr. Heike Nöthe for their editorial assistants, as well as Claudia Zschernitz and Nele Denzau for their help during the printing stage of the books.

Atlanta/Greifswald, July 2008

Stefan Lutz & Uwe T. Bornscheuer

List of contributors

Amir Aharoni

Department of Life Science and
the NIBN
University of Ben Gurion in the
Negev
POB 653
Beer-Sheva
84105
Israel
aaharoni@chem.ubc.ca

Miguel Alcalde

CSIC, Institute of Catalysis
Dept. of Applied Biocatalysis
28049 Madrid
Spain

Jennifer N. Andexer

Institute of Molecular Enzyme
Technology
Heinrich-Heine-University
Düsseldorf
Research Centre Jülich
52426 Jülich
Germany

Frances H. Arnold

Division of Chemistry and Chemical
Engineering
California Institute of Technology
210-41
1200 E California Blvd
Pasadena
CA 91125
USA
frances@cheme.caltech.edu

Ana Beloqui

CSIC, Institute of Catalysis
Dept. of Applied Biocatalysis
28049 Madrid
Spain

Blaise R. Boles

Department of Internal Medicine
Roy J. and Lucille A. Carver College of
Medicine
University of Iowa 440 EMBR
Iowa City
IA 52242
USA

Uwe T. Bornscheuer

Department of Biotechnology and
Enzyme Catalysis
Institute of Biochemistry
Greifswald University
Felix-Hausdorff-Str. 4
17487 Greifswald
Germany
uwe.bornscheuer@uni-greifswald.de

Dominique Böttcher

Department of Biotechnology and
Enzyme Catalysis
Institute of Biochemistry
Greifswald University
Felix-Hausdorff-Str. 4
17487 Greifswald
Germany

Martina Carbone

Division of Chemistry and
Chemical Engineering
California Institute of Technology
210-41
1200 E California Blvd
Pasadena
CA 91125
USA
martina@cheme.caltech.edu

Peggy Cebe

Department of Physics
Tufts University
Science and Technology Center
Room 208
4 Colby Street
Medford
MA 02155
USA

Alice Y. Chen

Department of Chemical Engineering
Stanford University
Stauffer III
381 North-South Mall
Stanford
CA 94305
USA

Wayne M. Coco

DIREVO Biotech AG
Nattermannalle 1
50829 Cologne
Germany
coco@direvo.com

Vincent P. Conticello

Department of Chemistry
Emory University
1515 Dickey Drive
Atlanta
GA 30322
USA
vcontic@emory.edu

Matthew P. DeLisa

120 Olin Hall
Department of Chemical and
Biomolecular Engineering
Cornell University
Ithaca
NY 14853
USA
md255@cornell.edu

Kevin K. Desai

Department of Chemistry and
Biochemistry
The Florida State University
213 Dittmer Laboratory
Tallahassee
FL 32306-4390
USA

Matthew DeSieno

Department of Chemical and
Biomolecular Engineering
University of Illinois at
Urbana-Champaign
600 South Mathews Avenue
Urbana
IL 61801
USA

Nicolas Doucet

Université de Montréal
Département de biochimie
CP 6128
Succursale Centre-Ville
Montréal
Québec
H3C 3J7 Canada

Jing Du

Department of Chemical and
Biomolecular Engineering
University of Illinois at
Urbana-Champaign
600 South Mathews Avenue
Urbana
IL 61801
USA

Jürgen Eck

B·R·A·I·N AG
Darmstaedter Straße 34-36
64673 Zwingenberg
Germany

Dale E. Edmondson

Departments of Biochemistry
and Chemistry
Emory University
Atlanta
GA 30322-4098
USA

Thorsten Eggert

Institute of Molecular Enzyme
Technology
Heinrich-Heine-University Düsseldorf
Research Centre Jülich
52426 Jülich
Germany
Present address
evocatal GmbH
Merowingerplatz 1a
40225 Düsseldorf
Germany

Neil Ferguson

Medical Research Council Centre for
Protein Engineering
Hills Road
Cambridge CB2 0QH
United Kingdom
and
Cambridge University
Chemical Laboratory
Lensfield Road
Cambridge CB2 1EW
United Kingdom

Manuel Ferrer

CSIC, Institute of Catalysis
Dept. of Applied Biocatalysis
28049 Madrid
Spain
mferrer@icp.csic.es

Alan R. Fersht

Medical Research Council Centre for
Protein Engineering
Hills Road
Cambridge CB2 0QH
United Kingdom
and
Cambridge University
Chemical Laboratory
Lensfield Road
Cambridge CB2 1EW
United Kingdom
arf25@cam.ac.uk

Adam C. Fisher

120 Olin Hall
Department of Chemical and
Biomolecular Engineering
Cornell University
Ithaca
NY 14853
USA

Susanne A. Funke

Institute of Molecular Enzyme
Technology
Heinrich-Heine-University
Düsseldorf
Research Centre Jülich
52426 Jülich
Germany
Present address
Institute of Neuroscience and
Biophysics, Molecular Biophysics
Research Center Jülich
52426 Jülich
Germany

Esther Gabor

B·R·A·I·N AG
Darmstaedter Straße 34-36
64673 Zwingenberg
Germany

Giovanni Gadda

Departments of Chemistry and
Biology
Georgia State University
The Center for Biotechnology and
Drug Design
Atlanta
GA 30302-4098
USA

Alison R. Gillies

Department of Chemical Engineering
Princeton University
Princeton
NJ 08544
USA

Peter N. Golyshin

Division of Microbiology
HZI – Helmholtz Centre for Infection
Research
38124 Braunschweig
Germany
and
Department of Biological Sciences
University of Wales
Bangor LL57 2DG
United Kingdom

Benjamin J. Hackel

Massachusetts Institute of Technology
Building E19-563
50 Ames Street
Cambridge
MA 02142
USA

Ulrich Haupts

DIREVO Biotech AG
Nattermannalle 1
50829 Cologne
Germany

Asael Herman

Department of Biological Chemistry
Weizmann Institute of Science
Rehovot 76100
Israel
and
Department of Pathology
University of Washington School of
Medicine
HSB K-058, BOX 357 705
Seattle
WA 98195-7705
USA

Oliver Hesse

DIREVO Biotech AG
Nattermannalle 1
50829 Cologne
Germany

Donald Hilvert

Laboratory of Organic Chemistry
E.T.H. Zurich
Altwiesenstrasse 64
CH-8093 Zurich
Switzerland
hilvert@org.chem.ethz.ch

Birte Höcker

Max-Planck-Institute for
Developmental Biology
Spemannstrasse 35
72076 Tübingen
Germany
birte.hoecker@tuebingen.mpg.de

Florian Hollfelder

University of Cambridge
Department of Biochemistry
Cambridge CB2 1GA
United Kingdom
fh111@cam.ac.uk

Alexander R. Horswill

Department of Microbiology
Roy J. and Lucille A. Carver
College of Medicine
431 Newton Rd
540 F. Eckstein
Medicinal Research Building
University of Iowa
Iowa City
IA 52242
USA
alex-horswill@uiowa.edu

Karl-Erich Jaeger

Institute of Molecular Enzyme
Technology
Heinrich-Heine-University Düsseldorf
Research Centre Jülich
52426 Jülich
Germany

Stefanie Jonas

University of Cambridge
Department of Biochemistry
Cambridge CB2 1GA
United Kingdom

Manu Kanwar

Department of Chemical and
Biomolecular Engineering
Johns Hopkins University
3400 N. Charles St.
Baltimore
MD 21218-2681
USA

David. L. Kaplan

Department of Biomedical
Engineering
Tufts University
Medford
MA 02155
USA

Peter Kast

Laboratory of Organic Chemistry
E.T.H. Zurich
Altwiesenstrasse 64
CH-8093 Zurich
Switzerland

Romas Kazlauskas

Department of Biochemistry,
Molecular Biology
and Biophysics, and The Biotech-
nology Institute,
University of Minnesota
1479 Gortner Avenue
Saint Paul
MN 55108
USA

Chaitan Khosla

Departments of Chemical
Engineering, Chemistry and
Biochemistry
Stanford University, Keck 337
Stanford
CA 94305
USA
khosla@stanford.edu

Andreas C. Kleeb

Laboratory of Organic Chemistry
E.T.H. Zurich
Altwiesenstrasse 64
CH-8093 Zurich
Switzerland

Marco Landwehr

Division of Chemistry and
Chemical Engineering
California Institute of Technology
210-41
1200 E California Blvd
Pasadena
CA 91125
USA

Yougen Li

Division of Chemistry and Chemical
Engineering
California Institute of Technology
210-41
1200 E California Blvd
Pasadena
CA 91125
USA

Klaus Liebeton

B·R·A·I·N AG
Darmstaedter Straße 34-36
64673 Zwingenberg
Germany

Stefan Lutz

Department of Chemistry
Emory University
1515 Dickey Drive
Atlanta
GA 30322
USA
sal2@emory.edu

Thomas J. Mansell

120 Olin Hall
Department of Chemical and
Biomolecular Engineering
Cornell University
Ithaca
NY 14853
USA

Glenna E. Meister

Department of Chemical and
Biomolecular Engineering
Johns Hopkins University
3400 N. Charles St.
Baltimore
MD 21218-2681
USA

Guido Meurer

B·R·A·I·N AG
Darmstaedter Straße 34-36
64673 Zwingenberg
Germany

Michelle Meyer

Division of Chemistry and
Chemical Engineering
California Institute of Technology
210-41
1200 E California Blvd
Pasadena
CA 91125
USA

Brian G. Miller

Department of Chemistry and
Biochemistry
The Florida State University
213 Dittmer Laboratory
Tallahassee
FL 32306-4390
USA
miller@chem.fsu.edu

Philippe Minard

Laboratoire de Modelisation et
Ingénierie des Protéines
Institut de Biochimie et
Biophysique Moléculaire et
Cellulaire
Université Paris-Sud - Bat. 430
91405 Orsay
France

Martin Neuenschwander

Laboratory of Organic Chemistry
E.T.H. Zurich
Altwiesenstrasse 64
CH-8093 Zurich
Switzerland

Frank Niehaus

B·R·A·I·N AG
Darmstaedter Straße 34-36
64673 Zwingenberg
Germany

Marc Ostermeier

Department of Chemical and Biomo-
lecular Engineering
Johns Hopkins University
3400 N. Charles St.
Baltimore
MD 21218-2681
USA
oster@jhu.edu

Melissa Patterson

Department of Chemistry
Emory University
1515 Dickey Drive
Atlanta
GA 30322
USA

Sonha C. Payne

Department of Chemistry
Emory University
1515 Dickey Drive
Atlanta
GA 30322
USA

Joelle N. Pelletier

Université de Montréal
Département de chimie &
Département de biochimie
CP 6128
Succursale Centre-Ville
Montréal
Québec
H3C 3J7 Canada
joelle.pelletier@umontreal.ca

Alexander Pisarchik

Department of Biochemistry,
Molecular Biology and Biophysics
University of Minnesota
1479 Gortner Avenue
St. Paul
MN 55108
USA

Manfred T. Reetz

Max-Planck-Institut für
Kohlenforschung
Kaiser-Wilhelm-Platz 1
45470 Mülheim an der Ruhr
Germany
reetz@mpi-muelheim.mpg.de

Jean-Louis Reymond

University of Berne
Department of Chemistry and
Biochemistry
Freiestrasse 3
3012 Berne
Switzerland
jean-louis.reymond@ioc.unibe.ch

Floyd E. Romesberg

The Scripps Research Institute
Department of Chemistry
10550 N. Torrey Pines Road
La Jolla
California
USA
floyd@scripps.edu

Gloria Saab-Rincon

Departamento de Ingenieria
Celular y Biocatalisis Instituto de
Biotecnologia Universidad
Nacional Autonoma de Mexico
Apdo Postal 510-3 Cuernavaca
Morelos 62250
Mexico

Martin Sagermann

Department of Chemistry and
Biochemistry and
Interdepartmental Program in
Biomolecular Science and Engineering
University of California Santa Barbara
Santa Barbara 4649 B PSB North
California 93106-9510
USA
sagermann@chem.ucsb.edu

Jeffery G. Saven

Department of Chemistry
University of Pennsylvania
231 South 34th Street
Philadelphia
PA 19104-6323
USA
saven@sas.upenn.edu

Marlen Schmidt

Department of Biotechnology and
Enzyme Catalysis
Institute of Biochemistry
Greifswald University
Felix-Hausdorff-Str. 4
17487 Greifswald
Germany

Claudia Schmidt-Dannert

Department of Biochemistry,
Molecular Biology and Biophysics
University of Minnesota
1479 Gortner Avenue
St. Paul
MN 55108
USA
schmi232@umn.edu

Patrice Soumilion

Laboratoire d'Ingénierie des
Protéines et des Peptides
Institut des Sciences de la Vie
Université catholique de Louvain
Place Croix du Sud 4-5, bte 3
1348 Louvain-la-Neuve
Belgium
patrice.soumilion@uclouvain.be

Alexander Steinbüchel

Institut für Molekulare Mikrobi-
ologie und Biotechnologie der
Westfälischen
Wilhelms-Universität
Corrensstraße 3
48149 Münster
Germany
steinbu@uni-muenster.de

Anna Steinle

Institut für Molekulare
Mikrobiologie und Biotechnologie
der Westfälischen
Wilhelms-Universität
Corrensstraße 3
48149 Münster
Germany

Michael Strerath

DIREVO Biotech AG
Nattermannalle 1
50829 Cologne
Germany

Seiichi Taguchi

Division of Biotechnology and
Macromolecular Chemistry
Graduate School of Engineering
Hokkaido University
N13W8, Kita-ku
Sapporo 060-8628
Japan
staguchi@isc.meiji.ac.jp

Dan S. Tawfik

Department of Biological Chemistry
Weizmann Institute of Science
Ullmann Bldg
Room 201a
PO BOX 26
Rehovot 76100
Israel
tawfik@weizmann.ac.il

Megan C. Thielges

The Scripps Research Institute
Department of Chemistry
10550 N. Torrey Pines Road
La Jolla
California
USA

Manuela Trani

Department of Chemistry
Emory University
1515 Dickey Drive
Atlanta
GA 30322
USA

Takeharu Tsuge

Department of Innovative and Engi-
neered Materials
Tokyo Institute of Technology
4529 Nagatsuta
Midori-ku
Yokohama 226-8502
Japan

Takuya Ueda

Department of Medical Genome
Sciences
Graduate School of Frontier Sciences
The University of Tokyo
FSB-401
5-1-5 Kashiwanoha
Kashiwa
Chiba 277-8562
Japan
ueda@k.u-tokyo.ac.jp