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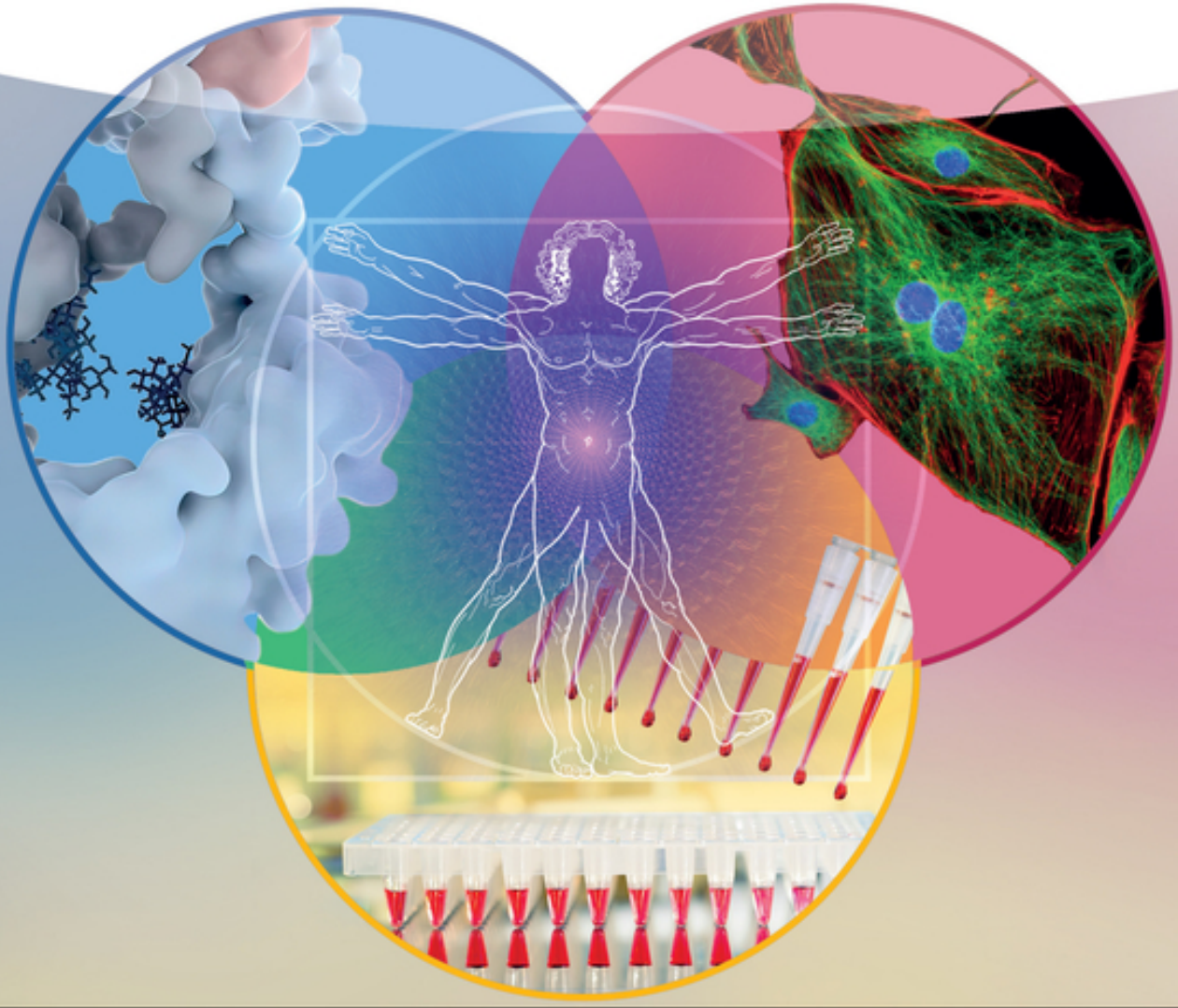
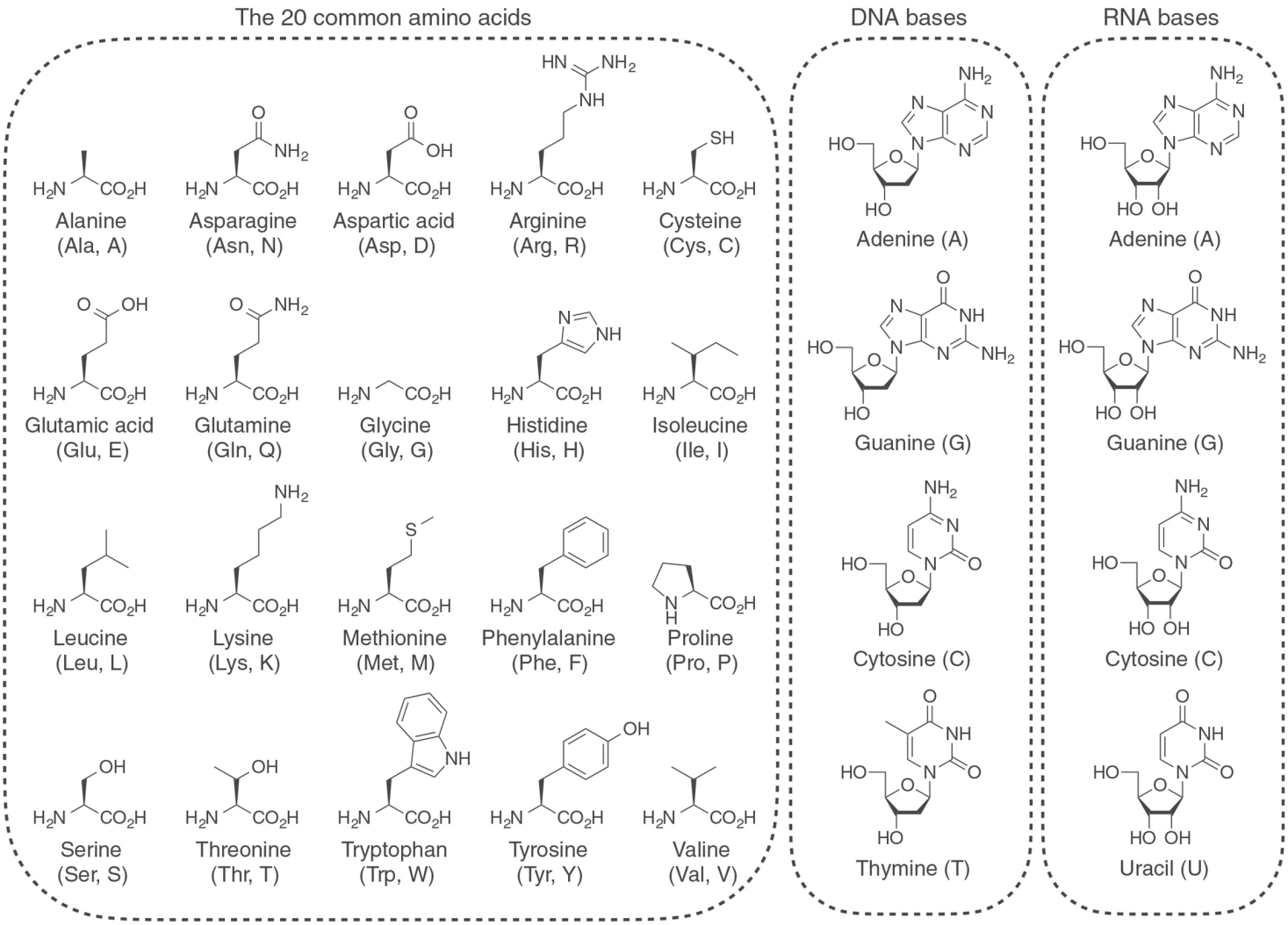
Edited by
Howard C. Hang, Matthew R. Pratt, and Jennifer A. Prescher

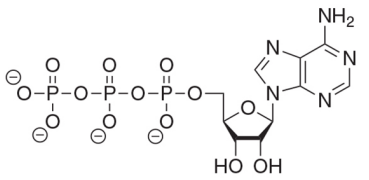
Advanced Chemical Biology

Chemical Dissection and Reprogramming of Biological Systems

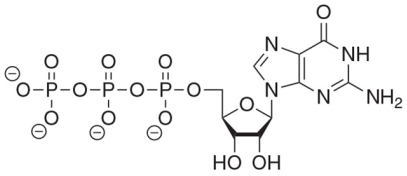



The 20 common amino acids
DNA bases
RNA bases
Alanine (Ala, A)
Asparagine (Asn, N)
Aspartic acid (Asp, D)
Arginine (Arg, R)
Cysteine (Cys, C)
Glutamic acid (Glu, E)
Glutamine (Gln, Q)
Glycine (Gly, G)
Histidine (His, H)
Isoleucine (Ile, I)
Leucine (Leu, L)
Lysine (Lys, K)
Methionine (Met, M)
Phenylalanine (Phe, F)
Proline (Pro, P)
Serine (Ser, S)
Threonine (Thr, T)
Tryptophan (Trp, W)
Tyrosine (Tyr, Y)
Valine (Val, V)
Adenine (A)
Guanine (G)
Cytosine (C)
Thymine (T)
Adenine (A)
Guanine (G)
Cytosine (C)
Uracil (U)

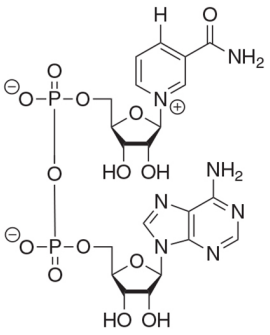
Common enzyme cofactors and substrates



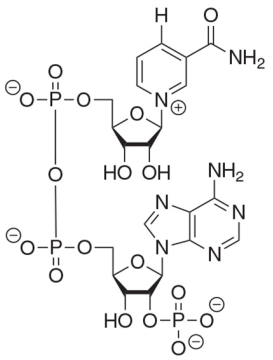
Adenosine triphosphate (ATP)



Guanosine triphosphate (GTP)



Nicotinamide adenine dinucleotide (NAD+)



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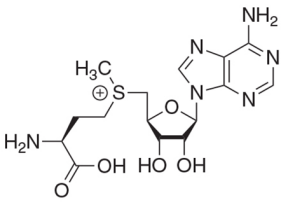
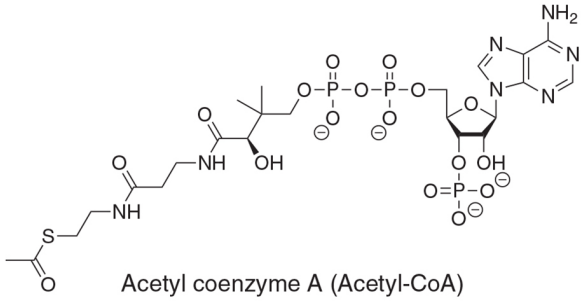
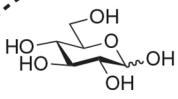


S-Adenosyl methionine (SAM)

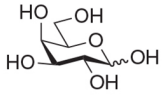


Acetyl coenzyme A (Acetyl-CoA)

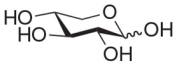
Mammalian monosaccharide building blocks



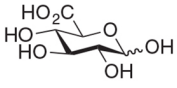
Glucose (Glc)



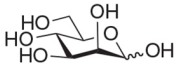
Galactose (Gal)



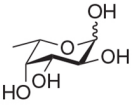
Xylose (Xyl)



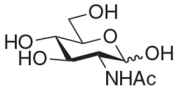
Glucuronic acid (GlcA)



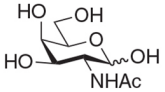
Mannose (Man)



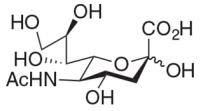
Fucose (Fuc)



N-Acetyl-Glucosamine (GlcNAc)

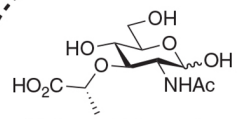


N-Acetyl-Galactosamine (GalNAc)

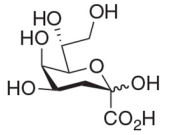


Sialic acid (NeuAc)

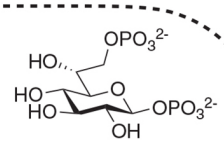
Example prokaryotic monosaccharide building blocks



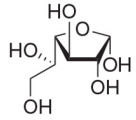
N-Acetyl-Muramic Acid (MurNAc)



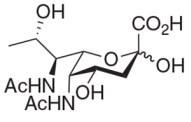
Ketodeoxyoctonic acid (KDO)



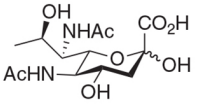
Heptose 1,7-bisphosphate



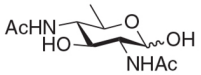
Galactofuranose



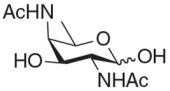
Pseudaminic acid



Legionaminic acid



Di-N-acetamindo D-bacillosamine (dNAcBac)



Di-2,4-N-acetamido-2,4,6-trideoxy galactose (d-DATDG)

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Edited by Howard C. Hang, Matthew R. Pratt, and Jennifer A. Prescher

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Cover image: © Shutterstock

Library of Congress Card No.: applied for

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library.

Bibliographic information published by the Deutsche Nationalbibliothek
The Deutsche Nationalbibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data are available on the Internet at <http://dnb.d-nb.de>.

Print ISBN: 978-3-527-34733-9
ePDF ISBN: 978-3-527-82629-2
ePub ISBN: 978-3-527-82630-8

Cover Design Adam Design, Weinheim, Germany
Typesetting Straive, Chennai, India

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Foreword

Carolyn R. Bertozzi

I came of age as a scientist during a time when the boundaries between the historically separate fields of chemistry and biology were being dismantled. The molecular biology revolution of the 1980s had brought newfound power to the life scientist, allowing biological systems to be engineered and manipulated to answer questions about molecular mechanism, rather than simply observed. High-resolution microscopy and structural biology techniques offered atomic views of biological molecules, complexes, and materials, bringing biology ever closer to the scale at which chemists operate. At the same time, chemistry was powering biology at record pace: solid-phase peptide and oligonucleotide synthesis were revolutionizing our understanding of these biomolecules' structures and functions, and also propelling advances in genome sequencing and engineering. The synthetic chemist's ability to synthesize complex natural products provided pharmacological tools that revealed the secrets of the cell, while analytical chemistry technologies, quite prominently mass spectrometry, provided unprecedented clarity on the molecular compositions of biological samples. The notion that chemists could design molecules to probe or perturb a biological process was becoming widely recognized among biologists, and likewise, historically intractable biological problems had become compelling challenges for chemists. In retrospect, my training years (i.e. the late 1980s and early 1990s) were a fantastic period for a young scientist to pursue research at the burgeoning interface of chemistry and biology!

Since those early days, I have watched the two fields coevolve to create the distinctive discipline we now call chemical biology. This evolution was not without friction. In the early days, very few labs possessed depth of knowledge and technical knowhow in both chemistry and biology. Indeed, it was the rare chemist who understood the needs of biology and the rare biologist who understood the power of chemistry; getting the two together as collaborators was key to progress in the field. Meanwhile, trainees who sought to develop skills in both disciplines were often misunderstood, or even worse, mischaracterized as "Jacks of all trades, masters of none." Pioneers at this exciting interface had to prove themselves separately as chemists and biologists while also creating the ethos of a distinctive new field.

Now, several decades into my own career as a chemical biologist, I am delighted to see our field playing a central role across academia and industry. We are the glue that binds chemists and biologists together, the bilingual interpreters that catalyze cross-pollination of ideas and technologies. And we make our own fundamental discoveries in biology that are uniquely enabled by our chemical tools, while also developing biological tools for better, greener, chemical processes. Many biopharma companies who were skeptical of our value a few decades back now host so-named chemical biology groups that cut across platforms and therapeutic areas. Our superpowers as multidisciplinary scientists are recognized, and we are rightfully in high demand.

While the professional practice of chemical biology has been codified, the mechanisms by which we train students in this discipline continue to evolve. Many of us academics teach courses in chemical biology that are rather *ad hoc*, often based on primary literature that happens to align with our interests. As the field has grown in scope and participation, so has the need for more structured and comprehensive resources on which such courses can be based. For this reason, I am delighted to celebrate this book, *Advanced Chemical Biology*, which covers a broad spectrum of exciting concepts and technologies and captures both the historic, defining moments in the field as well as its guiding principles. The topics cut across all the major biomolecule classes and highlight how chemical approaches can power fundamental research as well as clinical translation. The text illustrates applications in various branches of biology – neuroscience, immunology, cancer biology, and infectious disease – and showcases new therapeutic modalities arising from our unique brand of molecular engineering. The book's editors and contributors are leaders in chemical biology, and they have done all of us a great service. This book will be a valuable resource for both established chemical biologists and many future generations of trainees.

Preface

The field of chemical biology is expanding at a rapid pace, with continued advances in chemical methodologies and biological applications. The community of chemical biologists is also growing in number, with researchers now spanning a diverse set of backgrounds and interests. With this growth comes the need to train and educate newcomers to the field. Chemical biology courses have sprouted at institutions around the globe, and most do not use a standard text. We were motivated to fill this void, providing a book that is easily accessible to current and future generations of chemical biologists. This is no easy task, considering the breadth of the discipline and its continued evolution. Some unifying themes have emerged, though, that we hoped to capture in this book and provide a historical context for their development. To realize our vision, we reached out to leaders in the field for their input on generating a resource for the community. The end product is the compilation of the chapters between these covers.

Overall, the *Advanced Chemical Biology* textbook showcases how chemical tools and molecular methods have been used to gain insight into biological systems. The initial chapters highlight chemical biology in the context of the central dogma: how molecular-level thinking has enabled numerous discoveries relevant to DNA, RNA, proteins, and metabolites. Subsequent chapters feature transformative technologies developed within the community that continue to enable new pursuits. The final section of the book illustrates the impact of chemical biology in the broader scientific community, with examples from microbiology, immunology, neuroscience, drug discovery, and more. Collectively, these chapters underscore the breadth of discovery enabled by chemical approaches and provide a historical backdrop for the field.

Advanced Chemical Biology is designed for entry-level graduate students in chemical biology, although the text will serve as an excellent resource for students in a variety of chemistry- and biology-related fields, in addition to advanced undergraduates. Basic knowledge of organic chemistry and biochemistry, upon which much of chemical biology builds, is assumed. The chapters are not intended to be in-depth reviews on the subject matter; rather, they serve as basic primers for newcomers to the field. Each chapter begins with a brief introduction and historical context for the topic. The bulk of each chapter is then devoted to presenting key concepts and developments within chemical biology, drawing from a handful of landmark studies. Sample exam questions and slides for instructional use are also included. Since each chapter topic is not covered in-depth, we expect that instructors will supplement the materials in this book with additional examples and information to best suit their classes.

This textbook would not have been possible without the hard work and dedication of several individuals. We extend our sincere thanks to the authors of each chapter, whose work on this project coincided with the COVID-19 pandemic. Without their efforts and commitment, this book would have been impossible. We are also grateful to the team at Wiley for helping us to navigate the development of a teaching text during a quite unprecedented time. Last, we would like to thank the many colleagues and mentors who helped to spark our interests in the field and who continue

to guide our paths. We hope that this book similarly captivates the next generation of trainees and inspires them to continue to push the frontiers of chemical biology and scientific discovery.

11 July 2022

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About the Companion Website

Advanced Chemical Biology: Chemical Dissection and Reprogramming of Biological Systems is accompanied by a companion website:

www.wiley.com/go/hang

The website includes:

- Answers to Questions

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1

Introduction to Advanced Chemical Biology

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1.1 Introduction

As its name implies, the field of chemical biology employs chemical principles to dissect mechanisms in biology and potentially translate these discoveries into therapeutic approaches for health and disease. Chemical biology as a field evolved from and merged different specialized fields of investigation into a broader topic that encompasses many areas of research. One could argue that the origins of chemical biology date back to the discovery, characterization, and synthesis of small molecules to determine their mechanisms of action and production for therapeutic applications. Notably, studies in the late 1800s by Emil Fischer and coworkers led to the synthesis of indoles, peptides, and monosaccharides as well as their stereochemical determination [1], which was highlighted by the Nobel Prize in Chemistry in 1902. In addition, Paul Ehrlich and coworkers developed arsphenamine (Salvarsan) as antimicrobial treatment for syphilis in the early 1900s and pioneered the concept of chemotherapy as a "magic bullet" for disease treatment [2]. These two landmark examples established the foundation for the synthesis of small molecules, the determination of their structures and mechanisms of action as well as their therapeutic application. Many areas of chemistry and biology have evolved from these pioneering studies and have culminated in our current perspective on chemical biology. Notably, the design and synthesis of specific chemical probes and homogeneous biomolecules lies at the heart of chemical biology. It is also important to note that the advances in chemical biology have been enabled by many major areas of science such as physical and organic chemistry, biochemistry, structural biology, analytical chemistry as well as engineering and evolutionary approaches (Figure 1.1), which we highlight below.

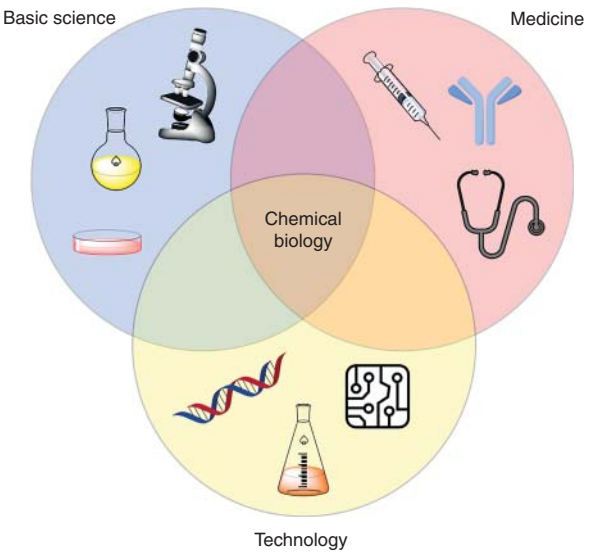

Figure 1.1 Chemical biology is at the nexus of basic science, medicine, and technology.

1.2 Enabled by Synthetic and Physical Organic Chemistry

The ability of chemists to understand reactivity of molecules and exploit these principles for synthesis has been transformative for science [3] and underlies much of the innovations in chemical biology [4, 5] (Figure 1.2). Indeed, innovations in organic chemistry

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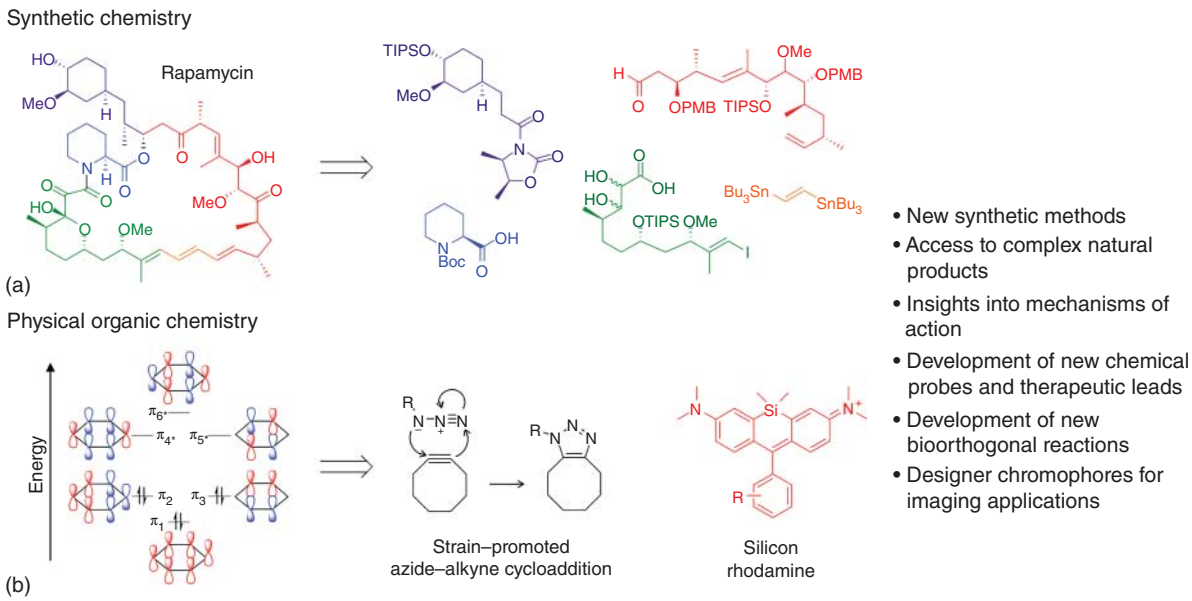

Figure 1.2 Impact of synthetic and physical organic chemistry on chemical biology. (a) Retrosynthetic analysis of complex natural product such as rapamycin. Source: Nicolaou et al. [6]/American Chemical Society. (b) Improved bioorthogonal reactions such as strain-promoted azide-alkyne cycloaddition [7] as well as new chromophores such as silicon rhodamine [8].

have greatly facilitated the synthesis of complex natural products (Figure 1.2a), small-molecule probes, and macromolecules for fundamental studies and therapeutic applications [4, 5]. For example, efficient methods for the chemical synthesis of nucleic acids have revolutionized molecular biology [9], facilitated the development of highly sensitive diagnostic methods [10], and supported the generation of precise vaccines [11] (Chapter 2). Moreover, the synthesis of short oligonucleotides has enabled structure–function studies, the rapid cloning of genes (Chapter 2) [10], and efficient programmable genome engineering [12] (Chapter 6). Likewise, the chemical synthesis of peptides [13], proteins [14, 15], and glycans [16, 17] have also provided important access to these biomolecules for structure–activity studies as well as the generation of diagnostics and therapeutics (Chapters 7, 8, 13, 15, 17, 24, 26, and 30). Of note, the site-specific installation of biophysical probes and posttranslational modifications onto peptides and proteins has revealed fundamental principles of protein folding, structure, and function (Chapters 7, 8, and 15). Alternatively, the synthesis of glycans has yielded homogeneous materials to explore their function as well as important imaging and diagnostic agents such as fluorine-18-2-fluoro-2-deoxy-D-glucose (F18-FDG) (Chapter 13).

Beyond the synthesis of biomolecules, advances in physical organic chemistry such as the hard–soft acid–base and molecular orbital theories (Figure 1.2b) [18] have led to the development of new chemical reactions and probes to explore biology. For example, understanding the relative reactivity of amino acid side chains with different chemotypes has yielded efficient bioconjugation methods for modifying native proteins (Chapter 14). Alternatively, the development of chemical reactions that are orthogonal to the endogenous reactivity in cells and yet compatible with biological conditions has afforded a variety of "bioorthogonal" reactions for the modification of diverse biomolecules and small molecules with unique functionality (Figure 1.2b) (Chapter 16). Moreover, understanding the stereo-electronic effects of chemical modifications on chromophores has yielded a wide range of imaging reagents for visualizing many biological processes in cells and animals (Figure 1.2b) (Chapters 17 and 18). These chromophores can also be tuned to bind different metals to explore their abundance and dynamics in biological systems (Chapter 19). Furthermore, the unique reactivity of different chemotypes can be harnessed for selective profiling of various redox states (Chapter 20) and biochemical activities of proteins (Chapter 21).

In addition to reaction and probe development, the total synthesis of complex natural products and their analogs has afforded important reagents to determine their molecular targets and mechanisms of action [19], which has led to more precise therapeutics for human diseases. A landmark example of these studies is the discovery, synthesis (Figure 1.2a), and target

identification of rapamycin, which revealed mammalian target of rapamycin (mTOR) [20, 21], as a key kinase that regulates cellular growth and metabolism (Chapter 25). Although rapamycin from *Streptomyces hygroscopicus* was originally explored as an anti-fungal agent, it exhibited potent immunosuppressive activity on T cells and was ultimately approved by the Federal Drug Administration (FDA) to mitigate the side effects of organ transplantation (Chapter 4). The subsequent characterization of mTOR as the mechanistic target of rapamycin [20, 21] and the discovery of its phosphatidylinositol 3-kinase-related kinase activity led to the development of more specific and potent mTOR kinase inhibitors to treat cancer and other metabolic diseases in humans (Chapter 30).

1.3 Guided by Biochemistry and Structural Biology

The design and development of specific chemical probes to perturb and visualize biological systems has been guided by innovations in biochemistry [22] and structural biology (Figure 1.3) [25, 26]. For example, the study of enzyme reaction mechanisms [22] allowed the development of specific chemical probes for activity-based protein profiling (ABPP) (Figure 1.3a) (Chapter 21). Alternatively, the advances in X-ray crystallography have allowed structure-based design of important small-molecule probes and therapeutics (Figure 1.3b). Moreover, the design of orthogonal "bump-and-hole" enzyme–substrate pairs (Chapter 22) was facilitated by X-ray structures of different enzymes and protein families. In addition, structural studies of large multi-domain protein complexes such as polyketide synthases (PKSs) have helped to deconvolute the biosynthesis of natural products and provided new opportunities to engineer these pathways (Chapter 24). More recently, advances in cryo-electron microscopy have shed light on the structures of membrane proteins and larger complexes [27], which has enabled the design and development of additional chemical probes and therapeutics. Furthermore, the establishment of robust protein structure prediction methods has provided important computational tools for exploring small molecule–protein interactions as well as *de novo* design of novel proteins with diverse functions [28].

1.4 Enhanced by Engineering and Evolution

As chemists and biologists began to understand the structure and function of biomolecules, this collaboration allowed the design of novel systems with improved or new functions (Figure 1.4). For example,

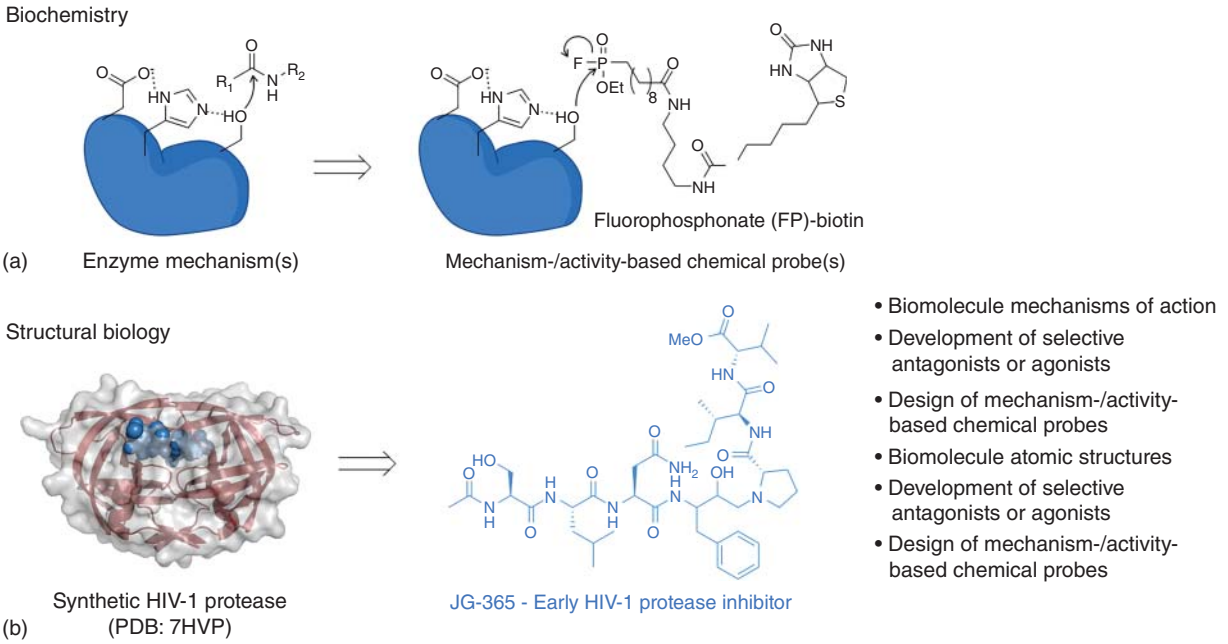

Figure 1.3 Impact of biochemistry and structural biology on chemical biology. (a) Understanding enzyme reaction mechanisms has afforded activity-based probes such as FP-biotin. Source: Liu et al. [23]/The National Academy of Sciences. (b) Structural biology and computational methods have enabled structure-based design of selective chemical probes and therapeutics such as HIV-1 protease inhibitor. Source: Swain et al. [24]/The National Academy of Sciences.

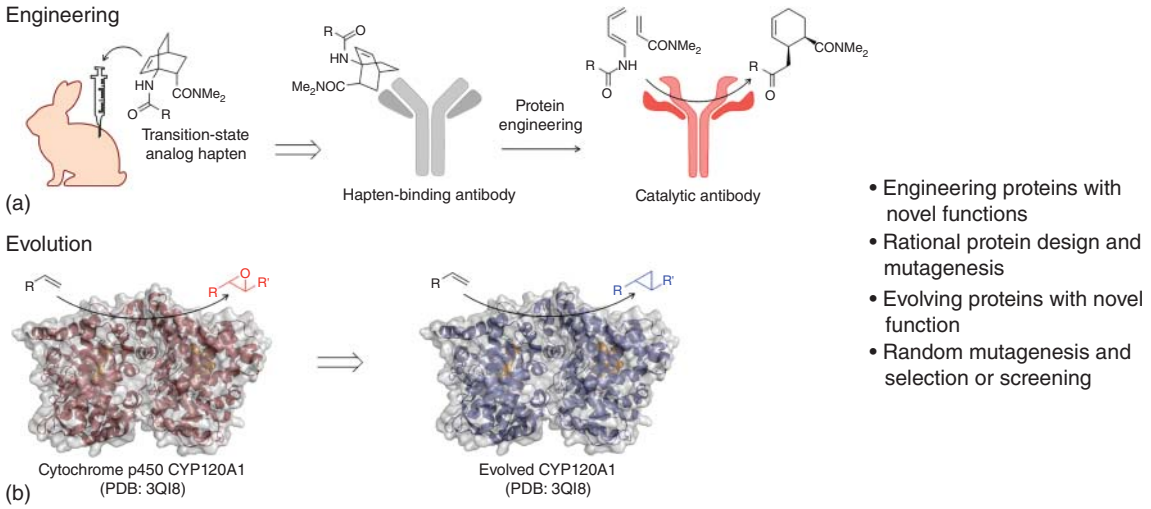

Figure 1.4 Examples of engineering and evolutionary approaches in chemical biology. (a) Advances in protein engineering have enabled the design and development of proteins with novel activity such as catalytic antibodies for stereoselective Diels–Alder reaction. Source: Adapted from Gouverneur et al. [29]. (b) Directed evolution has also afforded proteins with novel functions such as P450 enzymes with cyclopropanation activity. Source: Adapted from Coelho et al. [30].

protein-engineering methods were employed to generate catalytic antibodies that could execute chemical reactions like natural enzymes or entirely new reactions (Figure 1.4a) (Chapter 28). Alternatively, directed evolution approaches combining random mutagenesis in combination with high-throughput selection or screening methods were developed to identify unpredicted and novel protein variants with unique or improved properties (Figure 1.4b) (Chapter 9). Of note, protein engineering and directed evolution approaches have been employed to establish genetic codon expansion for the site-specific incorporation of non-canonical amino acids with unique reactivity into specific proteins and whole organisms (Chapter 15). Beyond these synthetic biology examples, protein engineering and directed evolution approaches have also been instrumental in generating fluorescent proteins (Chapter 17) and reporter enzymes (Chapter 18) with improved cellular and *in vivo* imaging properties.

1.5 Expanded by Analytical Chemistry and "Omics" Technologies

Chemical biology has also been significantly enabled and expanded upon with improved analytical methods and instrumentation (Figure 1.5). The development of rapid and inexpensive nucleic acid sequencing methods has been transformative for illuminating the genome of many organisms and has allowed comparative genomics of healthy and disease states (Figure 1.5) (Chapters 2–6). The extension of these methods to single cell analyses has revealed spatial and temporal phenotypes of diverse biological processes and is revolutionizing biology and medicine [31]. In parallel, the advances in mass spectrometry [32] and nuclear magnetic resonance spectroscopy [33] have greatly improved the detection and structural characterization of macromolecules and metabolites (Figure 1.5). For example, the high-throughput fragmentation and detection peptides by mass spectrometry along with accurate computational assembly methods have facilitated the large-scale comparative analysis of proteins [32] and their posttranslational modifications (Chapter 12). In addition, the union of mass spectrometry with chemical affinity probes and ABPP (Chapter 21) has facilitated the identification of small molecule–protein targets for improved pharmacology and drug development (Chapters 26 and 30). Furthermore, these significant advances in analytical chemistry have allowed the large-scale comparative analysis of cellular metabolites (Chapter 10) and lipids (Chapter 11) in cells, tissues, and whole organisms as well as complex natural products (Chapter 23). Collectively, these large-scale methods for analyzing the genome, transcriptome, proteome, and metabolome of cells and organisms are providing important methods for dissecting complex biology systems and diseases.

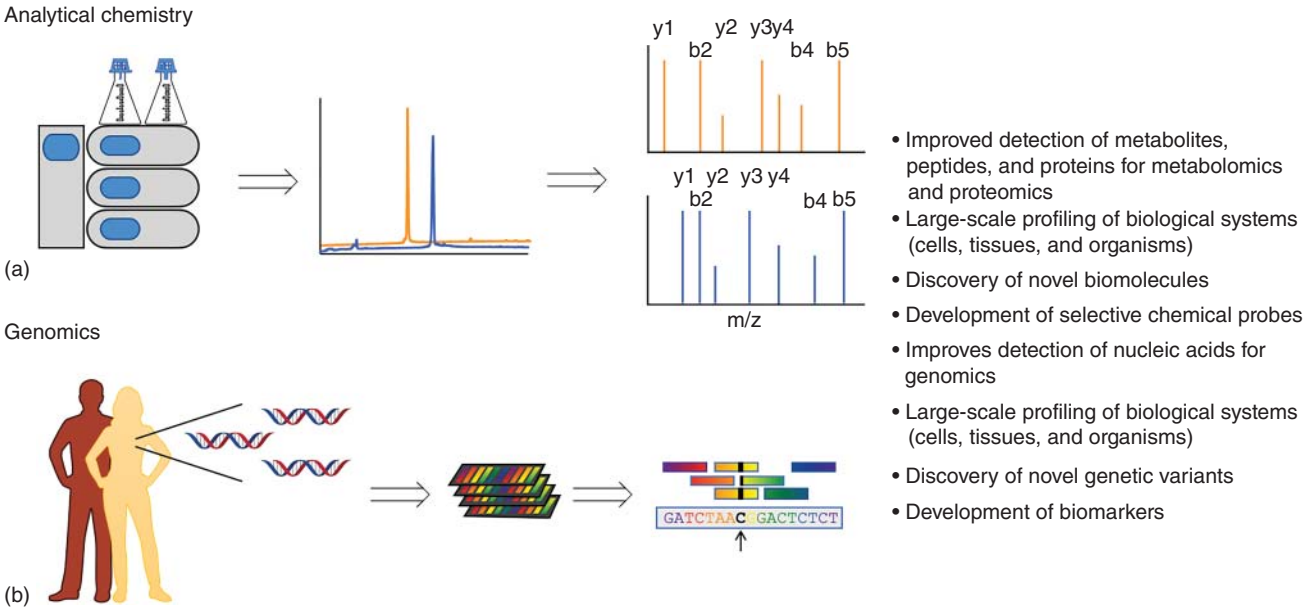

Figure 1.5 Impact of analytical chemistry and large-scale methods on chemical biology. (a) Better analytical methods have allowed improved detection of biomolecules for metabolomics and proteomics. (b) Enhanced nucleic acid detection and sequencing methods have significantly expanded the scope and impact of genomics.

1.6 Impact on Biological Discovery and Drug Development

Innovations in chemical biology have illuminated specific areas of biology and are fueling the development of new therapeutics. Since the original discovery of penicillin [34], chemical approaches and new probes have helped to elucidate fundamental biosynthetic pathways in bacteria and have facilitated the development of new antibiotics (Chapter 25). Likewise, chemical biology approaches have aided in the dissection of complex signaling pathways in eukaryotic cells and the determination of mechanisms of action and resistance for new small-molecule drug candidates (Chapter 26). Chemical biology has also helped to uncover important developmental pathways in whole organisms and characterize detrimental side effects of drug molecules (Chapter 27). Since the birth of immunology as field, chemistry has played a key role in establishing the principles of the adaptive immune response and has also afforded new tools for large-scale immune profiling as well as the next generation of adjuvant molecules (Chapter 28). Neuroscience has also benefitted from the advances in chemical biology, as the engineering of novel protein–ligand pairs has afforded methods for cell-specific perturbations and imaging *in vivo*, which has been instrumental in deconstructing neuronal circuits and modulating animal behavior (Chapter 29). Finally, the multitude of chemical biology approaches to discover novel bioactive small molecules and elucidate their mechanisms of action has greatly improved the overall pipeline for drug discovery (Chapter 30).

1.7 Outlook

We have been fortunate to witness and participate in the evolution of chemical biology as a multi-disciplinary field that integrates different fields of basic science to understand biology and disease. We greatly appreciate the remarkable contributions of the chapter authors and are grateful for their insightful perspectives on each area of chemical biology, which we hope will be helpful and inspire the next generation of scientists. As we look forward to the future, remarkable advances in synthetic chemistry continue to provide access to more complex molecules for investigation, while new and improved instrumentation from analytical chemistry will allow for more sensitive and high-throughput analyses of diverse biomolecules. Excitingly, machine-learning and artificial intelligence methods have already begun to provide new approaches to design and synthesize biomolecules

more efficiently and with novel properties [35]. The union of these advances with "omics" technologies should provide new opportunities to realize the promise of personalized medicine for different diseases. As we achieve new milestones in chemistry and biology for global health, we hope that new innovations in chemical biology will continue to expand beyond human health and provide key solutions for other major challenges facing our planet, including food security, energy production, and climate change.

References

1 Kunz, H. (2002). Emil Fischer – unequalled classicist, master of organic chemistry research, and inspired trailblazer of biological chemistry. *Angew. Chem. Int. Ed.* 41 (23): 4439–4451.

2 Stern, F. (2004). Paul Ehrlich: the founder of chemotherapy. *Angew. Chem. Int. Ed.* 43 (33): 4254–4261.

3 Corey, E.J. and Cheng X-m (1989). *The Logic of Chemical Synthesis*. New York: Wiley, 436 pages.

4 Schreiber, S.L., Kotz, J.D., Li, M. et al. (2015). Advancing biological understanding and therapeutics discovery with small-molecule probes. *Cell* 161 (6): 1252–1265.

5 Schreiber, S., Kapoor, T.M., Gn, W., and Wiley, I. (2007). *Chemical Biology: From Small Molecules to Systems Biology and Drug Design*. Weinheim: Wiley-VCH.

6 Nicolaou, K.C., Chakraborty, T.K., Piscopio, A.D. et al. (1993). Total synthesis of rapamycin. *J. Am. Chem. Soc.* 115 (10): 4419–4420.

7 Agard, N.J., Prescher, J.A., and Bertozzi, C.R. (2004). A strain-promoted [3+2] azide-alkyne cycloaddition for covalent modification of biomolecules in living systems. *J. Am. Chem. Soc.* 126 (46): 15046–15047.

8 Koide, Y., Urano, Y., Hanaoka, K. et al. (2011). Evolution of group 14 rhodamines as platforms for near-infrared fluorescence probes utilizing Photoinduced electron transfer. *ACS Chem. Biol.* 6 (6): 600–608.

9 Caruthers, M.H. (2013). The chemical synthesis of DNA/RNA: our gift to science. *J. Biol. Chem.* 288 (2): 1420–1427.

10 Mullis, K., Faloona, F., Scharf, S. et al. (1986). Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. *Cold Spring Harbor Symp. Quant. Biol.* 51 (Pt 1): 263–273.

11 Sahin, U., Kariko, K., and Tureci, O. (2014). mRNA-based therapeutics – developing a new class of drugs. *Nat. Rev. Drug Discovery* 13 (10): 759–780.

12 Doudna, J.A. (2020). The promise and challenge of therapeutic genome editing. *Nature* 578 (7794): 229–236.

13 Merrifield, B. (1986). Solid phase synthesis. *Science* 232 (4748): 341–347.

14 Thompson, R.E. and Muir, T.W. (2020). Chemoenzymatic semisynthesis of proteins. *Chem. Rev.* 120 (6): 3051–3126.

15 Dawson, P.E. and Kent, S.B. (2000). Synthesis of native proteins by chemical ligation. *Annu. Rev. Biochem.* 69: 923–960.

16 Pardo-Vargas, A., Delbianco, M., and Seeberger, P.H. (2018). Automated glycan assembly as an enabling technology. *Curr. Opin. Chem. Biol.* 46: 48–55.

17 Cheng, C.W., Wu, C.Y., Hsu, W.L., and Wong, C.H. (2020). Programmable one-pot synthesis of oligosaccharides. *Biochemistry* 59 (34): 3078–3088.

18 Anslyn, E.V. and Dougherty, D.A. (2006). *Modern Physical Organic Chemistry*. Sausalito, CA: University Science.

19 Nicolaou, K.C., Snyder, S.A., and Corey, E.J. (2003). *Classics in Total Synthesis*. Weinheim: Wiley-VCH, xix, 639 pages: ill p.

20 Schreiber, S.L. (1991). Chemistry and biology of the immunophilins and their immunosuppressive ligands. *Science* 251 (4991): 283–287.

21 Sabatini, D.M. (2017). Twenty-five years of mTOR: uncovering the link from nutrients to growth. *Proc. Natl. Acad. Sci. U.S.A.* 114 (45): 11818–11825.

22 Fersht, A. (1999). *Structure and Mechanism in Protein Science: A Guide to Enzyme Catalysis and Protein Folding*. New York: W.H. Freeman, xxi, 631 pages: illustrations p.

23 Liu, Y., Patricelli, M.P., and Cravatt, B.F. (1999). Activity-based protein profiling: the serine hydrolases. *Proc. Natl. Acad. Sci. U.S.A.* 96 (26): 14694–14699.

24 Swain, A.L., Miller, M.M., Green, J. et al. (1990). X-ray crystallographic structure of a complex between a synthetic protease of human immunodeficiency virus 1 and a substrate-based hydroxyethylamine inhibitor. *Proc. Natl. Acad. Sci. U.S.A.* 87 (22): 8805–8809.

25 Hendrickson, W.A. (1991). Determination of macromolecular structures from anomalous diffraction of synchrotron radiation. *Science* 254 (5028): 51–58.