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The State of the Art of Chemical Biology

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Bernhard Kräutler

Considering the eminent role of chemistry in helping to decipher and control life processes, a name of its own—"chemical biology"—has rightly been given to the thriving area at the interface with biology and medicine. It has become a molecule-based bridge that paves the way to a deep and logic interpenetration—on the molecular level—of structural, functional, and developmental approaches to the natural life sciences, including medicine.

As brought to our attention recently, "molecules have changed the world".[3] Indeed, low-molecular-weight organic compounds are an important and well-equipped tool box in the life sciences—on equal terms with and complementary to the (more typical) one of molecular and developmental biology—and medicine, which often focus on macromolecules and their interactions. Nevertheless, organic chemists have also adapted to the specific challenges of synthesizing complex compounds ("larger organic molecules", such as peptides, proteins, oligosaccharides, and oligonucleotides) and of building up biologically important supramolecular assemblies.

Let me single out the example of the "small molecule" vitamin B_{12} in kicking-off groundbreaking contributions to chemical biology. In the last decade, research with B_{12} has led to the discovery of gene-regulating RNA, the riboswitches (Figure 1),[4] of natural RNA-based logic elements,[5] and of unexpected symbiotic relationships among lower organisms.[6] Activated molecular transport through cell membranes in micro-organisms,[7] and intra- and intercellular cofactor sorting and trafficking in mammals have also seen major advances.[8]

On another level, chemical biological studies enrich the never-ending chapter of the molecular evolution of elementary life processes, for example, as spearheaded by the RNA-World hypothesis,[9] and how cofactors might have arisen and made their place in life.[10]

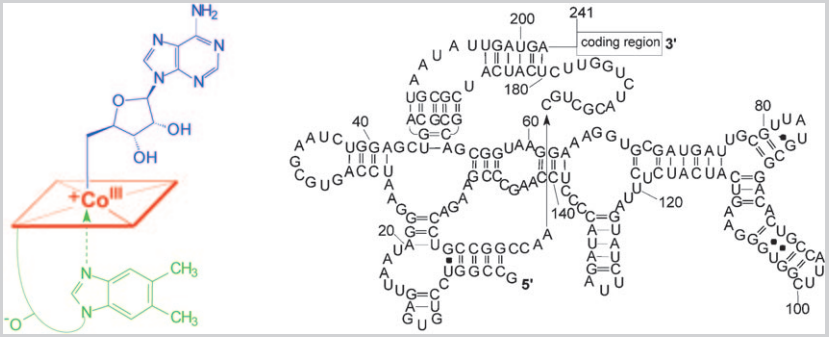


Figure 1. Symbolic formula of coenzyme B_{12} and sequence model of the B_{12}-riboswitch in the 5′-untranslated region of the *btuB*-gene of *E. coli* (adapted from ref. [4c]).

While it is difficult to foresee real breakthroughs in basic sciences, chemical biology is due to play a leading role in giving insights into ever more complex life phenomena, such as—possibly—(the molecular imprints of) behavior. However, molecular understanding of basic life processes has a strong technological impact (such as on information, communication, energy conversion, bio-, and food technology), as well as on the development of molecular tools for the diagnosis and therapy of diseases.

"Chemical biology is due to play a leading role in giving insights into ever more complex life phenomena"

Johannes Buchner

Horst Kessler

Chemical biology is a new term used to emphasize the importance of chemistry in exploring the potential of chemical structures to influence biological functions. Although this concept is far from being new—just think of Emil Fischer or Emil von Behring—in the last decades it has sparked new interest in this area and brought the idea into the focus of organic chemists. This was necessary, as many chemists had forgotten that chemistry is not only the science for creating new structures, but also for understanding their biological and physical properties. The repertoire of synthetic methods for preparing complex structures has expanded enormously in recent years. However, exciting new methods are found less often. The big challenge is now to "design properties" to understand biological functions; this is a complex issue because the structural dynamics and the dynamics of the interactions considerably hamper the search for small molecules to interfere with the function of large biomolecules (Figure 2). In 1981, Lord Alexander R. Todd stated:

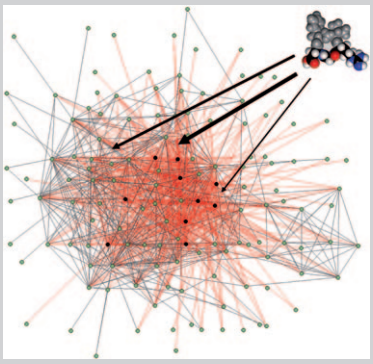


Figure 2. Interaction of a small-molecule with a biological network.

"Apart from consideration of the hydrogen bond, we organic chemists have really paid little attention to linkages other than the purely covalent. I believe that it will be the duty of organic chemists in the future to study the weak non-bonding interactions which are of enormous importance in the large natural macromolecules. Such studies will lead to a new blossoming of organic chemistry in the future."[1]

This still holds true. However, we now realize that extremely complicated multidimensional networks of interactions of a vast number of biomolecules determine biological chemistry and regulatory pathways. The elucidation of that will be *the* challenge for natural science. This is what "Chemical Biology" is all about.

"The big challenge is now to "design properties" to understand biological functions"

"Rich frontiers in research"

Chemical biology is a very attractive name for a field that applies the methods of chemistry to studying biology. It offers rich frontiers in research and technology to coming generations of chemists, as it did in the past, before the name was created. At the ETH Zürich, prominent early practitioners of chemical biology include Leopold Ruzicka, Vladimir Prelog, Albert Eschenmoser, and Duilio Arigoni. While chemists increasingly address biological systems such as proteins, nucleic acids, carbohydrates, biological membranes, and entire cells directly, investigations of well-designed model systems continue to fertilize the field. Biological mechanisms, such as enzyme-catalyzed transformations, protein–ligand interactions, self-assembly, selection, and replication are only fully understood if they can be reproduced by de novo-designed synthetic model systems. Furthermore, model studies have contributed to our understanding of biological systems, one prominent example being the discovery of cation–π interactions in complexation studies with synthetic cyclophane receptors in the Dougherty group (Figure 3). There are frequently limits to accuracy in biological studies due to the complexity of these systems, which often prevent quantification of the energetics of individual intermolecular interactions or the contribution of a single amino acid side chain to enzyme catalysis. Weak orthogonal dipolar interactions, such as C–F···C=O and C=O···C=O interactions, with free enthalpy increments of -0.3 to -0.6 kcal mol^{-1}, are a good example. While the relevance of these interactions was first recognized in enzyme–ligand complexation studies, their quantification required elaborate, structurally precisely defined model systems that could be investigated with great accuracy. It is therefore my strong belief that the successful future development of the field of chemical biology will continue to require the complementary study of both biological and synthetic model systems.

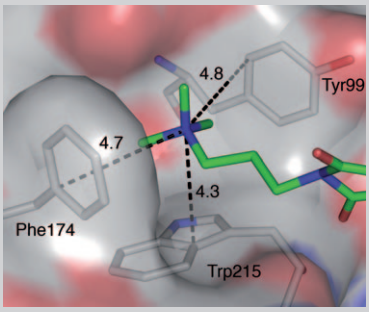


Figure 3. Cation–π interactions in the S4 pocket of the serine protease Factor Xa, as seen in a protein–ligand cocrystal structure.[2]

François Diederich

Rob Liskamp

Chemical *BIOLOGY* and *CHEMICAL* Biology

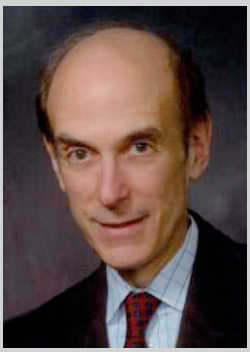
The last decades have witnessed tremendous developments in biology. These have stimulated the expansion of molecular approaches to the design and synthesis of bioactive and biomimetic molecules for studying and modulating relevant biological processes. As a result *chemical biology* has been shaped, which clearly offered the chemical tools to assist and facilitate further developments in biology. The interdependency of chemistry and biology in chemical biology has been shown to be of great synergistic value. A relatively obvious example is the development of click chemistry and the plethora of applications in biology ranging from purification tools to the efficient preparation of bioactive ligands. These and many other examples show the usefulness and enormous significance of chemistry for biology. Now, the time has come for the vast developments in biology to induce and stimulate new directions in chemistry. Early examples include the development of foldamers, such as the β-peptides. New chemical developments of (bio)materials will be inspired by biology, altered proteins will provide new catalysts, etc.

One of the greatest challenges for chemistry is to keep up with the pace of developments in biology. Biology, as illustrated by the emerging synthetic biology, is nowadays capable of the rapid construction or synthesis of not only complex proteins and DNA/RNA but also of modified organisms with desired properties. This speed is unsurpassed, and there is a large discrepancy with the speed for the synthesis of much smaller, but certainly still very complex molecules. Chemical synthesis on the one hand is very rewarding in terms of the insights one obtains into molecular structure and molecular properties as well as the versatility for the preparation of useful derivatives. On the other hand, it is very time consuming and needs hard work from many researchers. Thus, synthesis methods have to become even more efficient and faster. This can be partly achieved by learning from biology, using enzymes and/or catalysts, trying to devise chemistry that reduces the need for protecting groups or having very effective protecting-group strategies or adjusting and using biosynthetic routes. A lot is to gain here and is needed, because the non-chemist colleague wants to have compounds fast, because developments are fast, and competition is fierce.

"The interdependency of chemistry and biology in chemical biology has been shown to be of great synergistic value. Now, the time has come for the vast developments in biology to induce and stimulate new directions in chemistry."

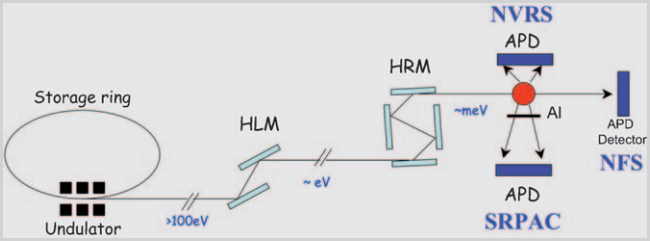
Furthermore, many "old" and recent discoveries in biology will offer handles on or point to inspiration for new chemistry. Questions as to why Nature has chosen certain approaches for molecular construction and why a particular molecule is as it is, might lead to the development of attractive "chemistry" alternatives. Examples could include the development of synthetic vaccines. It is important to realize that over a billion years, Nature's evolution has lead to impressive trees of evolution, but that does not mean that these trees have stopped growing. New braches will shoot, leading to organisms with new properties and capacities. In addition, new branches will shoot at the trees of molecular evolution, creating new molecules with new attractive properties.

Finally, developments during the last decades in, for example, synthetic chemistry and supramolecular chemistry have also been immense. Areas from the recent past in chemistry and chemical biology, which could not be realized or fully investigated, because crucial advancements had not yet taken place, can now be tackled, possibly with increasing success. One example would be the chemistry of catalytic mimics. Synthetic methods for their efficient construction are now much more advanced than, say, 20 years ago, when, for example, Cram, Breslow, Diederich, and their colleagues published their impressive molecular constructs; this will offer new challenging opportunities for re-entering these areas.



Stephen Lippard

The Fashioning of New Tools Drives Biological Chemistry



Not long after I completed graduate school in the mid-1960s, many chemistry departments in the USA systematically eliminated the subdiscipline of analytical chemistry. "We are all analytical chemists" was the refrain, so what was the point in devoting a portion of faculty appointments to analytical chemistry? Now, many decades later, this situation persists, although there remain a few very strong analytical units in selected chemistry departments. Progress in the field of biological chemistry, a term which I, as a chemist, prefer to chemical biology, is critically dependent on new tools of analysis. Examples that have changed the face of the discipline include gel electrophoresis to study proteins and nucleic acids, nucleic acid sequencing methodologies, numerous advances in X-ray diffraction methodologies, and the polymerase chain reaction. In this short piece I would like to highlight two analytical methods that are currently in advanced stages of development and are likely to have a major impact.

One example, pioneered by the Schultz laboratory at Scripps, is the ability to insert unnatural amino acids into any protein sequence in both prokaryotic and eukaryotic organisms by genetic engineering. The methodology utilizes a unique tRNA–codon pair and corresponding aminoacyl-tRNA synthetase. Many applications can be envisioned, but one of special appeal to me is to interrogate specific amino acids in an enzyme to define their roles in catalysis or other function, such as electron transfer.

A second analytical tool, one that I became aware of through the published papers of Cramer at UC Davis, is nuclear vibrational resonance spectroscopy, or NVRS. In this technique, which requires synchrotron radiation, vibrational sidebands of a Mössbauer transition are detected, revealing bonding details of adjacent ligands. For bioinorganic chemistry, ^{57}Fe is the metal of choice, allowing many important problems to be attacked that otherwise would have been intractable. The application of metal-focused spectroscopy enormously simplifies the analysis of complex systems such as biopolymers, illuminating the metal-coordination-sphere active site to the exclusion of unwanted background from elsewhere in the molecule.

"Progress in the field of biological chemistry... is critically dependent on new tools of analysis"

There are many other advances, such as single-molecule spectroscopy and nanotechnological methods, that cannot be covered in this short commentary. Thankfully, analytical chemistry remains a strong and an essential component in the laboratories of many investigators working within the biological chemistry community, and advances in physics, materials science, and other allied fields continue to offer exciting new methodological opportunities.

"Reducing the hurdles"

Of many major developments that have taken place in pharmaceutical research over the last decade, we should mention the development of novel miniaturized and parallelized analytical methods for the measurement of the physico- and biochemical properties of compounds relevant for their in vivo pharmacological behavior. These methodologies have contributed significantly to reducing the hurdles to bringing a drug candidate from preclinical discovery to the phase of the in vivo safety and profiling studies required for clinical development. They have generated a major paradigm shift from sequential to parallel optimization of lead-compound potency and properties. At the same time they have resulted in an unprecedented accumulation of new data that can now be analyzed in order to develop heuristic frameworks for a structure-based understanding and prediction of compounds' physico- and biochemical properties. Significant progress has been made in this regard; however, it is evident that the prediction of diverse compound properties is still in its infancy due to 1) the inherent complexity of the matter, 2) the lack of sufficiently extended, experimentally consistent, and structurally diverse data sets, and 3) missing data-mining tools that can cope with very large data sets in a multidimensional manner. A significant improvement in our understanding of structure–property relationships will enable structure-based property design and optimization in very much the same way as the continued improvement in intermolecular interaction concepts fostered structure-based molecular design regarding potency (and selectivity) for a given target of known 3D molecular structure. While this development still requires the generation of a lot of new data for specifically designed compound series to fill many essential gaps in the current data sets, a better understanding of structure–property relationships will not only have a significant impact on the speed and success of lead optimization programs, but also spur the design and synthesis of novel building blocks that will innovate medicinal chemistry. The outlook is fascinating.

Klaus Müller

"A much larger arsenal of side-chain and main-chain constrained amino acids is eagerly needed"

A deep understanding of the detailed nature and mechanism of physico-chemical interactions between two probes or between two host functionalities and a guest (e.g., a substrate) molecule depends heavily upon our ability to appropriately design and to successfully synthesize conformationally constrained 3D structural platforms (e.g., foldamers), the intercomponent geometry of which (either rigorously rigid or able to undergo destructuration, if required, but always precisely tunable) would be well defined.

As it is clear that none of the peptides entirely based on protein amino acids can produce truly *rigid* backbone templates, in the past few years several groups have concentrated their efforts on compounds rich in severely restricted backbone-modified amino acids (pseudopeptides, peptidomimetics). An appropriate choice of specific classes of building blocks will allow one to tailor 3D structures endowed with exactly pre-determined, intramolecular $C^{\alpha}\cdots C^{\alpha}$ distances.

However, in the search for rigid scaffolds, additional problems might arise from rotations about amino acid *side-chain* single bonds. Although a limited side-chain flexibility might in general be tolerated, or might even be beneficial, that arising from protein amino acids is definitely too large, and makes any conclusion quite approximate. In other words, by utilizing this type of side chain, any investigation inevitably suffers a range of uncertainty even larger than that saved from the restrictions imposed by rigidification of the backbone.

It is quite clear that, to further expand this research area, a much larger arsenal of side-chain and main-chain constrained amino acids is eagerly needed, and an active contribution from synthetic organic chemists is particularly welcome. The promising role of novel 3D structures as easy-to-prepare, cheap, and appropriately functionalized molecular scaffolds for applications in physical chemistry (radical···radical interactions; fluorescence quenching; electron transfer; precise determination of distances between probes by using new, but still to be calibrated, spectroscopic techniques) and organic chemistry (supramolecular assembly; receptor···guest interaction; asymmetric, redox and hydrolysis catalyses; macrocyclization; regioselective reaction) is rapidly emerging. The vitality and breadth of this basic science will not only contribute to eradicating the still existing cultural barriers between different branches of chemistry but also to deepening our knowledge on the fundamental reactions and processes of chemical biology.

Claudio Toniolo

Bruno Samorì

Tools and Approaches

Chemical biology, like molecular biology, is defined more by the set of technologies that it can bring into play to tackle biological problems, than by any particular research objective. Because of the complexity of biological systems, advancements in biology are constantly dependent on the approaches, methods and techniques that are made available to biologists by chemists, physicists, engineers, and mathematicians. We should therefore expect that the new trends in chemical biology will be strongly driven by the tools and approaches that will allow chemists to expand the scale and scope of biological problems they will tackle and, in particular, to be up to the two main tasks that seem to stand out for them right now.

"The new trends in chemical biology will be strongly driven by the tools and approaches that will allow chemists to expand the scale and scope of biological problems they will tackle"

The first task has emerged with the introduction of nanotechnological tools in biology, and of single-molecule methodologies in particular. They are now making it possible to monitor and measure, in real time, the myriad of mechanical forces that are generated in the biological world. They are disclosing the limits of a description of the cell nanoworld based on macroscopic definitions (such as pH or thermodynamics of equilibrium states, etc.) that have been developed through classical in-bulk chemistry involving Avogadro's number of molecules. They are unveiling how chemical transformations inside the cell are dominated by directionality and stochasticity. In the book *Physical Biology*,[11] Carlos Bustamante provides a very enlightening picture of this scenario and of the resulting need for a fundamental change in our conceptual approaches to our research, mostly with respect to the model of the cell as a reactor, where, according to classical biochemistry, chemical transformations are taking place by simple diffusions and random collisions of the reacting species. On this basis, we expect that chemical biologists will be up to the task of providing a more quantitative and comprehensive description of the biological systems at a molecular level by increasingly bringing into play nano- and single-molecule methodologies, and by fostering their development in order to apply them also inside living cells. It should be pointed out that single-molecule experiments do not necessarily rely on reductionist approaches, such as the one-compound–one-target paradigm, upon which chemical biology has heavily relied so far. In fact, many chemical transformations take place in the cell among reacting species that are present therein only in single or very few copies.

The second task has emerged with the renewed push towards a more holistic mentality, on following the pervading advent in biology of assays for biological fingerprints, and genomic profiles, and the consequent foundation of new hybrid disciplines, like systems biology and synthetic biology. The main quests that the former discipline addresses to chemistry seem to be mostly about new and more efficient activity-based probes, reactive tags, small molecules to probe signaling pathways, chemical-capture technologies, etc. Cross-fertilization with synthetic biology should be able to involve a broader range of chemical expertise in designing and building up biological systems, and then putting them together into a functional unit in order to learn how they work. This is a mentality we know very well in chemistry, and in this context the role of supramolecular chemists might be very central, provided that they decide to plunge their hands into the hierarchical complexity of the biological systems much deeper than they have so far. In a longer run, as nanotechnology teaches us, and as Carlos Bustamante suggests in the same book, the winning approach towards an increased understanding of how cells regulate and maintain their homeostasis will be that of converging and integrating the "top-down reductionist approaches with the bottom-up synthetic approach".[11]

"We expect that chemical biologists will be up to the task of providing a more quantitative and comprehensive description of the biological systems at a molecular level by increasingly bringing into play nano- and single-molecule methodologies"

Constant A. A. van Boeckel

Chemical Biology to Provide Valuable Instruments in the Toolbox of Drug Discovery

An important aspect of chemical biology in life sciences is its role in drug discovery. Despite the enormous knowledge gained over the last century about molecular targets in relation to diseases and molecular entities that modulate such targets, only relatively little is directly applied in current medication. Thus, in the western world fewer than 500 unique chemical entities, acting on fewer than 200 human molecular targets (mainly receptors and enzymes) are formulated in thousands of commercially available drugs. Clearly, there is a huge need for the identification of more validated drug targets and, in particular, new chemical entities that can be effectively used to cure diseases.

It seems we have a terrific toolbox in drug discovery containing "instruments" such as genomics, proteomics, knock-out models, RNAi, high-throughput screening (HTS), combinatorial chemistry, structural biology, monoclonal antibodies, etc. Chemical biology provides various novel instruments for the drug discovery toolbox. For instance, new conjugation technologies (e.g., click chemistry[12] and native chemical ligation[13]) are being developed to allow not only the preparation of tailor-made diagnostic tools for in vitro and in vivo studies, but also to prepare new constructs that combine special features of biologically active biomacromolecules.

Recently we published a novel conjugate between a heparin-like pentasaccharide and recombinant insulin. The pentasaccharide belongs to a family of synthetic heparin-like compounds[14] such as Arixtra®, which reached the market, and Idraparinux, now in phase 3 clinical trials for once-a week antithrombotic therapy. Due to its strong and specific interaction with antithrombin III in blood the pentasaccharide becomes a carrier of insulin, thereby prolonging the residence time of the small protein enormously.[15]

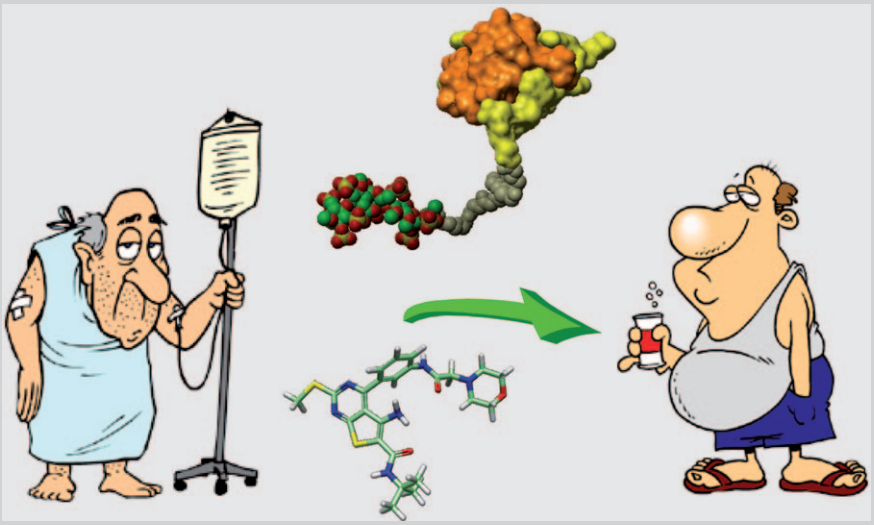
"It is fascinating that we can put so much information into small molecules"

In addition, one can think of designing various novel conjugates between well-defined oligosaccharides, proteins, antibodies, and nucleotides to enter a totally new space of molecular properties and result in improved pharmacokinetics, unprecedented mixed pharmacological profiles, new targeting opportunities, or leading to novel immunomodulating agents and synthetic vaccines.

On the other hand, for drug discovery it is of the utmost importance to condense many attractive biological and physicochemical properties in small molecules in order to get orally available drugs. In this respect, I like to refer to the first example[16] of a small molecule that mimics the action of a complex glycoprotein hormone (LH) acting on a G protein-coupled receptor (GPCR). With the aid of HTS and by applying the logics of medicinal chemistry we indeed succeeded in getting such unique molecules, which are also water soluble, nontoxic, and stabilized against metabolism: in other words excellent candidates for drug development. Apparently, a compact drug-like molecule with a $M_W \sim 500$ can turn the receptor into the same agonistic conformation as the big glycoprotein does with a M_W of about 28000.

It is fascinating that we can put so much information into small molecules following an intriguing game that comprises HTS, molecular modification, structural biology, physicochemical measurements, biological testing, and guided luck. The crucial question is how to utilize new developments in chemical biology to play this game faster, more predictably, and, of course, better!

Better tools should become available to design small molecules that display "specific complementarity" with the molecular target of choice, while, in the mean time, more emphasis should be placed on the enthalpy of binding rather than the entropy (i.e., creating high affinity molecules by increasing their lipophilicity).



Furthermore, it would be advantageous to develop more sensitive technologies to monitor target–drug interactions in vivo and to detect conformational changes in the target leading to either stimulation or inhibition.

Altogether I am convinced that chemical biology will help us to enter fascinating new areas of drug discovery and that we have a bright future ahead of us!

Herbert Waldmann

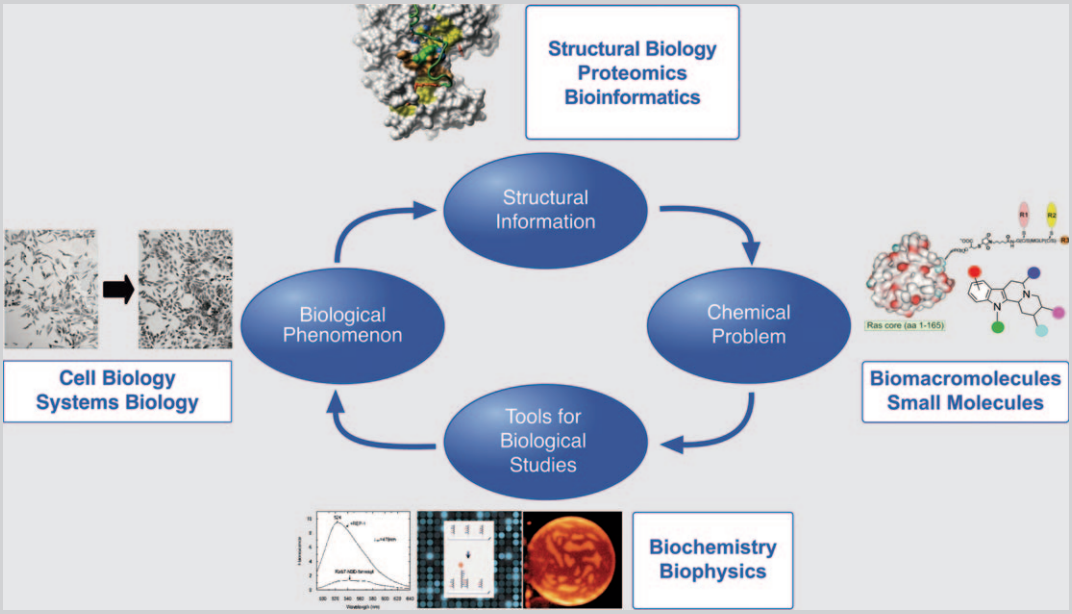
What's in a name?

Chemical biology—as the name suggests–-employs the methods and techniques of chemistry for the study of biological phenomena. As such it is a truly interdisciplinary science bridging chemistry, biology, and biophysics, in an ideal case under the roof of one research laboratory, but more frequently by means of collaboration between research groups with complementary expertise. The chemical biological approach to research in the life- and biosciences harbors the potential to gain insights into biological processes not accessible by means of biology techniques alone, and is, thereby, complementary to established biology methods.

Although seemingly a "no-brainer", appropriate valuation and recognition of the individual contributions of the subdisciplines might be a matter of debate. Thus, chemical biology is neither a kind of "applied chemistry" nor "another branch of biology", it is a truly interdisciplinary science requiring both components. While the rebirth of this science ("biochemistry" once used to carry many of the characteristics of contemporary "chemical biology") in the 1990s was driven to a substantial extent by organic chemists opening up to biology and attempting to address unsolved biological questions by means of organic synthesis as enabling science, in recent years the tendency to define chemical biology primarily as a branch of biology can be observed.

"Chemical biology is neither a kind of "applied chemistry" nor "another branch of biology", it is a truly interdisciplinary science"

Major challenges lie ahead of chemical biology research. The analysis of dynamic biological systems by means of selective, reversible, gradable, and conditional temporary perturbations (which cannot be achieved by means of genetic methods) provides one particularly relevant and timely example, and "systems chemical biology" clearly is on the horizon. To meet this challenge, the development of the chemical compounds needed to perturb dynamic biological systems and the development of the required biology techniques (e.g., new microscopy, targeting and visualization methods) need to go hand-in-hand in a truly trustful manner that fairly recognizes and values the contributions of both disciplines. In this interplay, it is the enabling ability to design, synthesize, characterize, and use tailor-made molecules (be they drug-like small molecules, modified proteins, oligo- or polynucleotides, saccharides, lipids, metal complexes or conjugates thereof or others, be they organic or inorganic) in well-chosen biochemical and biological experiments that defines the key expertise of chemical biology and distinguishes it from biochemistry and molecular biology. If research in chemical biology maintains this interdisciplinary character and balances the chemistry and the biology components well, it will establish itself as a key science in the study of complex and dynamic biological systems and phenomena.



Elizabeth M. Nolan

Christopher T. Walsh

Chemical Biology and Natural Product Discovery

Medicinally active natural products have functional group arrays and scaffold architectures that offer advanced platforms for the optimization of compound activity profiles. Over the past decade, however, natural products have fallen out of favor with medicinal chemists focused on high-throughput screens and syntheses because of their architectural and stereochemical complexity. On the other hand, the knowledge base for natural product biosynthesis has undergone a molecular renaissance, largely driven by the availability of bacterial and fungal genome sequences from producer organisms. One can now predict classes, and even scaffolds, of natural products from microbial genes and set about purposeful reprogramming for structural variations in even the most complex medicinally active scaffolds.

Biosynthetic gene clusters encode proteins necessary for 1) just-in-time synthesis of unusual monomeric building blocks, 2) polymerization of the building blocks on thiotemplated protein assembly lines, and 3) post-assembly tailoring of nascent peptide scaffolds (e.g., acylation, alkylation, glycosylation, and a variety of oxidative transformations). A decade's worth of analyses provide an understanding of the chemical logic and enzymatic machinery for polyketide (PK), nonribosomal peptide (NRP), and hybrid NRP-PK scaffold assembly and maturation.[17,18] Chemical structures of the encoded small molecules can sometimes be directly deduced from the gene sequences. Comparative genomics gives insights into the natural evolution of assembly lines, which occurs by mixing and matching domains and modules to make molecular congeners, and also provides guidance for re-engineering assembly lines and obtaining novel variants of natural products (e.g., in lipopeptide antibiotics such as daptomycin, glycopeptide antibiotics such as dalbavancin, and in polyketide macrolactones such as erythromycin and rapamycin).

"Examining underexplored niches should afford new discoveries."

Structural elucidation of multidomain fatty acid synthase, polyketide synthase, and nonribosomal peptide synthetase assembly line architectures has proceeded in parallel over the past decade.[19–21] These structures are remarkable achievements, providing a basis for understanding catalytic molecular machines and designing re-engineered synthetases. Defining core and optimal domains in PKS and NRPS assembly lines is necessary for designing swapping, deletion and addition strategies for the regioselective rerouting of a specific step in acyl chain elongations.

One surprising insight gleaned from the first complete genome sequences of streptomycete antibiotic producers is that bioinformatics predicts 20–25 biosynthetic gene clusters whereas only 10–15% of the encoded natural products have been detected in standard laboratory cultures. Twenty actinomycete genomes are currently being sequenced at The Broad Institute. If ~85% of these gene clusters are also cryptic under standard laboratory conditions, there will be great incentive to elucidate the regulatory mechanisms that turn-on conditional metabolic pathways, and to search for new antibiotics in familiar producers.

Novel approaches to screening for bioactive natural products will provide the means to discover unknown metabolites and increase structural an functional diversity. Examining underexplored niches should afford new discoveries. Traditionally, terrestrial actinomycetes have been prime targets, but two marine actinomycetes (*Salinospora*) genomes present a plethora of biosynthetic clusters and novel genetically encoded small molecules that include salinosporamide A (Figure 4).[22] Furthermore, the antibiotic abyssomicin (Figure 4), which exhibits an unusual molecular scaffold and blocks the first enzyme in the

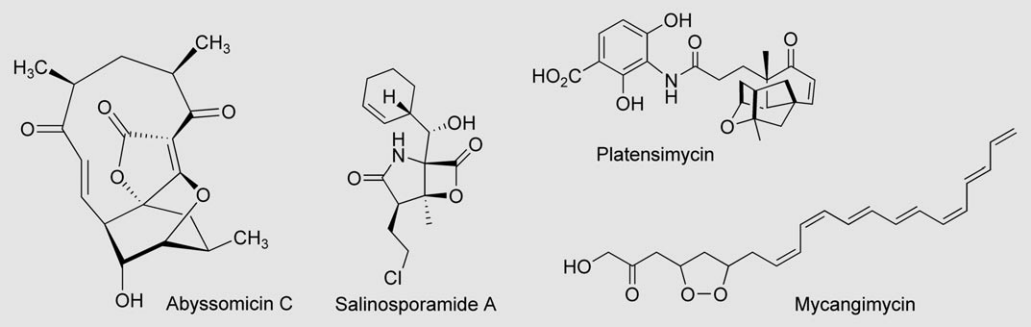


Figure 4. Some natural products isolated from acitnomycetes and streptomycetes.

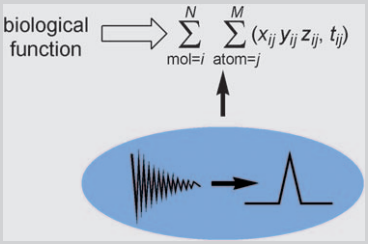
bacterial folate biosynthetic pathway, was isolated from an actinomycete recovered from a bottom-sediment sample collected from the Japanese Sea at a depth of about 275 m.[23] Deciphering a fascinating interplay of biological mutualisms between a beetle, a fungus, and a symbiotic actinomycete led to the discovery of a novel molecular scaffold in mycangimycin (Figure 4), produced by the actinomycete to selectively kill the harmful predatory fungus.[24]

Smart screens will likely turn up new molecular scaffolds. A recent notable effort was the discovery by the Merck infectious disease group of the antibiotic platensimycin (Figure 4).[9] They screened 250 000 natural product extracts against FabF, the chain-elongation enzyme Fin bacterial fatty acid biosynthesis, under conditions where an antisense RNA against the *fabF* target was included to make that enzymatic chain elongation step rate-determining. Both platensimycin and abyssimycin would be good starting points for semisynthetic elaboration for new classes of antibiotics.

It has been estimated that 90% of the actinomycetes-produced antibiotics remain undiscovered, and the metabolites most abundant in soil samples have already been found because of 50 years of industrial screening. One might have to sift through 10^7 bacterial strains to find the next blockbuster antibiotic scaffold from Nature, necessitating a contemporary reappraisal of microbial screen technology for high-throughput, miniaturization, and automatic dereplication of known molecules. Chemical biologists have many new medicinally active molecular scaffolds to discover in Nature, but will have to be sophisticated in both chemical and biological thinking to uncover them.

Harald Schwalbe

The Role of NMR Spectroscopy in Chemical Biology



Through the admittedly biased eyes of an NMR researcher, the research field of chemical biology seems to me most tightly connected to NMR spectroscopy when it comes to understanding how novel molecules, designed and skilfully synthesized, maintain, modulate, regulate, or interfere with cellular function or even change the function, morphology, and differentiation status of entire cells.

In other words, the structure–function relationship that leads to the understanding and prediction of cellular function is at the heart of chemical biology. Molecules exert function through their chemical properties, determined by the dynamic spatial arrangement of their atoms, their interactions with other molecules, and their localization within the cell. And nuclear magnetic resonance (NMR) spectroscopy is the most powerful method for investigating all aspects of the structure–function relationship of natural and designed novel molecules.

NMR spectroscopy can derive information about the conformational dynamics and interactions of molecules both in solution and in the solid state. Thermodynamic and kinetic information about the interaction of molecules can be derived on a per-atom basis, that is, the contributions of individual atomic groups to the enthalpy and entropy of an interaction can at least in part be delineated.

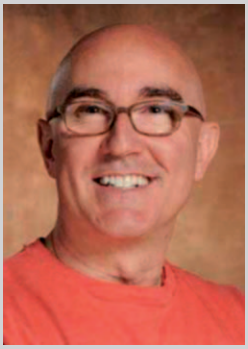
Liquid-state NMR spectroscopy, in which molecules are dissolved in a variety of different solvents and studied at ambient temperatures, can be applied to native states of proteins, but also to intrinsically unstructured proteins, as well as to proteins in their unfolded state and under nonphysiological conditions, including in organic solvents. NMR spectroscopy can detect the conformational dynamics, also referred to as conformational switching, of RNA molecules that coexist in different stable states.

"The structure–function relationship that leads to the understanding and prediction of cellular function is at the heart of chemical biology."

Solid-state NMR spectroscopy investigates molecules as powders or crystals and has become a powerful tool for the investigation of membrane proteins and their complexes with small peptide agonists, but protein amyloid fibers and polymers can also be investigated.

The technique can be applied to systems under defined conditions (in vitro), but proteins can also be investigated within cells (in vivo). NMR is also used for metabonomic analysis, which focuses on the identification of small molecules in biofluids and tissues, tracking changes that occur upon variation of the local environment or that are induced by specific knock-out variants of an organism. A chemical biologist is not only interested in defining the properties of a protein–ligand interaction, but also, how an entire cell or organism responds to external triggers. In this context, NMR has been applied to characterize the metabonome of the organism *C. elegans*. In combination with other techniques, especially mass spectrometry, NMR is a very valuable tool for this task.

NMR spectroscopy and chemical biology are now also bridging to functional magnetic resonance imaging (fMRI), where new molecules are being synthesized and applied to increase specific targeting to cells or beyond in imaging.



Stuart L. Schreiber

The Gap between Scientists' Aspirations and Society's Expectations

Many chemists and chemical biologists, as well as other life scientists, are interested in understanding human disease. But society is primarily interested in science eliminating suffering from disease. I suspect that the gap between these two might be much larger than is generally perceived. Human genetics, cancer genomics and developmental studies, for example, are identifying the causal genes in many diseases. Transcription factors, oncogenes such as K-RAS, and regulatory RNAs are examples—but these are all considered difficult if not impossible or "undruggable" targets, having little similarity to the ~500[30] targets of current pharmacopeia (Figure 1). In fact, most of the genes being identified as the root causes of diseases, as well as the processes that the genes suggest as treatments for disease—such as reprogramming in vivo or disrupting protein/protein or protein/DNA interactions—are in this category.

I believe that developing general processes that yield gold-standard small-molecule probes against difficult/impossible or undruggable targets is one way for academic research to bridge this gap (Figure 2). Probes are NOT drugs[31]—they are not exposed to human livers where they might be converted to toxic metabolites or hearts where they might inhibit an essential ion channel. Nevertheless, the concept of "undruggable" does not refer primarily to the challenge of overcoming pharmacological barriers; rather, it refers to the perception of extremely difficult molecular challenges being asked of small molecules. If a systematic process could be developed that yields small-molecule probes of difficult/impossible or undruggable targets and processes, our perception of what can be accomplished in drug discovery would change. Much hard work would still lie ahead in the pharmacological optimization process, but there would be greatly increased motivation to undertake this activity, knowing that the impossible molecular challenge is actually doable. Academic research, especially in chemistry and chemical biology, does not have to yield drugs in order to have a profound impact on drug discovery. Academic research that aims to achieve drug discovery by mimicking the current processes used by the pharmaceutical industry might have a greater impact if it were redirected to developing methods that change our view of what is doable.

"Academic research... might have a greater impact if it were redirected to developing methods that change our view of what is doable."

Figure 1. Causal disease genes tend to be on the long list of the impossible or undruggable (e.g., transcription factors, oncogenes such as K-RAS oncogene, regulatory RNAs), as are the processes that the disease genes suggest may be used to treat disease, such as reprogramming in vivo or disrupting protein–protein or protein–DNA interactions. Scientists and lay members of society have many reasons to be excited about the recent discoveries of genes that cause human disease. But are these genes on the path to the discovery of the revolutionary new therapeutics we all seek? Does the current drug-discovery process have a mechanism in place to exploit these new findings? Perhaps not. For example, it has been noted that current drugs target no more than hundreds of human genes, yet there are ~20000 human genes and >100000 individual functions of the products of these genes. The vast majority of the latter and, indeed, of the newly identified human disease genes, fall into the "impossible or undruggable" category. There is a gap between scientists' aspirations ("I want to illuminate the basis of a human disease") and society's expectations ("Please help eliminate our suffering from disease").

A systematic process for discovering small-molecule probes

By integrating methods from organic synthesis, chemical biology and genome biology, my colleagues and I at the Broad Institute are attempting to develop a systematic approach to the discovery of small-molecule probes of impossible or undruggable targets and processes.

Some of the elements of this approach are:

- A commitment to achieving a complete synthesis of a transformative small-molecule screening collection–––one whose members anticipate challenges, and therefore increase the probability of success, in the discovery of initial probes, their optimization, and the manufacture of the optimized variants.
- The development of screens for small molecules that achieve "the impossible" in physiologically relevant conditions; for example, niche-based screening using primary human cells and tissues that pre-

serves their essential physiological properties. Examples include the maintenance of 1) stemness by using various types of primary stem cells and 2) glucose-sensitive insulin secretion by using primary human pancreatic beta cells.

- The development of quantitative and high-throughput methods to identify the complete list of macromolecules with which biologically active small molecules interact in cells.
- The use of open data sharing and the development of powerful analytical tools that enable knowledge to be gained from small-molecule science.

This process has been in development over a ten-year period and has accelerated in the past several years. Already, it has contributed to the discovery of many small-molecule probes for extremely challenging targets—such as transcription factors, oncogenes, protein–protein and protein–DNA interactions, and chromatin—and processes—such as reprogramming in vivo. We aim to shine a bright light on human biology and to provide powerful new means for creating novel therapeutics that would be impossible to uncover otherwise.

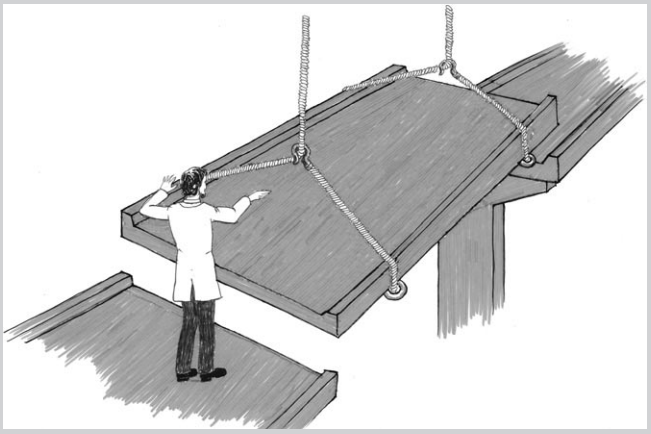
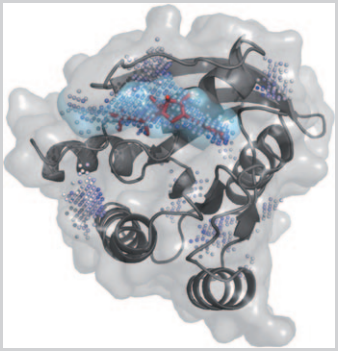


Figure 2. Developing gold-standard "small-molecule probes" against difficult/impossible or undruggable targets and processes is one way the public sector can bridge this gap. Small-molecule probes of targets/processes currently viewed as inaccessible to small-molecule modulation are not drugs, but they have the potential to change perceptions of what is possible in drug discovery.

Gisbert Schneider



During the past decade we have witnessed the birth, rise, and success of various "virtual screening" techniques that assist in the identification of bioactive agents and modeling of biological structures. Chemical biology has benefited from these technological advances in several ways. For example, we are now able to develop structural models of receptor–ligand interactions, design focused compound collections for bioactivity screening, and explore the behavior of macromolecular structures and complexes in much more detail. While, for a long time, the interaction of one ligand with one target has been in the focus of chemical biology research, the study of many-to-many interactions will become a central topic for chemical biologists for the next decade. For a deeper conceptual understanding of cellular-signaling processes, metabolic pathways and their regulation, and self-organizing macromolecular assembly—to name just some of the great challenges in chemical biology—it will be essential to think in terms of networks and interaction patterns. Technological advances in both experimental design and bioinformatics will undoubtedly allow innovative studies to be performed, but it is equally important to *think* outside the box in terms of higher-order interactions between molecular and cellular entities. This also means that it is no longer sufficient to analyze receptors, ligands, and reactions in a single contextual view. Chemical biology is carried out in vivo, in vitro, and in machina by cellular, biochemical, and computational experiments, and *ChemBioChem* provides a most suited forum for this exciting field of transdisciplinary research.

"The study of many-to-many interactions will become a central topic for chemical biologists"

Karl-Heinz Altmann

A Formative Decade for Chemical Biology

Chemical biology is the science of… well, chemical biology. Although chemical biology as a scientific approach has lived through a decade of expansive growth (not least reflected in the number of journals that now carry the term "Chemical Biology" in their title) and scientific achievements, there is no unifying definition of what it actually entails. And come to think of it, this may be rather natural and certainly not a bad thing, as it is just this lack of clear separation at the borderline of chemistry and biology that is at the heart of chemical biology's conceptual appeal and that has turned it into the powerful integrative force for research in the life sciences that we know today. According to Herbert Waldmann, who also contributes to this section, "Chemical Biology may be defined as the development of chemistry techniques for the study of biological phenomena",[26] but other, and broader, definitions have been put forward.[27] However, no matter which definition one prefers, it is indisputable that the core element of chemical biology as a branch of science is the use of chemistry (and chemicals) to interrogate, modify, and manipulate biological systems at the cellular and organismal level in a highly controlled manner.

"This lack of clear separation at the borderline of chemistry and biology that is at the heart of chemical biology's conceptual appeal and that has turned it into the powerful integrative force for research in the life sciences. Chemical biology cannot and should not be reduced to small-molecule-based "chemical genetics"."

Chemical intervention often takes the form of exposure of a biological system to a small molecule and analyzing the former's response, and it has been rightly argued that the specific modulation of protein function by small molecules offers a level of spatiotemporal resolution that cannot be achieved with other approaches, such as siRNA-based protein knockdown.[28] Nevertheless, chemical biology cannot and should not be reduced to small-molecule-based "chemical genetics". Many other chemistry-based approaches have been exploited in the quest for a molecular understanding of biological systems and attempts to redirect or even reinvent their functions. This includes the direct chemical modification of cellular components (e.g., cell-surface proteins), monitoring the effects of structurally modified biomacromolecules or of unnatural lipids on biological systems, or the use of photoactivatable ligands to study receptor function, to name just a few out of an abundant arsenal of existing approaches, and many more will undoubtedly be invented in the future.

It is the application of exogenous chemistry, the manipulatory aspect of the approach, that makes chemical biology go beyond classical biochemistry and biological chemistry, which have traditionally focused on the understanding of endogenous chemical processes in living systems (and very successfully so). One should not, however, establish new boundaries and in my view chemical biology should be all encompassing and include many of those scientific questions and approaches that have been associated with biochemistry or biological chemistry in the past. (If nothing else, this view is certainly in line with the scope of the research that is published in virtually all "chemical biology" journals.) The success of modern chemical biology research (although not generally appreciated, "chemical biology", if only unwittingly, has been practiced since the second half of the 18th century, as described in a very insightful recent commentary by Morrison and Weiss[29]) is inextricably linked to technological advances that have occurred since the mid-1990s in areas such as assay development, screening technology (including organismal screens in *C. elegans*, *Drosophila*, or zebrafish), array technology, imaging technology, protein-structure determination, mass-spectrometric analysis, or bioinformatics, but also in synthesis methodology and synthesis technology (e.g., combinatorial synthesis or automated parallel synthesis). Before this background, what are the most important discoveries that have been made in chemical biology research over the last decade? I think it would be pretentious to even try to answer this question, given the breadth of research taking place under the umbrella of chemical biology and the many stunning achievements that have been reported. A few things that come to (my personal, biased) mind are the multitude of small molecules that chemical biology has delivered with the ability to specifically modulate cellular or even organ function. Such molecules have provided many clues on the molecular mechanisms that underlie, for example, cell division or the cellular immune response, and this has also led to the delineation of new therapeutic strategies for the control of cancer growth, transplant rejection, or the treatment of autoimmune diseases. There is the chemistry that has allowed the controlled modification of cell surfaces with reactive groups, thus enabling the investigation of receptor function and cell–cell recognition processes. There are activity-based probes that have enabled functional proteomics profiling, and there is the invention of new nonradioactive molecular-imaging probes, including photosensitive receptor ligands that allow light-induced changes in receptor activation states. And there is much more.

Where do we go from here? What is the future of chemical biology, what should be its objectives, what should one try to accomplish? While it is difficult to formulate an all-encompassing answer to these questions, one of the ultimate defining goals of a future vision for chemical biology should be the identification of highly specific low-molecular-weight modulators

of each individual protein function in the cell, which would be invaluable tools for basic cellular as well as applied biomedical research. This objective was laid down previously by Stuart Schreiber, one of the world's foremost chemical biologists, in a 2005 commentary as one of four grand challenges that he believes should be addressed by future chemical biology (the others being the illumination of the origins of life and the creation of alternative life forms, the completion of the inventory of all naturally occurring small molecules, and the creation of an effective bridge between basic and clinical research by systematically linking genetic variation in cells to the ability of small molecules to effect phenotypic changes in the cell).[28] However, chemical biology should not stop here, but continue to investigate the biological impact of structurally modified versions of all types of cellular components, it should continue to develop and refine chemistry for the direct covalent modification of cells, and it should expand the scope of small-molecule-based functional modulation from proteins to nucleic acids. Last but not least, chemical biology should further strengthen its link with therapy-directed biomedical research, not only at the level of target identification, but also in areas such as medicinal chemistry, which would appear most natural, polypharmacology, and biomarker and diagnostics development.

Given the vitality of the field and the multitude of challenges ahead, I have no doubt that chemical biology will continue to deliver ground-breaking new findings during the next decade that will have a huge impact on our molecular understanding of biological systems and advance biomedical research to new frontiers. And *ChemBioChem* will continue to be one of the leading platforms for the publication of these results.

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