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GUEST EDITORIAL
A Decade of Bioorthogonal Chemistry

Inventing new chemical reactions is a long-standing passion of chemists, as reflected in the ever-popular field of reaction methodology. Historically, the environment in which newly crafted transformations would be performed imposed minimal limitations on reaction design; after all, solvents can be screened to find an optimal choice, temperature can be modulated, air and moisture can be excluded, catalysts can be added, competing functional groups can be protected, and byproducts can be siphoned away. Such flexibility permitted the use of diverse reagents and conditions, culminating in a vast compendium of synthetic transformations that has been wielded to produce myriad complex chemical structures. The target-driven synthetic chemist now enjoys an impressive reaction toolkit. But what if the challenge were inverted, wherein the target structure was relatively simple but the environment in which the necessary reactions must proceed was so chemically complex and uncontrollable that no two functional groups could combine reliably and selectively under such conditions?

Bioorthogonal chemistry, the topic of this special issue of *Accounts of Chemical Research*, addresses precisely this challenge, with reaction environments of interest ranging in complexity from aqueous solutions of biomolecules to live animals. Dating back just over a decade, the field was launched alongside the growing realization that the molecular details of biological processes can be most accurately understood by probing biomolecules within their native habitats, that is, in cells, or even better, live organisms. To interrogate biomolecules in such complex settings requires the means to selectively modify them with imaging probes, affinity reagents, or moieties that perturb function. In earlier decades, biology-derived technologies such as monoclonal antibodies and genetic fluorescent protein fusions enabled great strides toward elucidating the roles of specific proteins in dynamic cellular processes. Though their target selectivity is exquisite, even within environs as complex as laboratory animals, these biological tools were not amenable to every protein type; the large size of fluorescent proteins imposes too significant a structural perturbation for many targets, and antibodies are excluded from the intracellular space. Nor were antibodies and fluorescent protein fusions readily translated to nonprotein biomolecules. Glycans, lipids, nucleic acids, and metabolites, either alone or as posttranslational modifications, were largely refractory to *in vivo* monitoring.

With the limitations of conventional approaches to probing *in vivo* biochemistry becoming ever more apparent, chemists started contemplating whether target biomolecules could be rendered chemically unique within biological settings such that probe molecules might be delivered by the selective formation of covalent bonds. This was a maverick notion to be sure, given the obstacles that would be heaped upon the targeting reaction. To start with, the participating functional groups could not engage endogenous biological functionalities in competing side reactions. Moreover, the reaction would have to proceed rapidly in aqueous media at biocompatible pH and temperature, and for applications involving live cells or organisms, the reagents should be nontoxic.

Only 12 years ago, perusal of the synthetic chemist's compendium would yield not a single reaction that met all of these criteria, now recognized as defining attributes of bioorthogonality. The closest contenders were condensation reactions of aldehydes or ketones with hydrazides, hydrazines, and aminooxy compounds, α-effect nucleophiles that form relatively stable Schiff bases under mild aqueous conditions. In the late 1900s, these reactions found use in many bioconjugation applications, such as selective chemical modification of proteins, lipids, and glycans, and even cell-surface engineering. But in more complex biological settings wherein metabolites bearing aldehyde and ketone groups naturally reside, these early champions failed to achieve the highest level of bioorthogonality.

Toward the end of the second millennium, attention shifted to the invention of bioorthogonal reactions involving functionalities that are not found in Nature. In the vanguard of this movement were Roger Tsien and coworkers, who reported on the stunningly selective covalent reaction of synthetic bisarsenical fluorophores with proteins bearing a genetically introduced tetracysteine motif in live cells.[1] The bioorthogonality of the latter reaction

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partner derived from a unique combination of natural amino acids that was virtually absent from mammalian proteomes. Tsien's chemistry has since been harnessed for applications far beyond protein fluorescence imaging, as underscored by Schepartz and co-workers' Account in this issue describing the use of bisarsenicals for probing protein structure and function.

Though the bisarsenical/tetracysteine reaction predated the first use of the term "bioorthogonal" (see Sletten and Bertozzi, this issue), it solidified chemists' expectation that through careful design and tuning of reaction partners, the formidable mandate of selectivity imposed by biological systems could eventually be achieved. Thereafter, bioorthogonal reactions among entirely abiotic functional groups became the Holy Grail sought after by practitioners of this burgeoning area of chemical biology. The first success stories, and still most widely influential today, were bioorthogonal reactions of the azide, a rather miraculous functional group that is small and biostable and can behave either as a soft electrophile or as a 1,3-dipole, two modes of reactivity that are rare in Nature. The azide debuted as a partner in the Staudinger ligation with specially tailored phosphine reagents; then its use as a bioorthogonal functional group expanded with the advent of the Cu-catalyzed azide–alkyne cycloaddition, a paragon of click chemistry, and the more biofriendly Cu-free cycloaddition with strained cycloalkynes. Notably, the requirements of bioorthogonal chemistry relate closely to the concept of click chemistry (i.e., high selectivity and functional group tolerance) and any reaction that meets the demands of the former probably would classify as an example of the latter.

These first bioorthogonal chemistries have been transformative for chemical biologists seeking new ways to interrogate biomolecules *in vitro* and *in vivo*. Several Accounts in this special issue underscore the breadth of biological inquiry enabled by bioorthogonal reactions. Creative metabolic and genetic engineering strategies have made it possible to incorporate azides, alkynes, and many other abiotic functional groups into proteins, glycans, nucleic acids, lipids, metabolites, and various posttranslational modifications in cultured cells as well as live organisms. Once situated in a target molecule, the bioorthogonal functional group serves as a chemical reporter to which probes of all sorts can be delivered. As Tirrell and co-workers review in their Account, *de novo* protein synthesis can be probed using this chemical approach, and similarly, we (Sletten and Bertozzi) highlight applications of metabolic glycan labeling with azidosugars as a means to visualize spatiotemporal changes in cell surface glycosylation. As well, lipids on their own (Best and co-workers) and as posttranslational modifications (Hang and co-workers) have been probed in cells with bioorthogonal chemistry, and as He and co-workers report, the new chemistry can be used to detect putative epigenetic modifications of DNA. And it is not just the molecules themselves that can be revealed using bioorthogonal labeling methods. The activities of enzymes, as reflected in covalent active site labeling with bioorthogonal probes, can now be profiled in living systems (see Overkleeft and co-workers).

In addition to applications in basic research, bioorthogonal chemistry has proven to be a powerful new tool for biotechnology. Bioorthogonal reactions have enabled the production of site-specifically modified recombinant proteins (Davis and co-workers, Chen and co-workers), the assembly of semisynthetic proteins (McGrath and Raines), and the immobilization of proteins on surfaces for microarray applications (Waldmann and co-workers). In addition to their potential use as biotherapeutics, proteins modified with high precision using bioorthogonal reactions can form nanoassemblies with applications ranging from clinical diagnostics to energy storage materials, as summarized by Witus and Francis.

Another theme to emerge over the past decade is that some natural biomolecules are endowed with, or can be engineered to possess "latent bioorthogonality". That is, akin to Tsien's tetracysteine motif, biomolecules comprising all natural components can deliver bioorthogonality through unique combinations of structural elements. Cornish and co-workers provide examples wherein bioorthogonality of a protein target is engendered by fusing to it a noncovalent ligand-binding domain in proximity to a reactive nucleophilic side chain. This unique arrangement enables the ligand-mediated delivery of imaging probes adorned with reactive electrophiles to the engineered protein. Chang and co-workers illustrate how a bioorthogonal mode of reactivity of hydrogen peroxide, its oxidation of boronic acids, can be exploited to develop sensors for this important signaling molecule.

The above examples testify to the power of bioorthogonal chemistry in advancing biological research, but the field is, at its core, driven by new reaction methodology. Thus, of critical importance is the development of new bioorthogonal reactions, ideally among functional groups that are orthogonal to the current list. Some exciting developments on this front are presented in this issue, including new cycloaddition reactions (van Delft and co-workers, Devaraj

and Weissleder) and bioorthogonal reactions initiated with light (Lim and Lin). These recent achievements, together with earlier examples, underscore the importance of mechanistic thinking in designing reactions with the heightened selectivity required for performance in biological systems. In this regard, bioorthogonal chemistry offers a rich training ground for young chemists seeking to learn the fundamentals of organic reaction mechanisms while also pursuing applications in biology and beyond. A wealth of challenges and opportunities lie ahead, and the field is wide open for new innovations. Above all, we hope this special issue intrigues and inspires the rising generation of chemists and biologists.

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REFERENCES

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