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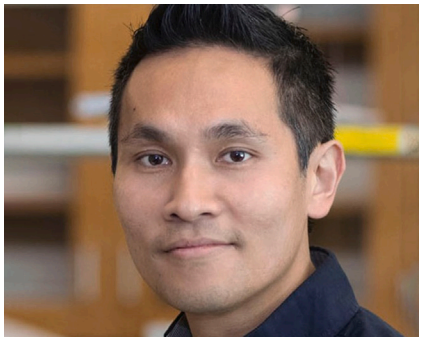
Cell Chemical Biology

Voices

Voices: Challenges and opportunities for bioorthogonal chemistry

Bioorthogonal chemistry was deservedly recognized with the 2022 Nobel Prize in Chemistry, having transformed the way chemists and biologists interrogate biological systems in the past twenty years. This Voices piece asks researchers from a range of backgrounds: what are some major challenges and opportunities facing the field in coming years?



Ku-Lung (Ken) Hsu
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A single-cell perspective on the potential of bioorthogonal chemistry

The chemical tools available to scientists today provide unprecedented opportunities for exploring chemical reactivity in diverse biological systems ranging from proteins in a test tube to living cells and organisms. Many of these methods for visualization, quantification, and chemical modulation are possible because of bioorthogonal chemistry and its enabling features. The ability to snap together azide- and alkyne-bearing compounds using ''click chemistry,'' for example, has provided chemical biologists and non-specialists the ability to conjugate, with relatively high efficiency, fluorophores, biotin, and other functional handles to a wide range of biological macromolecules including proteins, lipids, and glycans. The principles of bioorthogonal chemistry are simple and efficient, which has contributed to its widespread adoption in basic research and drug discovery.

An area of future opportunity is the application of bioorthogonal chemistry in the field of single-cell proteomics. While single-cell RNA sequencing technology is experiencing rapid advancement, the proteomic counterpart could benefit greatly from innovation to enhance signal-to-noise because unlike mRNA, proteins cannot be amplified. Bioorthogonal chemistry can offer creative solutions for boosting signal by, for example, the installment of functional and amplifiable tags into cells with high molecular precision. Additional opportunities include reactions with mass spectrometry-compatible bioorthogonal tags to probe cellular response and regulation at the single-cell scale. The successful implementation of bioorthogonal chemistry for single-cell proteomics could greatly impact our basic understanding of cell heterogeneity and provide a tractable path for translating these insights into therapeutic discovery.

Ben Schumann
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Chemical Glycobiology Laboratory, The Francis Crick Institute

Chemical biology is coming of age

The award of major prizes to leaders in the field is moving chemical biology into the spotlight it deserves, with notable consequences. If the focus thus far has been on the development of new reactions and modalities, we are seeing increasing and concerted uptake by biomedicine. The first applications of bioorthogonal chemistry in the clinic are a starting point and a prelude to the true potential of interdisciplinarity. To further advance the application of our tools, however, we must further drive the development and commercial availability of easy-to-use but, importantly, proven reagents and kits.

Personally, I am excited about the abilities that chemical tools offer to explore the integration of biomolecules in their immediate environment. Exploiting *proximity*, be it as degraders, molecular glues, sensors, or biotinylation techniques, has started to give us a glimpse beyond conventional steady-state concentration effects. Studying transient interactions, unstable intermediates and molecular conglomerates of large biological significance was intractable before but is now within reach. It is particularly gratifying to see the spotlight moving (back) to enzymology—there are so many

things we do not know about how enzymes collaborate, including in my favorite compartment, the secretory pathway!

Ellen Sletten
Department of Chemistry and Biochemistry, University of California, Los Angeles

The expanding applications of bioorthogonal

The word bioorthogonal, first appearing in the literature 20 years ago to describe the use of the azide to probe glycosylation (https://www.pnas.org/doi/10.1073/pnas.1632821100), has evolved into a concept that transcends many fields. Organic chemists continue to be intrigued with the classical application of bioorthogonal chemistry: fast covalent reactions that can occur within, yet do not interfere with, living systems. The past two decades have resulted in bioorthogonal chemical reactions with enhanced reaction kinetics, photo control, fluorogenic detection, and engineered release mechanisms. Covalent bioorthogonal chemistries are critical tools in many standard chemical biology methods and are particularly transformative for studying biomolecules that are not directly genetically encoded, as evidenced by their roots in glycosylation and growing applications toward studying lipidation. Recent implementation of bioorthogonal chemistries into high-throughput screens showcases the robustness of these reactions. Most excitingly, covalent bioorthogonal reactions have made it into the humans, where a click-and-release approach coined "click activated prodrugs against cancer (CAPAC^{TM})" targets chemotherapeutics to solid tumor sites (https://pubmed.ncbi.nlm.nih.gov/37034617/). There is no doubt that covalent bioorthogonal chemistry has transfigured how chemistry can impact biology and medicine; however, bioorthogonal has come to mean even more than just reliable, fast, covalent reactions. The concept of bioorthogonality is now considered when developing any method for performing, controlling, or detecting biological systems using unique chemical functionality. In light of the recent Nobel Prize recognition of the development of these chemistries, Kaitlin Hartung and I have highlighted the ever-expanding applications of bioorthogonal chemistry in a recent perspective. I look forward to watching the continuing evolution of bioorthogonal strategies.

Ekaterina Vinogradova
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Bioorthogonal chemistry enables global proteomic studies

The merger of chemical proteomic approaches with bioorthogonal chemistry recently enabled many exciting applications ranging from target identification of bioactive small molecules (metabolites, natural products, phenotypic screening hits, and drug leads), to classification of proteins based on their activity, and annotation of protein post-translational modifications (PTMs), including glycosylation, lipidation, and ADP-ribosylation.

Ongoing and future efforts in the field are aimed at the development of new stable and selective reagents for rapid bioorthogonal transformations *in vivo* and expansion of the toolbox of reversible chemistries for dynamic real-time chemical reporter development. At the interface with chemical proteomics, researchers are striving to develop new chemical probes for challenging protein targets and PTMs as well as cell biology tools to decipher functional consequences of chemically targeting specific sites on proteins. Identification of "silent" ligands that do not interfere with protein function and development of chemical probes with built-in mechanism for the release of the main binding element to produce minimalistic bioorthogonal handles is another intriguing direction that is currently gaining traction. Further advances of mass-spectrometry analysis platforms and development of AI approaches for more in-depth data analysis should also have a profound impact on the contribution of bioorthogonal chemistry and global proteomic studies to our understanding of human pathophysiology. Finally, strengthening collaborations between practitioners in chemistry, biology, and data science should enable further advances in the field with a growing appreciation from multiple research communities for the power of precise chemical tools being essential to solving important and unmet challenges in biology and biomedical sciences.

Peng Zou
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Bioorthogonal chemical ligation has created a panel of hybrid biosensors for monitoring cellular activities

Our understanding of the molecular mechanisms underlying cellular physiology depends critically on our capability of monitoring biochemical and biophysical signaling events in live cells and in real time. Fluorescent indicators are powerful tools for achieving such tasks with high spatiotemporal resolution, high molecular specificity, and low invasiveness. Since the discovery and development of fluorescent proteins, an array of protein-based indicators has been developed to optically record dynamic changes in cellular activities, which is often achieved through analyte-induced conformational changes that modulate the fluorescent protein emission. The genetic targetability of protein-based indicators has allowed cell type-specific measurement within complex tissue environments or even subcellular-specific recording in organelles. However, the photophysical properties of fluorescent proteins, such as the molecular brightness and photostability, are typically much worse than synthetic fluorophores.

To overcome these limitations, recent developments of bioorthogonal chemistry have enabled the creation of protein-small molecule hybrid indicators. For example, a bioorthogonal functional handle such as transcyclooctene is enzymatically installed to a specific site in the analyte-sensing protein scaffold, which is followed by reacting with a tetrazine-conjugated fluorophore. The resulting hybrid indicator combines the advantages of genetically encoded protein scaffold and the superior photophysical properties of synthetic dyes. In challenging applications, such as photon-starved kilo-hertz voltage imaging, hybrid indicators offer substantially higher signal-to-noise ratio than protein-based biosensors. Future improvement of hybrid indicators could benefit greatly from development of bioorthogonal chemistry that is compatible with live animals, which could facilitate *in vivo* imaging of cellular activities.

DECLARATION OF INTERESTS

Ku-Lung Hsu is a founder and scientific advisory board member of Hyku Biosciences. Ellen Sletten is a co-author on three patents related to bioorthogonal chemistry.