CLINICAL IMPLICATIONS OF BASIC RESEARCH

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Click Here for Better Chemistry

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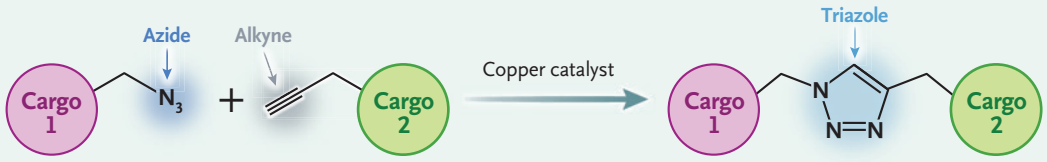
The drive to better understand living systems and the pursuit of more effective clinical tools have fueled a surge in research at the intersection of chemistry, biology, and medicine. A common motif in this work has been the modification of biologically active molecules to create probes for the interrogation of biologic systems and to produce diagnostics and therapeutics for the clinic. Yet the chemical manipulation of biomolecules — whether small molecules, nucleic acids, carbohydrates, lipids, proteins, or antibodies — is complicated by three challenges. First, nature uses only a handful of functional groups (e.g., amines and carboxylic acids), each with its own demands when it comes to reactivity. Second, a biomolecule can often contain multiple copies of a given functional group, and to make matters worse, some of these can be situated in the area responsible for its biologic activity. As a result, modifying a biomolecule at a single site without perturbing its function can be a daunting task. Third, many biomolecules are highly sensitive to temperature, solvent, and pH and can therefore be manipulated only under biocompatible conditions. Historically, these intrinsic obstacles have combined to make the synthesis of effective biomolecular tools arduous at best and impossible at worst.

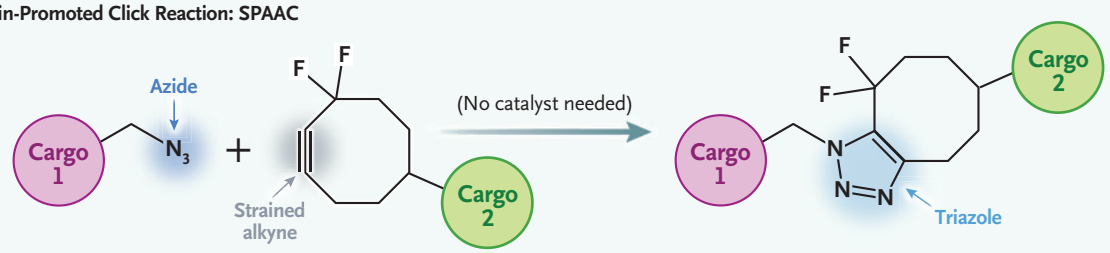
The 2022 Nobel Prize in Chemistry was awarded to Drs. Carolyn Bertozzi, Morten Meldal, and K. Barry Sharpless for their creation of a suite of new chemical transformations — "click chemistry" — that has revolutionized research at the interface of chemistry and biology. Click chemical reactions are fast, efficient, modular, clean, selective, and compatible with aqueous conditions. The name, coined by Sharpless, calls to mind molecular puzzle pieces that connect to one another (and only one another) simply and easily. Meldal and Sharpless independently and concomitantly developed what is known today as the canonical click reaction. In this transformation, an azide and alkyne undergo a cycloaddition reaction with the help of a copper catalyst to form a triazole ring, thereby linking together any cargoes attached to the members of this molecular duo (Fig. 1A).[1,2] One can easily imagine how this copper-catalyzed azide–alkyne cycloaddition (CuAAC) could be used to circumvent the aforementioned challenges: a biomolecule containing a single azide could easily be "clicked" to an alkyne-bearing partner under mild, aqueous conditions without the formation of unwanted byproducts. Beyond chemical biology, the CuAAC ligation has proved indispensable in fields ranging from drug development and materials chemistry to biomedical engineering and nanoscience.

Upon its discovery, the CuAAC reaction was an instant hit, but some conditions proved unsuitable for the ligation. In complex biologic systems, such as living organisms, it is just too difficult to get the azide, alkyne, and catalyst together in one place. A simpler, two-component version of the reaction that was developed by Sharpless and Meldal was needed. This is where Bertozzi's work comes in. Her variation on the cycloaddition still uses an azide but pairs it with a cyclic alkyne that is spring-loaded with what chemists call "ring strain" (Fig. 1B).[3] The release of this ring strain when the alkyne reacts with the azide gives the cycloaddition the push it needs to proceed without a copper catalyst. This transformation was dubbed the strain-promoted azide–alkyne cycloaddition (SPAAC). As a bioorthogonal click reaction — a transformation that can occur in biologic systems without perturbing them — the SPAAC reaction has enabled the use of click chemistry in previously inaccessible biologic environments. This was elegantly demon-

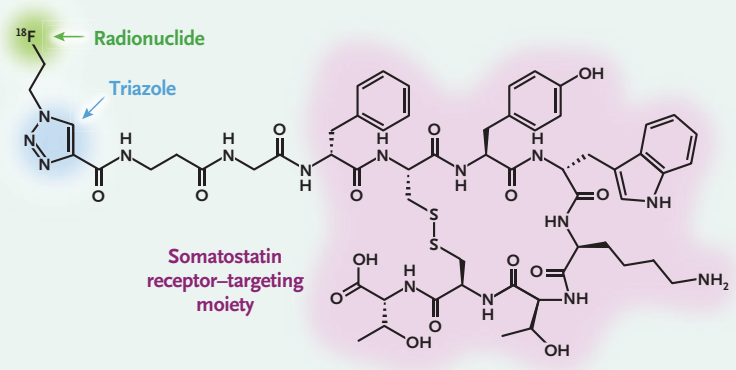
A Copper-Catalyzed Click Reaction: CuAAC

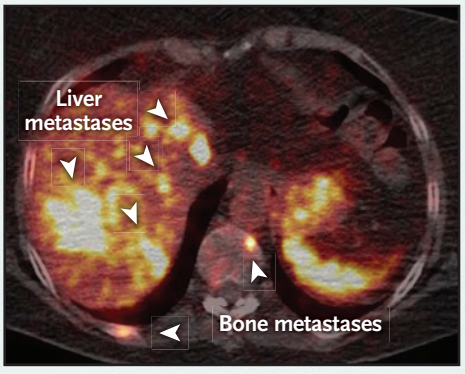


B Strain-Promoted Click Reaction: SPAAC



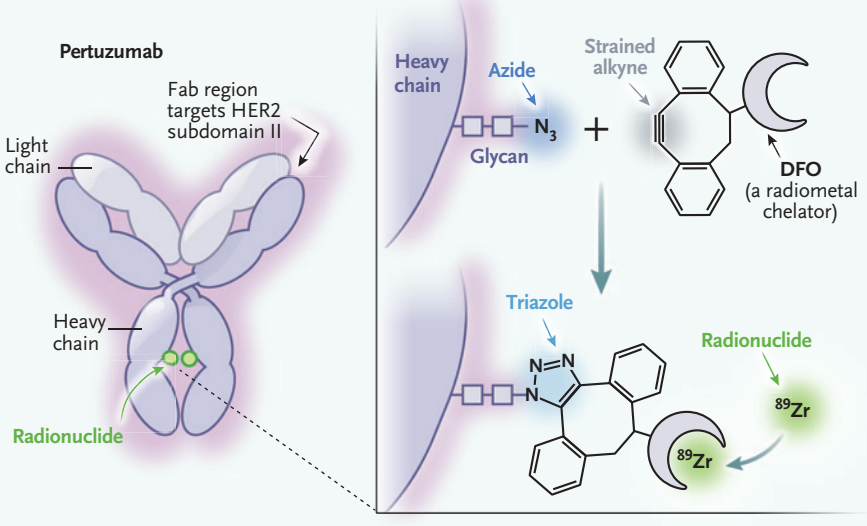
C CuAAC-Synthesized ^{18}F-FET-βAG-TOCA

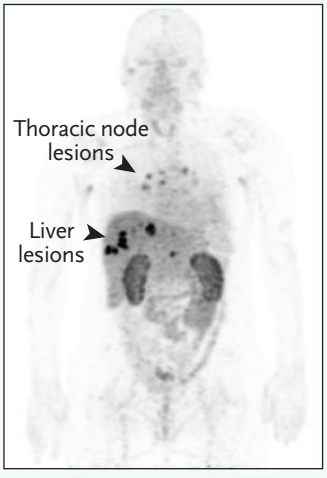




The radiolabeled imaging agent can be used to identify certain neuroendocrine tumors, such as in this PET-CT image of metastatic small-bowel neuroendocrine cancer.

D SPAAC-Synthesized Radiolabeled Monoclonal Antibody (^{89}Zr-^{ss}DFO-Pertuzumab) to Identify HER2-Positive Malignant Lesions





An immunoPET image of metastases in the liver and thoracic nodes.

Figure 1 (facing page). When Chemistry Clicks.
Panel A shows a schematic diagram of the copper-catalyzed azide–alkyne cycloaddition (CuAAC) reaction. Panel B shows a schematic diagram of the strain-promoted azide–alkyne cycloaddition (SPAAC) reaction. Panel C shows the structure of fluorine-18–labeled fluoroethyltriazole-Tyr^3–octreotate (^{18}F-FET-βAG-TOCA) and a transverse positron-emission tomographic–computed tomography (PET-CT) image (courtesy of Dr. E.O. Aboagye, Imperial College London) acquired with ^{18}F-FET-βAG-TOCA in a patient with metastatic small-bowel neuroendocrine cancer showing multiple liver metastases. Panel D shows the use of the SPAAC reaction to modify azide-bearing sugars incorporated into the heavy-chain glycans of pertuzumab with a chelator (desferrioxamine [DFO]) for subsequent radiolabeling with zirconium-89 as well as a coronal PET image (courtesy of Dr. Randy Yeh, Memorial Sloan Kettering Cancer Center) acquired 6 days after the administration of the radioimmunoconjugate to a patient with human epidermal growth factor receptor 2 (HER2)–positive breast cancer with multiple metastatic lesions in the liver and thoracic nodes. Fab denotes fragment antigen-binding.

strated by Bertozzi and colleagues when they treated live zebrafish embryos with azide-modified sugars.[4] As the embryos developed, these azide-bearing sugars were metabolically incorporated into the cell-surface glycans of various tissues, where they could then undergo SPAAC reactions with strained alkynes labeled with fluorophores to illuminate the emerging glycome of the zebrafish.

The advent of click chemistry has had a seismic effect on almost all areas of chemical science. Our field — radiopharmaceutical chemistry — is no exception. Click chemistry is a remarkably powerful tool for radiochemistry: the decay of radionuclides makes synthetic speed a priority, and both selectivity and water compatibility are critical because biomolecules are often used as platforms for radiotracers and radiotherapeutics. Over the past 15 years, the CuAAC and SPAAC reactions have been used to create radiopharmaceuticals labeled with everything from short-lived nuclides (e.g., carbon-11 and fluorine-18 [^{18}F]) for positron-emission tomography (PET) to long-lived isotopes (e.g., lutetium-177 and actinium-225) for targeted radionuclide therapy. Some of these agents have begun to appear in the clinic. In 2016, ^{18}F-labeled fluoroethyltriazole-Tyr^3–octreotate, a somatostatin receptor-targeting imaging agent that is synthesized with the CuAAC reaction, was translated for the PET imaging of neuroendocrine tumors in patients (Fig. 1C).[5] More recently, we have used the SPAAC reaction to create a variant of pertuzumab that is radiolabeled with zirconium-89 on the heavy-chain glycans (Fig. 1D). A trial of this probe for the antibody-based PET (immunoPET) imaging of human epidermal growth factor receptor 2–positive malignant lesions in patients is currently under way (ClinicalTrials.gov number, NCT04692831).

This Nobel Prize marks an exciting and consequential moment for click chemistry. In the laboratory, the trio of laureates have inspired the development of a host of new bioorthogonal click reactions, each with its own applications in chemistry, biology, and medicine. Importantly, click chemistry–based tools have increasingly entered the clinic. The road from chemical discovery to medical application may be long, but the technology that arose from the discoveries of Bertozzi, Meldal, and Sharpless is clearly well on its way.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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