Click chemistry with copper -- a biocompatible version

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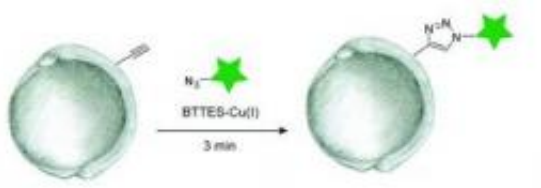

A version of click chemistry with an azide-based fluorophore (green star) and a BTTES-based copper catalyst was shown to be target specific, fast-working and safe for the fluorescent labeling og glycans in living cells. Credit: Yi Liu, Berkeley Lab Molecular Foundry

Berkeley Lab researchers have found a way to make copper-catalyzed click chemistry biocompatible. By adding a ligand that minimizes the toxicity of copper but still allows it to catalyze the click chemistry reaction, the researchers can safely use their reaction in living cells.

Biomolecular imaging can reveal a great deal of information about the inner workings of cells and one of the most attractive targets for imaging are glycans – sugars that are ubiquitous to living organisms and abundant on cell surfaces. Imaging a glycan requires that it be tagged or labeled. One of the best techniques for doing this is a technique called click chemistry. The original version of click chemistry could only be used on cells in vitro, not in living organisms, because the technique involved catalysis with copper, which is toxic at high micromolar concentrations.

A copper-free version of click chemistry that can safely be used in living organisms is available, but it is not always optimal in terms of reaction kinetics and target specificity. Now, a variation of click chemistry has been introduced that retains the copper catalyst of the original reaction - along with its speed and specificity – but is safe for cells in vivo.

Researchers with the Lawrence Berkeley National Laboratory (Berkeley Lab), in collaboration with researchers at the Albert Einstein College of Medicine at Yeshiva University in New York, have found a way to make copper-catalyzed click chemistry biocompatible. By adding a ligand that minimizes the toxicity of copper but still allows it to catalyze the click chemistry reaction, the researchers can safely use their reaction in living organisms. Compared to the copper-free click chemistry reaction, which can take up to an hour, the ligand-accelerated copper-catalyzed click chemistry reaction can achieve effective labeling within 3-5 minutes. The presence of the copper catalyst also enables this new formulation of click chemistry to be more target-specific with fewer background side reactions.

"The discovery of this new accelerating ligand for copper-catalyzed click chemistry should provide an effective complimentary tool to copper-free click chemistry," says Yi Liu, a chemist with Berkeley Lab's Molecular Foundry and the co-leader of this research with Peng Wu, of the Albert Einstein College of Medicine.

"While copper-free click chemistry may have advantages for whole animal imaging experiments such as imaging in mice," Liu says, "our ligand-accelerated copper reaction is better suited for enriching glycoproteins for their identification."

The ligand-accelerated copper-catalyzed reaction was used to label glycans in recombinant glycoproteins, glycoproteins in cell lysates, glycoproteins on live cell surfaces, and glycoconjugates in live zebrafish

embryos. Because a zebrafish embryo is transparent in the first 24 hours of its development, it allows labeled glycans to be detected via molecular imaging techniques, making it a highly useful model for developmental biology studies.

"Based on our results," says Peng Wu, "we believe that ligand-accelerated copper-catalyzed click chemistry represents a powerful and highly adaptive bioconjugation tool that holds great promise for further improvement with the discovery of more versatile catalyst systems."

Click chemistry, which was introduced in 2002 by the Nobel laureate chemist Barry Sharpless of the Scripps Research Institute, utilizes a copper-catalyzed azide-alkyne cycloaddition (CuAAC) reaction that makes it possible for certain chemical building blocks to "click" together in an irreversible linkage, analogous to the snapping together of Lego blocks. While the technique immediately proved valuable for attaching small molecular probes to various biomolecules in a test tube or on fixed cells, it could not be used for biomolecule labeling in live cells or organisms because of the copper catalyst.

In 2007, Carolyn Bertozzi, a chemist who holds joint appointments with Berkeley Lab, the University of California (UC) Berkeley, and the Howard Hughes Medical Institute, led a research effort that produced a copper-free version of click chemistry. In this version, glycans were metabolically labeled with azides - a functional group featuring three nitrogen atoms - via reactions that were carried out through the use of cyclooctyne reagents that required no copper catalyst. With their latest reagent, biarylazacyclooctynone (BARAC), Bertozzi and her group have provided a copper-free click chemistry technique that delivers relatively fast reaction kinetics and the bioorthogonality needed for biomolecule labeling. However, the technique can only be used on biomolecules that can be tagged with azides.

"Our bio-benign ligand-accelerated copper-catalyzed click chemistry reaction liberates bioconjugation from the limitation where ligations could only be accomplished with azide-tagged biomolecules," Liu says. "Now terminal alkyne residues can also be incorporated into biomolecules and detected in vivo."

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