A major shortcoming of the visualization of alkyne-tagged biomolecules with fluorescent azide probes through CuAAC reactions is the need to remove unreacted fluorescent probes. This is particularly problematic when imaging the intracellular environment, tissues of living organisms, or visualizing biomolecules in vivo. The difficulty of removing all unreacted fluorescent probes is also one of the major contributors to background signal and non-specific binding.

To overcome this shortcoming, the Carolyn Bertozzi group has designed fluorogenic azide probes that are activated by Cu-catalyzed or metal-free click chemistry. These azide probes are not fluorescent until they react with alkynes. Termed the CalFluors, these probes possess emission maxima that range from green to far-red wavelengths, and enable sensitive biomolecule detection under no-wash conditions. A number of reports showed that CalFluor probes are an indispensable tool for sensitive visualization of metabolically labeled molecules (glycans, DNA, RNA, and proteins) in cells, developing zebrafish, and mouse brain tissue slices under no-wash conditions.

**Selected References:**
