BERTOZZI RESEARCH LAB

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Research Interests

In the 21st century explorations at the interface of chemistry and biology will spawn new medicines and diagnostics that improve human health, technologies for probing natural biology and roadmaps for creating synthetic forms of life engineered to serve human needs. My lab focuses on creating new platform technologies that promote these endeavors, as well as applying chemical approaches to study systems that elude more conventional methods of biological inquiry. Examples of technologies that drive our research are bioorthogonal chemistries, site-specific protein modification methods and synthetic glycopolymers that emulate cell-surface glycoproteins. These tools and their applications in our lab, particularly in the area of glycoscience, are summarized below.

Bioorthogonal chemistry: Reaction methodology in the service of biology

We have a longstanding interest in developing chemical reactions that can be performed in living systems, i.e., cells, model organisms, and eventually, human patients. Termed "bioorthogonal chemistries", these reactions enable chemical modification of complex biological molecules in their native environments, with applications that include molecular imaging, in situ drug assembly, enzyme activity probing and interrogation of noncovalent complexes in cells. We explore the reaction manifolds of functional groups that do not exist in nature and have no inherent reactivity with natural components. Quintessential examples are reactions of azides with triaryl phosphines and strained alkynes, transformations that we call the Staudinger ligation and "copper-free click chemistry", respectively. These reactions have such high selectivity and efficiency that they can be performed in cells and organisms (i.e., *C. elegans*, zebrafish and mice). We aim to integrate such bioorthogonal reactivity into the design of smart probes for in vivo imaging, an effort that involves fundamental mechanistic and photophysical studies as well as innovative synthetic strategies. New bioorthogonal transformations are also under development, with a focus on exploiting or inventing functional groups that are orthogonal to current cohort.

In vivo imaging of glycans and other biomolecules

We have found bioorthogonal chemistry and other chemical approaches to be powerful allies in studies of glycobiology, a frontier area of biomedical science with limited experimental tools available for probing relationships of structure and function. Glycans decorate cell surfaces and secreted proteins and their structures change during dynamic physiological processes such as malignant transformation and stem cell differentiation. Yet, the precise biological functions of cell surface glycans, considered by many to be the "dark matter" of the cell, are largely unknown. Cancer-specific glycosylation patterns have been identified on tumor tissue *ex vivo*, prompting much excitement around their potential use as targets for molecular imaging and as disease biomarkers. To realize these possibilities, we developed a technique for glycan imaging that involves metabolic labeling with synthetic azidosugars followed by *in vivo* bioorthogonal reaction with optical or MRI contrast probes. We employ this chemical imaging platform to study glycomic changes associated with cancer, as well as during embryogenesis and microbial infection using zebrafish as a model organism.

Recently we pursued an analogous method for imaging bacterial peptidoglycan using azide- or alkyne-functionalized D-alanine analogs as metabolic labels. We are using this technique to probe spatiotemporal changes in peptidoglycan biosynthesis associated with infection by the intracellular pathogens such as *Mycobacterium tuberculosis* and *Listeria monocytogenes*. More broadly, we consider metabolic labeling with bioorthogonal "chemical reporters" as a general approach to interrogate biomolecules that might not be accessible to conventional genetic reporter strategies.

Glycoproteomics-based discovery of cancer and stem cell biomarkers

Given the strong correlation of altered glycosylation patterns with malignancy, glycosylated proteins may be an information-rich subset of the proteome from which cancer biomarkers can be discovered. We employ metabolic labeling with azidosugars as a means to tag specific classes of glycoproteins for enrichment from human tissue samples and subsequent identification by mass spectrometry. A challenge in this endeavor is defining sites of glycosylation on peptide digests derived from such complex samples. To facilitate this effort, we are creating isotopic labeling strategies and computational methods that enable the detection of glycosylated peptides independent of the mass of the pendant glycan. Collectively, these tools allow us to profile changes in protein glycosylation associated with human prostate cancer progression and embryoic stem cell differentiation.

Glycocalyx engineering toward probing cancer glycome evolution

While altered glycosylation patterns have long been identified as hallmarks of cancer, their functional significance with respect to tumor progression are not well understood. Two examples are overexpression of mucin glycoproteins¬ (densely glycosylated cell-surface molecules with unusual physical properties) and hypermodification of glycoproteins with the terminal sugar sialic acid. These glycosylation phenotypes are found on numerous cancer types with highly varied underlying driver mutations and their magnitude tends to correlate with tumor aggressiveness. To test hypotheses regarding the functional significance of cancer glycomes, we developed an approach to engineer the cell surface “glycocalyx” with chemically defined glycopolymers that emulate cancer-associated structures. Using living polymerization and chemoselective ligation chemistries, we synthesize glycopolymers functionalized with a biophysical probe on one end and a lipid capable of membrane insertion on the other. These biomimetic structures can be displayed on live cell membranes where they acquire functions analogous to natural mucin glycoproteins. With this glycocalyx engineering platform, we identified a role for hypersialylation in protection of tumor cells from innate immunosurveillance. Ongoing work focuses on the effects of mucin overexpression on cell adhesion and signaling.

Site-specific protein modification for enhanced biotherapeutics

Chemically modified proteins are a rapidly growing class of biologics, as illustrated by the dozens of antibody-drug conjugates and PEGylated proteins that populate the pipelines of major biopharma companies. A central challenge in generating chemically modified proteins is achieving control over the sites and stoichiometries of the modifications. We developed an operationally simple and scalable method to achieve site-specific protein modification via bioorthogonal chemistry. Termed the “aldehyde tag” method, we genetically encode the 5-residue motif (CTPxR) recognized by formylglycine (fGly) generating enzyme (FGE) at the desired site of protein modification. FGE cotranslationally converts cysteine to the aldehyde-functionalized fGly residue, producing proteins armed with a “chemical handle” for further modification. In parallel, we develop aldehyde-specific reactions for conjugation of cargo to fGly-proteins. The Pictet-Spengler ligation, for example, forms an irreversible C-C bond with protein-associated aldehydes and is now used for commercial production of antibody-drug conjugates. Ongoing work focuses on FGE engineering to achieve novel sequence specificities, mechanistic studies, and the generation of modified proteins for biotechnology applications.

New drug targets for *Mycobacterium tuberculosis* therapy

M. tuberculosis is the causative agent of human tuberculosis (TB) and the most lethal infectious pathogen in the world. While TB can be treatable with long-term multidrug regimens, new forms of drug resistant *M. tuberculosis* have arisen and disease continues to spread in low-resource settings. The full power of the pharmaceutical industry has not yet been harnessed toward a cure for TB, in part due to the economic realities of the disease’s demographics. Thus, philanthropic and academic institutions have an important role to play in anti-TB drug discovery and in accumulating the fundamental knowledge that underlies such efforts. We are interested in the sulfur assimilation pathway as a niche of metabolism that is essential for *M. tuberculosis* pathogenesis. We found that gene disruptions in this pathway compromise the ability to *M. tuberculosis* to defend itself against reactive oxygen and nitrogen species produced by host macrophages. Ongoing work in the lab focuses on small molecule drug discovery targeting sulfur assimilation enzymes, including structure-based and high-throughput screening approaches. As well, we are interested in metabolic systems related to cell wall biosynthesis as targets for anti-TB therapy.

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