Chemistry & Biology, Vol. 12, 617–620, June, 2005, ©2005 Elsevier Ltd All rights reserved. DOI 10.1016/j.chembiol.2005.06.004

Chemical Biology: Innovative Solutions for Diverse Challenges

Meeting Review

Joshua A. Kritzer* and Nathan W. Luedtke*
Department of Chemistry
Yale University
New Haven, Connecticut 06510

The Yale Chemical Biology Symposium is a day-long event that highlights new advances at the interface of chemistry and biology. Held annually since 1998, the symposium features presentations by acclaimed investigators from academic and industrial institutions as well as a poster session for student and postdoctoral presenters. The 2005 symposium held on May 13 featured Dr. Joshua Boger (Chairman, President, and CEO of Vertex Pharmaceuticals, Inc.) as the keynote speaker as well as Drs. Carolyn Bertozzi (UC Berkeley), Alice Ting (MIT), Peter Lansbury (Harvard), Patrick Loria (Yale), and Ronald Breaker (Yale). This meeting review summarizes the novel chemical biology approaches to fields as diverse as glycobiology, neurochemistry, drug discovery, molecular biophysics, and molecular genetics presented at the symposium.

The immense complexity of biological systems often demands the highly specific recognition and reactivity properties of small molecules to probe functional relationships. For example, with the revelation that the diversity of functional human proteins far exceeds the number of transcribed genes, scientists now have the daunting challenge of identifying, cataloging, and understanding the functional significance of a bevy of posttranslational modifications. Although some of these modifications, most notably phosphorylation, can be assessed by traditional biochemical techniques, chemical biologists have stepped up to the task of exploring more complex posttranslational modifications. Glycosylation may be the most chemically complex type of posttranslation modification, possessing both variable linkages and variable monosaccharide building blocks. Found on over 50% of all proteins [1], glycans are important for mediating protein stability, conformation, and catalytic activity and are absolutely critical for intercellular communication. The ability to identify and manipulate glycosylation sites is a central focus of the Bertozzi Laboratory (UC Berkeley). Dr. Carolyn Bertozzi explained that the study of O-linked glycosylation has been hindered by the lack of small-molecule inhibitors or affinity reagents for the glycosyltransferases (ppGalNacT) that install the initial N-acetyl galactosamine residue onto polypeptide substrates [2]. The Bertozzi Lab used a high-throughput screen to identify uridine-sugar conjugates that inhibit the activity of all 20 human isoforms of ppGalNAcT but are inactive against other cellular glycosyltransferases and nucleotide-utilizing enzymes [3]. These new inhibitors can be used to determine whether a particular protein is being glycosylated by a ppGalNAcT and to assess the effects of specific O-linked glycosylation. In a related endeavor, Dr. Bertozzi described the selective inhibition or activation of a single glucosyltransferase isoform by genetically splitting the enzyme into two functional domains and fusing each to a rapamycin-mediated heterodimerization domain (FKBP or FRB) [4]. The glycosyltransferase is reassembled in living cells upon addition of rapamycin, thus reactivating its catalytic activity [5]. This simple "on switch" can be applied to other enzymes, including sulfotransferases, and is currently being tested in model organisms.

In addition to providing new tools for deciphering protein modification and activity, one of the primary goals of chemical biology is to track the movements, actions, and interactions of biomolecules in their native context. Dr. Bertozzi explained how azide-modified monosaccharides can be incorporated into cell-surface proteoglycans in cell culture and in mice. A modified version of the Staudinger reaction is then used to covalently link the azide-modified cell-surface proteoglycan to a phosphine-containing imaging or capture reagent [6]. Amazingly, this Staudinger ligation can be performed in live mice with chemical yields of approximately 60% [7]. Future studies in the Bertozzi lab will move toward direct imaging of live animals by using proteoglycans modified with PET and NMR probes.

Although Dr. Bertozzi's methods take advantage of cellular metabolic pathways to introduce site-specific tags at glycosylation sites, the tagging and tracking of most proteins typically necessitates genetically fusing large proteins or short peptides to the protein of interest, often as recognition modules for small-molecule probes [8]. This approach is effective but has its disadvantages, most notably perturbation of protein function by the added domain or imperfect site specificity of the small-molecule probe. Dr. Alice Ting's laboratory (MIT) has been at the forefront of developing new techniques for tracking proteins in live cells. In her talk, Dr. Ting described a new strategy that involves genetically adding a 15-residue biotin acceptor peptide (AP) to a eukaryotic protein of interest [9]. The AP sequence is then biotinylated by the *E. coli* biotin ligase BirA with exceptional site specificity because BirA is functionally orthogonal to mammalian biotin ligases. Dr. Ting's method allows rapid, specific, and robust biotinylation of virtually any cell-surface protein in minutes, and use of a streptavidin-linked quantum dot probe allows tagged proteins to be monitored for hours without degradation of the signal because of dissociation or photobleaching. With the precision and speed of an enzymatic modification to provide extraordinary specificity, Dr. Ting's method is an ingenious twist on in vivo protein tagging.

In a striking demonstration of the new technique, Dr. Ting showed a movie clip tracking the movement of individual AMPA receptors along a neuron's surface, allowing direct observation that some AMPA receptors are highly mobile while others remain stationary [10]. She also discussed pulse-chase experiments carried

*Correspondence: joshua.kritzer@gmail.com (J.A.K.); nathan_luedtke@yahoo.com (N.W.L.)

out by performing BirA-mediated tagging and streptavidin labeling before and after stimulation of neuronal surface remodeling. The labeling was performed with a green organic dye before stimulation and with red quantum dots afterward, leading to a dual-color assay for turnover of AMPA receptor subunits that could be monitored in real time. Overall, the elegance of Dr. Ting's method, along with the striking visuals the method produces, should make it a critical tool for cell biologists interested in tracking cell-surface proteins.

A major theme of the Yale Chemical Biology Symposium was the application of chemical biology to drug discovery. Dr. Peter Lansbury's work has epitomized the creative application of chemistry to the biology of devastating human diseases, illustrating how "applied chemical biology" may be the most promising route to discovering new therapies. In his talk, Dr. Lansbury described mutagenesis experiments on superoxide dismutase 1 (SOD1), a protein whose misfolding and aggregation is implicated in the pathology of amyotrophic lateral sclerosis (ALS) [11]. His group found that mutations that fill a cavity in the SOD1 dimerization interface stabilize the dimeric form of SOD1 and prevent misfolding. This presented the intriguing possibility that small drug-like molecules that bind in this cavity could also prevent protein misfolding. Keenly focused on getting drug candidates into the clinic as soon as possible, Dr. Lansbury's group performed an in silico docking screen of a library of existing drugs, looking for molecules that docked into the dimerization interface. Of 100 predicted hits, 15 were shown to stabilize the dimeric form of SOD1 and prevent SOD1 aggregation [12]. These candidates are currently being optimized for potency and selectivity.

Dr. Lansbury's presentation also updated us on his current efforts targeting Parkinson's disease (PD) by following up on his initial report of small molecules that target ubiquitin-C-hydrolase L1 (UCH-L1) [13]. UCH-L1 is found in high levels in the brain, and it has been linked to PD by virtue of an unexpected dimerization-dependent ubiquitin ligase activity [14]. Dr. Lansbury outlined a potential mechanism for UCH-L1 action by showing that UCH-L1 is farnesylated and membrane anchored in both mammalian tissues and cultured cells. He hypothesized that farnesylation and subsequent membrane localization of UCH-L1 triggers its dimerization and ubiquitin-ligase activity. Among the evidence presented, perhaps the most compelling was that farnesyltransferase inhibitors prevent the death of dopaminergic neurons in cell-culture models of PD. In validating UCH-L1 as a drug target, Dr. Lansbury has provided ammunition for those who, like Lansbury himself, are willing to attack PD at the molecular level.

Target validation is perhaps the greatest hurdle in developing new drugs, as the keynote speaker, Dr. Joshua Boger (Chairman, President, and CEO of Vertex Pharmaceuticals, Inc.), reminded the audience. Vertex's highly successful strategy for drug discovery begins with a validated protein target and integrates structural biology, computational design, and medicinal chemistry to engineer a small-molecule drug. As a case study, Dr. Boger described the development of VX-680, a specific Aurora kinase inhibitor currently in phase I clinical trials for patients with solid tumors. Dr. Boger allowed that kinase active sites are quite similar, but insisted that specific inhibition of kinases by small molecules is "not a lemonade-from-lemons proposition." It simply requires precise tailoring of small molecules to fit a structurally distinct portion of the target kinase.

Vertex's approach consists of an iterative design process with well-chosen small-molecule scaffolds. In the first stage, high-throughput screening of a scaffold library against the target typically yields weak hits as lead compounds. Even at this early stage, Vertex evaluates the pharmacokinetics of initial hits to judge their potential as drugs. High-throughput crystallography is then used to decipher how the scaffold fits into the ATP binding pocket of the enzyme. Vertex has generated 50–100 kinase•inhibitor crystal structures per year since 1999. Potential variants of each scaffold are evaluated with a rapid computational docking algorithm, which winnows the pool of possible lead derivatives from nearly 10^{12} to several thousand; these can then be evaluated experimentally. Dr. Boger admitted that "affinity is the easy part," implying that once Vertex identifies a promising scaffold, the design and testing cycle (termed "morphing") reliably turns out a high-affinity ligand for the desired target. During this process, the experimental data for all drug targets, scaffolds, and derivatives are maintained in an integrated database. This promotes "target hopping," which occurs when a scaffold originally developed for one kinase is adapted for another based on clues from the database. Throughout his talk, Dr. Boger stressed the importance of obtaining high quality data on both kinase specificity and pharmacokinetics so that only those candidates with high potency and minimal side effects are advanced into clinical trials. Dr. Boger concluded by predicting that in the future, cancers will be classified not by their tissue of origin or their aggressiveness but by their responses to potent and specific small molecules.

Vertex has been a pioneer in structure-based drug design, demonstrating the cumulative power of hundreds of crystal structures of target•inhibitor complexes and rapid docking algorithms. Although such techniques have become a staple of modern drug design, static snapshots and rigid docking exercises cannot fully describe the functions of complex biomolecules or their responses to binding events. Thus, characterizing the dynamic motions of biomolecules is essential to understanding fundamental aspects of their function. Dr. Patrick Loria's laboratory (Yale University) uses advanced NMR techniques and site-directed mutagenesis to examine the relationships among enzyme motion, substrate binding, and catalysis. The backbone motions of RNase A bound to the high-affinity substrate mimic "pTppAp" were recently quantified with NMR spin-relaxation measurements in the Loria Lab. Interestingly, backbone motions within RNase A are not altered upon pTppAp binding, and the NMR data demonstrated that, rather than causing a conformational change, ligand binding alters an already existing equilibrium between two discrete conformations of RNase A [15]. This result contrasts with ligand-induced conformation changes often observed in other systems and may represent a general feature of high-affinity substrate binding and enzyme inhibition. In a

separate series of experiments, Dr. Loria studied how the motions of a molecular "hinge" region of triosephosphate isomerase (TIM) that opens and closes the active site can affect substrate binding and catalytic turnover. Mutations of residues at the hinge that join the loop region to the rest of the enzyme modestly alter substrate binding but dramatically increase molecular motions in the hinge region for both the *apo* and substrate bound forms of the enzyme. This flexibility allows access to nonproductive conformations, leading to a profound loss in catalytic activity. Dr. Loria concluded that although the dynamic opening and closing of enzyme active sites is essential for catalysis, hinge-like motions are sufficiently well-defined so that too much flexibility abolishes catalytic activity.

Proteins are not the only biomolecules whose functions cannot be fully understood with static pictures. The dynamics and flexibility of RNA are critical for its ability to mediate both information storage and chemical catalysis. Given scenarios involving a prebiotic "RNA world" in which RNA served as the evolutionary precursor to metabolic proteins, one might expect RNA to have evolved regulatory binding sites for simple metabolites. Dr. Ron Breaker (Yale University) gave an account of the discovery and exploration of "riboswitches," RNA motifs that rely on propagation of conformational changes to control gene expression [16]. The journey began with in vitro selection experiments showing that the activity of a self-cleaving RNA enzyme can be allosterically regulated by small molecule binding [17]. Dr. Breaker explained how such sophisticated RNA function led to speculation that natural systems might utilize RNA•small-molecule binding interactions to control gene expression. A search of the literature revealed a number of well-characterized prokaryotic genes that were regulated through product feedback but lacked an identified protein modulator. Suspected riboswitches were identified as highly conserved sequences in the 5′ untranslated regions of these genes and were subsequently assessed for metabolite binding by inline probing, a method that relies on differential rates of phosphodiester bond scission in structured versus unstructured regions of RNA [18]. These experiments showed that certain regions within the suspected riboswitches exhibit changes in RNA flexibility and geometry upon addition of the metabolite, revealing a binding event at these sites. The resulting RNA•small-molecule complexes modulate gene expression by numerous mechanisms at the level of either transcription or translation [19]. Riboswitches can even exhibit cooperative substrate binding that leads to highly sensitive transcriptional control [20]. Currently, there are 11 different classes of known riboswitches controlling perhaps 2% of all gene expression in *B. subtilis*. No evidence has yet been found for the presence of riboswitches in higher-order organisms, which led Dr. Breaker to see prokaryotic riboswitches as targets for new antibiotics. The Breaker group used a newly reported crystal structure of the guanine riboswitch [21] to rationally design guanine derivatives that exhibit promising antibiotic activity. Combined with the recent insights into how aminoglycoside antibiotics selectively bind to and inhibit prokaryotic ribosomal RNA, the discovery of riboswitches has demonstrated that RNA is an important and underutilized drug target.

Conclusions

The 2005 Yale Chemical Biology Symposium made it clear that chemical biology's reach is expanding into a wide variety of fields. In areas as diverse as drug discovery, neurochemistry, and molecular genetics, small-molecule-based approaches are becoming the methods of choice for careful profiling and specific manipulation of biological processes. In both industry and academia, chemical biology has provided indispensable tools that can address even the most complex challenges in biology and human disease.

Acknowledgments

Financial support from the National Science Foundation Predoctoral Fellowship Program (J.A.K.) and National Institutes of Health (GM 074401) are gratefully acknowledged.

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