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The Three Cornerstones of Chemical Biology: Innovative Probes, New Discoveries, and Enabling Tools

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Keystone Symposia have a long history of serving as catalysts for the advancement of biomedical and life sciences by connecting scientists within and across disciplines. Thus, it was a natural extension of the central Keystone mission to hold the first ever Keystone Symposia on "Chemical Biology and Novel Tools in Pharmacology" on February 12−16, 2012. Organized by **Laura Kiessling** (University of Wisconsin, Madison), **Jason Gestwicki** (University of Michigan), and **Kevan Shokat** (University of California, San Francisco), this conference brought scientists from the international chemical biology community together in Santa Fe, New Mexico to communicate new ideas and technologies as well as recent progress in research. Unlike the majority of Keystone Symposia, this meeting was not focused on a particular aspect of biology but rather nucleated chemists and biologists across a wide range of disease areas and biological topics, providing an especially vibrant forum for the discussion of cross-platform ideas. As evidence of the breadth of the community, representatives from 10 countries, including academic, industrial, and government researchers and more than 40 graduate students, came together for this meeting.

The conference schedule was filled with impressive presentations from experts across multiple areas of chemical biology (Figure 1). Throughout the program's various sessions, three overarching themes emerged: (A) Design and Discovery of New Molecules and Probes, (B) New Advances in Biology Identified Using Chemical Approaches, and (C) Development of Novel, Enabling Methods (Figure 2). Select highlights are described here to illustrate the breadth and depth of these concepts.

DESIGN AND DISCOVERY OF NEW MOLECULES AND PROBES

Emerging Strategies for Identifying New Chemical Matter. Chemical probes remain one of the most powerful and visible "products" of chemical biology. Not surprisingly then, one major theme of the meeting was expanding the sphere of available probes via mining the diversity of natural products. For example, the keynote address was given by **Christopher Walsh** (Harvard Medical School) and highlighted the rich reservoir of chemical matter present in natural products. Dr. Walsh presented his elucidation of the assembly of the thiazolylpeptide antibiotics, including plantazolicin A, goadsporin, and the patellamides. Work from the Walsh group has shown that the precursors to these antibiotics are genetically encoded, ribosomal polypeptides that are translated, cleaved by proteases, extensively modified, and then cyclized.[1,2] One of the most interesting features of the biosynthesis of the thiocillin scaffold is a condensation between two dehydroalanines derived from phosphothreonines, in what may represent a concerted aza-Diels−Alder reaction or a stepwise condensation. This final biosynthetic step results in the formation of a central pyridine ring while simultaneously and neatly completing the macrocycle.[3,4] Importantly, the Walsh group also found that a helical leader peptide is essential for recognition by the biosynthetic cluster that carries out this series of complex, post-translational transformations. Thus, an intriguing extension of this work might be to engineer leader sequences that could be used to introduce structural diversity into other polypeptides.

In addition to Dr. Walsh's advances in understanding complex natural product biosynthesis, **Nathan Magarvey** (McMaster University) and **Frank Schroeder** (Cornell University) discussed innovative analytical solutions to expedite the identification of active natural products. Magarvey described a method that utilizes bioinformatics coupled with mass spectrometry to rapidly identify whether a newly isolated natural product has been previously identified. The need for this type of platform is becoming increasingly urgent because the most common molecules are prone to being repeatedly reidentified, diverting resources from the discovery of new chemical scaffolds. Departing from the typical activity-guided purification of natural products, Schroeder applied NMR- and MS-based comparative metabolomics to identify active small molecules, such as new ascarosides, within mixtures of metabolites produced by *C. elegans*.[5,6] A key aspect of this work is that Schroeder's group uses worm mutants to create control and test metabolite pools, which significantly focuses the search and enriches for molecules with activity related to the desired phenotypes. Together, these methods are enabling smarter and more biology-driven discovery of new bioactive molecules.

Chemical Biology and Its Role in Drug Discovery. As an extension of work on the discovery of new small molecules, chemical biologists are oftentimes at the forefront of developing novel strategies and ideas in drug discovery. **Kevan Shokat** (University of California, San Francisco) illustrated one clever example of this concept in his description of the paradigm shift from single targets toward optimal polypharmacology. Polypharmacology is a concept in which a compound purposefully modulates a complement of targets, allowing new opportunities for using a drug in multiple indications and sometimes reducing the risk of resistance. But how does one go about finding small molecules with optimal polypharmacology? Dr. Shokat highlighted his group's pioneering efforts toward these goals.[7,8] In one example, they collaborated with the Cagan group to screen known promiscuous kinase inhibitors in *Drosophila* larvae

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Keynote Speaker
Christopher Walsh, Harvard Medical School, USA
Biosynthetic Morphing of Peptide Backbones and Side Chains into Heterocyclic Scaffolds

Chemical Microbiology
Nathan Magarvey, McMaster University, Canada
Mining Microbiomes for Genetically Encoded Bioactive Small Molecules
Stephen del Cardayre, LS9, Inc., USA
Engineering Microbial Fatty Acid Metabolism for Sustainable Fuel and Chemical Production
Suzanne Walker, Harvard Medical School, USA
Structure, Mechanism, and Inhibition of Human O-GlcNAc Transferase
Laura Kiessling, University of Wisconsin, Madison, USA
The Chemistry and Biology of Mycobacterial Polysaccharide Assembly
Douglas Weibel, University of Wisconsin-Madison, USA
Bacteria Control Intracellular Organization by Straining Their Cytoplasmic Membrane
Howard Hang, Rockefeller University, USA
Chemical Reporters for Dissecting Host-Pathogen Interactions

Chemical Communication
Sean Cutler, University of California, Riverside, USA
Sidestepping Genetic Redundancy with Small Molecules
Cameron Currie, University of Wisconsin-Madison, USA
Drugs from Bugs of Bugs
Erin Carlson, Indiana University, USA
Chemical Probes to Explore Histidine Kinase Signaling
John Kozarich, ActivX Biosciences, Inc., USA
Toward a Functional, Chemoproteomic Interrogation of Kinome and Nucleotide Binding Space

Probing and Perturbing Cell Signaling
Timothy Mitchison, Harvard Medical School, USA
How Does Taxol Work as a Medicine?
Carsten Schultz, European Molecular Biology Laboratory Heidelberg, Germany
FRET Reporters for Proteases
Jing Huang, University of California, Los Angeles, USA
Small Molecule Regulators of Aging
Tom Muir, Princeton University, USA
Discovery of New "Supercharged" Inteins: Prospects for Protein Engineering
Kevan Shokat, University of California, San Francisco, USA
Chemical-Genetic Investigations of Protein and Lipid Kinase Signaling

Probing and Perturbing Cell Signaling II
Ulrike Eggert, King's College London, UK
A Chemical Approach to Understanding Cell Division
Rebecca Heald, University of California, Berkeley, USA
Importazole, a Small Molecule Inhibitor of Nucleocytoplasmic Transport and Cell Division
Matthew Pratt, University of Southern California, USA
O-GlcNAc as a Link Between Metabolism and Survival in Cancer
Morgan Huse, Memorial Sloan-Kettering Cancer Center, USA
A Photochemical Approach to Lymphocyte Signaling Dynamics
Luke Lavis, Howard Hughes Medical Institute, USA
Targeting Small Molecules with Cellular Specificity using Enzyme–Substrate Pairs
E. James Petersson, University of Pennsylvania, USA
Thioamides: Minimalist Chromophores for Monitoring Protein Dynamics

Protein Quality Control
Jason Gestwicki, University of Michigan, USA
Molecular Chaperones and Protein Folding: Chemical Tools for Probing Quality Control
Julian Adams, Infinity Pharmaceuticals Inc., USA
Saridegib: Natural Product-Derived Inhibitor of the Hedgehog Pathway and Clinical Development to Treat Cancer
Raquel Lieberman, Georgia Institute of Technology, USA
Pharmacological Chaperone Development for the Glaucoma-Associated Olfactomedin Domain of Myocilin
Kurt Deshayes, Genentech, Inc., USA
Peptide Sensors of Angiogenesis
Eric Strieter, University of Wisconsin, USA
Strategies for Synthesizing Defined Ubiquitin Polymers

Chemical Biology in Model Organisms
Charlie Boone, University of Toronto, Canada
Linking Bioactive Compounds to Cellular Targets by High-Throughput Chemical-Genetic Profiling in Yeast
Frank Schroeder, Boyce Thompson Institute at Cornell University, USA
C. elegans as a Model Organism for the Study of Small Molecule Signaling
Carolyn Bertozzi, University of California, Berkeley, USA
Bioorthogonal Chemistry for Glycoprofiling and Beyond
Jeffery Kelly, The Scripps Research Institute, USA
The Oligosaccharyl Transferase (OST) Enzyme Prefers to N-glycosylate Sequences That Also Result in Native State Protein Stabilization Upon Glycosylation.

Session chairs: Ronald Woodard, University of Michigan, USA, **Susan Clugston,** Cubist Pharmaceuticals, USA, **Jeffrey Henderson,** Center for Women's Infectious Diseases Research, Washington University, USA, **Sharon Xiaoning Zhao,** Amgen Inc, USA, **Susan Wiedner,** Pacific Northwest National Laboratory, USA

Meeting coordinators: Laura Kiessling, Jason Gestwicki, Kevan Shokat

Figure 1. Program and list of speakers for the Keystone Symposia on "Chemical Biology and Novel Probes in Pharmacology".

harboring a RET tyrosine kinase mutation.[9] Unexpectedly, this screen showed that EGFR inhibitors are active in this model, and this finding has led to new clinical trials for EGFR inhibitors in Ret^{MEN2}-driven multiple endocrine neoplasia. With growing appreciation of polypharmacology in the pharmaceutical industry, elegant chemical biology approaches, such as the ones used by Shokat and Cagan, provide a framework for designing more biology-driven screening platforms. **Timothy Mitchison** (Harvard) provided another key example of how chemical biology concepts are transforming how we think about drug discovery. He explored the question of how paclitaxel and kinesin 5 inhibitors give rise to different sustained apoptotic responses.[10] His work points to a key role for single cell pharmacodynamics and the rates of cell cycle progression in dictating drug efficacy in tumor systems. His mixture of chemical ideas with cell biology is a powerful example of how chemical biology can be used to make drug discovery efforts more informed, biology-driven, and ultimately, more effective.

Additional talks highlighted strategies to address common hurdles encountered throughout the drug development process. **Raquel Lieberman** (Georgia Institute of Technology) discussed a screening strategy based on measuring protein stability to find molecules that bind to non-enzyme targets. Her group utilized this strategy to identify compounds that stabilize myocilin, a protein that is misfolded and mistrafficked in open angle glaucoma.[11] Moving downstream in the drug development pipeline, **Charlie Boone** (University of Toronto) highlighted cutting-edge methods for target identification. He used large-scale synthetic lethal screens in *Saccharomyces* to place the activity of small molecules into specific biological pathways and processes.[12,13] As target identification is one of the key challenges in cell-based and phenotypic screens, this method is promising to revolutionize our ability to narrow the search for protein targets. Similarly, **Jing Huang** (UCLA) described her group's work on Drug Affinity Responsive Target Stability (DARTS), a method for finding protein targets using differential susceptibility to proteases.[14] Finally, **Carolyn Bertozzi** (University of California, Berkeley) discussed a novel approach to site-specific protein modifications, using a formylglycine generating enzyme to region-specifically install an aldehyde functionality on the protein.[15] This technique involves engineering a 5-residue consensus sequence into a suitable region of the protein of interest, such as a disordered loop, then expressing the resulting protein in cells expressing a formylglycine generating enzyme.[16] This technology permits bio-orthogonal coupling reactions that allow site-specific incorporation of post-translational modifications, such as glycosylation or drug conjugates. Thus, this clever chemical biology method has immediate pharmaceutical applications in the production of therapeutic antibodies and other protein-based therapeutics.

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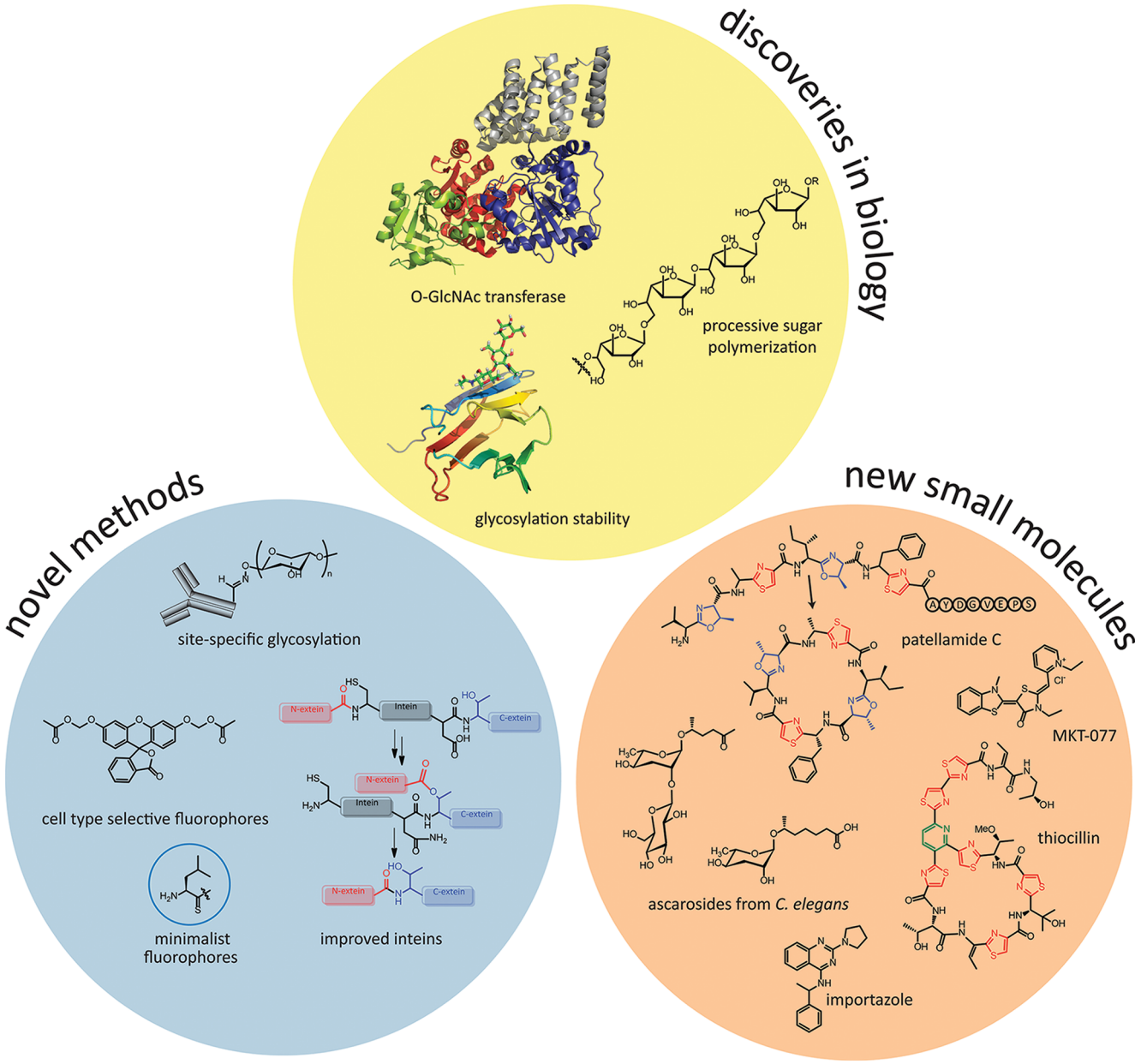

Figure 2. Major themes of the Keystone meeting. A few representative topics in each broad category are illustrated. See text for additional examples.

NEW ADVANCES IN BIOLOGY IDENTIFIED USING CHEMICAL APPROACHES

Small Molecules Enable Our Understanding of Biology. After hearing so many exciting strategies for the identification of new chemicals, it was then satisfying to also learn of examples in which new small molecules were used to make important biological insights. In one such example, **Sean Cutler** (University of California, Riverside) discussed using small molecules to understand cellular pathways that are too highly redundant to study by traditional genetic mechanisms.[17] His group identified the small molecule pyrabactin from a screen for compounds that phenocopy the plant hormone abscisic acid (ABA) by inhibiting seed germination. The target of ABA was unknown and conventional efforts to identify it had been unsuccessful. However, studies into the target of pyrabactin using a yeast two hybrid approach demonstrated that it bound PYR1, the first member of a novel class of proteins. Further studies revealed that pyrabactin induced complex formation between PYR1 and a type 2C protein phosphatases, inhibiting its activity. It was further shown that ABA exhibited the same mechanism of action as pyrabactin but was less specific. ABA was able to activate a variety of isoforms of PYR1, called PYL proteins, perhaps explaining why target identification studies were more successful using pyrabactin compared to previous efforts using ABA.[18,19,17] In a similar manner, **Ulrike Eggert** (King's College, London) described how her group used 1-phenyl-2-palmitoylamino-3-morpholino-1-propanol (PPMP) to uncover a role for glycosphingolipids in cytokinesis and that the compound XZ1 can be used to probe connections between endocytic sorting and cytokinesis.[20] These are just some of many examples from the Keystone Symposia in which understanding the mechanism of action of a small molecule uncovered valuable insights into biological systems. In other examples, **Rebecca Heald** (University of California, Berkeley) has used the compound importazole to reveal new methods for targeting Ran GTPases.[21] By exploiting

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his identification of small molecule probes of multiprotein complexes, **Jason Gestwicki** (University of Michigan) used the anticancer drug MKT-077 and other small molecules to identify a key role for heat shock protein 70 (Hsp70) in controlling the turnover of microtubule-binding protein tau (MAPT).[22,23]

Chemical tools also play a large role in elucidating the logic of biological systems by overcoming classic technical hurdles. For example, **Morgan Huse** (Memorial Sloan-Kettering Cancer Center) illustrated how using a photochemically activated caged ligand for the T cell receptor (TCR) allowed spatial and temporal control over TCR stimulation. The spatial and temporal control allowed Dr. Huse and colleagues to identify a novel role for diacylglycerol (DAG) in cytoskeletal polarization during T cell activation.[24,25] Similarly, **Howard Hang** (Rockefeller University) was able to utilize novel reporters of palmitoylation to identify IFITM3 palmitoylation as an important factor in its ability to counteract influenza infection within dendritic cells.[26]

Naturally Occurring Examples of Chemistry in Action. Throughout the Keystone Symposia, the power of chemistry was carefully leveraged to study and understand multiple biological systems. However, some of the most eye-catching examples where those in which the tables were turned: biology was shown to use clever chemistry to achieve a desired outcome. For example, **Cameron Currie** (University of Wisconsin) described a fascinating example of three-way mutualism between leaf-cutter ants, a fungus that the ants cultivate as a food source, and *Actinobacteria* that live off the exoskeletons of the ants and secrete antibiotics that protect the fungal food source from bacterial contamination.[27] Currie's story provided a particularly tangible illustration of nature's ingenuity. This theme was further emphasized by **Douglas Weibel** (University of Wisconsin) who described how curvature strain in the lipid membrane accommodates the localization of lipids and membrane-associated proteins. Weibel demonstrated that the membrane lipid cardiolipin, shaped like a "badminton shuttlecock", rapidly diffuses to cellular poles when spherical bacteria are forced to assume rod-like shapes, elegantly controlling the physical chemistry of the membrane and assisting protein localization.[28] Further, **Laura Kiessling** (University of Wisconsin, Madison) discussed her group's contributions to understanding mycobacterial polysaccharide assembly by the galactosyltransferase GlfT2. GlfT2-catalyzed polymerization exclusively produces chains of 10 and 20 furanose units with regio-specificity alternating between 1,5 and 1,6 linkages. Remarkably, this polymerization occurs without the aid of a template.[29] After demonstrating that the polymer elongation occurs processively, Kiessling proposed an entropically driven tethering mechanism for the control of length,[30] which could have general applicability in other non-templated biological polymerizations.

Finally, an exquisite example of the logic and chemistry of biological systems was presented by **Jeffery Kelly** (Scripps Research Institute). Approximately one-third of proteins in the eukaryotic proteome are synthesized in the secretory pathway, and the majority of these are N-glycosylated. While it is well-known that glycosylation stabilizes proteins, the molecular forces underlying that stabilization have not been elucidated. Kelly's group found that the first GlcNAc residue confers an advantage to the kinetics and energetics of folding by stabilizing the native state.[31,32,33] This stabilization is rooted in the interactions between GlcNAc1 and an aromatic residue at the n-2 position. These so-called enhanced aromatic sequons are also efficiently glycosylated by oligosaccharyltransferase. Taken together, this work re-emphasizes the idea that chemical inspiration can often be found in nature.

DEVELOPMENT OF NOVEL, ENABLING METHODS

Chemical Tools with the Potential for Broad Application. Another underlying theme of the meeting was the development and improvement of new chemical tools. This area has long been a foundational aspect of chemical biology, producing such widely used methods as bump-hole technology, bio-orthogonal chemistry, non-natural amino acids, and others. Presentations at the Keystone Symposia clearly showed that this traditional cornerstone of the field is alive and thriving. **Carsten Schultz** (EMBL) touched on his group's efforts to develop genetically encoded FRET probes by utilizing either small molecules or installing mTurquoise and variants of the yellow fluorescent protein Venus into optimal positions within target proteins.[34] **Tom Muir** (Princeton University) described the identification and optimization of improved, "supercharged" inteins.[35,36] Inteins are proteins with autoprocessing domains that are spliced and then ligated to give a traceless peptide bond between two originally noncontiguous polypeptides. These self-splicing proteins are naturally occurring in microbes and are frequently leveraged in modern chemical biology and protein semisynthesis. However, a common technical challenge is the slow reaction rates of the most commonly used inteins, such as MxeGyrA. Muir and colleagues conducted an inclusive and systematic search of natural inteins and, remarkably, found that nearly all of them are faster than MxeGyrA.[35] This work opens up a new suite of research-ready inteins for use in a wide range of applications. **E. James Petersson** (University of Pennsylvania) built on the FRET and intein technology highlighted by the aforementioned talks, applying them in his development of clever "minimalist chromophores". In this approach, Petersson installed a thioamide within the peptide backbone using expressed protein ligation[37] and then strategically introduced a fluorophore using unnatural amino acid incorporation (refs 38−40 and unpublished results), producing a fluorescence reporter suitable for monitoring subtle changes in protein structure. Likewise, a variety of chemical tools were described that allow for greater spatial and temporal control of activity. For example, **Luke Lavis** (Janelia Farm Research Campus, Howard Hughes Medical Institute) presented an approach of utilizing enzyme−substrates pairs to selectively activate dyes and drugs within a specific cell type expressing the required enzyme pair.[41] **Erin Carlson** (Indiana University) developed a powerful antibody-free probe for histidine phosphorylation, providing a tool for exploring the biology of this enigmatic family of signaling factors. **John Kozarich** (ActivX) described new probes for identifying ATP/ADP-binding proteins in complex mixtures.[42] That technology is promising to greatly accelerate how we identify and profile kinase inhibitors, among other potential uses. **Kurt Deshayes** (Genentech) described the development of peptide-based imaging agents, based on well-folded 2- and 3-helical bundles, which have surprisingly good pharmacokinetic properties.[43] This work showed a surprising role for protein platforms as delivery vehicles for imaging modalities that are suitable for use *in vivo*. Finally, **Eric Strieter** (University of Wisconsin) described a clever thiol−ene coupling approach for creating mixed-linkage ubiquitin polymers, providing a method for answering some of the most pressing questions in protein homeostasis.[44]

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CONCLUSIONS

The first Keystone Symposium on Chemical Biology and Novel Tools in Pharmacology was praised for its ability to bring together scientists from a variety of backgrounds with a shared interest in developing, understanding, and using chemical biology. Academic, government, and industrial researchers alike were actively engaged in a dialogue regarding the broad potential of chemical biology. Although modern chemical biology has now branched into many far-flung corners of biomedical science, it was refreshing, invigorating, and perhaps most importantly, comforting to come together as a community and rediscover why chemical biology remains a vibrant field.

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REFERENCES

(1) Walsh, C. T., Malcolmson, S. J., and Young, T. S. (2012) Three ring posttranslational circuses: insertion of oxazoles, thiazoles, and pyridines into protein-derived frameworks. *ACS Chem. Biol. 7*, 429–442.

(2) Wieland Brown, L. C., Acker, M. G., Clardy, J., Walsh, C. T., and Fischbach, M. A. (2009) Thirteen posttranslational modifications convert a 14-residue peptide into the antibiotic thiocillin. *Proc. Natl. Acad. Sci. U.S.A. 106*, 2549–2553.

(3) Acker, M. G., Bowers, A. A., and Walsh, C. T. (2009) Generation of thiocillin variants by prepeptide gene replacement and in vivo processing by Bacillus cereus. *J. Am. Chem. Soc. 131*, 17563–17565.

(4) Bowers, A. A., Acker, M. G., Koglin, A., and Walsh, C. T. (2010) Manipulation of thiocillin variants by prepeptide gene replacement: structure, conformation, and activity of heterocycle substitution mutants. *J. Am. Chem. Soc. 132*, 7519–7527.

(5) Pungaliya, C., Srinivasan, J., Fox, B. W., Malik, R. U., Ludewig, A. H., Sternberg, P. W., and Schroeder, F. C. (2009) A shortcut to identifying small molecule signals that regulate behavior and development in Caenorhabditis elegans. *Proc. Natl. Acad. Sci. U.S.A. 106*, 7708–7713.

(6) von Reuss, S. H., Bose, N., Srinivasan, J., Yim, J. J., Judkins, J. C., Sternberg, P. W., and Schroeder, F. C. (2012) Comparative metabolomics reveals biogenesis of ascarosides, a modular library of small molecule signals in C. elegans. *J. Am. Chem. Soc. 134*, 1817–1824.

(7) Apsel, B., Blair, J. A., Gonzalez, B., Nazif, T. M., Feldman, M. E., Aizenstein, B., Hoffman, R., Williams, R. L., Shokat, K. M., and Knight, Z. A. (2008) Targeted polypharmacology: discovery of dual inhibitors of tyrosine and phosphoinositide kinases. *Nat. Chem. Biol. 4*, 691–699.

(8) Knight, Z. A., Lin, H., and Shokat, K. M. (2010) Targeting the cancer kinome through polypharmacology. *Nat. Rev. Cancer 10*, 130–137.

(9) Vidal, M., Wells, S., Ryan, A., and Cagan, R. (2005) ZD6474 suppresses oncogenic RET isoforms in a Drosophila model for type 2 multiple endocrine neoplasia syndromes and papillary thyroid carcinoma. *Cancer Res. 65*, 3538–3541.

(10) Orth, J. D., Kohler, R. H., Foijer, F., Sorger, P. K., Weissleder, R., and Mitchison, T. J. (2011) Analysis of mitosis and antimitotic drug responses in tumors by in vivo microscopy and single-cell pharmacodynamics. *Cancer Res. 71*, 4608–4616.

(11) Burns, J. N., Orwig, S. D., Harris, J. L., Watkins, J. D., Vollrath, D., and Lieberman, R. L. (2010) Rescue of glaucoma-causing mutant myocilin thermal stability by chemical chaperones. *ACS Chem. Biol. 5*, 477–487.

(12) Costanzo, M., Baryshnikova, A., Bellay, J., Kim, Y., Spear, E. D., Sevier, C. S., Ding, H., Koh, J. L., Toufighi, K., Mostafavi, S., Prinz, J, St Onge, R. P., VanderSluis, B, Makhnevych, T, Vizeacoumar, F. J., Alizadeh, S, Bahr, S, Brost, R. L., Chen, Y, Cokol, M, Deshpande, R, Li, Z, Lin, Z. Y., Liang, W, Marback, M, Paw, J, San Luis, B. J., Shuteriqi, E, Tong, A. H., van Dyk, N, Wallace, I. M., Whitney, J. A., Weirauch, M. T., Zhong, G, Zhu, H, Houry, W. A., Brudno, M, Ragibizadeh, S, Papp, B, Pál, C, Roth, F. P., Giaever, G, Nislow, C, Troyanskaya, O. G., Bussey, H, Bader, G. D., Gingras, A. C., Morris, Q. D., Kim, P. M., Kaiser, C. A., Myers, C. L., Andrews, B. J., and Boone, C. (2010) The genetic landscape of a cell. *Science 327*, 425–431.

(13) Parsons, A. B., Brost, R. L., Ding, H., Li, Z., Zhang, C., Sheikh, B., Brown, G. W., Kane, P. M., Hughes, T. R., and Boone, C. (2004) Integration of chemical-genetic and genetic interaction data links bioactive compounds to cellular target pathways. *Nat. Biotechnol. 22*, 62–69.

(14) Lomenick, B., Hao, R., Jonai, N., Chin, R. M., Aghajan, M., Warburton, S., Wang, J., Wu, R. P., Gomez, F., Loo, J. A., Wohlschlegel, J. A., Vondriska, T. M., Pelletier, J., Herschman, H. R., Clardy, J., Clarke, C. F., and Huang, J. (2009) Target identification using drug affinity responsive target stability (DARTS). *Proc. Natl. Acad. Sci. U.S.A. 106*, 21984–21989.

(15) Hudak, J. E., Yu, H. H., and Bertozzi, C. R. (2011) Protein glycoengineering enabled by the versatile synthesis of aminooxy glycans and the genetically encoded aldehyde tag. *J. Am. Chem. Soc. 133*, 16127–16135.

(16) Carrico, I. S., Carlson, B. L., and Bertozzi, C. R. (2007) Introducing genetically encoded aldehydes into proteins. *Nat. Chem. Biol. 3*, 321–322.

(17) Park, S. Y., Fung, P., Nishimura, N., Jensen, D. R., Fujii, H., Zhao, Y., Lumba, S., Santiago, J., Rodrigues, A., Chow, T. F., Alfred, S. E., Bonetta, D., Finkelstein, R., Provart, N. J., Desveaux, D., Rodriguez, P. L., McCourt, P., Zhu, J. K., Schroeder, J. I., Volkman, B. F., and Cutler, S. R. (2009) Abscisic acid inhibits type 2C protein phosphatases via the PYR/PYL family of START proteins. *Science 324*, 1068–1071.

(18) Fujii, H., Chinnusamy, V., Rodrigues, A., Rubio, S., Antoni, R., Park, S. Y., Cutler, S. R., Sheen, J., Rodriguez, P. L., and Zhu, J. K. (2009) In vitro reconstitution of an abscisic acid signalling pathway. *Nature 462*, 660–664.

(19) Nishimura, N., Hitomi, K., Arvai, A. S., Rambo, R. P., Hitomi, C., Cutler, S. R., Schroeder, J. I., and Getzoff, E. D. (2009) Structural mechanism of abscisic acid binding and signaling by dimeric PYR1. *Science 326*, 1373–1379.

(20) Atilla-Gokcumen, G. E., Bedigian, A. V., Sasse, S., and Eggert, U. S. (2011) Inhibition of glycosphingolipid biosynthesis induces cytokinesis failure. *J. Am. Chem. Soc. 133*, 10010–10013.

(21) Soderholm, J. F., Bird, S. L., Kalab, P., Sampathkumar, Y., Hasegawa, K., Uehara-Bingen, M., Weis, K., and Heald, R. (2011) Importazole, a small molecule inhibitor of the transport receptor importin-beta. *ACS Chem. Biol. 6*, 700–708.

(22) Rousaki, A., Miyata, Y., Jinwal, U. K., Dickey, C. A., Gestwicki, J. E., and Zuiderweg, E. R. (2011) Allosteric drugs: the interaction of antitumor compound MKT-077 with human Hsp70 chaperones. *J. Mol. Biol. 411*, 614–632.

(23) Wisen, S., Bertelsen, E. B., Thompson, A. D., Patury, S., Ung, P., Chang, L., Evans, C. G., Walter, G. M., Wipf, P., Carlson, H. A., Brodsky, J. L., Zuiderweg, E. R. P., and Gestwicki, J. E. (2010) Binding of a small molecule at a protein-protein interface regulates the chaperone activity of hsp70-hsp40. *ACS Chem. Biol. 5*, 611–622.

(24) Quann, E. J., Merino, E., Furuta, T., and Huse, M. (2009) Localized diacylglycerol drives the polarization of the microtubule-organizing center in T cells. *Nat. Immunol. 10*, 627–635.

(25) Huse, M. (2011) Lymphocyte polarity, the immunological synapse and the scope of biological analogy. *Bioarchitecture 1*, 180–185.

(26) Yount, J. S., Moltedo, B., Yang, Y. Y., Charron, G., Moran, T. M., Lopez, C. B., and Hang, H. C. (2010) Palmitoylome profiling reveals S-palmitoylation-dependent antiviral activity of IFITM3. *Nat. Chem. Biol. 6*, 610–614.

(27) Currie, C. R., Wong, B., Stuart, A. E., Schultz, T. R., Rehner, S. A., Mueller, U. G., Sung, G. H., Spatafora, J. W., and Straus, N. A. (2003) Ancient tripartite coevolution in the attine ant-microbe symbiosis. *Science 299*, 386–388.

(28) Renner, L. D., and Weibel, D. B. (2011) Cardiolipin microdomains localize to negatively curved regions of Escherichia coli membranes. *Proc. Natl. Acad. Sci. U.S.A. 108*, 6264−6269.
(29) May, J. F., Levengood, M. R., Splain, R. A., Brown, C. D., and Kiessling, L. L. (2012) A processive carbohydrate polymerase that mediates bifunctional catalysis using a single active site. *Biochemistry 51*, 1148−1159.
(30) May, J. F., Splain, R. A., Brotschi, C., and Kiessling, L. L. (2009) A tethering mechanism for length control in a processive carbohydrate polymerization. *Proc. Natl. Acad. Sci. U.S.A. 106*, 11851−11856.
(31) Culyba, E. K., Price, J. L., Hanson, S. R., Dhar, A., Wong, C. H., Gruebele, M., Powers, E. T., and Kelly, J. W. (2011) Protein native-state stabilization by placing aromatic side chains in N-glycosylated reverse turns. *Science 331*, 571−575.
(32) Hanson, S. R., Culyba, E. K., Hsu, T. L., Wong, C. H., Kelly, J. W., and Powers, E. T. (2009) The core trisaccharide of an N-linked glycoprotein intrinsically accelerates folding and enhances stability. *Proc. Natl. Acad. Sci. U.S.A. 106*, 3131−3136.
(33) Price, J. L., Powers, D. L., Powers, E. T., and Kelly, J. W. (2011) Glycosylation of the enhanced aromatic sequon is similarly stabilizing in three distinct reverse turn contexts. *Proc. Natl. Acad. Sci. U.S.A. 108*, 14127−14132.
(34) Piljic, A., de Diego, I., Wilmanns, M., and Schultz, C. (2011) Rapid development of genetically encoded FRET reporters. *ACS Chem. Biol. 6*, 685−691.
(35) Shah, N. H., Vila-Perello, M., and Muir, T. W. (2011) Kinetic control of one-pot trans-splicing reactions by using a wild-type and designed split intein. *Angew. Chem., Int. Ed. 50*, 6511−6515.
(36) Vila-Perello, M., and Muir, T. W. (2010) Biological applications of protein splicing. *Cell 143*, 191−200.
(37) Batjargal, S., Wang, Y. J., Goldberg, J. M., Wissner, R. F., and Petersson, E. J. (2012) Native chemical ligation of thioamide-containing peptides: development and application to the synthesis of labeled α-synuclein for misfolding studies. *J. Am. Chem. Soc.*, DOI: 10.1021/ja2113245.
(38) Goldberg, J. M., Batjargal, S., and Petersson, E. J. (2010) Thioamides as fluorescence quenching probes: minimalist chromophores to monitor protein dynamics. *J. Am. Chem. Soc. 132*, 14718−14720.
(39) Goldberg, J. M., Wissner, R. F., Klein, A. M., and Petersson, E. J. (2012) Thioamide quenching of intrinsic protein fluorescence. *Chem. Commun. 48*, 1550−1552.
(40) Goldberg, J. M., Speight, L. C., Fegley, M. F., and Petersson, E. J. (2012) Minimalist probes for studying protein dynamics: thioamide quenching of selectively excitable fluorescent amino acids. *J. Am. Chem. Soc. 134*, 6088.
(41) Tian, L., Yang, Y., Wysocki, L. M., Arnold, A. C., Hu, A., Ravichandran, B., Sternson, S. M., Looger, L. L., and Lavis, L. L. (2012) Selective esterase-ester pair for targeting small molecules with cellular specificity. *Proc. Natl. Acad. Sci. U.S.A. 109*, 4756−4761.
(42) Patricelli, M. P., Nomanbhoy, T. K., Wu, J., Brown, H., Zhou, D., Zhang, J., Jagannathan, S., Aban, A., Okerberg, E., Herring, C., Nordin, B., Weissig, H., Yang, Q., Lee, J. D., Gray, N. S., and Kozarich, J. W. (2011) In situ kinase profiling reveals functionally relevant properties of native kinases. *Chem. Biol. 18*, 699−710.
(43) Fedorova, A., Zobel, K., Gill, H. S., Ogasawara, A., Flores, J. E., Tinianow, J. N., Vanderbilt, A. N., Wu, P., Meng, Y. G., Williams, S. P., Wiesmann, C., Murray, J., Marik, J., and Deshayes, K. (2011) The development of peptide-based tools for the analysis of angiogenesis. *Chem. Biol. 18*, 839−845.
(44) Strieter, E. R., and Korasick, D. A. (2011) Unraveling the complexity of ubiquitin signaling. *ACS Chem. Biol. 7*, 52−63.