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The Interface of Chemistry and Biology Is Actually a Continuum

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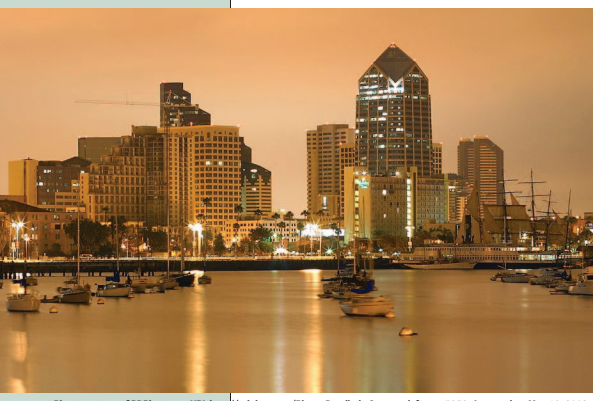

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The increasingly successful cohabitation of the chemical and biological sciences was on display at the 2008 Experimental Biology meeting held April 5−9 in San Diego. The most tangible evidence of this merger was supplied by the "Chemical Biology" sessions, which were organized by *ACS Chemical Biology* Editors Laura Kiessling and Anna Mapp. These sessions focused on important topics in the field (Table 1), and they provided a representative microcosm of the broader influence of chemistry at the meeting. In addition, more general themes transcended the individual biological topics and provided concepts of broader interest, such as subcellular aspects of protein function and the design principles of efficient chemical probes. In this Meeting Report, brief highlights of some of the individual accomplishments are provided in the context of their overarching thematic relevance.

Along the continuum of chemical and biological methods, biochemistry and structural biology continue to be key components of the chemical biologist's arsenal. Innovative applications of these tools were exemplified in talks from John May (Kiessling group, University of Wisconsin) and Junyu Xiao (Xu group, University of Michigan). May showed that the mycobacterial galactofuranosyltransferase (GlfT) is a polymerase that functions by an unexpected processive mechanism. Galactofuranose residues form the galactan polysaccharide in the cell-wall core of *Mycobacterium tuberculosis*. The authors found that GlfT elongates synthetic acceptors to produce polysaccharides that rival the naturally occurring galactan in length. Using a series of clever biochemical experiments, they showed that GlfT uses a tethering mechanism (rather than a template) to control polymer length. The timing and regulation of this process likely have fundamental ramifications on the viability of the causative agent in tuberculosis.

In a distinct biological system, Xiao used structural biology and biochemistry to provide mechanistic insights into the regulation of the multivesicular body pathway. With his colleagues, he showed that the C-terminal domain of a yeast regulatory protein, Vta1, directly stabilizes the crucial ATPase, Vps4 (*1*). Both Vps4 and Vta1 had been shown, *via* genetic experiments, to be important for ESCORT-III mediated vesicle budding, but the molecular details had not been clear. The insights provided by the authors' structural approach expose an additional level of regulation and coordination for vesicle assembly, with implications for subcellular trafficking and diabetes.

Adding innovative chemistry to classic methods provides new opportunities for asking previously inaccessible questions. One of the most elegant examples of this concept comes from the laboratories of Dennis Dougherty and Henry Lester (California Institute of Technology). At the Experimental Biology 2008 meeting, Lester led an excellent discussion of the use of non-natural amino acids to explore fundamental questions of protein function and allostery. In this system, the gene of interest, which con-

tains an amber codon (UAG) at a specific site in the coding sequence, is injected into *Xenopus* oocytes. A chemically modified, amber-suppressing tRNA is coinjected, such that the non-natural amino acid is incorporated into the resulting gene. This method has proven to be general, and a wide range of ion channels and receptors have been modified, including the nicotinic acetylcholine receptor (nAChR). By placing fluorinated residues into various locations in these channels, the Lester and Dougherty groups have made the remarkable discovery that a cation$-\pi$ interaction with a conserved tryptophan side chain in the agonist-binding site is a key determinant of activation (*2*). In addition, they showed exciting results using a series of constricted proline derivatives to identify key allosteric mechanisms in nAChR (*3*). These findings provide clear examples of how non-natural amino acids can be used to ask sophisticated physical chemistry questions with clear implications for drug discovery and receptor biology.

Lei Wang (Salk Institute) followed this discussion with an exciting glimpse into how this system can be applied as a genetically encoded technology. Specifically, he reported his group's efforts to incorporate non-natural amino acids into proteins in yeast, mammalian cells, and stem cells (*4, 5*). This goal has two main barriers: expression of prokaryotic tRNA in eukaryotic cells and evolving a mutant aminoacyl-tRNA synthetase specific for the desired analog. Because prokaryotes and eukaryotes differ significantly in tRNA transcription and processing, the Wang group employed internal leader *pol* III promoters to express bacterial tRNAs in yeast. In addition, they created a yeast strain that is deficient in nonsense-mediated mRNA decay, a surveillance mechanism in eukaryotes to degrade mRNA containing premature stop codons. Strikingly, they found that non-natural amino acids could be incorporated ~300-fold more efficiently in this engineered strain than in wild-type yeast. A transferring strategy was used to solve the second barrier: mutant synthetases were evolved in yeast and then transferred into mammalian cells. Using these new approaches, they successfully incorporated non-natural amino acids in cultured mammalian cells including HEK293 cells, primary neurons, and stem cells. In preliminary biological studies, they applied these tools to the study of the K^+ channel Kv1.4. By selectively placing restrictively bulky side chains in desired positions, the Wang group mapped regions required for channel function, a finding that had not been possible using conventional mutagenesis.

Spatiotemporal Control at the Subcellular Level. One of the missing aspects of the genomic and proteomic revolution is the regulatory role of spatial localization. Most omic techniques are performed on dissociated cellular lysates, and the procedures used to generate these lysates abolish subcellular and spatial information. However, spatial information has been shown to dictate selectivity in numerous pathways, so a need exists for new methods that enable studies of protein function and localization in natural environments. Toward this goal, a series of excellent talks focused on supplying new fluorophores and peptide-based probes that directly report on enzyme function with unprecedented spatiotemporal resolution. For example, Ron Raines (University of Wisconsin) has developed a series of clever, masked fluorophores. In one application, rhodamine was modified with a "trimethyl lock", which upon hydrolysis of an ester undergoes rapid lactonization and dramatic enhancement in fluorescence intensity (*6*). Moreover, this masked-fluorophore approach has also proven versatile, with many spectral variants described (*7*). The Raines group has coupled a fundamental understanding of physical organic chemistry with in-depth knowledge of the key qualities that are desired in a probe to yield a useful series of reagents.

In another example, Jin Zhang (Johns Hopkins University) provided a compelling synopsis of her group's fluorescent kinase reporter strategy (*8*). Briefly, a matched phosphorylation substrate and a phosphoaminoacid-binding domain were fused to a FRET pair. Kinase activity on the substrate brings together the two domains and enhances the FRET signal. After a series of impressive technical improvements, the most recent reporters provide robust, real-time monitoring of protein kinase A (PKA) function. Her group has applied this A-kinase activity reporter tool to study kinase activity in stimulated mammalian cells and to quantitatively explore the relationships between cAMP and PKA function (*9*). With continued improvements in fluorescent protein technologies, these probes will likely become increasingly sensitive reporters that are widely applicable to other protein–protein interactions.

To target a distinct set of protein functions, May Morris (CRBM-CNRS, University of Montpellier, France) presented a series of mitotic kinase and phosphatase sensors. These sensors are based on environmentally sensitive fluorophores appended to conformationally or structurally selective mimics of protein interfaces and are introduced into living cells thanks to cell-penetrating peptides. These tools provide sensitive monitoring of specific interactions with partners or substrates in live cells and subcellular resolution of protein function. As evident from these talks and the poster sessions, there is a growing and recognized need for new fluorescent probes, each designed to report on biological processes with minimal invasiveness and maximal resolution and sensitivity. These tools address the "where" and "when" questions of protein function and, when combined with omic methods, provide a more comprehensive picture.

To fully understand a system, it is beneficial to examine (and even manipulate) the

TABLE 1. Experimental Biology 2008 "Chemical Biology" Symposium Sessions

	New Strategies for Imaging Protein Localization and Dynamics	
J. Zhang	Johns Hopkins U.	"Dynamic visualization of signaling activities in living cells"
B.R. Martin and B.F. Cravatt	Scripps Research Institute	"Proteomic visualization of dynamic palmitoylation"
J.T. Groves	U.C. Berkeley	"Imaging the mechanisms of signal transduction"
M.C. Morris, J. Scheper, M. Pellerano	U. Montpellier	"Sensors of mitosis"
S.Y. Breusegem, J.T. Blaine, R. B. Doctor, N.P. Barry, M. Levi	U. Colorado Health Sci. Center	"Microvillar protein trafficking and dynamics imaged by TIRF microscopy in living cells"
R.T. Raines	U. Wisconsin-Madison	"Latent fluorophores for biomolecular imaging"
	Chemical Perspectives in Neurobiology	
H. Lester	Caltech	"Chemical-scale studies of neuroreceptors and ion channels"
L. Wang, W. Wang, J.K. Takimoto, Q. Wang	Salk Institute	"Efficient incorporation of nonnatural amino acids in yeast, mammalian cells, and stem cells"
B.M. Olivera	U. Utah	"Using Conus venom peptides to understand nervous systems and discover drugs"
F.L. Robinson, I.R. Niesman, K.K. Beiswenger, J.E. Dixon	U.C. San Diego	"Loss of inactive phosphatase Mtmr13 leads to a Charcot-Marie-Tooth type 4B2 peripheral neuropathy in mice"
J.E. Gestwicki	U. Michigan	"Small molecules, chaperones and neurodegenerative disease"
E. Isacoff	U.C. Berkeley	"Optical probing of neuronal membrane proteins"
	Small Molecule Control of Protein Folding and Assembly	
A.K. Mapp	U. Michigan	"Dissecting protein complexes with small molecules"
J. Xiao, H. Xia, J. Zhou, I. Azmi, B.A. Davies, D.J. Katzmann, Z. Xu	U. Michigan	"Structural basis of Vta1 function in the multivesicular body sorting pathway"
A.Z. Ansari	U. Wisconsin-Madison	"Engineering small molecules that nucleate assembly of protein complexes"
J.F. May, R.A. Splain, C. Brotschi, L.L. Kiessling	U. Wisconsin-Madison	"The polymerase activity of a mycobacterial galactofuranosyl-transferase suggests a novel mechanism for template-independent processive polymerization"
L.R. Comstock and J.M. Denu	U. Wisconsin-Madison	"Analogs of sirtuin metabolite as chemical probes of nudix hydrolases"
T.W. Muir, R. McGinty, M. Pratt, C. Chatterjee, E. Schwartz	Rockefeller U.	"Making and breaking ubiquitin"
	Chemical Probes and Their Use in Identifying New Therapeutic Targets	
R. Peterson	Harvard Med. School	"Connecting development and disease pathways through zebrafish chemical biology"
J.E. McDunn, H. Kashiwagi, S. Vangveravong, P.O. Simon Jr., K.C. Chan, P. Goedegebuure, R.S. Hotchkiss, R.H. Mach, W.G. Hawkins	Washington U.	"Targeted delivery to tumors in vivo with sigma-2 ligands"
H.E. Blackwell	U. Wisconsin-Madison	"Synthetic ligands that disrupt bacterial quorum sensing pathways and outcomes"
K. Sefah, Y. Li, Z. Tang, W. Tan	U. Florida	"DNA aptamer as molecular recognition probe to selectively differentiate tumor cells"
C.M. Connelly, R. Grosely, R. G. MacDonald	U. Nebraska	"Bivalent mannose 6-phosphate-based ligands for the mannose 6-phosphate/insulin-like growth factor II receptor as potential antitumor agents"
L.L. Kiessling	U. Wisconsin-Madison	"Small molecule probes of mycobacteral cell wall assembly"

function of components in a native environment. While fluorescent probes are a powerful platform, the complexity of this problem has also fueled development of additional technologies. For example, both Jay Groves and Ehud Isacoff (University of California, Berkeley) discussed methods that incorporate aspects of engineering. The Groves group has discovered critical aspects of T-cell receptor (TCR) regulation using hybrid interfaces between live cells and engineered substrates combined with advanced microscopy applications, including total internal reflection fluorescence. Recognition of peptide antigens by the TCR triggers coordinated movements of the receptor and its coreceptors into organized spatial patterns. The Groves group uses hundreds of micropatterned TCRs to study the forces that govern these movements, and they have made a number of exciting discoveries (*10*), including the observation that receptor movements are coupled (with a similar flow of ~20 nm/s) and that the valency of the ligand interactions are sufficient to determine sorting order. Moreover, they showed evidence that lipid "rafts" may be more heterogeneous than previously thought, containing previously undefined microclusters.

Isacoff's group, together with the Trauner laboratory, is using clever light-regulated ion channels to gain control over neuronal function (*11*). In this system, photoswitchable tethered ligands (PTLs) are tethered to the receptor surface in positions adjacent to the active site. Light of the appropriate wavelength reversibly switches the azobenzene core of the PTL from an "off" to "on" state and, thereby, controls channel activity in the absence of native stimuli. When incorporated into the target receptor, these light-

gated channels can be used in increasingly complex ways. For example, a light-gated ionotropic glutamate receptor (LiGluRs) has been used in dissociated, primary neuronal cultures, complex 3D cultures, and even adult zebrafish. These studies are beginning to reveal the intricate circuitry of neuronal systems, which in many ways represents the most spatially hierarchical of biological systems.

Together, these examples (and others) provide evidence of the growing diversity of solutions to the problem of spatiotemporal control. These tools continue to improve, and it seems likely that this area will continue to be a major area of emphasis in chemical biology. Challenges that were discussed include the need for improved fluorescence intensity, advanced activity-dependent reporters, upgraded transfection efficiency, and the expanded availability of bio-orthogonal chemistries. In addition, generality will continue to be a goal, such that many different systems can be interrogated by these technologies. Finally, the information derived from these experiments needs to be coupled with genomic and proteomic data sets to provide integrated models. The goal is to map existing protein−protein networks and signaling pathways onto large-scale, subcellular colocalization data sets to filter out artificial interactions and produce more descriptive networks.

Chemical Probes: Diverse Strategies and Lessons from Nature. A core aspect of chemical biology has always been chemical inhibitors. These probes permit rapid inactivation of protein function and, therefore, are the tools of choice for studying loss-of-function phenotypes. In addition, optimized probes are often interesting launching points for drug discovery. There is not one "correct" way of identifying chemical probes and, currently, a combination of high-throughput screening, molecular design, computational methods, and medicinal chemistry is typically employed. Good examples of this approach, which also typify the diversity of solutions, were on display in talks from Laura Kiessling (University of Wisconsin), Randy Peterson (Harvard Medical School), Helen Blackwell (University of Wisconsin), and Lindsay Comstock (Denu group, University of Wisconsin). The Kiessling group has used a fluorescent, high-throughput screen to identify potential new therapeutics directed against cell wall biosynthesis in *Mycobacterium tuberculosis* (*12*). Their chemical library included custom-built, structure-guided compounds, along with more diverse collections (*13*). Importantly, during their studies, they found potent chemical leads and also identified exciting new mechanisms of enzyme function, including an unprecedented covalent flavin-galactose adduct. The Blackwell group designed and assembled a focused collection of N-acylated homoserine lactones and their derivatives. This collection is not only producing new inhibitors (and, interestingly, activators) of bacterial Biofilm signaling but also helping to define the substrate selectivity information that governs intra- and interspecies communication in prokaryotes (*14*). In another example, the Peterson group is using whole-animal zebrafish models to identify compounds that impact hematopoiesis and bone morphogenic protein signaling. They have been able to perform screens directly in a whole organism, which creates a higher bar to discovery but also accelerated compound maturation because the "hits" must be membrane-permeable and metabolically stable. In turn, these tools are both leads for drug discovery in leukemia and anemia and chemical probes for studying development (*15*). Finally, Comstock and Denu are using structure- and substrate-based design to uncover probes of the nudix hydrolases, enzymes that are critical for sirtuin-mediated gene regulation and that cleave pyrophosphate bonds to regulate the levels of acetylated ADP-ribose (*16*). Each of these groups has uncovered powerful chemical probes that will be used to reveal key molecular and regulatory features of their system of interest. In addition, these success stories are a good reminder that promising compounds can be discovered in multiple ways and that the best strategies often use a combination of screening and iterative design. Finally, it is clear from these talks (and others throughout the meeting) that chemical probes remain one of the most important "products" of chemical biology.

Because of evolutionary pressures, the most robust probes are sometimes those that are co-opted directly from natural systems. This paradigm was exemplified by Baldomero Olivera's work on conotoxins (*17*). The Olivera group has been studying fish-hunting snails that employ a class of peptide-based toxins, called conotoxins, to manipulate the nervous system of their prey. Work by Olivera and others has characterized the targets of some individual conotoxins, and a few of these compounds are now in use in the clinic for pain and other indications. Interestingly, they have also found that each snail's venom is a mixture of hundreds of individual conotoxins, and many of these peptides have unique and nonoverlapping molecular targets. Moreover, it is the combinatorial action of multiple conotoxins that yields the paralyzing phenotype and, from this work, it is obvious that nature has been using compound mixtures long before this practice became popular in high-throughput screening or therapeutics. Perhaps most importantly, these mixtures can also be used in purely discovery experiments to map neuronal networks. Conotoxins are known to cause severe paralysis, so the identification of each of the molecular targets in a mixture can provide insight into the key receptors required for normal neuromuscular function.

Chemical Mimicry. Mimicry is the ultimate form of flattery, and this concept has a strong place in chemical biology. Often, the complexity and redundancy of biological systems complicates attempts to understand the individual components while

chemical mimics can provide reductionist models in which to collect clear results. A few excellent talks at the 2008 Experimental Biology meeting made use of this approach, while also adding new twists. In one excellent example, Brent Martin (Cravatt group, Scripps Research Institute) presented an exciting new method for the identification of proteins that are post-translationally modified by palmitoyl groups. Protein *S*-palmitylation is important for reversible membrane anchoring and regulation of key signaling proteins. To identify sites of palmitylation in mammalian cells, Martin and Cravatt used a commercially available compound, 17-octadecynoic acid, in an entirely new way. Although this compound is sold as an inhibitor of fatty acid ω-hydroxylases, it has an ω-terminal acetylene that can be used in orthogonal coupling reactions with a biotin-azide using copper(I)-catalyzed cycloaddition chemistry. Using this probe and multidimensional protein identification technology (MudPIT), Martin and his colleagues found several hundred palmitylated proteins, including >20 small GTPases. This work advances our understanding of lipid post-translational modifications. Moreover, this work is an excellent example of how observations from nature (e.g., the natural substrates of palmitoyl transferases), combined with some judicious choices in chemical reagents, can be used to solve complex problems.

Small-molecule intervention at the site of protein−protein interactions has become an active area of research, and recent examples provide evidence that inhibitors of these contacts can be generated by mimicking one of the interacting partners (*18*). My group presented a variation on this idea, which is based on our work on small molecules that act on heat shock protein 70 (Hsp70) (*19*). *In vivo*, Hsp70 makes an important protein−protein contact with a co-chaperone, Hsp40, which stimulates Hsp70s chaperone activities. Using chemical screens, the Gestwicki group found a dihydropyrimidine that functionally replaces Hsp40. This compound binds at the site of Hsp70−Hsp40 interaction, stimulates Hsp70 function through the endogenous allosteric pathway, and partially rescues an Hsp40 loss-of-function mutation in yeast. Thus, this "artificial co-chaperone" functionally replaces the native protein−protein contact in a stimulatory (rather than inhibitory) fashion. Although the generality of this finding is not yet clear, the results provide evidence for a new method of chemical mimicry.

Both Aseem Ansari (University of Wisconsin) and Anna Mapp (University of Michigan) provided details of their group's development of artificial transcription factors (ATFs). ATFs are synthetic or semisynthetic reagents used to selectively probe and modulate transcription. Like natural transcription factors, ATFs are modular, and they are assembled from a DNA-binding group, which controls the recruitment to specific promoter sequences, and a transcriptional activation domain, which assembles the permissive polymerase complex. Unlike the natural system, however, these tools are synthetic, so the individual recognition properties can be carefully controlled. Using ATFs with polyamide-based DNA-binding domains, the Ansari group has defined the nucleotide recognition code for these reagents (*20*). In addition, they have rigorously approached the design criteria of these tools and have uncovered optimal linker characteristics and the temperature dependence for binding. In complementary work, the Mapp group has identified potent ATFs that incorporate isoxazolidines as transcriptional activation domains (*21*). Using their collection of derivatives, they have revealed that amphipathic topology is a critical structural component. This is interesting because natural activation domains are also known to have amphipathic character, and they have found that their ATFs and the natural examples bind similar regions on components of the transcriptional complex. Together, the work of the Ansari and Mapp groups is revealing the design principles underlying the generation of potent ATFs. At the same time, these chemical mimicry studies are helping to elucidate the features that might be important for natural regulatory mechanisms.

One of the problems with studying natural systems is their heterogeneity, redundancy, and complexity. In some cases, progress might be made by reducing the system to its components and then isolating the contributions of each part. This idea was expertly illustrated by Tom Muir (Rockefeller University), who discussed his group's work on homogenously modified histones. Histones are extensively regulated by post-translational modifications, including acetylations, methylations, and ubiquitinations, and in turn, these appendages govern transcriptional competence. The problems are that there are thousands of possible combinations and, thus, histones isolated from natural sources are heterogeneous, and the biological role of an individual modification is not easy to discern. Using a chemical ligation approach, the Muir group has been able to produce chemically defined preparations of histone 2B (H2B) (*22*). This resulting H2B contains a single ubiquitin at lysine 120, and the Muir group has used this reagent to provide mechanistic insight into histone function. Histone modification remains one of the most active areas of science (with ~3500 papers in the last year), and it remains a fertile arena for chemical approaches. The example provided by the Muir group provides a successful framework for further studies.

The Spread of Chemical Biology. In addition to the formal "Chemical Biology" sessions, parallel symposia incorporated characteristic aspects of the field in areas as diverse as protein misfolding, nuclear hormone receptor biology, antibiotic strategies, and G-protein-coupled receptors. Together, these sessions reaffirmed that the

blurry lines between chemistry and biology are increasingly porous and that chemical methodologies are being leveraged to provide new insights throughout the most important biological systems. However, another theme is that the spread of chemical biology is not yet complete. Many speakers directly called for increased efforts to generate chemical probes for their systems of interest. This idea might be best illustrated by an excellent session called "Drug Discovery in Academic Settings" organized by Jeff Conn (Vanderbilt University). Included in this series were seminars by John Lazo (University of Pittsburgh), Hugh Rosen (Scripps Research Institute), and Colleen Niswender (Vanderbilt University), leaders in the National Institutes of Health-sponsored Molecular Libraries and Screening Centers Network sites and other academic probe discovery programs. The existence (and popularity) of these centers is indicative of the desire for new chemical probes within the biology communities. As outlined by the speakers, one challenge is to continue to answer this call with innovative, cost-effective, and robust approaches.

Brief Synopsis and Major Themes. What can we take away from the Experimental Biology 2008 Meeting? A detailed examination of all the experimental findings is not practical here (nor is it the goal), but the reader is encouraged to focus on a few major philosophical underpinnings. First, attendees were given the satisfying impression that biology is being well served by its association with organic chemistry, engineering, and other disciplines. Certain methods, such as fluorescent probes, have become integral to the modern biological community because these tools allow their users to ask increasingly sophisticated questions—including those that are not within the reach of classic methodologies. Put another way, a chemical probe is only as successful as the biological insights it allows, and the truly powerful methods are simple, elegant, and generally useful. As a field, chemical biology seems situated to support the greater scientific endeavor through its continued contributions in this area.

Second, one of the hallmarks of the chemical biology field is the scope of subjects discussed. An attendee at a given symposium will likely be exposed to organic synthesis, molecular biology, patch-clamp methods, biochemistry, genetic screens, NMR, crystallography, and a host of other methods. Truly, the interface between chemistry and biology has become increasingly vague, to the point where a symposium of this kind is best described by a continuum between the two fields. The challenge, as always, is to navigate the continuum while incorporating new ideas and giving freely of one's own expertise.

Finally, if intellectual advances emerge from the chance meetings of typically disparate concepts, then these sessions were fertile soil. Similarly, we hope that this Meeting Report can impart the feeling of excitement that arises from learning new things and placing them in context with one's intellectual base. Often, scientists may be well versed in their own area, they may have a firm grasp on their area's literature, and, within a narrowly focused session, they might have a good idea of what the speaker is going to present. However, "Chemical Biology" sessions are intellectual buffets that provide a filling meal for those who are open to trying new things. Perhaps this property is what makes chemical biology so effective as a recruiting tool for excellent young students.

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