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EMBL Conference on Chemical Biology 2008

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The tremendous progress made in the use of molecular biological techniques over the past several decades has been the main driving force in expanding our knowledge of biology at the molecular level. This development has pushed classical approaches, such as those based on synthetic chemistry, somewhat into the background. There has even been a tendency to regard contributions from this area as being dispensable. This attitude has dramatically changed again, with the emergence of the field termed "Chemical Biology". It is now widely appreciated that synthetic chemistry in combination with modern biological methods and computational chemistry can make unique contributions to the outstanding problems in fundamental biological and medically oriented research.

Many facets of this truly interdisciplinary field were discussed during a symposium on Chemical Biology, held from October 8th to 11th 2008 at the European Molecular Biology Laboratory (EMBL) in Heidelberg (Germany). The organizers, Maja Köhn, Joe Lewis and Carsten Schulz from EMBL, put together a program of more than 40 presentations by eminent speakers covering many aspects of chemical biology, including tools for molecular imaging, computational approaches, screening methods, molecular engineering, and novel synthetic methods. 250 scientists attended the symposium to learn about these methods and approaches being applied to solve biological problems.

The still growing importance of chemical biology was also reflected by the number of journals now dedicated to this field such as this journal, *Nature Chemical Biology*, *Chemistry & Biology*, *ACS Chemical Biology*, and new additions such as the *Journal of Chemical Biology*. Representatives of these journals were present in Heidelberg to hear more about recent highlights and to see where the field is heading in the future.

Since chemical biology thrives on contributions from two classical fields, chemistry and biology, being applied to biological questions, it has become a new central discipline in many chemistry and biology departments.

Computational approaches have already aided researchers in drug development for several years and should become even more powerful tools for identifying lead structures and for the design of new drugs in the future. Results from in silico experiments are good starting points for the development of focused compound libraries and for identifying new drug targets. The symposium was opened by Malcolm Walkinshaw from the University of Edinburgh, UK, describing a computational approach to identify and test small molecules against antiparasitic drug targets. This area is not well covered by large pharmaceutical companies. Computational approaches based on data base mining as well as on virtual docking have led to promising starting points for combinatorial synthetic chemistry aimed at cyclophilin A mimetics and for targeting cyclin analogues in protozoan parasites.[1] Gabriele Cruciani (University of Perugia, Italy) complemented this approach with methods to analyze and compare proteins and ligands in silico. He used molecular interaction fields (MIFs) to identify potential binding sites for small molecule features. These sites can subsequently be used by the FLAP (Fingerprint for Ligands and Proteins) software to virtually screen pharmacophores that could fit into these "protein pockets".[2]

The difficulties encountered when trying to identify new lead compounds in the pharmaceutical industry and new ways to chart chemical and biological space according to target families were described by Karl-Heinz Baringhaus (Sanofi–Aventis Deutschland GmbH). Substructure and similarity searching in combination with virtual screening in target-family-related compound libraries have led to the identification of ion-channel modulators. In order to apply such techniques in an academic setting, scientists have to rely on publicly available compound records and related biology data. Bernd Wendt from EMBL described an approach to identify compounds related to a specific lead structure in the public data base PubChem. Even though this data base covers more than 19 million compound records, the much smaller number of relevant biological data sets has limited this approach until now.

Many potential drug candidates fail during clinical development due to inadequate pharmacokinetic properties or off-target side effects. Igor Tetko (Helmholtz Zentrum München, Germany) suggested improvements in how to predict in silico ADME properties or toxicity of these compounds.

The ability to tackle complex questions related to changes in protein localization in connection with dynamic post-translational modifications was impressively demonstrated by Herbert Waldmann from the Max Planck Institute of Molecular Physiology (Dortmund, Germany). He described an approach based on the semisynthesis of lipidated Ras proteins in combination with live-cell imag-

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ing methods that led to an understanding of how *S*-palmitoylation and depalmitoylation of Ras control its localization on cell membranes.[3] The option to chemically modify lipid modifications of different Ras isoforms allowed the inhibition of depalmitoylation, for example, by the use of thioether instead of thioester linkages, and eventually led to the identification of an enzyme that could be responsible for Ras (de-)palmitoylation, acyl protein thioesterase 1. This protein is now under investigation as a potential drug target that could be used to regulate the important oncogene Ras, which has so far eluded most drug-development efforts.

Tom Muir from the Rockefeller University (New York, USA) surprised the audience by talking about his work on quorum sensing in staphylococci and not his efforts to synthesize post-translationally modified histone proteins, as previously announced. This process is mediated by small cyclic autoinducing peptides (AIPs) that contain a thiolactone structure. Chemical synthesis of different AIPs allowed their interaction with their cognate AgrC receptors (I–IV) to be studied and provided information about the amino acids in the receptor that determine receptor specificity. Further studies with genetically engineered receptors provided new insights into receptor activation and cross reactivities of AIPs.[4] These findings nicely demonstrated how chemical biology can be used to understand bacterial warfare as well as to gain information that can be of therapeutic value when used to control the virulence of staphylococci.

Chemical modifications of biomacromolecules allow control over biologically important interactions. Oliver Seitz (Humboldt University, Berlin, Germany) made the case for using chimeric (bio-)molecules to control protein–protein and protein–nucleic acid interactions. He presented an approach to control SH2 binding to a peptide–PNA chimera by hybridization-dependent switching of peptide conformation[5] and new tools for chemical protein synthesis by using native chemical ligation.

Thomas Carell (LMU München, Germany) has been interested in DNA repair for quite some time, and he described chemistry to introduce lesions into DNA as well as new insights into the mechanism of how glycosylases specifically recognize such lesions. His latest results include structural and mechanistic information on a DNA (6–4) photolyase. The following talk by Michal Hocek (AS CR, Prague, Czech Republic) also dealt with functionalizing nucleic acids by using a cross-coupling reaction to obtain aminophenyl- and nitrophenyl-labeled nucleoside triphosphates and their subsequent incorporation into DNA by a polymerase reaction.[6] Such nucleobase modifications can be used as electrochemical labels in DNA hybridization and sequencing. Elmar Weinhold (RWTH Aachen, Germany) presented new aspects of an enzymatic approach for sequence-specific DNA labeling. This approach is based on chemically modified variants of the cofactor *S*-adenosylmethionine (SAM) that can be utilized to transfer either small reporter groups or the entire cofactor, including base modifications, onto DNA molecules.[7]

Recognition of carbohydrates by proteins represents another example of highly specific recognition between different classes of biomacromolecules. Peter Seeberger (ETH Zürich, Switzerland) presented in his talk fast, automated synthesis approaches and applications of complex carbohydrates specific to certain parasites that cause devastating diseases such as malaria. Glycosylphosphatidylinositol (GPI) structures on surfaces of the parasite *Plasmodium falciparum* play a crucial role during the infection of red blood cells, and a vaccination strategy that raises antibodies against these GPIs could impart protection against infection.[8] The development of glycan microarrays was also described as a potential diagnostic tool.[9] Such glycoarrays were also a topic of Chi-Huey Wong's (Academia Sinica, Taipei, Taiwan) talk, who used them for high-throughput analysis of protein–glycan interactions.[10] His talk was a tour de force, showing the audience many of the successful strategies for glycoprotein synthesis carried out in his laboratory and their application in diagnosis and drug development. In order to improve our understanding of glycosyltransferases and to set up new screening systems for inhibitors of this class of enzymes, Gerd Wagner (University of East Anglia, Norwich, UK) presented the synthesis and initial applications of novel sugar nucleotides. His versatile synthesis allows quick access to nucleobase-modified UDP and GDP sugars that can provide a strong fluorescence signal in glycosyltransferase assays.[11]

Hagan Bayley (Oxford University, UK) showed how "soft" micromachines can be constructed based on the ability of small water droplets covered with a lipid monolayer to form networks in a hydrocarbon environment.[12] The connection between these droplets is formed by a lipid bilayer that can also be functionalized by incorporation of proteins. Engineered variants of α-hemolysin, the preferred membrane pore in Bayley's laboratory, were used to build droplet networks that can respond to light or act as an electrical circuit.

High-throughput screening and its success, or lack thereof, is always a matter of debate between scientists in academia and industry. During this meeting, one session was devoted to problems and challenges related to this approach, with special emphasis on screening facilities set up either by academic institutions alone or in collaboration with industry. Facilities from Hamburg, Germany (European Screening Port), Cambridge, USA (Broad Institute of Harvard and MIT), Sutton, UK (Institute of Cancer Research) and Dundee, UK (University of Dundee) presented their approaches to high-throughput screening and how scientists can take advantage of these facilities. These presentations were complimented by two contributions from industry presenting new assay technologies (Roger Bosse, Perkin–Elmer LAS Inc.) and drug discovery strategies (Dirk Eberhard, Cellzome, Germany). Heino Prinz (Max Planck Institute of Molecular Physiology, Dortmund, Germany) suggested a biochemical explanation and mathematical analysis of screening hits with apparent nonstoichiometric binding, a phenomenon commonly observed in HTS campaigns.[13]

Tobias Meyer from Stanford University (USA) described approaches to understanding the flow of information in cells by using tools for in vivo imaging in

combination with chemical perturbations and RNA interference. He covered a wide area of different cell functions, such as control of cell migration, oscillation in concentration of signaling molecules such as calcium, and how small polybasic protein domains can recruit proteins to membranes. These data should eventually lead to the very heart of systems biology: a quantitative model of the cellular control systems.[14] In order to achieve this goal, scientists need to shed light on many signaling pathways in cells and require a highly variable tool box to do so. Kai Johnsson from the Ecole Polytechnique Fédérale de Lausanne, Switzerland, develops such tools in his laboratories. In his talk, he focused on combining well-established calcium-sensitive dyes with his O6-benzylguanine derivatives for labeling proteins containing a SNAP tag and on multiprotein labeling using the newly developed CLIP tag.[15] Gerard Marriott (University of Wisconsin, Madison, USA) uses and develops optical switches to observe low abundant proteins in cells and animals. He described the development of a new imaging approach termed optical lock-in detection (OLID) that provides impressive results even in the presence of high background signals.[16]

Yasuteru Urano from the University of Tokyo, Japan, described how synthetic modifications of fluorophores can be used to make them responsive to changes in the concentrations of analytes such as oxygen, nitric oxide and glutathione or in pH. He further presented examples for the detection of the level of these molecules inside living cells.[17]

Several talks dealt with the chemical biology of phosphoinositides, which are lipid-anchored secondary messengers at the heart of many signaling pathways. Tamas Balla from the NIH (Bethesda, USA) presented tools to detect these molecules inside cells as well as an elegant way to control their cellular levels by chemically induced protein–protein dimerization.[18] An even more versatile way to manipulate phosphoinositides was developed by Carsten Schultz by establishing synthetic access to membrane-permeable phosphoinositide prodrugs. Using these tools he "bypassed" receptor tyrosine kinase-induced PIP_3 production and could show that, for the EGF receptor (which normally signals through this pathway), PIP_3 generation is sufficient for internalization without the need for receptor activation. This theme was completed by Barry Potter (University of Bath, UK), who presented synthetic strategies for potent analogues of the downstream secondary messenger IP_3.

The power of molecular labeling was further demonstrated by Carsten Hoffmann (University of Würzburg, Germany) who accomplished the double labeling of the α2A-adrenergic receptor; this allowed the detection of conformational changes of this GPCR upon activation by FRET.[19] Adriano Henriques (ITQB, Portugal) discussed structural data for the bacterial enzyme transglutaminase. These findings can help to develop this enzyme into a tool for site-specific protein modification. Scott Stenson (Janelia Farm Research Campus, USA) presented the protein engineering of a ligand-activated ion channel that would only respond to synthetic ligands. If expressed in a cell- or tissue-specific manner, for example, in transgenic mice, this approach could allow an unprecedented chemical control of neuronal activity.

Glenn Prestwich (University of Utah, USA) turned the attention of the audience to the extracellular processes that are required, for example, for wound healing. By chemical crosslinking of hyaluronan-based hydrogels, special properties of the normal extracellular matrix can be mimicked; this substantially facilitates tissue regeneration.These materials are now in development as medical devices for humans and animals.[20]

Henning Mootz (Technische Universität Dortmund, Germany) introduced improvements in split-intein technology using the Ssp DnaB and Mxe GyrA inteins and their application for introducing a prelabeled cystein-based tag into proteins. Mechanistic studies of the DnaB artificial split intein system have shed more light on side reactions during splicing.[21] Luc Brunsfeld from the Technical University Eindhoven (The Netherlands) exploited the intein technology to site-specifically introduce phosphorylated residues into the ligand binding domain of the estrogen receptor (ER). Using these semisynthetic ER variants he could show that phosphorylation modulates cofactor binding.

Temporal control is of paramount importance in understanding the dynamics of living systems. Among the best methods for achieving such control are photoreleasable reagents ("caged" compounds) that can be activated on the microsecond timescale. Maurice Goeldner from the University of Strasbourg (France) introduced the audience to this concept and presented novel photocleavable chemical groups that are amenable to two-photon photolysis.[22]

In the evening session, Vern Schramm from the Albert Einstein College (Bronx, USA) took the audience on an exciting journey into biochemical catalysis. Using a set of isotope-labeled nucleotide analogues, he determined a set of kinetic-isotope-effect constants for the rate-determining step of the enzyme purine nucleoside phosphorylase. From this he was able to construct a model for the transition state of the catalyzed reaction[23] and to design transition-state mimics with low picomolar affinities for this clinically relevant enzyme.

Gregory Verdine (Harvard University, USA) addressed a pressing unmet pharmacological problem, the inhibition of protein–protein interactions. He presented the stabilization of α-helical peptides by olefin metathesis-mediated crosslinking of side chains.[24] These analogues have a more stable active conformation, are more resistant to proteolytic degradation and—most importantly—have been shown to penetrate cell membranes. It would be interesting to elucidate the underlying mechanism for this remarkable property and to explore how general it is for this type of peptide modifications.

On the final day, Michael Famulok (LIMES Institute, Bonn, Germany) presented the use of aptamers to screen for small-molecule protein binders that would have been difficult to identify by traditional means. He applied this approach to cytohesins, which are members of the guanine-exchange factor family and for which no small-molecule probes had been available before. He continued by using the identified ligands for a thorough biological characteriza-

tion of cytohesins.[25] He showed that these proteins are involved in insulin signaling and that cytohesin inhibition can mimic a longevity phenotype that resembles a calorie-restriction paradigm. Athanassios Giannis from the University of Leipzig, Germany, presented the total synthesis of the steroid derivative cyclopamine, which causes a developmental defect leading to vertebrates (sheep, cows, fish) with only one eye. Renato Bauer outlined the diversity-oriented synthetic program at the Memorial Sloan–Kettering Cancer Center (New York, USA), focusing on the rapid transition-metal-catalyzed generation of polycyclic scaffolds starting from stereochemically defined enynes.

Proteome-wide knowledge is essential for the understanding of biological systems. As a pioneer in this field, Benjamin Cravatt (Scripps Institute, La Jolla, USA) introduced the audience to the activity-based protein-profiling approach that takes advantage of the unique reactivity of a certain subset of proteins. This was exemplified—with numerous biological applications—for the large family of hydrolases. Cravatt further presented an electrophoresis–LC-MS-coupled procedure for proteome-wide analysis of proteolysis events.[26] Genome-wide profiling was also the topic of Andres Jäschke (University of Heidelberg, Germany), who presented photoreactive capture probes with the aim of identifying novel RNA–small molecule interactions. Kirti Sharma (Max Planck Institute of Biochemistry, Munich, Germany) pursued a combined affinity purification–MS analysis approach for the profiling of the kinase proteome and extended this to a mass spectroscopy-based quantification of kinase inhibitor affinities.

George Reid (EMBL, Heidelberg, Germany) reported on the fast, cyclic nature of gene transcription at estrogen-receptor-responsive promoters. Importantly, he showed recent data indicating that these rapid changes in transcriptional activity correlate with the presence of chromatin-modifying enzymes and with the CpG methylation status of the promoter region. These results indicate that the epigenetic mark of CpG methylation might be much more dynamic than previously thought.[27]

The EMBL Conference on Chemical Biology 2008 has impressively demonstrated how the successful collaboration and mutual understanding of the chemical and biological communities can lead to very exciting science. Several creative chemical projects that were inspired by biological processes were introduced as well as many examples of biological progress that would have been unattainable without the integration of tailor-made chemical approaches. In this conference, the organizers succeeded in getting together many of Europe's leading figures as well as several key international representatives in the field of chemical biology. The science that was presented during the three days of the conferences can be regarded as one of the highest quality compilations that can be found in this field. It definitely whets the appetite and curiosity for the next EMBL Conference on Chemical Biology scheduled for September 2010.

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