Chemical biologists gather in Heidelberg

Maja Köhn & Carsten Schultz

Chemical biology is well defined at its core—chemistry helping to answer biological questions—yet the boundaries are rather fuzzy. What are the differences between chemical biology and pharmacology? Is intracellular imaging a branch of chemical biology, and what about screening libraries? At Chemical Biology 2008, held in Heidelberg in October, participants heard presentations covering all these topics and more.

Sometimes new research areas are ignited by a single conference or a series of meetings. This is not the case for chemical biology. The field has been around for some years now, and it is generally accepted as being important, especially to complement many other areas of biological research. Indeed, professorships in chemical biology are filled around the world. However, if you want to meet more than a couple of colleagues at a time, tough luck. So far, there has been the occasional symposium within big chemistry or biology events such as the annual American Chemical Society or European Life Scientist Organization meetings where a session chair gathers a small number of interesting speakers in chemical biology. This has now changed: this past October, we, along with Joe Lewis, the head of the European Molecular Biology Laboratory (EMBL) Chemical Biology Core facility, organized a full conference dedicated to chemical biology in its entirety. One of the motivations was simple—we wanted to get to know some of the most prominent scientists in the field. In addition, we hoped that bringing together the different areas of the field would spur discussions, learning, ideas, inspiration and collaborations.

Accordingly, Chemical Biology 2008, held 8–11 October 2008 in Heidelberg, was designed to span the entire spectrum of topics from high-content screening and modern drug development to the generation of molecular tools, new synthetic methods, fluorescent probes and live cell imaging. This reflects not only the diversity of the field but also its roots. Most of the participants of Chemical Biology 2008 were chemists by education. The big questions that chemical biologists step up to answer are, however, coming from biology.

Accordingly, it became very obvious from many of the presentations that the heart of research in the field is based on collaboration, collaboration, collaboration. For instance, Herbert Waldmann (Max Planck Institute (MPI) Dortmund) presented collaborative work identifying the factors controlling proper localization of Ras G proteins, which required chemists to prepare artificially lipidated versions of these proteins (**Fig. 1a**) as well as inhibitors of protein lipidation and biologists to analyze the cellular behavior of these chemical perturbations with spatial and temporal resolution[1]. Over the years, four groups at the MPI in Dortmund spanning organic chemistry, protein chemistry, imaging and cell biology contributed to the work. Below, we highlight some areas of significant advancement in chemical biology presented by the speakers in Heidelberg.

Protein and peptide engineering

Chemical biology often includes a large engineering and technology development component, as exemplified, for instance, by a new general glycosyltransferase assay presented by Gerd Wagner (University of East Anglia). Another prominent example of a technology developed in chemical biology is chemical protein ligation. Oliver Seitz (Humboldt University Berlin), Henning Mootz (Dortmund University of Technology) and Luc Brunsveld (Technische Universiteit Eindhoven) presented new developments, mechanistic studies and applications of this technique. Seitz reported the optimization of the thioester synthesis, an essential step in native chemical ligation, through a self-purification strategy and also described the use of penicillamine as a precursor of valine in a ligation-desulfurization reaction for ligation at valine[2]. In addition, he gave an overview of his work on the development of switchable enzymes as well as on DNA and RNA detection. Mootz provided insights into the mechanism of protein splicing for the use of split inteins in protein semisynthesis[3] and the development of a prelabeled cysteine tag for protein labeling through protein trans-splicing. An impressive example of this powerful technique was described by Brunsveld, who spoke about how nuclear receptor functioning is regulated via post-translational modifications. He introduced a methodology for generating phosphorylated ligand-binding domains of receptors to study the influence of phosphorylation on cofactor binding.

One of the leading experts in the field of chemical ligation, Tom Muir (Rockefeller University), discussed the intersection of peptide synthesis with quorum sensing. He described his investigation of the interaction of bacterial virulence factors—cyclic autoinducing peptides (AIPs, **Fig. 1b**)—with their cognate accessory gene regulator receptor (AgrC). By using a new linker for Fmoc-based thioester synthesis, Muir's group could rapidly synthesize AIPs for AgrC inhibition[4].

New paths toward therapeutics

The development of synthetic methods and new approaches for discovering effector molecules and drugs has been, and still is, an important driving force in chemical biology. Chemical biologists have established innovative methodologies to find small molecules that influence cellular systems and protein interactions,

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and have also provided input into new drug-design approaches. Accordingly, sessions were dedicated to new approaches in drug and target discovery including the development of new synthetic methods, high-throughput screening and *in silico* drug design.

Peter Seeberger (ETH Zurich; soon to be at the MPI Golm) presented the newest advances in the field of oligosaccharide solid-phase synthesis. He also described fascinating applications for these synthesized polysaccharides in the development of vaccines against malaria as well as for mechanistically dissecting and blocking inflammation in this disease[5]. Chi-Huey Wong (Academia Sinica) gave an overview of his work on glycoproteins. This included the synthesis and application of glycans as probes for glycoproteomics through methodologies such as glycan microarrays, with applications such as a new potential diagnostic tool for breast cancer (**Fig. 1c**)[6]. He presented case studies of carbohydrates as potential immunogens for HIV vaccine development and as potential antiviral agents. An enyne cyclization approach using sulfinamide lynchpins to obtain a multiscaffold library was introduced by Renato Bauer (Memorial Sloan-Kettering Cancer Center). Athanassios Giannis (University of Leipzig) explained the significance of the hedgehog signaling pathway in development by showing pictures in which treatment with cyclopamine, an antagonist of the hedgehog signaling pathway, resulted in one-eyed sheep. He continued by presenting the total synthesis of cyclopamine and exo-cyclopamine, an impressive accomplishment, and discussed the medical relevance of these compounds.

Further evidence for the importance of chemical biology to the pharmaceutical field was given by Gregory Verdine (Harvard University), Vern Schramm (Albert Einstein College of Medicine), Glenn Prestwich (University of Utah) and Benjamin Cravatt (The Scripps Research Institute). Verdine introduced his approach to drugging the undruggable: protein-protein interactions. His solution to the challenge was 'stapled' peptides, in which the α-helix was stabilized through macrocyclization and α-methylation (**Fig. 1d**). Verdine gave convincing examples of the activity of these peptides in cancer-relevant applications: for example, peptides targeting negative regulators of p53 (ref. 7), binders of BCL-2 domains and even inhibition of the transcription factor MAML. A deep insight into the catalytic mechanism of enzymes, and particularly into the transition state, was provided by Schramm. Using purine nucleoside phosphorylase, he demonstrated the importance of in-depth structural knowledge, as the synthesis of true transition-state analogs led to therapeutically relevant inhibitors with low-picomolar binding constants and high specificity between enzyme homologs of different species (**Fig. 1e**)[8]. Prestwich presented a medicinally relevant project of a different kind: he described fascinating applications of hyaluronan-based hydrogel, for example, in wound healing or in controlling the growth and differentiation of stem cells[9]. Cravatt gave an overview of his work on activity-based protein profiling (ABPP) and particularly in using this technique to pinpoint new cancer targets. In addition, he introduced a new technique called protein topography and migration

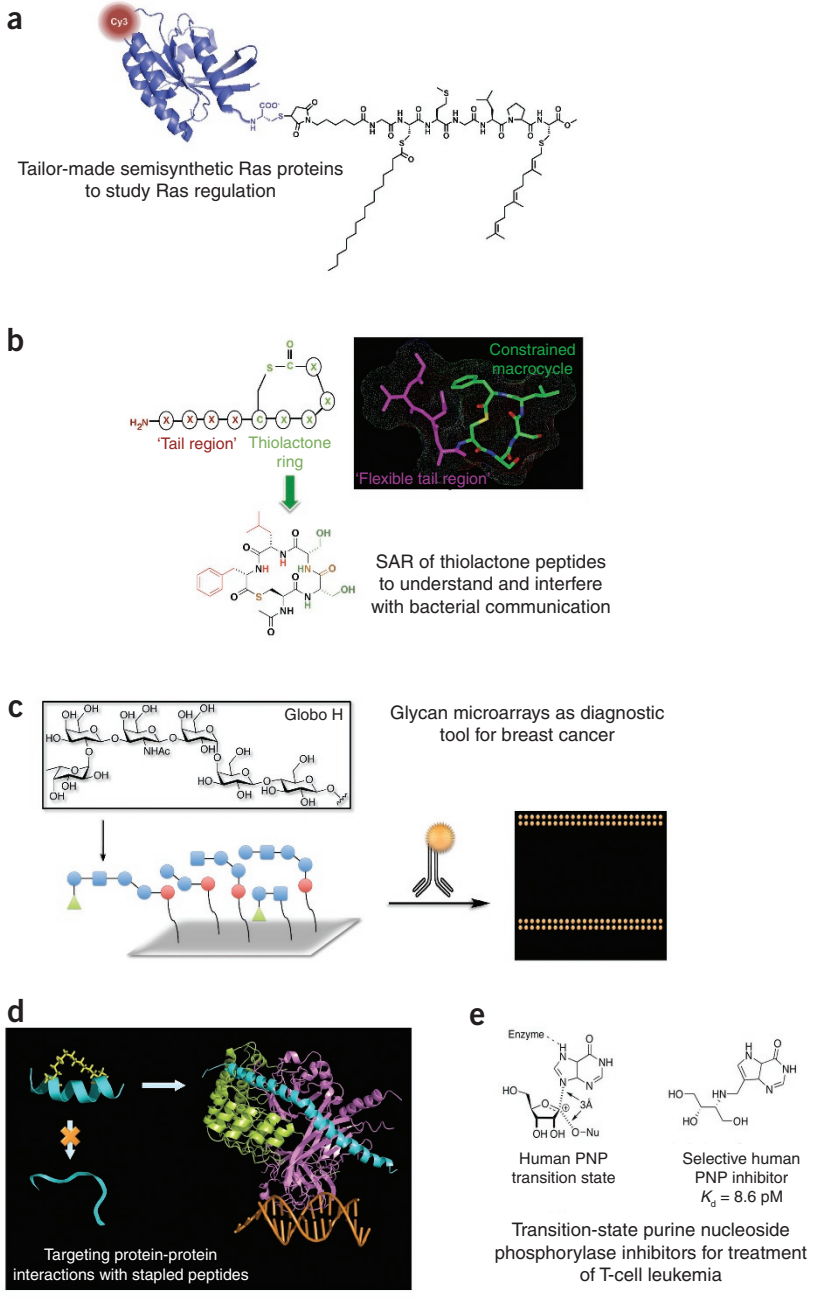

Figure 1 A wide variety of molecular tools were shown to be instrumental for new findings in biology. (**a**) Artificial lipidated C-terminal peptides that were essential for studying membrane-associated Ras function. (**b**) Cyclic autoinducing peptides mimicking bacterial virulence factors. (**c**) Glycoproteomics made possible by glycoprotein microarrays. (**d**) 'Stapled' peptides as potential drug candidates with preassembled conformation. (**e**) Super–strong-binding transition-state analogs designed based on detailed structural information. These approaches exemplify how creative work with small and large molecules is one of the hallmarks of chemical biology.

analysis platform (PROTOMAP) to identify cleavage events in apoptosis as apoptotic markers[10]. Kirti Sharma (MPI Munich) presented an approach to identifying targets using small molecules in which resin-bound kinase inhibitors were used to fish for kinases in cell lysates. Analysis of the data was carried out through a combination of quantitative mass spectrometry and several biochemical assays.

Figure 2 The Chemical Biology 2010 venue will be the new Advanced Training Center at EMBL Heidelberg (model by Bernhardt and Partners).

Searching for small molecules

Several presentations focused on ways to search for new small-molecule binders of target proteins. Michael Famulok (LIMES Institute) showed how his group uses an aptamer displacement screen to find small-molecule inhibitors. He demonstrated the great versatility of this technology by presenting its application to HIV-1 reverse transcriptase as well as to cytohesins[11], which are cytoplasmic guanine nucleotide exchange factors. Philip Gribbon (European ScreeningPort) introduced his institute, a public-private partnership designed to fill the gap between academic results and the pharmaceutical industry, with a particular focus on underexploited targets. The ESP has access to a screening library of 250,000 compounds and can use a plethora of screening techniques. Alykhan Shamji (Broad Institute) reviewed technology platforms, such as small-molecule microarrays, that are used at the Chemical Biology Program of the Broad Institute for the identification of small molecules against 'undruggable' targets and processes with impact on research and medicine. He described novel applications of these technologies, for example, screening in cancer stem cells, and stressed that the screening results are made publicly available through Chembank (http://chembank.broad.harvard.edu/). Wynne Aherne (Institute of Cancer Research) presented approaches for the discovery of cancer therapeutics[12]. Her group and collaborators follow drug candidates from high-throughput screening to phase 1 clinical trials. She also discussed the advantages and disadvantages of biochemical versus cell-based screens. Julie Frearson (University of Dundee) highlighted the university's high-throughput facilities and their focus on parasitic diseases. Industrial screening efforts, assay applications and drug discovery strategies were described by Roger Bosse (Perkin-Elmer) and Dirk Eberhard (Cellzome). Finally, Heino Prinz (MPI Dortmund) discussed non-stoichiometric binding phenomena in high-throughput screening.

Drug design

The session on *in silico* drug design was opened by Malcolm Walkinshaw (University of Edinburgh), who presented structure-based data-mining approaches using the unique docking program LIDAEUS[13]. Virtual screening of a database of 5 million available compounds followed by biochemical testing of *in silico* hits yielded promising inhibitors against parasitic immunophilins. Walkinshaw also reported on the identification of allosteric pockets of proteins by analyzing different conformational forms of target structures. A new docking algorithm, FLAP, was introduced by Gabriele Cruciani (University of Perugia). The algorithm combines the use of molecular interaction fields from GRID with pharmacophore fingerprints. By showing results from several case studies, Cruciani demonstrated the use of FLAP for selectivity studies, off-target interaction analysis and structure-based virtual screening. Karl-Heinz Baringhaus (Sanofi-Aventis) described his company's target-family approach for lead finding. A chemical biology space was constructed for a particular target family on the basis of chemical and biological data. Application of similarity searches and three-dimensional virtual screening methods led to target family–directed compound libraries for synthesis and testing. Baringhaus reported on the discovery of modulators for ion channels and G protein–coupled receptors using this approach. Bernd Wendt (EMBL Heidelberg) described how to run lead optimization in the absence of proprietary chemical and biological data. Starting from a lead structure, the PubChem database was mined for structure-activity relationships. He showed that resulting topomer CoMFA models could provide important direction to the drug design process. Lipophilicity is an important property of current *in silico* ADME (absorption, distribution, metabolism and excretion) and toxicology models. Igor Tetko (Helmholtz Zentrum München) presented how estimation of the accuracy of prediction can save drug development costs using as an example logP prediction with the ALOGPS program (http://www.vcclab.org). He also demonstrated how prediction errors can be reduced by applying local corrections to global models.

Imaging tools for cell biology

Hours before the conference started, Osamu Shimomura, Martin Chalfie and Roger Y. Tsien were announced as this year's Nobel prize winners for chemistry. The prize was given for the identification and development of fluorescent proteins and, accordingly, we were happy to have this important area represented with a session devoted to new visualization approaches. In a keynote lecture, Tobias Meyer (Stanford University) presented various imaging approaches to visualizing signaling events in migrating cells[14]. In addition, he shared with the audience the discovery of the store-operated direct calcium entry from the extracellular space into the endoplasmic reticulum. The community of researchers dedicated to labeling proteins in living cells was represented by Kai Johnsson (École Polytechnique Fédérale de Lausanne), Adriano Henriques (Universidade Nova de Lisboa), Elmar Weinhold (Aachen University) and Carsten Hoffmann (University of Würzburg). Johnsson introduced alkylguanine transferase–based multiprotein tagging and described the first covalent attachment of a calcium sensor to a protein of interest in living cells. New methods of protein tagging might arise from the work of Henriques and co-workers, who presented structural studies of a microbial/Tg1 transglutaminase, an enzyme that can transfer functionalized primary amines to protein-bound glutamine residues. Weinhold presented the synthesis of analogs of the

cofactor *S*-adenosyl-L-methionine for efficient DNA labeling through DNA methyltransferases. Hoffmann described how biarsenite-based FlAsH labeling of tetracysteine motifs can be applied to scrutinize G protein–coupled receptor behavior under native conditions in the plasma membrane.

Yet other essential imaging tools were presented by Gerard Marriott (University of Wisconsin; soon to be at the University of California, Berkeley) and Yasuteru Urano (University of Tokyo). Marriott achieved much-desired improvements in the signal-to-noise ratio of images by using very fast optical switches. In correlating image acquisition with the switching frequency, non-switching fluorescence is eliminated, thereby vastly reducing background fluorescence. This technique will be particularly useful for imaging low-abundance molecules in cells. Urano and his co-workers are addressing the heart of optical imaging—the fluorophore—by working systematically to improve existing fluorophores and develop new ones. The latter include fluorophores that are switchable through electron-density changes induced by pH variation as well as reporters for glutathione *S*-transferase[15].

Imaging is crucial for studying intracellular signaling molecules. Two talks by Tamas Balla (US National Institutes of Health) and Carsten Schultz (EMBL Heidelberg) focused on this topic. Balla presented ways in which chemical dimerizers such as rapamycin and its analogs can be used effectively to bring lipid-modifying enzymes to membranes, where their substrates reside[16]. Translocation thus acts as an inducible switch that can quickly perturb signaling pathways and is easily traceable by live cell imaging. Schultz's goal was similar: to rapidly induce changes in second-messenger concentrations by using membrane-permeant and photoactivatable derivatives of phosphoinositides to manipulate signaling events. By imaging receptor tyrosine kinase internalization, his group demonstrated the effects of 3-*O*-phosphorylated phosphoinositides on endocytosis. It was a short step from phosphoinositides to inositol polyphosphates. Barry Potter (University of Bath) presented the syntheses of new, potent inositol 1,4,5-trisphosphate receptor ligands as well as docking studies thereof, with the goal of making small molecules to interfere with calcium signaling in living cells.

More chemical probes of biology

The development of chemical tools and their use in basic biological research is one of the classic areas of chemical biology. In sections predominantly devoted to the area of (poly)nucleotides, Thomas Carell (Ludwig-Maximilians-Universität Munich) updated us on newly discovered mechanisms in DNA repair. His approaches ranged from chemical synthesis of relevant model compounds mimicking DNA lesions to structural biology, providing insight into the mechanisms by which glycosylases and photolyases tackle DNA lesions[17]. New functionalized nucleotides for enzymatic incorporation into DNA were presented by Michal Hocek (Academy of Sciences of the Czech Republic). The modified DNA was used *in vitro* to study hybridization or in DNA sequencing. Andres Jäschke (University of Heidelberg) develops techniques for the identification of riboswitches—RNA molecules that bind to small metabolites—from biological samples. He showed how to convert the metabolites into photoaffinity probes using trifunctional building blocks equipped with an affinity tag for isolation and a photo-cross-linking group. Meanwhile, the analysis of dynamic epigenetic DNA methylation patterns in estrogen receptor–responsive promoter regions was correlated with the cyclic nature of gene transcription by George Reid (EMBL Heidelberg). Maurice Goeldner (University Louis Pasteur Strasbourg) led the symposium away from nucleotides into the area of photoswitchable molecules. Goeldner gave a good overview of photoactivatable groups, so-called 'cages', and demonstrated applications of a novel class of nitrophenylethyl cages to the cellular adhesion motif RGD[18]. In addition, he reported on two-photon nitrophenylethyl cages for glutamate, which have excellent quantum yield and two-photon cross-sections. Hagan Bayley (Oxford University) is interested in molecular machines formed from small water droplets covered with a lipid monolayer in a very hydrophobic environment. When proteins are incorporated, the resulting droplet networks may respond to light or transport an electrical current. Scott Sternson (Howard Hughes Medical Institute Janelia Farm) introduced another important tool for cell biology: engineered ion channels that are specifically triggered by synthetic ligands using a bump-hole strategy. Applications of this approach in whole animals will provide new possibilities to modulate neuronal or tissue activity.

On to Chemical Biology 2010

With Chemical Biology 2008, we tried to provide an overview of this broad field. This was reflected in the high quality of the presentations, the strong participation of editors from the most relevant journals, and the over 250 participants and over 150 poster presentations. Chemical Biology 2010 will be held 22–26 September 2010 and will take place at EMBL's new Advanced Training Center (**Fig. 2**). With this next conference, we will continue to be committed to high quality and will further expand the time for poster sessions and interactions between the many members of the chemical biology community.

1. Rocks, O. *et al. Science* **307**, 1746–1752 (2005).
2. Haase, C., Rohde, H. & Seitz, O. *Angew. Chem. Int. Ed.* **47**, 6807–6810 (2008).
3. Ludwig, C., Schwarzer, D. & Mootz, H.D. *J. Biol. Chem.* **283**, 25264–25272 (2008).
4. George, E.A., Novick, R.P. & Muir, T.W. *J. Am. Chem. Soc.* **130**, 4914–4924 (2008).
5. Kamena, F. *et al. Nat. Chem. Biol.* **4**, 238–240 (2008).
6. Wang, C.C. *et al. Proc. Natl. Acad. Sci. USA* **105**, 11661–11666 (2008).
7. Bernal, F., Tyler, A.F., Korsmeyer, S.J., Walensky, L.D. & Verdine, G.L. *J. Am. Chem. Soc.* **129**, 2456–2457 (2007).
8. Taylor, E.A. *et al. J. Am. Chem. Soc.* **129**, 6984–6985 (2007).
9. Prestwich, G.D. & Kuo, J.W. *Curr. Pharm. Biotechnol.* **9**, 242–245 (2008).
10. Dix, M.M., Simon, G.M. & Cravatt, B.F. *Cell* **134**, 679–691 (2008).
11. Hafner, M. *et al. Nature* **444**, 941–944 (2006).
12. Rayter, S. *et al. Oncogene* **27**, 1036–1044 (2008).
13. Taylor, P. *et al. Br. J. Pharmacol.* **153**, S55–S67 (2008).
14. Inoue, T. & Meyer, T. *PLoS ONE* **3**, e3068 (2008).
15. Fujikawa, Y. *et al. J. Am. Chem. Soc.* **130**, 14533–14543 (2008).
16. Varnai, P., Thyagarajan, B., Rohacs, T. & Balla, T. *J. Cell Biol.* **175**, 377–382 (2006).
17. Maul, M.J. *et al. Angew. Chem. Int. Ed.* **47**, 10076–10080 (2008).
18. Petersen, S. *et al. Angew. Chem. Int. Edn. Engl.* **47**, 3192–3195 (2008).