Rudinger Award Lecture. Abstract number: 506
Through the Looking Glass - a New World of Proteins Enabled by Chemical Synthesis
S.B.H. Kent, K. Mandal, R. Okamoto, M. Avital Schmilovici, S. Liu
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Our most recent developments in chemical synthesis of proteins will be described, including a fully convergent total synthesis of EPO (Suhuai Liu). We have used chemical synthesis to determine novel Xraystructures of a series of otherwise recalcitrant proteins and a glycoprotein by crystallization of the (quasi)racemates. Design, convergent chemical synthesis, and structure of protein molecules with covalent topologies not yet discovered in nature will be described. Finally, chemical protein synthesis has enabled single molecule fluorescence and other spectroscopic studies of the mechanism of enzyme catalysis in the HIV-1 protease.

Zervas Award lecture.
Peptides as Asymmetric Catalysts and Templates for the Formation of Ag-Nanoparticles
Helma Wennemers, University of Basel, Basel, Switzerland

PL1. Abstract number: 593
Testing the 'Histone Code' Hypothesis Using Synthesis
T.W. Muir, R McGinty, D Chatterjee
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DNA in eukaryotic cells is packaged into chromatin. The fundamental unit of this chromatin is the nucleosome that is made up of 146 base pairs of DNA wrapped around a histone core octamer containing two copies of each histone (H2A, H2B, H3 and H4). The packaging of these nucleosomes into higher-order chromatin structures is a key determinant of gene expression making histone biology fundamental to all processes within a living organism. An ever-increasing body of information indicates that posttranslational modification of histone proteins is one way local chromatin structure is manipulated. These modifications affect their influence through changes in nucleosome structure, as well as recruitment of additional protein factors that mediate downstream functions. One such modification, ubiquitylation, occurs on histones 2A and 2B, has been implicated in gene transcription and in methylation of lysines 4 and 79 of histone 3. The mechanism by which this ”crosstalk’ occurs is unclear, primarily due to the inability to generate or isolate homogeneous ubiquitylated nucleosomes for biochemical studies. Here, we report the semisynthesis of homogeneous ubiquitylated H2B and its incorporation into nucleosomes and nucleosome arrays. Access to this ”designer chromatin”has allowed functional dissection of methylation of K79 by the methyltransferase, hDot1. Mechanistic insights from these studies will be discussed.

PL2. Abstract number: 597
The Dynamic Ras Cycle
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The Ras-proteins are farnesylated and palmitoylated membrane bound GTPases that hold a prominent position in one of the major signal transduction cascades of all human cells. They function as molecular switches translating growth factor-derived signals into biological responses, in particular cell growth and differentiation. Mutations in Ras are found in ca. 30 % of all human tumours making this oncogene product one of the most relevant targets for the development of anti-cancer drugs.
Interference with Ras signalling and localization is of major interest both from a basic science point of view as well as for potential pharmaceutical application.
In the lecture the identification of the dynamic cycle of Ras palmitoylation and depalmitoylation which orchestrates Ras localization on and signalling from the plasma membrane and the Golgi will be presented. This research included the development of methods for the synthesis of tailor-made Ras proteins and the knowledge-based development of small molecule inhibitors of Ras depalmitoylation as well as their application in live cell imaging as key techniques.
[1] Reviews: (a) L. Brunsveld, J. Kuhlmann, K. Alexandrov, A. Wittinghofer, R. S. Goody, H. Waldmann, Angew. Chem. Int. Ed., 2006, 45, 6622-6646; (b) M. H. Gelb, L. Brunsveld, C. A. Hrycyna, S. Michaelis, F. Tamanoi, W. C. Van Voorhis, H. Waldmann, Nat. Chem. Biol., 2006, 2, 518-528.
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DOI: 10.1016/j.cell.2010.04.007.
[5] F. J. Dekker, O. Rocks, N. Vartak, S. Menninger, R. Balamurugan, S. Wetzel, S. Renner, M. Gerauer, C. Hedberg, B. Schölermann, J. W. Kramer, D. Rauh, G. J. Coates, L. Brunsveld, P. Bastiaens, H. Waldmann, Nat. Chem. Biol., 2010, DOI: 10.1038/nchembio.362.

PL3. Abstract number: 583
Discovery of Glucagon-based Peptides with Broadened Molecular Pharmacology For the Treatment of Diabetes and Obesity
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Glucagon, glucagon-like peptide-1 (GLP-1) and gastric inhibitory peptide (GIP) are three gut hormones involved in the maintenance of glucose homeostasis and of sizable importance to the clinical management of diabetes. These three peptides exhibit specific association with their native receptors through structural elements within different regions of their amino acid sequences to confer differential pharmacology. We explored the structure-activity relationship of these three hormones through the use of single residue substitutions, hybrid peptides and backbone stabilization. As a result, we have identified a set of novel peptides which exhibit high potency and balanced activity across these three receptors. The structural basis for this change appears to be a combination of local positional interactions and a change in conformation to a more helical form. We have previously reported the combinatorial efficacy of receptor agonism at the glucagon and GLP-1 receptors to achieve potent satiety inducing and lipolytic effects in a single peptide of sustained duration of action (1). With this new set of gut hormone hybrid peptides we addressed the appreciable uncertainty pertaining to the relative wisdom of agonism or antagonism at the GIP

receptor for the treatment of diabetes and obesity. To explore the combinatorial efficacy of GIP pharmacology, we tested select peptides from a series of high potency analogs with differential dual receptor activity and found them to rapidly normalize adiposity and glucose tolerance levels in diet induced obese mice. These in vivo observations establish a basis for testing of these novel gut hormones in human subjects.

1. Day, J.W. et al. (2009) Nature Chemical Biology 5 (10), 749-57.

PL4. Abstract number: none

From peptides to proteins: Analysis of the hierarchic organization of proteins and de novo design of protein-like architectures.

W.F. DeGrado

The folding of water-soluble and membrane proteins reflects the hierarchic assembly of peptide-like segments into a structural and functional unit.

This talk will focus on the principles governing this assembly process, with particular reference to the analysis and design of: 1) natural and designed membrane proteins including the M2 proton channel from influenza A virus and artificial channel peptides; 2) metalloproteins; 3) nanostructured materials.

PL5. Abstract number: 582

Peptides Hormones for GPCRs: Tools and Drugs in Therapy and Diagnosis

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Peptides hormones play an important role in the regulation of activity. Most of them bind to G-protein coupled receptors and transmit activity via G-protein mediated signaling systems. Many peptides are involved in important regulatory mechanisms like the regulation of food intake (e. g. neuropeptide Y, PP, ghrelin), the modulation of pain and anxiety (NPFF, NPY) or the regulation of the sleeping (orexin). Additionally tumours frequently express receptors that selectively bind peptides. Owing to their manifold possibilities to modify peptides several analogues have been shown to target tumours and have been used in selective labelling.

Here strategies to modify peptides will be outlined including possibilities to increase stability, to analyze biodistribution and to use peptides in the regulation of food intake, in tumour diagnosis and as therapeutic shuttles.

I1. Abstract number: 575

Folding and Defolding of Helices Control Biological Functions

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In recent years it became clear that not all biological proteins are folded or contain only folded domains. We show folding and defolding of helices is often used to regulate the function of proteins.

The first example given here will be the CH1 domain of IgG antibodies which controls the folding and secretion of antibodies by its structural state. The structure of a folding intermediate of the CH1 domain explains differences in immunoglobulin amyloidogenicity between CL , VL , and β2m (β2 microglobuline). There, fibril for-mation is prevented by a short helix that seems to function as internal chaperon.

The second example is the DNA binding domain of p53 (DBD) where the helical parts do not only control binding to DNA (recognition of DNA strand breaks), but also mediate binding to BclxL which leads to the release of pro-apoptotic Bax/Bak and mediates the cytosolic pro-apoptotic function of p53. The same helices within p53DBD bind to the molecular chaperon Hsp90. The low stability and high flexibility of these helices is an essential part of the biological function of the proteins.

This is also the case for the C-terminal domain of the major ampullate protein of the spider silk ”spidroin”, which regulates controlled silk fibre assembly. We have determined the structure of this folded intertwisted homodimeric domain and could show that its unfolding upon defined stimuli (salt, shear stress, disulfide reduction) controls the formation of spider silk by providing correct alignment of the repetitive sequence elements of the silk spidroin, a prerequisite for stable inter-chain hydrogen bonds and the formation of crystalline β-sheets within the fibre.

I2. Abstract number: 596

Peptide Marine Natural Products, from a Challenge for the Chemists to a Base for the Development of New Drugs

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The role of natural products in drug discovery has suffered *ups* and *downs* during the last years. Recently and as the number of classical drugs is not increasing enormously, it looks that pharmaceutical companies are looking again towards the nature for inspiring new drugs. In particular, marine ecosystems have demonstrated to be a wide source of biological and chemical biodiversity. It is encouraging to note that during recent years the first three marine products have arrived on the market, thereby demonstrating the ”proof-of-concept”that the marine ecosystem is a source of new pharmaceutical compounds. Among the natural products, pharmaceutical industries had rekindled the interest in peptides, due to the current novel technological accomplishments, strategy developments, advances in the areas of formulation and enhanced drug delivery technology of peptides. Today, more than 40 peptides are in the pharmaceutical world market, and more than a hundred are in several clinical phases.

From a chemical point of view, peptides from marine source show unique features: *N*-and *O*-methylation, methoxylation, aza derivatives of hydroxyamino acids, and tricyclic units such as 1,2,3,3a,8,8a-hexahydro pyrrolo[2,3-b]indole-2-carboxylate (HPIC), formed by internal cyclization of a Trp residue. The presence of several *N*-methyl and β-branches in a row introduce extra difficulties in the elongation of the peptide chain.

In this presentation, synthetic methods developed in our laboratory for the preparation of the modified amino acids and for their incorporation (new coupling reagents) into the peptide chain will be discussed taking as example the Mayotlide, which has been recently isolated and contains a rather unusual *N-C* bond between the *C3a* of HPIC and the indole *N* of a second Trp residue, and other cyclic peptides from marine origin.

I3. Abstract number: 228

New Dimensions in Azapeptides and their Applications as CD36 Lignds for Treating Age-Related Macular Degeneration

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Azapeptides are peptide analogs, in which one or more of the alpha-amino acids is replaced by a semicarbazide surrogate. Rigidity about the urea and hydrazine of the semicarbazide induces the aza-residue to situate itself in the central positions of a β-turn conformation in the azapeptide, as determined by X-ray crystal analysis and computation. Furthermore, the semicarbazide introduces increased chemical stability and enzyme resistance to azapeptides, which have been used to develop drugs with improved pharmacokinetic properties such as prolonged duration of action. The synthesis of azapeptides has, however, been challenging, due to the inherent difficulties of making substituted hydrazines. In efforts to develop ligands for the CD36 scavenger receptor as targets for treatments of age-related macular degeneration, the leading cause of adult blindness, we have pursued the synthesis of azapeptides and have developed a submonomer approach featuring modification of a semicarbazone moiety. Alkylation, conjugate addition and arylation of the protected aza-glycine residue have now provided entrance to a new dimension of aza-amino acid residues, possessing side-chains that could not be obtained by previous methods. Our presentation will discuss the broad scope and utility of this new method for preparing novel aza-peptides as well as their potential as biologically active CD36 ligands.

I4. Abstract number: 571

Foldamers: Expanding The Chemical Space

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Our group has developed helical foldamers - oligomers that adopt stable helical folded conformations - derived from aromatic amino acids.1 Some of these folded objects have shown unprecedented conformational stability,2 and constitute convenient building blocks to elaborate synthetic, very large (protein-sized) folded architectures.3 They possess a high propensity to assemble into double, triple and quadruple helices.4 Cavities can be designed within such synthetic molecules that enable them to act as artificial receptors5 including for chiral guests. Water soluble analogues of these foldamers show promise in nucleic acid recognition.6

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I5. Abstract number: 579

Molecular Recognition at Protein Surfaces

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Both from a basic science perspective as well as from a drug design point of view there is no doubt that proteins can be considered as privileged targets for binding of small ligands. In this context the design of ligands able to disrupt protein-protein interactions is emerging as an even more relevant issue. The breakthrough concept that proteins function as a contact network rather than as independent individuals is not only one of the most important advances in our comprehension of living systems, but also translates to a new era in drug discovery. The few reported examples of diseases caused by ”impolite”protein social behavior certainly represent only the tip of the iceberg. Therapeutic intervention through molecules designed to selectively modulate the strength and specificity of protein-protein interactions is becoming a reality. This will not only feature molecules with inhibitory capacity: equally or even more interesting are those compounds which can rescue pre-established interactions or structures whose loss results in disease.

Protein-protein interactions are the result of an ensemble of exquisitely regulated molecular recognition events that take place at protein surfaces. This can be referred to as a ‘protein recognition code’. In order to understand protein-protein interactions and to achieve the efficient design of molecules with the capacity to modulate these protein-protein interactions, it is necessary to decipher this molecular recognition code, the language that proteins use to communicate. Unfortunately, progress in this field is highly unsatisfactory. Indeed, we are not completely illiterate, in the sense that we know the letters of this alphabet. They are the non-covalent interactions, such as hydrogen bonds, electrostatic interactions, π-cation interactions, Van der Waals forces, and the others. However, we could be compared with a child who is learning to read and attempts Dickens’s Oliver Twist.

In this context, a comparison of binding in gas phase and solution can pave the way towards a better understanding of molecular recognition at protein surfaces. Peptide ligands are highly suited for these types of studies. The comparison of the relative affinities of collections of ligands with the same molecular weight facilitates the interpretation of data from MS because it is not necessary to make corrections that are not very reliable with the present state of the technique.

I6. Abstract number: 595

Electronic Effects on Protein Structure

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In 1951, Linus Pauling first reported on the hydrogen bonds between backbone amides that are common in α-helices and β-sheets. We have discovered another intimate interaction between backbone amides. This interaction arises from the delocalization of a lone pair of electrons *(n)* from an oxygen atom to the anti-bonding orbital (π*) of the subsequent carbonyl group. The

signature of this n'π* interaction is most evident in the pyramidalization of the acceptor carbonyl group. Our *ab initio* calculations predict significant n'π* interactions in certain regions of the Ramachandran plot. We have validated these predictions by a statistical analysis of a large, non-redundant subset of protein structures determined to high resolution. We find n'π* interactions to be especially abundant in common secondary structures such as α-, 3_{10}-, and polyproline II helices, and twisted β-sheets. n)(π Pauli repulsion attenuates the n'π* interaction with olefins and compromises their utility as peptidomimetics. In addition to their evident effects on peptide and protein conformation, n'π* interactions could play important roles in protein folding and function, and merit inclusion in computational force fields.

I7. Abstract number: 594
Solving the a-conotoxin folding problem
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Many organisms including snakes, spiders, scorpions, cone snails, fish and some mammalian species have evolved venom as either a defence mechanism or a weapon for prey capture[1]. These venoms typically contain a complex cocktail of bioactive disulfide-rich small polypeptide antagonists (10-120 amino acids) which target a wide range of receptors1 including ion channels[2], GPCRs2, transporters[3] and enzymes. Of interest to drug designers is their high potency combined with their resistance to many proteases[4].
Of particular interest are venoms from the *Conidae*, with smaller polypeptide chains of 10-30 amino acids that are highly constrained by two to four disulfide bridges and structurally well-defined. Their high potency and *exquisite selectivity* for ion channels and receptors has led to one registered drug by Elan (Prialt) and two drug candidates[2,3] from our laboratories.
In this presentation I will outline the use of selenocysteine in a supported phase method to direct native folding and produce α-conotoxins with improved biophysical properties. By replacing complementary cysteine pairs with selenocysteine pairs on an amphiphilic resin, we were able to chemically direct all five structural subclasses of α-conotoxins exclusively to their native folds. The α-selenoconotoxins exhibited similar or improved potency at rat diaphragm muscle and α3β4α7 and α?$_2$β?δγn ACHRs expressed in *Xenopus* oocytes plus exceptional stability in plasma. Together, these results underpin the development of more stable and potent nicotinic antagonists suitable for new drug therapies, and highlight the application of this technology more broadly to disulfide-bonded peptides and proteins.
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