Miklos Bodanszky session

MB01

Introduction

Martinez, Jean
Max Mousseron Biomolecule Institute (IBMM), CNRS, Montpellier, FRANCE

MB02

VIP almost 40 years later: Diverse functions and therapeutic promise

Said, Sami I.
State University of New York, Stony Brook, Departments of Medicine, Physiology & Graduate Progr. Pharmacology, UNITED STATES

Soon after Viktor Mutt and I, in 1970, reported its isolation from porcine duodenal extracts, Miklos Bodanszky successfully synthesized the Vasoactive Intestinal Peptide (VIP), and characterized its chemical nature. Intensive research by numerous investigators over the years since has revealed VIP to have multiple biological effects, important physiological influence in many organ systems, and strong promise as a therapeutic agent for a number of varied human disorders. Produced mainly in neurons of the central and peripheral nervous systems, VIP is therefore widely distributed throughout the body. Its biological actions include: Relaxation of vascular and non-vascular smooth muscle, inhibition of smooth muscle cell proliferation, suppression of inflammation, modulation of immune function, and anti-apoptotic, pro-survival effects. Among its likely physiological roles, deduced in part from studies of mice lacking the VIP gene and the use of specific antibodies, VIP serves as: A co-transmitter, with NO and CO, of non-adrenergic, non-cholinergic smooth muscle relaxation; a modulator of inflammation, apoptosis, and smooth muscle cell proliferation; and a promoter of cell survival.

Based on the preceding information, as well as on the use of the peptide in experimental models of disease, and in some instances on early clinical trials, VIP and its analogs may prove to be novel and effective agents for the treatment of a variety of human disorders. These include: Bronchial asthma; pulmonary hypertension; Acute Lung Injury/Acute Respiratory Distress Syndrome (ARDS); Multi-Organ Dysfunction Syndrome (MODS); inflammatory/auto-immune disorders, such as rheumatoid arthritis and inflammatory bowel disease; cardiovascular disorders; neurodegenerative diseases, including Alzheimer's disease; and small-cell lung cancer.

After some delay, the pace of progress in this area seems to be accelerating. There is thus reason to hope that the promise fueled by much work, inspired and supported by Viktor Mutt and Miklos Bodanszky, will soon be fulfilled.

MB03

Potent and selective peptide agonists for human melanocortin receptors 1b and 5

Bednarek, Maria A
Merck Research Laboratories, UNITED STATES

α-Melanocyte-stimulating hormones (αMSH), through interactions with the cell surface receptors (melanocortin receptors 1, 3, 4 and 5; MC1,3-5R), elicits numerous physiological functions in the CNS and periphery. Of those receptors, MC1R and MC5R have been predominantly found on the surface of several types of skin and immune cells, in a number of endocrine and exocrine glands, and others. To elucidate and differentiate the role of MC1R and MC5R in some skin disorders, and immunomodulatory and anti-inflammatory actions of αMSH, selective ligands for are necessary.

In the structure of the endogenous agonist - αMSH, the His^{6}-Phe^{7}-Arg^{8}-Trp^{9} segment has been recognized as critical to molecular recognition at hMC5R and hMC1bR (an isoform of the human MC1aR with virtually identical pharmacological properties). The same segment has also been crucial for potency of various synthetic analogs of αMSH at hMC1b,5R. One of the most broadly used analogs of αMSH is a cyclic peptide, Ac-Nle^{4}-cyclo-(Asp^{5}-His^{6}-D-Phe^{7}-Arg^{8}-Trp^{9}-Lys^{10})-NH_2, designated MTII. Herein, the role of Trp^{9} in the interactions of MTII with the hMC1b,5R was examined through the ligand structure -function studies. The side chain of Trp^{9} was found to be not critical for high agonist potency at the hMC1b,5R; however, it was essential for potency of the MTII peptides at the human melanocortin receptors 3 and 4 (hMC3,4R). Several analogs of MTII are reported which are potent agonists at hMC1bR or hMC5R, and of high receptor subtype selectivity.

MB04

Peptides Containing Novel Tyrosine Analogues: Pharmacological Tools, Systemically Active Opioid Analgesics and Cell-Penetrating, Mitochondria-Targeted Antioxidants

Schiller, Peter W.
Clinical Research Institute of Montreal, CANADA

Syntheses of novel analogues of 2',6'-dimethyltyrosine (Dmt) lacking the amino group and, in some cases, containing a -$CONH_2$ function in place of the phenolic hydroxyl group or various substituents at the β-carbon were developed. Substitution of these analogues for Tyr^{1} in opioid peptides resulted in highly selective δ- or κ-opioid antagonists, the first opioid peptide-derived high affinity μ opioid antagonist and a mixed κ agonist/μ antagonist. The dermorphin-derived tetrapeptide H-Dmt-D-Arg-Phe-Lys-NH_2 ([Dmt^{1}]DALDA) is a highly selective μ opioid agonist showing subnanomolar potency in vitro, stability against enzymatic degradation and a long elimination half-life, capable of producing a potent, centrally mediated analgesic effect when given i.th. or s.c. Conjugates of [Dmt^{1}]DALDA linked to a δ opioid antagonist

either directly or via a short spacer turned out to be the first potent and systemically active opioid analgesics with a demonstrated mixed μ agonist/δ antagonist profile and the expected low propensity to produce analgesic tolerance. Interestingly, [Dmt[1]]DALDA (SS-02) and the structurally related non-opioid peptide H-D-Arg-Dmt-Lys-Phe-NH_2 (SS-31) were found to be taken up by cells and to partition into the inner mitochondrial membrane. These so-called SS-peptides, consisting of alternating aromatic and basic amino acid residues and containing Dmt as antioxidant moïety, were shown to scavenge and reduce mitochondrial reactive oxidant species (ROS) production and to block the mitochondrial permeability transition, thereby inhibiting oxidative stress-induced cell death. Their therapeutic potential was demonstrated in animal models of amyotrophic lateral sclerosis and Parkinson's disease, and their ability to protect against ischemia-reperfusion induced tissue injury.

MB05

Purification of Synthetic Peptides by Reverse Osmosis and Crystallization

Becker, Calvin[1]; Califano, Jean-Christophe[1]; Chang, Jane[2]; Napier, James[1]; Sachs, Howard[1]; Viswanath, Shekhar[1]; Pal, Agnes[1]; Sheikh, Ahmad[1]; Tolle, John[1]
[1]Abbott Laboratories, UNITED STATES; [2]U.S. FDA, UNITED STATES

This presentation is dedicated to Professor Miklos Bodanszky, my Ph.D. advisor and friend. The purification and isolation steps are often bottlenecks in large-scale peptide synthesis processes. Chromatography is routinely used to purify peptides and great advances have been made in the support media and equipment with regard to selectivity and capacity. Further advances can be expected as the demand for synthetic peptides continues to grow. The focus of or research effort has been the development of processes that involve minimal chromatography, are easily scaled, readily transferred to a manufacturing setting, and result in products with consistent impurity profiles. Reverse osmosis (RO) is a very manufacturing-friendly technique that is used in a number of industries at large scale. We have been able to employ RO as a simple purification and ion exchange method in several peptide processes. Typically, this results in products that still require some further purification to reduce the level of closely related impurities. We have developed crystallization methods for several moderately sized peptides, the result of which are the complete avoidance of chromatographic purification, as well as highly consistent purities and yields. Both R.O. and crystallization were used in the purification process for ABT-510, a nonapeptide angiogenesis inhibitor in Phase 2 clinical trial. Details of these techniques, which have been demonstrated at multi-kg scale, will be presented.

MB06

Closing words

Hudecz, Ferenc
Eötvös Loránd University, Department of Organic Chemistry, Budapest, *HUNGARY*

Professor Miklos Bodanszky (1915-2007)

Professor Miklos Bodanszky, a chemist, one of the forefathers of modern peptide science, died on February 7th, 2007 in Princeton, NJ. He made significant contributions to the file of peptide chemistry, peptide antibiotic synthesis and process research.

Dr. Bodanszky introduced new methods for the synthesis of peptides and small proteins with a broad variety of biological activities. He devised a new strategy, now widely accepted, for the construction of peptide chains from their building components and applied it in the synthesis of peptide hormones and their analogs: oxytocin, vasopressin, vasoactive intestinal peptide and secretin. He was the author of numerous scientific papers and of several books dealing with peptide chemistry.


Prof. Dr. Miklos Bodanszky

A native of Budapest, Hungary, Miklos Bodanszky received his doctorate at the Technical University of Budapest, where he later became lecturer in medicinal chemistry. He left Hungary at the time of the 1956 uprising and came to the United States to join Professor V. du Vigneaud in the Department of Biochemistry at Cornell University Medical College in New York City. Subsequently, he formed and led a peptide research group at the Squibb Institute for Medical Research. From 1966 until his retirement in 1983 he taught at Case Western Reserve University in Cleveland, Ohio, where he was the Charles Frederic Mabery Professor of Research in Chemistry. He was the first recipient of the Alan Pierce Award (now the Merrifield Award), was honored by scientific societies in the U.S. and abroad, and was named a foreign member of the Hungarian Academy of Sciences. After retirement, he returned to Princeton, N.J., where he continued to contribute to the literature of peptide chemistry. His wife, Agnes, who was also his coworker and frequent coauthor, died in 1989. He is survived by his daughter, Dr. Eva Bodanszky.

Highlights of synthetic peptide chemistry

S02-1 Inv

Design, synthesis and use of scaffold based peptidomimetics

Luthman, Kristina; Dahlén, Kristian; Dyrager, Christine; da Silva Andersson, Krystle; Friberg, Annika; Pemberton, Nils; Saitton, Stina; Saxin, Maria; Tullberg, Marcus; Wallén, Erik; Grötli, Morten
University of Gothenburg, SWEDEN

Peptides are considered important in several disease states and therefore peptide receptors and processing enzymes have become interesting targets for drug discovery efforts. As peptides are not usually useful as drugs in themselves, compounds with improved pharmacokinetic properties are needed. Such compounds are called peptidomimetics.

One approach towards the development of peptidomimetics is to use a molecular template or scaffold to which important pharmacophoric groups, supposedly amino acid side chains, can be attached. Conformationally rigid structures can also provide important information about the bioactive conformation of peptides by mimicking peptide secondary structures, e.g. β-sheet and β- or γ-turns.

We are interested in the development of general scaffolds useful in mimetics of biologically active peptides or oligopeptide sequences. We have identified several different heterocyclic ring systems as interesting scaffolds, e.g. 4-chromanone,[1] pyridine[2] and 2,5-diketopiperazine[3] derivatives. To explore the use of such scaffolds efficient synthetic protocols have been developed which allow regioselective introduction of a variety of substituents in different positions.

The presentation will cover the design and synthetic aspects as well as biological applications of the different types of scaffold based peptidomimetics.

References:

1.K. Dahlén et al. *J. Org. Chem.*, **2006**, *71*, 6863-6871
2.S. Saitton et al. *J. Med. Chem.*, **2004**, *47*, 6595-6602
3.M. Tullberg et al. *J. Org. Chem.*, **2007**, *72*, 195-199

S02-2

Solid-phase synthesis of lantibiotics: Synthesis and structure of an analogue of the C-terminus of Nisin

Nicolau, Daniel[1]; Driscoll, Paul[2]; Gaffney, Piers[3]; Tabor, Alethea[1]
[1]UCL Department of Chemistry, UNITED KINGDOM; [2]UCL Department of Biochemistry, UNITED KINGDOM; [3]Imperial College Department of Chemistry, UNITED KINGDOM

The lantibiotics are a family of antimicrobial peptides, characterised by complex structures featuring multiple thioether bridges (from the residues lanthionine and methyl lanthionine) and other residues, such as dehydro amino acids and other bridges (1). Nisin is the best studied Type I lantibiotic. It has a dual mode of action on bacterial membranes. NMR studies have shown that the C-terminus (rings A and B) recognises and specifically bind lipid II (a key component of the biosynthesis of bacterial membranes)(2), this is then followed by pore formation by the nisin/lipid II complex, mediated by C-terminus (rings C, D/E). Both parts of nisin appear to possess antimicrobial action, both separately and in synergy, and the structure of the intermediate hinge region also affects the activity. The biosynthetic pathways responsible for the production of the lantibiotics are now well understood(1): Ser or Thr residues in a precursor peptide are dehydrated to give Dha or Dhb residues, which are then cyclised with Cys residues to form the thioether bridges. Based on these pathways, a number of biomimetic strategies for the synthesis of lantibiotics have been used, however these are not always suitable for the synthesis of multiply bridged peptides (3). We have recently developed methodology for the synthesis of lanthionine-bridged peptides based on the use of orthogonally protected lanthionine, and have previously reported the synthesis of analogues of ring C of nisin(4). In this presentation we now report the extension of this approach to give lanthionine-containing peptides with multiple bridges, and present the synthesis and structure of analogues of the C-terminus of nisin (rings A and B).

References:

1 C. Chatterjee, M. Paul, L. Xie, W. A. van der Donk Chem Rev., 105, 633-683 (2005).
2 S. Hsu et al Nature Struct Mol Biol, 11, 963-967 (2004).
3 Y. Zhu, M. D. Gieselman, H. Zhou, O. Averin, W. A. van der Donk Org Biomol Chem, 1, 3304-3315 (2003).
4 S. Bregant, A. B. Tabor J. Org. Chem.

S02-3

Solid phase synthesis of alkene dipeptidosulfonamide isosteres using olefin cross metathesis and the incorporation into amyloidogenic amylin(20-29)[1]

Brouwer, Arwin J.; Elgersma, Ronald C.; Rijkers, Dirk T. S.; Liskamp, Rob M. J.
Department of Medicinal Chemistry and Chemical Biology, Faculty of Science, Utrecht University, NETHERLANDS

The replacement of a backbone amide bond in peptides is a strategy which has been widely used to study peptide backbone interactions as well as for stabilization of peptides towards enzymatic degradation. Two amide bond surrogates that mimic the amide bond are the (E)-alkene dipeptide isostere and the sulfonamide. The sulfonamide increases the flexibility of the backbone, is resistant to enzymatic degradation and is conveniently accessible.[2] A disadvantage of peptidosulfonamides might be the presence of an additional carbon atom in each amino sulfonic acid residue, which is needed for stability reasons. As a result, there is no exact match of a peptidosulfonamide and its parent peptide. By combining the alkene dipeptide isostere with the sulfonamide a new peptidomimetic was designed and synthesized: the alkene dipeptidosulfonamide isostere. This isostere has the same backbone length as the parent (di)peptide, and also contains a sulfonamide moiety. The synthesis is based on a cross metathesis (CM) reaction between two allylic building blocks, which can be performed either in solution or on the solid phase. This CM method was found to be also applicable to the solid phase synthesis of alkene dipeptide isosteres. It was decided to incorporate a leucine-derived alkene dipeptidosulfonamide into the peptide sequence of human amylin(20-29) (SNNFGAILSS), a highly amyloidogenic peptide. Previously it was shown that aggregation phenomena of this sequence are highly sensitive toward modification of the peptide backbone.[3] It was found that the alkene dipeptidosulfonamide isostere delayed the fibril formation and altered the secondary structure. In the present contribution the synthesis as well as a structure-aggregation behavior study of this class of amylin derivatives will be presented.

References:

1. A.J. Brouwer et al., Bioorg. Med. Chem. Lett., 2008, 18, 78.
2. A.J. Brouwer et al., Synthesis 2000, 1579.
3. R.C. Elgersma et al., Org. Biomol. Chem. 2006, 4, 3587.

S02-4

Synthesis of Cyclic Peptide Chitinase Inhibitors: Natural Products with Chemotherapeutic Potential

Dixon, Mark[1]; Nathubhai, Amit[1]; Andersen, Ole[2]; van Aalten, Daan[2]; Eggleston, Ian[1]
[1]University of Bath, UNITED KINGDOM; [2]University of Dundee, UNITED KINGDOM

Many pathogenic organisms need to hydrolyse chitin, a homopolymer of β(1,4)-linked N-acetyl-D-glucosamine, at key points in their life cycles. Chitin is the main structural component of insect exoskeletons, the cell walls of fungi, and is also found in the eggshells of parasitic nematodes. Molecules able to inhibit chitinase enzymes in these organisms have considerable potential as novel fungicides, insecticides, and nematocides. The recent discovery of the human acidic mammalian chitinase and its apparent role in inflammatory disorders such as asthma has led to renewed interest in this field, but the lack of readily available broad spectrum inhibitors has until now severely limited research in this area. We will describe new, efficient and flexible solid phase syntheses of two families of potent chitinase inhibitors, derived from the cyclic pentapeptide natural products, argifin and argadin. [1,2] These permit assembly, cyclisation, and key side chain derivatisations to be performed entirely upon the solid phase. The unusual modified Arg residues of both peptides are installed via an orthogonally protected Orn, thus avoiding side reactions observed on final deprotection in previous combined solid phase/solution approaches. SAR data for designed analogues vs typical fungal and mammalian chitinases will be presented, along with recent data on the X-ray structure-led dissection of the argifin scaffold. (3) This has allowed us to identify, synthesise and evaluate, minimal active fragments from argifin which may serve as starting points for a new generation of drug-like, peptidomimetic inhibitors.

References:

1. M. J. Dixon, O. A. Andersen, D. M. F. van Aalten, and I. M. Eggleston, Bioorg. Med. Chem. Lett., **15**, 4717-4721, 2005;
2. M. J. Dixon, O. A. Andersen, D. M. F. van Aalten, and I. M. Eggleston, Eur. J. Org. Chem. **22**, 5002-5006, 2006.
3. O. A. Andersen, A. Nathubhai, M. J. Dixon, I. M. Eggleston, and D. M. F. van Aalten, Chem. Biol., 2008, in press.

Peptide ligation, conjugation and tailoring

S03-1

Dynamic ligations with peptide electrophiles allow to identify protein-binding fragments and to develop protein ligands

Rademann, Jörg; Schmidt, Marco; El-Dahshan, Adeeb
Leibniz Institute for Molecular Pharmacology, GERMANY

Specific protein ligands are crucial for the modulation of protein activities in medicinal chemistry and chemical biology. Recently, we have prepared various peptidyl bis- and tris-electrophiles from amino acids using of phosphoranes as polymeric carbanion equivalents.(1) We now found such peptidyl electrophiles useful in Dynamic Ligation Screening (DLS), enabling the rapid and site-directed identification of protein binders by template-assisted fragment assembly. (2) DLS was conducted with libraries of 200-6000 fragments. A fluorogenic substrate competed with an equilibrium of nucleophilic fragments and the directing peptide electrophile.(2) Decreased initial rates of product formation in the enzyme assay indicated the inhibitory activity of the reversibly formed ligation product. Via an iterative scanning of different binding sites on the protein surface, moderately active peptide ligands could be transformed into entirely non-peptidic inhibitors with low μM inhibition (KI). The concept was established for the development of a non-peptidic SARS coronavirus main protease (SARS-CoV Mpro) inhibitor. This enzyme has been identified as a drug target of SARS being essential for replication of the virus inside the infected host cell. The thermodynamics of protein-assisted fragment ligation were studied for caspase 3, a cellular switch for apoptosis, the programmed cell death. As a result, a model for the additivity of binding contributions of ligated fragments is proposed. The method can be further extended to other protein targets such as phosphatases4. and to protein-protein interactions.

References:

1. a) A. El-Dahshan, S. Weik, J. Rademann Org. Lett. 2007, 9, 949-952
1. b) S. Weik et al. ChemMedChem 2006, 1, 445-457.
1. c) S. Weik, J. Rademann, Angew. Chem. Int. Ed. 2003, 115, 2491-2494.
2. M.F. Schmidt et al. Angew. Chem. Int. Ed. 2008 DOI: 10.1002/anie.200704594.
3. S.I. Al-Gharabli et al. ChemBioChem 2006, 7, 1048-1055. 4. K. Hellmuth et al. Proc. Nat. Acad. Sci. USA 2008, in print.

S03-2

Semisynthesis of Membrane-Associated Prion Proteins

Olschewski, Diana[1]; Seidel, Ralf[1]; Becker, Christian F.W.[2]
[1]Max-Planck Institut für molekulare Physiologie, GERMANY;
[2]Technische Universität München, GERMANY

Transmissible spongiform encephalopathies (TSEs) such as Creutzfeld Jakob disease (CJD) are rare fatal neurodegenerative disorders that are characterized by the accumulation of a misfolded isoform (PrPsc) of the cellular prion protein (PrPC). PrPC is an N-glycosylated protein attached to the outer leaflet of the plasma membrane by a C-terminal glycosylphosphatidylinositol (GPI)-anchor, where it segregates into cholesterol- and spingomyelin-rich microdomains (1). This membrane localization seems to be crucial for the de novo generation of infectious PrPsc (2). In order to mimic the influence of membrane attachment on folding and conversion, synthesis strategies based on expressed protein ligation (EPL) and the process of protein trans-splicing (3) were devised that allowed incorporation of palmitoylated and fluorescently labeled peptides as well as of glycosylphosphatidylinositol (GPI) anchors at the C-terminus of PrP. Following these synthesis strategies, murine PrP was modified with chemically synthesized peptides containing lipid moieties that mimic the GPI anchor and fluorescent labels for localization studies. Synthesis of these peptides was accomplished by solid phase peptide synthesis (SPPS) The resulting C-terminally modified mPrP variants were successfully folded and transferred into liposomes as well as N2a cells (4). Chemically synthesized, native GPI anchors are currently tested for incorporation into mPrP via EPL as well.

References:

1. P. Critchley, J. Kazlauskaite, R. Eason, T. J. T. Pinheiro, Biochem. Biophys.Res.Commun. 2004, 313. 559-567.
2. G. S. Baron, B. Caughey, J.Biol.Chem. 2003, 278. 14883-14892.
3. T. Durek, C. F. Becker, Biomol.Eng 2005, 22. 153-172.
4. D. Olschewski, R. Seidel, M. Miesbauer, A. S. Rambold, D. Oesterhelt, K. F. Winklhofer, J. Tatzelt, M. Engelhard, C. F. W. Becker, ChemBiol 2007, 14, 994-1006.

S03-3

Fmoc solid-phase synthesis of C-terminal peptide thioesters by formation of a pyroglutamyl imide moiety

Tofteng, A. Pernille[1]; Conde-Frieboes, Kilian W.[2]; Hoeg-Jensen, Thomas[2]; Jensen, Knud J.[1]
[1]University of Copenhagen, Faculty of Life Sciences, DENMARK;
[2]Novo Nordisk A/S, DENMARK

C-Terminal peptide thioesters are required for native chemical ligation and other chemoselective reactions. Thioesters are labile to the secondary amines commonly used for removal of Fmoc groups, and the development of methods for solid-phase synthesis of peptide thioesters compatible with Fmoc chemistry have been a major challenge over the past decade. Numerous approaches have been published and methods for reliable synthesis of peptide thioesters with a C-terminal glycine have been established. However, Fmoc based methods for the syntheses of peptide thioesters with a C-terminal chiral amino acid are less reliable. The most versatile approach uses a modification of Kenner's safety catch linker which requires a very strong activation using trimethylsilandiazomethane followed by a thiol displacement. Here we present a novel method for the synthesis of C-terminal peptide thioesters. A C-terminal glutamic acid residue with a highly acid-labile side-chain protecting group is first anchored to a solid support. The desired peptide is then assembled on-resin, followed by selective removal of the glutamic acid side-chain protecting group with dilute TFA. Activation of the deprotected carboxylic acid, e.g. PyBrop/DIEA in NMP, resulted in formation of the pyroglutamyl imide moiety on-resin. Nucleophilic displacement of the pyroglutamyl imide leaving group by treatment with thiol released the peptide as the thioester from the solid support. We have successfully used this method for the synthesis of a range of peptide thioesters. We believe this new method has the potential to become a general tool for the synthesis of peptide thioesters.

S03-4

Doubling of cyclopeptides and cyclodepsipeptides: a new topological approach towards more active compounds

Ruíz-Rodríguez, Javier; Albericio, Fernando; Spengler, Jan
Institute for Research in Biomedicine (IRB Barcelona), SPAIN

Cyclization is the most common geometrical manipulation in peptide chemistry. Two functional groups (e.g. N- and C-terminus) of a peptide are connected by a new bond. The cyclized peptide obtained can exhibit much higher receptor affinity and better stability towards degrading enzymes. Optimization of these properties is crucial in peptide science. Thus, what about other types of peptide geometry manipulation? Both halves A and B of a cyclic peptides type cyclo-AB can interact with macromolecules to the same degree. A connection of two cycles via a C-C single bond to the double cyclic structure, AB-BA, should result in a differentiation of the topology into a "buried region" (B-B) and a "solvent-exposed region" (the A-ends of AB-BA). When A represents a receptor-interacting sequence and B a conformationally constraining peptidic sequence, the A-sequence should consequently have the capacity to interact with higher propensity with macromolecules (receptors) than the B-sequence, which should be less available for catabolising macromolecules (proteases). The test this hypothesis, we performed a first synthesis for "doubled" sansalvamides, and a first depsipeptide synthesis of double-cycles with S,S-tartaric acid and S,S-hydroxy aspartic acid as core building blocks. the cyclic 5-membered anticancer depsipeptide sansalvamide A (1) was chosen as a model compound. The structures of these "bi-cycles" were confirmed by NMR and the anticancer activity of the tartaric acid derivative was found to be one order of magnitude higher than the single cycle natural product.

References:

1. Belofsky, G. N.; Jensen, P. R.; Fenical, W. Tetrahedron Lett. 1999, 40, 2913-2916.

S03-5

Side-Chain Assisted Peptide Ligation

Brik, Ashraf
Ben-Gurion University, ISRAEL

Peptide ligation strategies that rely on intramolecular acyl transfer to form an amide bond have been of great interest for accessing synthetic proteins. Unarguably, Native Chemical Ligation (NCL), assisted by the N-terminal cysteine residue to facilitate S → N acyl transfer, is the most effective method for protein synthesis. However, the requirement of cysteine at the ligation junction hampers its use in a variety of protein systems. Mimicking the cysteine function in the ligation pathway with a removable auxiliary is a promising approach to assist S → N acyl transfer at various amino acid junctions. Presented here is a new peptide ligation method that uses an auxiliary attached to the side-chains of different amino acids (Ser, Thr, Asp, Glu) to facilitate an efficient ligation at non-cysteinyl junction. Following the ligation reaction, without product isolation, the auxiliary can be rapidly removed under basic conditions to generate the unmodified peptide. * Marina-Yamit Lutsky, Natalia Nepomniaschiy and Ashraf Brik, Chemical Communications, 2008.

Libraries and peptidomimetic applications

S04-1

Modulating Biology with Cryptic Cell Penetrating Peptides

Howl, John; Jones, Sarah
Research Institute in Healthcare Science, School of Applied Sciences, University of Wolverhampton, UNITED KINGDOM

Numerous cell penetrating peptides (CPP) have been utilised as vectors for the intracellular delivery of diverse bioactive cargoes. The utility of this strategy requires highly efficient CPP with minimal toxicity and negligible biological activity. However, general consideration of the multi-domain architecture of many human proteins indicates that cell penetrant cryptic sequences could mimic and/or modulate their functions. Our starting point for the development of biologically-active cryptic CPP was the QSAR-based prediction algorithm recently described by Ülo Langel's group. This strategy has enabled the study of cryptic CPP in which multiple pharmacophores for cellular penetration and biological activities are discontinuously organised within the primary sequence. The term rhegnylogic is used to distinguish this class of CPP from the more usual sychnologic combination of CPP and cargo. Cryptic CPP sequences are located within both amino and carboxyl helical domains of cytochrome c (Cyt c), a signalling protein integral to apoptotic events. Detailed characterisation of these peptides has identified a series of efficient CPP vectors that differentially target intracellular organelles. These molecular properties mimic the propensity of Cyt c to translocate biological membranes as a mediator of both mitochondrial apoptosis and inflammation. Moreover, chimeric combination of Cyt c-derived CPP and other biologically-active sequences has generated potent apoptogenic peptides. Related studies have identified a cryptic CPP within nitric oxide synthase, $eNOS^{492-507}$, located within a helical domain known to tightly bind calmodulin. Significantly, this peptide potently inhibits the proliferation, migration and tube-forming capacity of primary endothelial cells and displays anti-angiogenic properties in vivo. In summary, these and other data confirm that cryptic CPP can modulate many aspects of cell biology and are, therefore, a valuable new class of bioactive peptide.

S04-2

From Peptide to Non-Peptide Metalloconstructs: Dynamic Combinatorial Libraries of Oxorhenium Coordinates for the Selection of New Cyclophilin Inhibitors

Clavaud, Cécile; Le Gal, Julien; Thai, Robert; Heckenroth, Marion; Stricane, Charlotte; Lelait, Marie-Anne; Moutiez, Mireille; Dugave, Christophe
CEA / iBiTecS / SIMOPRO, FRANCE

Peptide metalloconstructs often display interesting properties and bioactivities. The self-assembly of short peptides through oxorhenium coordination by a recurrent NS2/S motif gives libraries of peptide mimics of general formula [A–NS2•ReO•S–B] that could bind to the active site of cyclophilin hCyp-18, an important human peptidyl-prolyl isomerase (PPIase). In a preliminary study, we ensured that grafting of an oxorhenium core on reference substrates did not alter significantly their affinity for hCyp-18. Firstly, two libraries of 112 and 176 peptide-oxorhenium complexes were synthesized and systematically screened as ligands of hCyp-18. Two coordinates were shown to bind specifically to cyclophilin with affinities higher by one order of magnitude relative to a reference substrate. We also demonstrated that the self-assembly of mixtures of peptide-oxorhenium coordinates can be carried out in various solvents including buffers. As anticipated, these complexes were sensitive to high concentrations in glutathione (GSH) which substitutes de B–SH moiety. In a second step, we explored the cyclophilin-mediated selection of new oxorhenium coordinates from a set of 192 possible complexes. For this purpose, 12 modules A–N(SH)2 and 16 modules B–SH were incubated with [ReO•(gluconate)2] and hCyp-18, together with increasing concentrations in GSH. LC-MS analysis of the mixtures showed that oxorhenium coordinates, which tightly bind to cyclophilin, were partially protected against GSH substitution, whereas complexes, which do not bind to the enzyme, readily dissociated. Three complexes were selected and thoroughly characterized. These complexes bind to the cyclophilin active site 1000-fold better than the reference peptide and are able to inhibit the PPIase activity with IC50 up to 0.2 µM. A kinetic study of the assembly of these complexes strongly suggests the existence of two distinct modes of selection by the enzyme.

S04-3

Peptides as carbohydrate mimetics

Sewald, Norbert[1]; Bächle, Dirk[1]; Loers, Gabriele[2]; Guthöhrlein, Eckhart[1]; Schachner, Melitta[2]
[1]Bielefeld University, GERMANY; [2]Hamburg University, GERMANY

Carbohydrates are involved in a broad variety of physiological or pathological processes, which basically rely on molecular recognition events. Despite the tremendous methodological advancements in carbohydrate synthesis, the development of carbohydrate-based therapeutics comprises multi-step syntheses and always has to cope with unfavourable ADME parameters of oligosaccharides. Peptides in general do not display any similarities to carbohydrates, but can be synthesized in a straightforward fashion by solid phase synthesis. Structural variations can easily be introduced in a combinatorial way. Peptides that functionally and/or structurally mimic oligosaccharides provide a straightforward and elegant approach to interfere with the interaction between complex carbohydrates and their receptors. A cyclopeptide library was synthesized on the basis of the linear sequences TFKLSETTLEYY and TFQLSTRTLPFS previously discovered in phage display studies as functional mimetics of the HNK-1 trisaccharide. This oligosaccharide plays a major role e.g in developmental processes in the nervous system. The cyclopeptide library comprised cyclic hexapeptides based on the partial sequences TFKLSE, LSETTL, TTLEYY, TFQLST, LSTRTL, and RTLPFS in a spatial screening approach. Cyclic peptides can be designed in such a way that conformation and three-dimensional orientation of the side chain functional groups that are responsible for the contacts to a receptor is highly predictable. The affinity of all peptides to the selection antibody recognizing HNK-1 was determined in surface plasmon resonance studies. For some peptides considerably increased affinity compared to the linear control was observed with K_D values in the low micromolar range. The most active peptides also significantly stimulate neurite outgrowth of murine motoneurons. This proves that they act as functional mimics of the HNK-1 oligosaccharide.

S04-4

Non peptide mimetics: A new generation of drugs

Matsoukas, John
University of Patras, GREECE

The discovery of Losartan a non peptide Angiotensin II Receptor antagonist was announced in 1989 during the Gordon Research Conference on Angiotensin and the Renin – Angiotensin – System (RAS). The drug was discovered in the Laboratories of Dupont and the announcement at the Conference was the approval for Clinical trials which led to the first Angiotensin II nonpeptide Receptor antagonist followed by another eight Sartans, now in medical use. Previous Angiotensin II peptide antagonists such as Sarilesin and Saralasin failed to become drugs due to its peptide nature rendering them susceptible to proteolytic enzymes which hydrolyze them. The announcement was the result of many years work on Angiotensin and the RAS System, since it was discovered 80 years ago. Breakthroughs in this evolution was the discovery of Captopril by Miguel Ondetti in 1975 and Losartan by Timmermans in 1989. In this lecture the main steps followed in our laboratories in Patras are mentioned which led to our Sartan, named Elsartan. Briefly the main steps are: 1. Peptide (The tool), 2. Peptide Model (The ligand – receptor interaction), 3. Cyclic Peptide (The drug lead), 4. Non-peptide mimetic (The Drug). Also, the strategic steps are described, in the design and synthesis of non peptide mimetics for Myelin Epitopes (MBP, PLP, MOG) implicated in Multiple Sclerosis, for Thrombin Receptor Activating Peptides (TRAP) implicated in Angiogenesis and Cancer and Gonadotropin Releasing Hormone (GnRH) implicated in Fertility and Cancer.

Natural peptides

S05-1 Inv

Biosynthesis of cyanobacterial peptide toxins

Sivonen, Kaarina
Department of Applied Chemistry and Microbiology, Viikki Biocenter, University of Helsinki, FINLAND

Cyanobacteria produce a wide variety of toxins as well as biomedically interesting bioactive compounds. The cyclic hepatotoxic peptides microcystins and nodularins are the best known. These toxins have caused number of animal poisonings and are a risk for human health. Microcystins and nodularins are specific inhibitors of eykaryotic protein phosphatases and act as tumour promoters. In addition, several bioactive cyclic and linear peptides have been characterized from cyanobacteria and display a number of bioactivities including serine protease inhibition and cytotoxicity. Many of these compounds contain non-proteinogenic amino acids and are synthesised on non-ribosomal peptide synthetases. Microcystins and nodularin are products of a mixed non-ribosomal peptide and polyketide synthetase. The microcystins and nodularin gene clusters encode peptide synthetases, polyketide synthatases and tailoring enzymes. Studies on the evolution of microcystin/nodularin synthetase genes suggest that these genes are ancient and that present non-toxic strains have lost the genes and toxin production. The cyclic anabenopeptilides, and anabaenopeptins as well as linear spumigins are synthesised on non-ribosomal peptide synthetases. Recently the complete genome of Anabaena sp. strain 90 revealed ribosomal peptide synthesis of a novel family of cyclic peptides by planktonic cyanobacteria. This genome project also showed that 5% of the genome was dedicated to biosynthesis of bioactive compounds and that all the gene clusters are carried on the chromosome. Cyanobacteria are a rich source of bioactive compounds for drug leads. They produce peptides by ribosomal and non-ribosomal peptide synthesis pathways and could provide enzymes to be used in combinatorial biosynthesis or semisynthesis to produce novel compounds in future.

References:

Sivonen, K. and T. Börner. 2008. Bioactive compounds produced by cyanobacteria. In: “The cyanobacteria: Molecular Biology, Genomics and Evolution”, Herraro, A. & E. Flores (Eds.). p. 159-197. Caister Academic Press, Norfolk, U. K.

Sivonen, K. 2008. Pathogenesis: Cyanobacterial toxins. In: “The Encyclopedia of Microbiology”, 3rd Edition, Schaechter M. (Ed.), 18 p. Elsevier (in press).

S05-2

The biosynthesis and evolution of cyclotides: macrocyclic peptides from plants, with applications in drug design

Craik, David[1]; Saska, Ivana[1]; Gruber, Christian[1]; Gillon, Amanda[2]; Anderson, Marilyn[2]
[1]University of Queensland, AUSTRALIA; [2]La Trobe University, AUSTRALIA

The cyclotides [1,2] are a recently discovered family of plant-derived proteins that have applications in drug design and agriculture. They occur in plants from the Violaceae (violet), Rubiaceae (coffee) and Cucurbitaceae (cucurbit) families and have a diverse range of biological activities, including uterotonic, anti-HIV, antimicrobial, and insecticidal activities, the latter suggesting that their natural function is in plant defense. Individual plants express suites of 10-100 cyclotides at high levels (2g/kg wet plant weight). Cyclotides typically comprise 30 amino acids, have a head-to-tail cyclized backbone and incorporate three disulfide bonds arranged in a cystine knot topology. In this motif an embedded ring in the structure formed by two disulfide bonds and their connecting backbone segments is penetrated by a third disulfide bond. The combination of this knotted and strongly braced structure with a circular backbone renders the cyclotides impervious to enzymatic breakdown and makes them exceptionally stable. The cyclotides are the largest of several groups of naturally occurring circular proteins that have been discovered over recent years (2). Their stability and compact structure makes them an attractive protein framework onto which bioactive peptide epitopes can be grafted to stabilize them. Cyclotides are matured from larger precursor proteins via a series of processing events, including excision, and head-to-tail peptide ligation via an asparaginyl endoproteinase. Folding is facilitated by a protein disulfide isomerase (PDI). This presentation will describe the discovery and characterization of the precursor proteins, PDI and the biosynthetic process.

References:

1. Craik D J, Cemazar M, Daly N L: The cyclotides and related macrocyclic peptides as scaffolds in drug design. Curr. Opin. Drug Discovery and Development 2006, 9, 251-260
2. Craik D J: Seamless proteins tie up their loose ends. Science, 2006, 311, 1561

S05-3

First Example of a Cardioactive (CCAP) Peptide in Venom: CCAP-vil Defines a New Conopeptide Superfamily

Moller, Carolina[1]; Melaun, Christian[2]; Marks, Vered[1]; Sanaz, Dovell[1]; Diaz, Mary[3]; Frank, Marí[1]
[1]Florida Atlantic University, UNITED STATES; [2]Justus-Liebig Universität, GERMANY; [3]The University of Edinburgh, UNITED KINGDOM

The venom of cone snails is a remarkable source of bioactive peptides. Here we describe the first example of an exogenous CCAP peptide as part of the venom of Conus villepinii, a deep-water Western Atlantic cone snail species. Cardioactive peptides (CCAPs) were first isolated from the shore crab Carcinus maenas. These endogenous peptides have been isolated from other invertebrates and insects. CCAP's are multifunctional neuropeptides involved in circulatory function, ecdysis behavior and visceral organ activity. One of the principal roles of the CCAP neuropeptide is that it has a cardioacceleratory effect when it is applied in vitro and a cardiotonic effect when it is applied in vivo. This discrepancy may result from the modulation of the cardiac CCAP signaling pathway by other molecules that are present in the whole organism but absent in the isolated cardiac tissue or cells. We initially isolated a peptide from the venom of Conus villepinii whose precursor we determined that belong to the CCAP family (CCAP-vil). We determined the 3D structure of the CCAP-vil peptide by NMR, which was shown to be a loop flanked by the disulfide bridge, with two turns and several intermolecular hydrogen bonds. This structure suggests potential cell-penetrating features that might be important for the peptide activity. Assays using mammalian myocytes show that CCAP-vil is cardiotonic, as it decreases hearts contraction by 58%. No other studies on the cardiac effects of CCAPs in mammals have been reported so far. These results suggest that the effects on systolic Ca2+ and contraction may be due to the action of the peptide on the ryanodine receptor (RyR) activity. The fact that these effects are time-dependent also suggests that the peptide action may require binding to a target protein that affects Ca2+ release via RyR.

S05-4

A novel nucleolar localization signal sequence derived by structural minimization of an animal toxin

Radis-Baptista, Gandhi[1]; G. de la Torre, Beatriz[2]; Andreu, David[2]
[1]Dept of Experimental and Health Sciences, Pompeu Fabra Univ, Spain/Dept of Biochemistry, Fed Univ of Pernambuco, BRAZIL; [2]Department of Experimental and Health Sciences, Pompeu Fabra University, Barcelona, SPAIN

Localization sequences are specific sorting signals present in proteins, which direct them to precise cellular compartments. For instance, nuclear localization signals (NLSs), typified by cluster(s) of basic residues (e.g. –KKKRK-), are found in nuclear proteins selectively recognized by nuclear import machinery. Cell penetrating peptides (CPPs), on the other hand, are natural or synthetic sequences, also cationic and usually amphipathic, capable of translocating cell membranes and often localizing to the nucleus of eucaryotic cells. Although cationic and amphipathic peptides are plentiful in animal venoms, the number of reported examples of animal toxins with membrane translocating properties is limited. In one of such cases, we have dissected the highly folded, 3-disulfide native structure of an amphiphatic toxin with a view to defining the pharmacophore responsible for membrane translocation and nuclear localization. Using a minimalist synthetic approach in conjunction with *in vivo* time-lapse confocal microscopy, we have successfully identified a novel 13-residue peptide sequence with exquisite nucleolar localization (NrL) properties. Fluorescent tagged peptide initiates uptake as early as 15 min, reaching maximal penetration rate and compartmentalization at 45-60 min. In addition, the uptake process and nucleolar localization are shown to be cell cycle-dependent. Cytotoxicity assay with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) shows the peptide to be not harmful for HeLa cells up to 100 μM. These results are interesting as the nucleolus is a preeminent nuclear compartment that, aside from its role in ribosomal RNA biogenesis, contains a number of proteins involved in cell cycle regulation.

S05-5

The Reproductive Role of P12 from Male Accessory Sexual Glands

Chen, Yee-Hsiung[1]; Gandhi, P. S. Sudhakar[1]; Ou, Chung-Mao[1]; Huang, Mei-Shan[2]
[1]Institute of Biochemical Sciences, College of Life Science, National Taiwan University, TAIWAN; [2]Institute of Biological Chemistry , Academia Sinica, TAIWAN

We have purified and identified a 5.6 KDa Kazal-type trypsin inhibitor (P12) in mouse seminal vesicle fluid. It shows high affinity to the plasma membrane overlaying the anterior acrosome of mouse spermatozoa (Kd = 70nM, 1.49x106 sites/cell) to suppress the spermatozoal Ca2+-uptake. It gives very slight impact on the spermatozoal status represented by the cell population in uncapacitated, capacitated and acrosome-reacted stages. Arg19 is the reactive site for protease inhibitor, and Tyr21 or/ and Asp22 is essential for the sperm-binding. There are three epitopes located in residues 5-10, residues 41-47, and residues 51-56, and they are three-dimensional distant from both Arg19 and Tyr21/Asp22. Recently, we have succeeded in specifically crosslinking P12 to its binding site on mouse spermatozoa by the photo affinity technique, using P12 conjugated with Sulfo-HSAB (N- Hydroxysulfosuccinimidyle- 4-azidobenzoate), a photo affinity labeling reagent. The spermatozoal protein complexed with P12 has been demonstrated to be £\-enolase by proteomic study. The presence of £\-enolase on the sperm surface has been also confirmed by immunocytochemical staining using the antibody against enolase. In addition, we have found that the £\-enolase antibody is able to inhibit the P12-sperm binding. Meanwhile, we have resolved the protein components of mouse uterine fluid into three fractions, Fr I-III by gel chromatography on a G-100 Sephadex column, and demonstrated predominant distribution of trypsin-like proteases in Fr III. Hydrolysis of the trypsin substrate N-benzoyl-phe-Val-Arg p-nitroanilide by Fr III can be inhibited to a certain extent by P12, indicating that mouse uterine fluid contains trypsin-like protease which can be specially inhibited by P12. Finally, the ability of Fr III to remove P12 on the sperm surface has been demonstrated.

Peptide biochemistry

S06-1 Inv

Designed armadillo repeat protein libraries for the selection of peptide binders

Plückthun, Andreas
University of Zürich, Department of Biochemistry, SWITZERLAND

Armadillo repeat proteins are abundant eukaryotic proteins involved in several cellular processes, including cellular signaling and cytoskeletal regulation. They are characterized by an armadillo domain, composed of tandem armadillo repeats of approximately 42 amino acids, which mediate interactions with peptides or parts of proteins in extended conformation. The conserved binding mode, observed for different targets, makes armadillo repeat proteins attractive candidates for the generation of modular peptide binding scaffolds. Taking advantage of the large number of repeat sequences available, a consensus-based approach combined with a computational optimization of the hydrophobic core was used to derive soluble, highly expressed, stable, monomeric designed proteins with improved characteristics in comparison with natural armadillo proteins. The designed sequences constitute then the starting point for the generation of designed armadillo repeat protein libraries for the selection of peptide binders, taking advantage of their modular structure and their conserved binding mode.

S06-2

Design, synthesis and activity of phosphorus containing peptides as inhibitors of bacterial ureases

Berlicki, Lukasz[1]; Grabowiecka, Agnieszka[1]; Vassiliou, Stamatia[2]; Yiotakis, Athanasios[2]; Kafarski, Pawel[1]
[1]Department of Bioorganic Chemistry, Wroclaw University of Technology, POLAND; [2]Department of Organic Chemistry, University of Athens, GREECE

Urease (E.C. 3.5.1.5) is an enzyme catalyzing the last step of nitrogen metabolism — hydrolysis of urea into ammonia and carbamate 1.. This protein is highly important for the survival of pathogenic bacteria *Helicobacter pylori* and *Proteus vulgaris* in digestion and urinal tracts, respectively (2). The release of ammonia upon urea hydrolysis by urease causes the increase of local pH providing microenvironment suitable for bacterial existence. *Helicobacter pylori* may cause stomach ulcers and finally stomach cancer, while *Proteus* species are responsible for urinal tracts inflammations. Thus, inhibitors of urease are potential drugs against these diseases. Among several known inhibitors of urease, phosphoramidates are the most potent. Phosphorus acid diamide — enzymatic reaction transition state analogue is the simplest example of this group of compounds. The main disadvantages of this class of compounds are their low stability in aqueous solutions and low selectivity. On the basis of crystal structure of *Helicobacter pylori* urease novel class of urease inhibitors — phosphonic and phosphinic peptides were designed. Proposed scaffold gives the possibility of several modifications in order to enhance both inhibitory activity and selectivity towards pathogen protein. Subsequently, several phosphonic, P-methyl- and P-hydroxymethyl-phosphinic compounds were synthesized. Evaluation of their potency in vitro against bacterial ureases showed high activity, with inhibitory constants for most active compounds being in nanomolar range.

S06-3

Transduction of Optimized, Monomeric Peptide Aptamers Targeting Stat3 Prevents Proliferation and Induces Apoptosis in Cancer Cells

Kunz, Christian; Weiß, Astrid; Groner, Bernd; Heinz, Corina
Insitute for Biomedical Research, GERMANY

Signal transduction events often involve the assembly of protein complexes dependent upon modular interactions. The inappropriate assembly of modular components plays a role in oncogenic transformation and can be exploited for therapeutic purposes. Selected peptides embedded in a scaffold protein, so-called peptide aptamers, can serve as competitive inhibitors of intracellular protein functions in cancer cells. Therapeutic application depends on binding specificities and affinities, as well as on the production and purification characteristics of the peptide aptamers and their delivery into cells. We carried out experiments to improve the properties of the scaffold. We found that the commonly employed bacterial thioredoxin scaffold is suboptimal for therapeutic purposes, since it aggregates during purification and is most likely immunogenic in humans. In contrast, a modified version of the human thioredoxin (hTrx) can be efficiently produced in bacteria and purified with high yield. We removed five internal cysteines of hTrx to circumvent aggregation during purification, which, we found, is a prerequisite for efficient uptake into cells. By inserting our previously characterised peptide aptamer DD3 into the modified hTrx scaffold, binding properties of the aptamer to the dimerization domain of the transcription factor Stat3 were retained. Addition of a protein transduction domain (PTD), consisting of nine arginines, results in a fusion protein, which is taken up by cultured cells. We show that the transduced aptamers prevent nuclear import of phosphorylated Stat3. As a result, treatment of various cancer cells, expressing constitutively activated Stat3, with purified peptide aptamers strongly inhibits Stat3 signalling, causing cell growth arrest and inducing apoptosis. Our data show, that optimization of the scaffold offers new perspectives to overcome the technical difficulties encountered with the development of therapeutically active peptide aptamers.

S06-4

Detection and modification of intracellular calpain activity by cell penetrating peptide conjugate

Bánóczi, Zoltán[1]; Farkas, Attila[2]; Alexa, Anita[2]; Tantos, Ágnes[2]; Tompa, Péter[2]; Friedrich, Péter[2]; Hudecz, Ferenc[1]
[1]Research Group of Peptide Chemistry, Eötvös L. University, HUNGARY; [2]Institute of Enzymology, Biological Research Center, Hungarian Academic of Science, HUNGARY

Calpains are intracellular cysteine proteases and are of considerable interest due to their involvement in numerous physiological and pathological events. Our aim is to study the intracellular activity and function of calpains. For this, new cell-penetrating calpain substrate and calpain activator peptides were developed. Our early results suggested that Dabcyl-TPLKSPPPSPR-EDANS acts as an efficient subtrate of isolated calpains (1). This compound is specific for calpain even in cell lysate, but unfortunately has poor cell-uptake. Thus we have designed its new dervivatives with cell-penetrating capacity. In order to achieve this we have elongated the sequence at the C-terminal with heptaarginine unit, Dabcyl-TPLKSPPPSPRE(EDANS)R7. For preservation the necessary distance between the two FRET partners, we inserted a Glu residue between the substrate and heptaarginine (2). We found that this conjugate was even better substrate of Calpain B than the unconjugated one and could enters COS-7 cells efficiently. Specific activation of intracellular calpain may be a suitable tools to study its function. For this, we synthesised a new group of cell-penetrating calpastatin-peptide conjugates. The calpastatin A or C subunit related peptides with calpain activatory effect were covalently conjugated to penetratin via amide, thioether, or disulfide bond (3). Our results show that conjugates with different bond possess essentially the same level of activation and also could internalise into COS-7 cells. We also noticed that even the conjugate with disulfide linkage are stable and activate m-calpain after intracellular translocation under the conditions studied. Thus, these conjugates seem to be appropriate as molecular tools to activate intracellular m-calpain and to study calpain functions in living cells.

References:

1. Tompa, P. et al. J. Biol. Chem. 279, 20775–20785, 2004
2. Bánóczi, Z. et al. Bioconjugate Chem. (submitted), 2008 3. Bánóczi, Z. et al. Bioconjugate Chem. 18, 130-137, 2007

Peptides as drugs

S07-1

A leptin receptor glycopeptide agonist for leptin replacement therapy

Otvos, Laszlo[1]; Cassone, Marco[1]; Terrasi, Marianna[1]; Scolaro, Laura[1]; Wade, John[2]; Knappe, Daniel[3]; Hoffmann, Ralf[3]; Henry, Belinda[4]; Clarke, Iain[4]; Surmacz, Eva[1]
[1]Temple University, UNITED STATES; [2]Howard Florey Institute, AUSTRALIA; [3]Leipzig University, GERMANY; [4]Monash University, AUSTRALIA

Leptin, a hormone produced by adipose tissue, regulates energy balance in the hypothalamus and is involved in fertility, immune response and carcinogenesis. The existence of disorders related to leptin deficit or overabundance calls for the development of drugs acting on the leptin receptor (ObR). We synthesized individual arms of three proposed ObR-binding leptin fragments (sites I-III), their reportedly antagonist analogs, and a peptide chimera composed of two site II arms. To assess the pharmacological utility of leptin fragments, we studied their ability to stimulate the growth of ObR-positive and ObR-negative cells. The combined site II construct and site III derivatives selectively reversed leptin-induced growth of ObR-positive cells at mid-nM concentrations. However, these peptides appeared to be partial agonists as they activated cell growth in the absence of exogenous leptin. A designer site III analog, featuring non-natural amino acids at terminal positions to decrease proteolysis and a blood-brain barrier (BBB) penetration-enhancing carbohydrate moiety, proved to be a full agonist to ObR, i.e., it stimulated proliferation of different ObR-positive but not ObR-negative cells in the presence or absence of leptin. This glycopeptide bound to isolated ObR and activated ERK 1/2 signaling in ObR-positive MCF-7 cells at 200 – 500 nM concentrations. The glycopeptide was stable in mouse serum, readily crossed epithelial/astrocyte cell layers, and was distributed into the brain of Balb/c mice after intraperitoneal administration. Finally the efficacy of the glycopeptide was tested on typical leptin functions in sheep. Voluntary food intake of program-fed animals was reduced by 25% and central infusion increased post-prandial thermogenesis in muscle by 3 degrees C without an effect on core body temperature. These characteristics suggest a potential pharmaceutical utility of the designer site III glycopeptide in leptin-deficient diseases.

S07-2

Novel non-peptide ghrelin receptor ligands based on 1,2,4-trisubstituted triazoles

Moulin, Aline[1]; Blayo, Anne-Laure[2]; Demange, Luc[2]; Galleyrand, Jean-Claude[2]; Locatelli, Vittorioi[3]; Torsello, Antonio[3]; Perrissoud, Daniel[4]; Fehrentz, Jean-Alain[2] ; Martinez, Jean[2]
[1]Max Mousseron Biomolecule Institute (IBMM), FRANCE; [2]IBMM, FRANCE; [3]University of Milano, ITALY; [4]Aeterna-Zentaris, GERMANY

The central actions of ghrelin include stimulation of appetite and GH secretion. Such properties support the hope that ghrelin receptor antagonists could be useful for the treatment of obesity. However for an efficient anti-obesity activity, a ghrelin antagonist should counteract the orexigenic effect of ghrelin but not the GH secretagogue effect, since GH deficiency is frequently associated with increased adiposity. Starting from a triazole scaffold, we have designed and prepared a series of novel small molecules with high binding affinity for the cloned human GHS-R1a and we have investigated their effects on food intake and GH secretion in animal models. The systematic screening of over 250 novel compounds for their ability to displace radiolabelled ghrelin and to activate or to inhibit calcium uptake in cells transiently expressing GHS-R1a led to the characterization of several compounds acting as GHS-R1a ghrelin full agonists, partial agonists or antagonists. Our results showed that non-peptide compounds characterized as in vitro GHS-R1a ghrelin antagonist or partial agonist were able to inhibit food intake without altering in vivo GH secretion. The dissociated effect of the novel ghrelin receptor ligands on food intake and GH secretion supports the role of different subtypes or signaling pathways of the ghrelin receptor in the control of these functions. Thus this report supports the feasibility of a specific pharmacological modulation of the ghrelin effect on appetite.

S07-3

The Proline-Rich Antimicrobial Peptide Dimer A3-APO and its Single-Chain in vivo Metabolite Represent a new Paradigm in Microbiology, Pharmacology and Drug Development

Cassone, Marco[1]; Vogiatzi, Paraskevi[1]; Wade, John D[2]; Rozgonyi, Ferenc[3]; Szabo, Dora[4]; Kocsis, Bela[4]; Otvos, Laszlo, Jr[1]
[1]Sbarro Institute for Cancer Research and Molecular Medicine, Temple University, Philadelphia, UNITED STATES; [2]Howard Florey Institute, University of Melbourne, Victoria 3010, AUSTRALIA; [3]Microbiology Laboratory, Dept of Dermatology, Dermatooncology and Venerology, Semmelweis University, Budapest, HUNGARY; [4]Institute of Medical Microbiology, Semmelweis University, Budapest, HUNGARY

Structure-activity relationship studies of native proline-rich antimicrobial peptides together with biochemical evidence points to a multiple mode of action including disintegration of bacterial membranes and inhibition of the bacterial heat shock protein, DnaK. Molecular modeling of the bioactive protein indicates that the peptide binding site is located at the D-E helix region of the carboxy-terminal multihelical lid, a domain directly involved in dimer formation and needed for the protein refolding activity of DnaK. A series of peptide dimers were designed from which A3-APO was selected for its highest efficacy against several Enterobacteriaceae as well as a few Gram-positives such as Staphylococcus saprophyticus. The Minimal Inhibitory Concentrations of A3-APO in undiluted broth ranged from 1 to 8 mg/L for E. coli, K. pneumoniae, S. typhimurium and S. saprophyticus strains, and was independent of Multi-Drug Resistance (MDR) status. A3-APO was tested in CD-1 female mice pre-treated with 18 mg/kg cisplatin for four days then infected intraperitoneally (ip) with LD90 amount of the extended spectrum ß-lactamase producing, fluoroquinolone-resistant E. coli 5770 strain. A total ip dose of 3x10 mg/kg each in four hour intervals led to blood sterilization and survival improvement, similar to imipenem added at a higher dose. Through inactivating of resistance enzymes, A3-APO was able to recover in vitro the lost activity of conventional antibiotics including chloramphenicol, β-lactams, sulfonamides or trimethoprim with a partial or full synergic effect. However, the synergy appeared to be individual strain and drug combination-dependent. While A3-APO remains the most effective amongst the many designer dimers studied, a single chain, natural metabolite identified in vivo showed a 2-4 fold increase in activity against some test strains, and appears to be an ideal candidate for preclinical development based on its microbiological, pharmacological and cost/benefit.

S07-4

New Approaches to the Design, Synthesis and Biochemical and Biophysical Evaluation of Heteromultivalent Ligands for Detection and Treatment of Cancer

Hruby, Victor[1]; Josan, Jatinder[2]; Vagner, Josef[3]; Fernandes, Steve[4]; Handl, Heather[4]; Xu, Liping[4]; Lynch, Ronald[5]; Mash, Eugene[2]; Gillies, Robert[6]
[1]University of Arizona, Departments of Chemistry, BIO5, and Biochemistry and Molecular Biophysics, UNITED STATES; [2]University of Arizona, Department of Chemistry, UNITED STATES; [3]University of Arizona, Department of Chemistry and BIO5, UNITED STATES; [4]University of Arizona, Biochemistry and Molecular Biophysics, UNITED STATES; [5]University of Arizona, Department of Physiology, UNITED STATES; [6]University of Arizona, BIO5 and Biochemistry and Molecular Biophysics, UNITED STATES

Many cancers including pancreatic cancer and melanoma cancer are highly resistant to treatment especially following metathesis. Furthermore, it has become increasingly clear that essentially all cancers have multiple phenotypes so any single approach to treatment will fail for many persons with that cancer. To overcome these problems we are taking a novel approach which directly addresses the cancer state. In particular we are targeting cancer cells with a single ligand that contains an imaging agent and/or a therapeutic agent and two or more different ligands that target two or more cell surface proteins (receptors, cytokines, ion channels, enzymes, etc.) that distinguish a cancer cell from normal cells. This requires the development of scaffolds that will place the ligands at sufficient distance from each other so as to crosslink two or more different cell surface proteins, and imaging and/or therapeutic agents such that it (they) will not interfere with binding. Both thermodynamic and kinetic considerations are critical for obtaining useful ligands. We will report on the design, synthesis, biochemical, biophysical and imaging of several multimeric, multiheterovalent ligands which demonstrate for the first time crosslinking of two different cell surface proteins and the viability of this approach.

Acknoledgements: Supported in part by grants from the U.S. Public Health Service, National Institutes of Health, National Cancer Institute, BIO5, and the Arizona Biological Research Commission.

S07-5

Can biotherapeutics ever be cheap enough for developing countries? Anti-HIV chemokines as a case study

Offord, Robin[1]; Hartley, Oliver[2]
[1]Mintaka Foundation for Medical Research, SWITZERLAND; [2]Université de Genève, SWITZERLAND

Women and girls in developing countries, the group now most at risk from HIV infection, urgently need substances (wrongly but universally called ‘microbicides’) for intravaginal application before intercourse to prevent transmission of the disease.

We developed a potent microbicide lead known as PSC-RANTES, a 69-residue protein based on the CCR5 ligand RANTES (CCL5). It is still the only published example of a substance tested in the standard macaque vaginal challenge model to protect all animals in a dose group even when used alone. However, it contains non-coded amino acids, and its manufacture requires synthetic steps. Total synthesis would never permit its production at a cost per dose low enough to permit worldwide use.

Using a novel phage-display technique we have identified new RANTES analogues that show equivalent potency to PSC-RANTES but, consisting only of coded residues, can be produced by microbial fermentation or other biological routes.

For a Phase I trial, where some tens of grams only are required and cost would not yet be the determining issue, it would be quite practical to express the wanted molecules intracellularly, as soluble protein or inclusion bodies. Downstream processing is normally the principal element that determines cost, and for the multi-ton production that would be required if these molecules came into general use, an expression system that leads to secretion of the product into the culture medium would probably be best. This is done at the scale of hundreds of thousands of tons every year in the production of food-grade GMP enzymes for the detergent industry.

To be viable as a solution for developing countries, calculation suggests that it would be necessary to produce our molecules at a maximum cost of goods of approximately $2/gram. This figure seems at first sight to be wildly discrepant with the perceived cost of common biotherapeutics, but we will present experimental results tending to correct that perception.

Peptide pharmacology

S08-1

Chimeric opioid-tachykinin ligands as a new prospective analgesics in chronic pain

Kosson, Piotr[1]; Carr, Daniel B[2]; Misicka, Aleksandra[3]; Lipkowski, Andrzej W[1]
[1]Medical Research Centre Polish Acad Sci, POLAND; [2]New England Medical Centre, UNITED STATES; [3]Department of Chemistry Warsaw University, POLAND

Multidrug therapies became routine approach in modern medical treatment protocols. However, using combinations of drugs has disadvantages, including differences in pharmacological profiles of single drug component. Therefore, over twenty years ago we have proposed development of multitarget medicines as a new avenue of drug discovery. Identification of numerous endogenous components that participate in the formation, transmission, modulation and perception of pain signals offers numerous strategies for the development of new analgesics. One of them is hybridization of opioid pharmacophores with tachykinin receptor ligands. Tachykinins, like substance P (SP) produces both hyperalgesia and, at low doses, a naloxone-sensitive analgesia. Very likely, these opposite effects of SP in the spinal cord, are mediated through activation of various self-regulatory mechanisms. Modulation of tachykinin receptor system is probably significant component of tolerance and dependence development. Therefore, various new opioid agonist-tachykinin antagonist and opioid agonist-tachykinin agonist have been synthesized and tested to develop new medicines for chronic pain treatment. The communication will present new group of opioid agonist-tachykinin agonist that express strong analgesic activity even after peripheral application. These new compounds are interesting candidates for treatment of chronic pain because they express very low tolerance development properties.

Acknowledgements: Presented studies have been supported in part with EU grant Normolife (LSHC-CT-2006-037733)

S08-2

Determination of GPCR structures and activation mechanisms with reactive peptide probes.

Arsenault, Jason[1]; Clément, Martin[2]; Beaulieu, Marie-Ève[1]; Holleran, Brian[1]; Leduc, Richard[1]; Guillemette, Gaétan[1]; Lavigne, Pierre[1]; Escher, Emanuel[1]
[1]Department of Pharmacology, Université de Sherbrooke,, CANADA; [2]Department of Pharmacology, University of Sherbrooke, CANADA

The G-protein coupled receptor family is the most important family of regulatory proteins in the genome and, hence, the most important drug target family of conventional and future drug therapy. In spite of this, structure-based or target derived drug design is still hampered for GPCRs since their structures are extremely difficult to access and their activation mechanisms even more. Thus far, reactive peptides probes have allowed identifying the immediate receptor environment of the ligands of aminergic, proteinergic and peptidergic GPCRs, among them, the human Angiotensin II receptor AT1. The determination of several such contact points in this receptor has allowed proposing the first molecular structures of this receptor but also in others. In the present contribution, the combined application of different photochemical probes, together with the chemical selectivity of such probes, their introduction in several positions of a peptide ligand, as well as iterative mutagenesis approaches, have allowed to obtain not only molecular structures of such peptide ligand bound receptors but also the thermodynamic behaviour and information about structural changes associated to GPCR activation. These activation mechanisms are addressed with constitutively active and constitutively inactive receptor mutants and reactive peptide ligands of agonistic as well as antagonistic nature. Supported by grants from CIHR.

S08-3

3D-Pharmacophore Based Virtual Screening: Discovery of Opioid GPCR Lead Compounds.

Bryant, Sharon[1]; Balboni, Gianfranco[2]; Guerrini, Remo[3]; Salvadori, Severo[3]; Marczak, Ewa[1]; Lazarus, LH[1]
[1]National Institute of Environmental Health Sciences, UNITED STATES; [2]University of Cagliary, Depart. of Toxicology, ITALY; [3]University of Ferrara, Dept. Pharm. Sci. & Biotech. Center, ITALY

3D-chemical featured based pharmacophores were developed and used for screening several compound databases with the aim of identifying active molecules targeted for G-protein coupled opioid receptors. Previously developed models proposing bioactive conformations of potent opioid ligands containing 2',6'-dimethyltyrosine (Dmt), 1,2,3,4-tetrahydroisoquinoline carboxylic acid (Tic) and Dmt-Tic-Bid (1H-benzimidazole-2-yl) were utilized to develop various pharmacophores based on previously designed molecular models and using the program LigandScout. The pharmacophores were deployed as 3D-search queries using Catalyst to screen the Derwent World Drug Index, National Cancer Institute and ChemDiv databases. Seven pharmacophores retrieved hits from the databases. Three hits were tested experimentally. One exhibited high μ-opioid receptor affinity (Kiμ = 0.075 nM); the others displayed moderate μ-affinities (Kiμ = 4.26 & 6.03 μM) and no competitive binding to δ-opioid receptors. This approach enabled the validation of the proposed Dmt-Tic opioid pharmacophore as well as identification of lead compounds for development of opioid therapeutics. Furthermore, 3D-chemical featured based virtual screening offers a rapid and effective means to identify new lead compounds when the bioactive forms of ligands and protein target structures are unknown. Compared to high through-put screening the hit rate for identifying active compound leads using 3D-chemical featured based pharmacophore virtual screening was increased 1000-fold.

S08-4

Non-opioid beta-endorphin receptor: localization and function

Navolotskaya, Elena; Kovalitskaya, Yulia; Sadovnikov, Vladimir Branch of Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences, Science Avenue 6, RUSSIAN FEDERATION

We have synthesized the beta-endorphin-like decapeptide SLTCLVKGFY (referred to as immunorphin {IMN}) corresponding to the sequence 364-373 of the heavy chain of human IgG(1-4) and found it to be a selective agonist of non-opioid (naloxone-insensitive) beta-endorphin receptor on human T lymphocytes, mouse peritoneal macrophages, rat brain synaptic membranes, and human T-lymphoblastoid cell line Jurkat. The study of biologic activity of IMN has revealed that it enhances the concanavalin A-induced proliferation of human T lymphocytes in vitro, activates mouse peritoneal macrophages in vitro and in vivo, and stimulates the growth of human T-lymphoblastoid cell lines Jurkat and MT-4. We have recently identified non-opioid beta-endorphin receptors on the membranes isolated from rat adrenal cortex. IMN at concentrations of 0.1-1000 nM was found to inhibit the adenylate cyclase activity in adrenocortical membranes, while intramuscular injection of IMN at doses of 1-100μg/kg was found to reduce the secretion of corticosterone from the adrenals to the bloodstream.

S08-5

Structure Prediction of a G Protein-Coupled Receptor — The Neuropeptide Y Receptor

Günther, Robert[1]; Lindner, Diana[1]; Meiler, Jens[2]; Beck-Sickinger, Annette G.[1]
[1]Leipzig University, Institute of Biochemistry, Faculty of Biosciences, Pharamacy, and Psychology, GERMANY; [2]Vanderbilt University, Center for Structural Biology, UNITED STATES

G protein-coupled receptors (GPCRs) play a crucial role in biochemical communication processes. These membrane-bound receptors are characterised by seven trans-membrane helices connected via three intracellular (ICL) and three extracellular loops (ECL). Upon binding of the ligand at the extracellular regions, the signal is transduced into the cell.

Approximately 60 % of all drugs currently on the market act on GPCRs. However, little is known on the detailed receptor-ligand interaction due to the lack of structural data. Up to now, the three-dimensional structures of only two GPCRs have been experimentally elucidated: bovine rhodopsin(1) and the human β2-adrenergic receptor(2). Both structures were crystallised with a (covalently) bound inverse agonist, that shows no interaction with the ECLs.

The vast majority of endogenous ligands of GPCRs most likely interact with the receptors via the extracellular regions. Thus, profound knowledge on the structure of the flexible ECLs is desired in order to understand the mechanism of GPCR signalling.

Based on the 3-dimensional structure of rhodopsin and the recently published crystal structure of the β2-adrenergic receptor, we present a 3D-model of a GPCR with special focus on the flexible ECLs. These loops were explicitly modelled employing the Rosetta de novo modelling approach(3). Significant similarities with the loop structures of rhodopsin and the β2-adrenergic receptor were found.

Our novel 3-dimensional model of the GPCR (Y-receptor) will be discussed in light of recent experimental data.

References:

1. Palczewski K, et al. (2000) *Science* **289**, 739-45
2. Cherezov V, et al. (2007) *Science* **318**, 1258-65
3. Rohl CA, et al. (2004) *Proteins: Struct Funct Bioinf* **55**, 656-7

SO8-6

GPC-Receptors not Ligands Decide on Binding Mode in Multi-Receptor/Multi-Ligand Systems

Lindner, Diana; van Dieck, Jan; Günther, Robert; Hofmann, Hans-Jörg; Beck-Sickinger, Annette G. Leipzig University, GERMANY

Many G protein coupled receptors belong to families of different receptor subtypes, which are recognized by a variety of distinct ligands. To study such a multi-receptor/multi-ligand system we investigated the Y-receptor family. This family consists of four G protein coupled Y receptors in human (hY1R, hY2R, hY4R, and hY5R) and is activated by the so-called NPY hormone family, which itself consists of three native peptide ligands named neuropeptide Y (NPY), pancreatic polypeptide (PP), and peptide YY (PYY). We recently could show that one conserved Asp-residue is essential for ligand binding in all four Y-receptors, but binds the endogenous ligands in a different mode by interacting with different ligand Arg-residues (1). As the hY5R shows high affinity for all ligands, we performed an extensive mutagenesis study and identified several novel important residues. By matching them with the important ligand residues we were able to discover a novel interaction point between hY5R and NPY, and tested whether PP and PYY bind to the receptor in the same manner. The interaction between NPY(Arg25) and hY5R(Asp2.68) as well as between NPY(Arg33) and hY5R(Asp6.59) is maintained in the binding of PYY and PP to hY5R, but different to the PP–hY4R and NPY–hY1R contact points. Therefore, we provide evidence that the receptor subtype and not the ligand decides on the binding mode of a ligand. Furthermore, the first hY5R model was set up on the basis of the crystal structure of bovine rhodopsin. We can show that most of the residues identified to be critical for ligand binding are located within the now postulated binding pocket.

References:

1. N. Merten et al. (2007) J. Biol. Chem 282, 7543-7551.

Protein misfolding and amyloid peptides

S09-1 Inv

Life on the Edge: The Nature and Origins of Protein Misfolding Diseases

Christopher M. Dobson
University of Cambridge, Department of Chemistry
Lensfield Road, Cambridge CB2 1EW, UK

Natural proteins are a highly select group of molecules, and their properties have a number of very special characteristics when compared to random sequences of amino acids, one of which is the ability to fold to unique and often highly intricate structures [C.M. Dobson, *Nature* 426, 884-890 (2003)]. This characteristic has enabled biological systems to generate a vast range of functions and an astonishing degree of specificity in their chemical processes. Great progress has been made recently in defining the conceptual basis and fundamental principles that underlie the folding of natural proteins. Of particular significance have been approaches that bring together biochemical and biophysical experiments with computer simulations to define the characteristics of the ensembles of protein structures that are populated *in vitro* at different stages of the folding process of individual proteins [D.M. Korzhnev, X. Salvatella, M. Vendruscolo, A.A. Di Nardo, A.R. Davidson, C.M. Dobson and L.E. Kay, "Low Populated Folding Intermediates of the Fyn SH3 Domain Characterized by Relaxation Dispersion NMR", **Nature 430**, 586-590 (2004)].

In addition, the roles of a wide variety of cellular processes associated with the folding of proteins *in vivo* are being unravelled, leading to an increasingly detailed understanding of the life cycles of proteins from their synthesis and degradation.

Because proteins are involved in every chemical process taking place within living systems, the failure of proteins to fold, or to remain correctly folded, can give rise to serious cellular malfunctions that frequently lead to disease. One particularly important group of such diseases is associated with the aggregation of misfolded proteins into remarkable thread-like structures known as amyloid fibrils [T.P. Knowles, A.W. Fitzpatrick, S. Meehan, H.R. Mott, M. Vendruscolo, C.M. Dobson and M.E. Welland, "Role of Intermolecular Forces in Defining Material Properties of Protein Nanofibrils", **Science 318**, 1900-1903 (2007)], and includes disorders ranging from Alzheimer's disease to late-onset diabetes, conditions that are becoming increasingly common in our aging populations. The manner in which the normal soluble forms of peptides and proteins can convert into these pathogenic amyloid structures is being uncovered by a wide variety of *in vitro* experimental studies along with theoretical simulations and bioinformatics studies [C.M. Dobson and F. Chiti, *Annu. Rev. Biochem.* 75, 333-366 (2006)]. As with folding, these studies are increasingly being linked to events occurring *in vivo* using a variety of strategies. Of particular interest are experiments an simulations designed to link the principles of misfolding and aggregation to the effects of such processes in model organisms such as *Drosophila* (the fruit fly) [L. M. Luheshi, G.G. Tartaglia, A.C. Brorsson, A.P. Pawar, I.E. Watson, F. Chiti, M. Vendruscolo, D.A. Lomas, C.M. Dobson and D.C. Crowther, "Systematic In Vivo Analysis of the Intrinsic Determinants of Amyloid-beta Pathogenicity", **PLoS Biol. 5**, e290 (2007)]. This talk will draw together some of the ideas that are emerging from recent work in our laboratory including evidence for the extremely narrow boundary between normal and aberrant behaviour [Tartaglia et al., *Trends Biochem. Soc*. 32, 204-206 (2007)], and how this concept sheds light on the origin, current proliferation and potential means of prevention of many of the diseases associated with misfolding.

S09-2 Inv

Elucidating molecular mechanisms on and off the pathway to amyloid deposition

Miranker, Andrew
Yale University, UNITED STATES

Polypeptide chains have a generic capacity to self-associate into linear, non-covalent assemblies termed amyloid fibers. Such fibers give the appearance of violating basic principles of protein folding because they can serve as templates of their own propagation. Regardless of the identity of the starting precursor, the resultant amyloid structures share a number of properties. These include insolubility, protease resistance, chemical resistance and cytotoxicity. Proteins are therefore under selective pressure to avoid this phenomenon and are most commonly associated with diseases of advancing age, such as Alzheimer's, and diseases associated with medical therapy, such as dialysis related amyloidosis (DRA). In our laboratory, we investigate fibrillogenesis as a biologically relevant chemical reaction. Therefore, understanding the mechanism of assembly requires insight into the structures, energetics and pathways which relate different intermediate states. In general, insights are elusive due to the transient and heterogeneous nature of fibrillogenesis. We are nevertheless able to shed light by combining a range of biophysical approaches and focusing on protein systems ranging from peptide models to globular proteins. The commonality among such investigations reveals the rules governing this class of polymeric assembly.

S09-3 Inv

Alzheimer Peptides. The membrane-induced random coil-to-β-structure transition

Seelig, Joachim
University of Basel, SWITZERLAND

Biologically important peptides such as the Alzheimer peptide Aβ(1–40) display a reversible random coil-to-β-structure transition at anionic membrane surfaces. We present a new method to quantitatively analyze the thermodynamic parameters of the membrane-induced β-structure formation. We have used the model peptide $(KIGAKI)_3$ and eight analogues in which two adjacent amino acids were substituted by their d-enantiomers. The positions of the d,d pairs were shifted systematically along the three identical segments of the peptide chain. The β-structure content of the peptides was measured in solution and when bound to anionic lipid membranes with CD spectroscopy and isothermal titration calorimetry. The thermodynamic parameters were found to be linearly correlated with the extent of β-structure formation, characterized by an enthalpy change of $DH_\beta = -0.23$ kcal/mol per residue, an entropy change of $DS_\beta = -0.24$ cal/molK per residue and a free energy change of $DG_\beta = -0.15$ kcal/mol residue.(1) We have further synthesized peptides containing the KIGAKI module with a chain length varying between n = 6 to 30. The above parameters are valid for peptides with a chain length of n >12. For short chains with n = 12 β-structure formation becomes unfavorable with $DG_\beta = 0.08$ kcal/mol per residue,$DH_\beta = -0.23$ kcal/mol and$DS_\beta = -0.24$ cal/molK. The residual free energy of membrane-induced β-structure formation for long peptides is close to that of membrane-induced α-helix formation.(2)

References:

1. Meier, M. and Seelig, J. Thermodynamics of the Coil-to-β-Sheet Transition in a Membrane Environment. J. Mol. Biol. 369, 277–289 (2007)
2. Meier, M. and Seelig, J. Length Dependence of the Coil-to-β-Sheet Transition in a Membrane Environment. J. Am. Chem. Soc. 130, 1017-1024 (2008)

S09-4

Retro-Enantio *N*-Methylated Peptides as β-Amyloid Aggregation Inhibitors

Grillo-Bosch, Dolors[1]; Carulla, Natalia[2]; Cruz, Montse[1]; Sanchez, Laia[1]; Madurga, Sergio[3]; Rabanal, Francesc[4]; Giralt, Ernest[1]
[1]Institute for Research in Biomedicine Barcelona, SPAIN; [2]ICREA researcher at Institute for Research in Biomedicine Barcelona, SPAIN; [3]Dept Physical Chemistry/ University of Barcelona, SPAIN; [4]Dept. Organic Chemistry/ University of Barcelona, SPAIN

Current treatments for Alzheimer's disease (AD) offer only symptomatic benefits to patients. Thus, the development of new treatments to actually slow, stop or cure the disease is greatly needed. The fact that β-amyloid peptide (βA) aggregation is central to AD, together with an increasing knowledge on amyloid fibril structure and dynamics, makes inhibition of the aggregation process of βA an attractive and promising target for AD therapy. Here we present a new peptide, *inr*D, designed using the retro-enantio approach in tandem with the use of *N*-methylated amino acids and show that disrupts β-amyloid aggregation and decreases βA cytotoxicity while proteolytically stable. In vitro and in silico studies have revealed that our strategy could be very promising not only for designing molecules to treat Alzheimer's disease, but also for designing ligands for a protein surface having a β-sheet surface-patch.
In conclusion, *inr*D is a very promising lead compound to treat AD, owing to its ability to interfere with βA aggregation and decrease βA cytotoxicity, while being stable to proteases. Docking and molecular dynamics calculations indicate that although *inr*D does not maintain the native side-chain pairing observed in βA molecules within the fibril, it can establish an effective hydrogen-bonding pattern with the fibril. Our results with *inr*D are testament of the value that the retro-enantio approach can have for obtaining peptides able to interact with β-sheet proteins. This observation may have major implications for the design of bioactive peptides for myriad therapeutic indications, including those targeted at disrupting protein-protein interactions.

Lipid chemistry and protein folding

S10-1 Inv

Oxidatively stressed lipids and polypeptide misfolding in human disease

Axelsen, Paul
Departments of Pharmacology, Biochemistry, Biophysics, and Medicine, University of Pennsylvania School of Medicine Philadelphia, Pennsylvania, USA

Evidence of elevated oxidative stress has been found in many human diseases, including all forms of Alzheimer's Disease. The substances in the brain most vulnerable to oxidative stress are ordinary membrane lipids, and they yield highly reactive metabolic products when subjected to oxidative stress. Recent investigations have demonstrated that polypeptides known as amyloid beta (Abeta) proteins can dramatically accelerate oxidative stress and lipid damage in membranes. In turn, the reactive products of oxidative lipid damage covalently modify Abeta proteins, converting them into a form that promotes the aggregation of unmodified Abeta proteins and accelerates amyloid fibril formation. As a consequence, the normal metabolic degradation pathways for membrane lipids become key components of a chemical amplification mechanism that is triggered by Abeta proteins and that results in a reduction of Abeta protein solubility and accelerated fibrillogenesis.

In addition to promoting the formation of amyloid fibrils, this amplification mechanism can exert multifaceted neurotoxicity by generating free radicals, depleting antioxidant reserves, altering transmembrane signals, and elaborating compounds with known propensities to cause chemical modification of proteins and nucleic acids. Because this type of mechanism is inhibited by free sulfhydryl groups, it may explain the well-known inverse relationship between the risk of Alzheimer's disease and the number of Cys residues in isoforms of lipoprotein E.

S10-2

Hairpin Peptide Inhibitors of Amyloid Fibril Formation

Andersen, Niels
University of Washington, UNITED STATES

Amyloid fibril formation is associated with at least 17 human diseases; among these, the human-amylin(hAM)-derived deposits in type II diabetes were the discovery system and hAM aggregation is one of the more thoroughly studied systems. To date, inhibitors of beta-aggregate and fibril formation have been polyphenols, mutants of hAM, or short peptide related to the hAM(22-29) sequence, NFGAILSS. We now report that stable beta-hairpin scaffolds displaying Trp and Tyr residues are effective inhibitors, delaying the onset of both the CD changes associated with beta structure formation and the nucleation time and net enhancement of the fluorescence observed with added Thioflavin-T (ThT). Under our test conditions (8 microM hAM, 2% HFIP in 5mM phosphate buffer, pH 7), hAM begins to display an increase in beta structure by CD at 40 min with a constant maximal value from 85 – 220 min. ThT fluorescence also indicates a circa 50 min onset time with a rapid (<20 min) rise to the full response. Inhibition (delayed onset and reduction in the maximal fluorescence enhancement) has been observed with a number of hairpins; those with two Trp residues on a single face of the hairpin are more potent. Of these, our best inhibitor to date, KKLTVWIpGKWITVSA (p = D-Pro), increases the onset time more than 2-fold at equimolar concentrations. At 4 molar equiv., the onset time is greater than 320 min and ThT fluorescence levels out at < 40% of the control value. In analogy to the report by Prof. Ghosh (JACS 2006, p. 14456) that a beta-sheet protein with added Tyr and Trp residues inhibits fibril formation by the Alzheimer-related Abeta peptide; we expect that designed beta-hairpin scaffolds will be more generally applicable and will afford new insights into the recognition phenomena of amyloidogenesis.

S10-3

PrP(106-126) does not interact with membranes in physiological conditions. A biophysical study with model membranes.

Henriques, Sónia[1]; Pattenden, Leonard[2]; Aguilar, Mibel[2]; Castanho, Miguel[1]
[1]Instituto de Medicina Molecular, University of Lisbon, PORTUGAL; [2]Monash University, AUSTRALIA

Prion diseases result from a post-translational modification of the physiological Prion Protein (PrPC) into a scrapie isoform (PrPSc), which accumulates in the central nervous system and is responsible by progressive neuronal loss. Its fragment 106-126 was reported to be the most amyloidogenic region and to maintain pathological features of PrPSc. A role in neurodegeneration was proposed based on the modulation of membrane properties and channel formation. If these processes are important, peptide-membrane interactions would be a key feature to the toxicity of PrPSc. The interaction of PrP(106-126) with model membranes were examined using surface plasmon resonance and fluorescence methodologies. Different parameters relevant to characterisation of peptide-membrane interactions including: membrane charge, viscosity, lipid composition, pH and ionic strength, were studied. We report that PrP(106-126) has a low affinity for lipid membranes under physiological conditions without evidence for membrane disturbances. Membrane insertion and permeabilization only occurs under conditions where strong electrostatic interactions operate. These results show that toxic effects of PrP(106-126) cannot be explained by cell membrane leakage and support the hypothesis that the physiological prion protein, PrPC, mediates PrP(106-126) toxic effects in neuronal cells.

S10-4

Human cystatin C interactions with amyloidogenic molecules

Juszczyk, Paulina[1]; Szymanska, Aneta[1]; Rodziewicz-Motowidlo, Sylwia[1]; Jankowska, El¿bieta[1]; Kolodziejczyk, Aleksandra S.[1]; Sladewska, Anna[1]; Spodzieja, Marta[1]; Paraschiv, Gabriela[2]; Przybylski, Michael[2]; Grzonka, Zbigniew[1]
[1]University of Gdansk, Faculty of Chemistry, Department of Organic Chemsitry, POLAND; [2]University of Konstanz, Laboratory of Analytical Chemistry and Biopolymer Structure Analysis Department of Chemistry, GERMANY

The devastating effect of amyloidogenic proteins and peptides is still not fully understood. Numerous experiments determined many physiological ligands of amyloidogenic proteins. The search for the new molecules binding to amyloids could help to understand the aggregation pathway, cellular toxicity of amyloids or neuroprotective properties of protein ligands. Therefore, it necessitates the development of sensitive analytical methods that enable to clarify details of interactions between amyloidogenic molecules. Recent developments show that selective proteolytic excision combined with mass spectrometric peptide mapping (Epitope-Excision-MS) present high potential for the determination of epitope for antigen-epitope mapping and for the identification of antibody paratope sequences. In this work we present a novel affinity method for protein-peptide interaction studies that enabled identification of the interactions between), serum amyloid A (SAA) andβhuman cystatin C and the amyloid beta peptide (A anti-cystatin C antibody. We report here the identification of the molecular interaction, epitope binding sites and primary structure of recognized by SAA and ,βcystatin C fragments. For the identification of the epitope from A antibody and its paratope binding structure on cystatin C, proteolytic epitope extraction/excision-MS protocols were applied using different proteolytic enzymes. Our results might be of paramount importance for the development of new inhibitors for aggregation processes of all examined cystatin C ligands. The determination of the interacting sites could also be useful in designing new tools for diagnostics in many neurodegenerative disorders. Our studies will allow to speculate whether amyloidogenic molecules association is bona fide phenomenon or just accident. Acknowledgement: University of Gdansk grant BW to Paulina Juszczyk.

Peptide-lipid interactions

S11-1

Structural Studies of Large Fragments of G-Protein Coupled Receptors

Naider, Fred[1]; Becker, Jeff[2]; Neumoin, Alexey[3]; Zerbe, Oliver[3]; Zou, Chao[3]
[1]CUNY, New York, UNITED STATES; [2]2Department of Microbiology, University of Tennessee, Knoxville, UNITED STATES; [3]Institute of Organic Chemistry, University of Zurich, SWITZERLAND

We present our structural studies of larger fragment of G-protein coupled receptors. One system of interest is the Ste2p receptor, for which fragments containing the 7th TM helix as well as TM1-C1-TM2_E1 have been biosynthetically produced by our collaborators Prof. Naider and Becker. The second system to be presented comprises constructs derived from the Y4 receptor, a GPCR targeted by peptides from the NPY family. Herein, we present data on constructs comprising the N-terminal (extracellular) domain and on a construct that contains the N-terminal domain fused to TM1-C1-TM2. The polypeptides have been expressed in isotopically labelled form in E. Coli. Their structures in solution are determined in the presence of phospholipid micelles using high-resolution NMR techniques. NMR is also utilized to establish the way these polypeptides are embedded in the detergent micelles. The resonances of both backbone and sidechain resonances have been almost completely assigned using triple-resonance NMR experiments. This challenging work needed optimization of procedures in all aspects of the work, e.g. in in labeling procedures but also in the spectroscopic work. In particular sample conditions and the detergent had to be extensively optimized. The present data now reveal secondary structure, and details of tertiary structure start to emerge. Progress and problems of the spectroscopy of large GPCR fragments in membrane mimetics will be reviewed. It will be discussed, in which way structures possibly differ from those of the entire receptors.

S11-2

Informatics-based structure design for obtaining hypolipidemic short peptides

Ryuji, Kato[1]; Kani, Kei[1]; Kaga, Chiaki[1]; Takeshita, Toshikazu[1]; Okochi, Mina[1]; Morikawa, Kensei[2]; Yamashita, Yuka[2]; Nagaoka, Satoshi[2]; Honda, Hiroyuki[1]
[1]Nagoya University, JAPAN; [2]Gifu University, JAPAN

Naturally derived peptides with hypolipidemic effects are one of the most ideal biocompatible ingredients for designing preventative functional food for patients suffer from cardiovascular diseases. One strategy to design hypolipidemic peptide is to increase the affinity to bile acid, which result in the inhibition of cholesterol absorption in small intestine. Several peptides had already been reported to suppress cholesterol absorption in vitro and in vivo by having greater binding affinity to bile acids. However, classical approaches to screen candidate peptides from some selected natural products require greater time and expense. If we could previously understand the common structural rule among objective peptides, we could concentrate and reduce the expense of screening by searching specific products that involve the investigated peptide rules in their proteins. For this purpose, we combined peptide array technique with bioinformatic methodologies (hierarchical clustering / prediction models) to understand the common physicochemical structural rule hidden in short peptides that strongly binds to bile acid. Both in clustering and prediction, we effectively introduced amino acid indices to understand the common physicochemical properties of short peptides. As a result, by extracting the physicochemical rules that discriminates peptides with greater affinity and no affinity to bile acid, we succeeded in finding novel peptide sequences and rules to design hypolipidemic short peptide ingredients for functional food.

S11-3

Membrane Interaction of NPY in the Presence of Negatively Charged and Zwitterionic Phospholipids

Thomas, Lars; Scheidt, Holger A.; Bettio, Andrea; Beck-Sickinger, Annette G.; Huster, Daniel; Zschörnig, Olaf
University of Leipzig, GERMANY

Neuropeptide Y (NPY) is one of the most abundant peptides in the central nervous system of mammals. In micellar solution, NPY features an unstructured and flexible N-terminus and a C-terminal α-helix. The interaction of NPY with liposomes was studied using the intrinsic tyrosine fluorescence of NPY and an NPY-fragment comprising amino acids 18-36. The vesicular membranes were composed of phosphatidylcholine and phosphatidylserine at varying molar ratios. From the experimentally measured binding curves the free hydrophobic Gibbs energy for the peptide transfer from aqueous solution to the lipid membrane was calculated to –30 kJ/mol. This agrees well with the model that only the helical segment of NPY interacts with the phospholipids. The hydrophobicity scale predicts a theoretical value of around –25 kJ/mol for membrane binding of this segment. The effective charge of the peptide depends on pH value and is about half of its theoretical net charge. The results were confirmed using the fluorescence of the NPY-analog [Trp32]-NPY. Further, the position of NPY's helix in the membrane was estimated from the intrinsic tyrosine fluorescence of NPY, from quenching experiments with spin labelled phospholipids using [Trp32]-NPY, and from 1H MAS NMR relaxation measurements using spin labelled [Ala31,TOAC32]-NPY. The results suggest that the immersion depth of NPY into the membrane is triggered by the membrane composition. The helix of NPY is located in the upper chain region for zwitterionic membranes but its position is shifted to the glycerol region in negatively charged membranes. For membranes composed of phosphatidylcholine and phosphatidylserine an intermediate position of the helix is observed.

S11-4

Unintended human exposure to toxic microbial ionophoric peptides

Andersson, Maria[1]; Mikkola, Raimo[2]; Jääskeläinen, Elina[2]; Shaheen, Ranad[2]; Kruglov, Alexey[3]; Teplova, Vera[3]; Grigorjeev, Pavel[4]; Saris, Nils-Erik[2]; Salkinoja-Salonen, Mirja[2]
[1]Dept of Applied Chemistry and MicrobiologyUniversity of Helsinki, FINLAND; [2]Dept of Applied Chemistry and Microbiology, University of Helsinki, FINLAND; [3]4Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, RUSSIAN FEDERATION; [4]Institute of Cell Biophysics, Russian Academy of Sciences, RUSSIAN FEDERATION

Many microbially produced substances are known to affect the functioning of mitochondria. Unintended human exposure to such substances has received little attention inspite of the potential great influence of mitochondrial functioning on health. Cereulide is a cyclic peptide, 1153 g/mol, causing emetic food poisonings at concentrations of 0.01 to 1 µg/g food. It is a lipophilic (log Kow > 6) ionophore specific to K+, driving electrogenic influx of K ions into mitochondria at exposure concentrations of ≤ 1 ng/ml. This leads to depolarisation of mitochondria in primary cells (porcine pancreatic beta cells, boar spermatozoa, human NK-cells and T-cells) and many cell lines. Enniatin, a cyclic peptide produced by several species of Fusarium fungi is K+ ionophore like cereulide but about 100 × less potent. Enniatin occurs in Finnish grains at concentrations from 0 to up to 50 mg/kg. Cereulide producing Bacillus cereus were commonly found in infant food formulas and potatoes purchased from consumer markets. Neither the toxin, nor the producer strains, are inactivated by cooking. Producers of cereulide also occurred in indoor materials of buildings that suffered from aged moisture damage, together with Bacillus amyloliquefaciens, highly toxic in cell assays. These contained a novel lipopeptide, 1197 g/mol, amylosin. This contains a polyene tail that may anchor the peptide into biological membranes. It forms cation channels in biological membranes, with selectivity to K+:Na+:Ca2+ of 26:15:3.5. Exposure to 250 ng/ml of amylosin collapsed the mitochondrial membrane potential in somatic cells and spermatozoa. Amylosin was produced by several Bacillus species connected to food poisoning. Mitochondriotoxic peptaibols were also present in water damaged buildings contaminated with Trichoderma harzianum, T. longibrachiatum or Acremonium sp. Thus human exposure to toxic microbial peptides may occur via indoor air as well as via bacterially contaminated food.

S11-5

The synthesis of covalent assemblies of model peptides to study membrane proteins

Meijneke, Tania[1]; Rijkers, Dirk T.S.[2]; Liskamp, Rob M.J.[3]; Killian, J. Antoinette[1]
[1]Utrecht University, Chemical biology and organic chemistry, NETHERLANDS; [2]Utrecht University, Medicinal chemistry on chemical biology, NETHERLANDS; [3]Utrecht University, Medicinal Chemistry and Chemical Biology, NETHERLANDS

Membrane proteins play a major role in health and disease. Since their structure and function is highly influenced by the surrounding lipids, often simple model systems are used to study the general principles of protein-lipid interactions. Examples of a system in which properties of both peptide and lipids can be systematically varied are the WALP peptides (Ac-GW2(LA)8LW2A-NH2) in vesicles of varying lipid-content. The well-defined á-helical WALP peptides mimic the features of natural membrane proteins, nicely incorporate in membranes and can be characterized by various biophysical methods (e.g. solid state 2H-NMR). However, most proteins have multiple membrane spanning segments. To mimic such systems and to study the effect of oligomerization on peptide-lipid interactions we decided to design WALP dimers and tetramers. Firstly, three cysteine-containing WALP analogs, that can be oxidized to form covalent dimers, were synthesized. Several experiments were performed, using SDS-PAGE or HPLC to monitor the dimerization reaction. Secondly we synthesized a tetrameric assembly of the WALP peptide, based on the TASP approach of Mutter and using 'click' chemistry to attach the helices to a scaffold. The scaffold was a cyclic peptide with four alkyne-functionalized lysines. The WALP peptide was equipped with an N-terminal azide and a C-terminal tail to facilitate solubilisation and characterization of the reaction product. A tetramer (MW=14 kDa) was obtained, as shown by SDS-PAGE, GPC and MALDI. The properties of the dimers and tetramers will be tested in a membrane environment (e.g. secondary structure and mode of incorporation). Next their interactions with lipids will be compared to those of monomers (e.g. efficiency of lipid flip-flop and lipid chain order)

Antimicrobial peptides

S12-1

Synergism Between Temporins in Killing of Gram-negative bacteria and in Neutralizing Lipopolysaccharide-Activation of Macrophages

Mangoni, Maria Luisa[1]; Rosenfeld, Yosef[2]; Epand, Raquel F.[3]; Barra, Donatella[4]; Epand, Richard M.[3]; Shai, Yechiel[5]
[1]La Sapienza University, ITALY; [2]The Weizmann Institute of Science, ISRAEL; [3]McMaster University, Hamilton, CANADA; [4]La Sapienza University of Rome, ITALY; [5]The Weizmann Institute of Science, ITALY

The increasing emergence of multidrug-resistant microbes has urgently required the discovery of new antibiotics with a new mode of action, and naturally occurring antimicrobial peptides (AMPs), which are produced by almost all forms of life, represent promising candidates. There is compelling evidence that unlike conventional antibiotics, most AMPs interact and increase the permeability of the bacterial membrane as part of their killing mechanism. However, before reaching it, they need to cross their cell wall that, in Gram-negative bacteria, is surrounded by the lipopolysaccharide (LPS)-outer membrane, which forms a very efficient barrier against a variety of hydrophilic and hydrophobic molecules. LPS also possesses inflammatory properties which can result in a fatal phenomenon known as septic shock. Therefore, the ability of a peptide to display both antimicrobial and anti-endotoxin activities makes it an attractive compound for therapeutic application. In Amphibia, temporins are among the shortest (10 to 14 residues) AMPs, with up to ten isoforms within the same specimen. Recently, we have shown that some of the temporins have a synergistic effect in killing of bacteria, suggesting an important strategy to overcome bacterial resistance imposed by the LPS layer. Here we show that this effect is highly dependent on the type of LPS composing the bacterial outer layer. In addition, we show that the same temporins synergize in neutralizing the endotoxin effects of LPS, by inhibition of TNF-α release (considered to be a primary mediator of endotoxemia) from macrophages stimulated with LPS from various species of Escherichia coli. Furthermore, we show the potential mechanism of this synergism by means of spectroscopic and thermodynamic studies. Besides improving our knowledge on the peptide-LPS interaction, such studies should contribute to the development of new peptide-based anti-infective and/or antisepsis therapeutics.

S12-2

Biochemical characterization and strong in vivo activity of a novel antimicrobial peptide specific for Gram-negative bacteria

Pini, Alessandro; Falciani, Chiara; Bindi, Stefano; Iozzi, Sara; Brunetti, Jlenia; Bernini, Andrea; Niccolai, Neri; Rossolini, Gian Maria; Bracci, Luisa
University of Siena, ITALY

We recently identified a non-natural peptide sequence showing a strong antimicrobial activity (Pini et al., 2005, Antimicr Agents Chemother). This peptide (QKKIRVRLSA; called M6) was synthesized in the branched Multiple Antigen Peptide form, that we had previously demonstrated to induce general peptide resistance to proteolysis, making this kind of molecules very suitable for in vivo use (Bracci et al., 2003, J Biol Chem; Falciani et al., 2007, Chem Biol Drug Des). The branched peptide M6 was characterized for its activity against a number of bacteria showing a strong specificity for Gram negative species. The peptide resulted particularly active against Escherichia coli, Pseudomonas Aeruginosa, Klebsiella pneumoniae and some other enterobacteriacee, including many multi-drug resistant isolates. M6 showed a poor toxicity for eukaryotic cells, it bound LPS and DNA and it did not produce appreciable haemolysis even upon prolonged incubation. We also evaluated M6 acute toxicity (LD50 around 125 mg/Kg for i.p. administration) and we demonstrated that it is not immunogenic upon repeated injections in animals (Pini et al., 2007, J Pept Sci). The peptide was also analysed for its secondary structure by Nuclear Magnetic Resonance in the presence of SDS micelles that mimic negatively charged bacterial membranes. M6 folds as an α-helix interacting with membranes essentially with a side of the helix. M6 is the shortest antimicrobial peptide structured as an α-helix described so far. We also report results on M6 in vivo activity in models of sepsis induced by E. coli and P. aeruginosa. These experiments, carried out with i.p. administration of M6, showed that the peptide is able to prevent animal death and to neutralize sepsis symptoms when used in doses comparable to traditional antibiotics and compatible with a clinical use. These results make the branched M6 peptide a strong candidate for the development of a new antibacterial drug.

S12-3

Synergistic pore-formation in lipid membranes by the antimicrobial peptides PGLa and magainin 2 studied with solid-state NMR

Strandberg, Erik; Tremouilhac, Pierre; Wadhwani, Parvesh; Ulrich, Anne S.
Karlsruhe Institute of Technology, GERMANY

A solid-state NMR method has been used to study the orientation in a lipid membrane of the cationic α-helical antimicrobial peptides PGLa and magainin 2 (MAG). These peptides, found in the skin of the African frog *Xenopus laevis*, have broad spectrum antimicrobial activity. It has been observed that the activity is synergistically enhanced in mixtures of both peptides, and the formation of PGLa/MAG heterodimers has been proposed. Using solid-state ^{2}H-NMR on 3,3,3-^{2}H-alanine labeled PGLa and MAG, the orientation of the peptides in a lipid membrane was determined with high accuracy. We find that each peptide alone binds flat to the membrane surface at low concentration. For PGLa at higher concentration a somewhat more tilted orientation is found, which is attributed to homodimer formation, but the tilt is not large enough to let the peptide span the lipid bilayer. However, in a PGLa/MAG (1:1) mixture both peptides obtain a more upright orientation, spanning the membrane. This suggests that PGLa/MAG heterodimers form stable pores through the membrane, which can explain the synergistic activity.

S12-4

Antimicrobial peptides mode of action through spectrocopic techniques and molecular simulation

Jean-Francois, Frantz[1]; Desbat, Bernard[2]; Elezgaray, Juan[2]; Vacher, Pierre[3]; Metz-Boutigue, Marie-Hélène[4]; Dufourc, Erick[2]
[1]Bordeaux 1/IECB/UMR 5248, FRANCE; [2]Université bordeaux1/IECB/UMR5248, FRANCE; [3]Université bordeaux2/Institut bergonié/INSERM EMI 347, FRANCE; [4]Université louis pasteur strasbourg/ISERM U 575, FRANCE

The antimicrobial peptide Cateslytin (bCGA-344-RSMRLSFRARGYGFR-358) is a five-positively charged arginin rich peptide known to inhibit the release of catecholamine in chromaffin granules. Although it is able to rapidly cross the bacterial membrane and stop the bacterial growth, the mechanism of action has not been studied yet. In order to better understand both targeting and selectivity of this peptide towards microorganisms, model membranes with neutral or negative global net charge have been chosen to respectively mimic bacterial or mammalian membranes. Structural studies have been performed using polarised ATR-FTIR, circular dichroïsm and high resolution NMR. Membrane dynamics has been followed using deuterium labelled lipids and solid state NMR. Patch clamp experiments were also performed on lipid vesicles to measure channel conductivity. All-atom molecular dynamics on hydrated peptide-lipid membrane systems was also used to assess the interaction from the atomic level. Main results from this interdisciplinary approach are three-fold. i) Electric current passages through membranes demonstrate permeation akin to pore formation. ii) Peptide-induced formation of rigid domains mainly made of negatively charged lipids is found. iii) Peptide antiparallel β-sheets are observed preferentially with negatively charged lipids. The general picture leads to the proposal that membrane destabilization/permeation is promoted by rigid domains stabilised by peptide β-sheets.

S12-5

Design of highly active and protease resistant antibacterial peptides as well as identification of their bacterial targets.

Czihal, Patricia[1]; Otvos Jr., Laszlo[2]; Hoffmann, Ralf[1]
[1]Institute for Bioanalytical Chemistry, Center for Biotechnology and Biomedicine, Leipzig University, GERMANY; [2]Sbarro Institute, Temple University, Philadelphia, PA 19122, UNITED STATES

The incidence of serious bacterial and fungal infections is increasing despite remarkable advances in antibiotic therapy. At a time of the rapid emergence of drug-resistant bacterial strains, there is an urgent need to develop new antimicrobial compounds with novel modes of action. Thus antimicrobial peptides (AMPs) as part of the innate immune system appear very promising. Among these proline-rich antibacterial peptides are potential therapeutic molecules as they act stereospecifically on bacterial targets by a bacteriostatic rather than a lytic process. Here, we describe the design of novel peptidomimetics to (i) obtain superior antimicrobial activities against pathogenic, multi-resistant Gram-negative bacteria (*Escherichia coli, Klebsiella pneumoniae, Salmonella typhimurium, Pseudomonas aeruginosa*), (ii) increase the resistance against proteases and peptidases, and (iii) reduce the eukaryotic cell toxicity. The best peptide derivatives had minimal inhibitory concentrations (MIC) in the ng/mL range against several Gram-negative bacteria. Interestingly, the MIC-values were independent of antibiotic resistances indicating a novel mode of action. Additionally, the newly designed analogs killed the bacteria much faster than the native AMPs but still in a non-lytic process. We could even extend the activity spectrum to Gram-positive bacteria and design peptides that enter *Staphylococcus aureus* for example. Whereas the native peptides were metabolized in serum relatively fast, we could stabilize all labile bonds and thereby increase the half-lives up to 6 hours (25% aqueous serum) without losing the antimicrobial activity. All chemical modifications had no effect on the eukaryotic cell toxicity, as studied for COS-7 and HeLa cells and the hemolytic rate of red blood cells for example. Furthermore, we could identify two bacterial targets that nicely explain the activity spectrum and non-toxicity towards mammalian cells.

Peptide materials

S13-1

Naposomes: A new class of peptide containing supramolecular aggregates as target selective delivery systems

Accardo, Antonella; Tesauro, Diego; Benedetti, Ettore; Pedone, Carlo; Morelli, Giancarlo
CIRPeB - Center for Research on Bioactive peptides - Univeristy of Naples "Federico II", ITALY

The use of peptides as targeting tools has been validated in a wide number of applications. Radiolabeled peptides are used in nuclear medicine techniques to perform imaging or to deliver radiotherapeutic doses to cancer tissues, overexpressing cell membrane receptors such as those for somatostatin. A more complex and challenging objective would be to adopt a similar scheme to deliver supramolecular aggregates, such as micelles or liposomes, to a tissue of interest. We have developed new mixed aggregates with enhanced properties(1,2) (Naposomes) as potential target selective nanovectors of drugs and/or contrast agents. Naposomes are obtained by supramolecular aggregation of two monomers. The first monomer containing a chelating agent able to coordinate a paramagnetic or a radioactive metal ion and bearing a lipophilic tail and a second monomer containing a bioactive peptide linked to a similar lipophilic tail. The aggregates are selectively driven by the exposed bioactive peptide on the chosen biological target. The aggregates (micelles, vesicles or liposomes) entrap on their inner compartment or in the phospholipid bilayer, a pharmaceutical active principle. Naposomes could act as: i) Target-selective vehicles for drug-delivery; ii) Target-selective vehicles of contrast agents for imaging techniques; iii) Target-selective vehicles for simultaneous delivery of a drug (in the inner compartment) and a contrast agent (on the aggregate surface, for its visualization); iv) Target-selective delivery of a drug and a therapeutic active radionuclide. Supramolecular aggregates exposing peptides such as CCK-8, 7-14 Bombesin and Octreotide, on their external surface have been developed. A complete characterization of their chemical-physical properties and the in vitro and in vivo behaviour as target selective delivery systems for drugs and contrast agents is presented.

References:

1. G. Morelli et al. JBIC 2007, 12, 267-276 (2) G. Morelli et al. ChemMedChem, 2008, in press.

S13-2

Photocurrent Generation in Peptide-based Self-Assembled Monolayers Functionalized with Electron Transfer Antenna Chromophores

Gatto, Emanuela[1]; Stella, Lorenzo[1]; Formaggio, Fernando[2]; Toniolo, Claudio[2]; Venanzi, Mariano[1]
[1]University of Rome Tor Vergata, ITALY; [2]University of Padova, ITALY

Bio-hybrid devices based on the integration of biological molecules and metal substrates are currently actively explored for potential applications in the areas of molecular recognition, biological sensing, and molecular electronics. Among the various types of available biomaterials, helical oligopeptides have shown peculiar properties in terms of long-range efficiency and directional character of the electron transfer process. We have recently demonstrated that self-assembled monolayers (SAM) formed by conformationally-constrained hexapeptides suitably functionalized with antenna chromophores and covalently linked on gold microelectrodes are able to generate electronic current after photoexcitation (1). The deposition of the peptide layer modifies the gold surface potential, allowing for fine tuning of the electronic conduction properties of the substrate. In particular the electrostatic field associated to the helix macrodipole strongly affects the efficiency and the direction of the electron transfer along the peptide chain and the response of the electrode to the applied potential through the peptide/gold heterojunction. Furthemore, a new approach has been employed for the construction of a mixed SAM, exploiting favorable helix-helix dipolar interaction. By use of this approach, a helical peptide has been intercalated into an already-formed helical peptide SAM covalently linked to the substrate. The two peptides are both functionalized, but with different photoactive groups, so that it is possible to switch the direction of the generated current by selective excitation of each of the two chromophores. All of these results confirm the very good electron mediating properties of helical peptides and make them very promising materials for the development of molecular devices based on peptide wires.

References:

1. E. Gatto, L. Stella, F. Formaggio, C. Toniolo, L. Lorenzelli and M. Venanzi, J. Pept. Sci. (2008) 14, 184-191.

S13-3

Cell Adhesive Laminin Peptides for Tissue Engineering

Nomizu, Motoyoshi; Otagiri, Dai; Fujimori, Chikara; Hozumi, Kentaro; Kikkawa, Yamato
Tokyo University of Pharmacy and Life Sciences, JAPAN

Tissue engineering aims to create functional tissues or organ-replacements using materials and cells. Materials provide a support or scaffold for tissue formation. Extracellular matrix components including laminin, collagen, and fibronectin, and their active peptides are potential candidates for affording the cell binding activities to materials. Laminin, a major of the basement membrane matrix component, has diverse biological activities including promotion of cell adhesion, migration, neurite outgrowth, and angiogenesis. Our goal is to identify cell type-specific active sequences from the laminins and to use the biologically active peptides for biomaterials. We have identified various active peptides in the laminins using a systematic screening with more than 2,000 synthetic peptides. These peptides recognized various cellular receptors and have the potential ability to serve as bio-adhesiveness for tissue engineering. We have previously demonstrated that several active laminin peptide-conjugated chitosan membranes enhanced the biological activity and promoted cell adhesion in a cell-type specific manner. Here, we demonstrate that a most active laminin peptide (AG73: RKRLQVQLSIRT)-conjugated chitosan membrane can deriver cells and is applicable for keratinocyte transferring to wound bed. When human keratinocytes were seeded onto the membranes, more than 70% of the cells attached within 2 hrs. The membranes carrying keratinocytes were stable enough for handling with forceps and were inverted onto the muscle fascia exposed on the trunk of nude mice. Keratinocyte sheets were observed after 3 days and colonies appeared after 7 days on the fascia of host mice. These cells were multilayered on day-3 and expressed various keratinocyte markers. These results suggest that the AG73-conjugated chitosan membrane is useful as a therapeutic formulation and is applicable as a cell delivery system. The peptide-chitosan approach may be a powerful tool for tissue engineering.

S13-4

Peptides as Nanomaterials: Self-Assembly and Technological Applications of Peptide Nanotubes, Nanospheres, Hydrogels and other Nanostructures

Gazit, Ehud
Tel Aviv University, ISRAEL

Our works on the mechanism of aromatic peptide self-assembly, lead to the discovery that the diphenylalanine recognition motif of the Alzheimer's "nano-forests". Reches M, & Gazit E (2003) Casting Metal Nanowires Within Discrete Self-Assembled Peptide Nanotubes. Science 300, 625. Reches M & Gazit E (2004) Formation of Closed-Cage Nanostructures by Self-Assembly of Aromatic Dipeptides. Nano Lett. 4, 581. Yemini M, Reches M, Rishpon J, & Gazit E (2005) Novel Electrochemical Biosensing Platform Using Self-Assembled Peptide Nanotubes. Nano Lett. 5, 183. Mahler A et al. (2006) Novel Self-Assembled Gel Biomaterial Composed of Modified Aromatic Dipeptide. Adv. Mater. 18, 1365. Carny O, Shalev D, and Gazit E (2006) Fabrication of Coaxial Metal Nanowires Using Self-Assembled Peptide Nanotube Scaffold. Nano Lett. 6, 1594 Reches M and Gazit E (2006) Controlled Patterning of Aligned Self-Assembled Peptide Nanotubes. Nature Nanotechnol.1, 195. Adler-Abramovich L & Gazit E (2008) Controlled pattering of peptide nanotubes and nanospheres using inkjet printing technology. J. Pep. Sci. 14, 217.

S13-5

Solid phase peptide-carbene and peptide-phosphine transition metal catalysts in asymmetric synthesis and "Green" chemistry

Meldal, Morten; Worm-Leonhard, Kasper; Christensen, Christian A.
Carlsberg Laboratory, DENMARK

In Nature metalloproteins play a crucial role in complex biochemical transformations while displaying exquisite regio- and enantio-selectivity. More importantly the protein framework coordinates the catalytic metal and ensure substrate match and lower activation energy of the reaction to provide very high turnovers, which in turn facilitates the efficient biochemical transformation at low concentration of the catalytic protein. These properties can advantageously be mimicked in the field peptide and peptide-organic chemistry to putatively create catalysts for "green" chemistry. By engineering the peptide scaffold with one or several ideal ligands for a variety of transition metals, e. g. Pd, Cu, Rh, Fe, Ni, Ru etc. artificial enzyme like compounds, displaying selectivity and turnover for general organic chemistry transformations may be obtained. This presentation describes the synthesis and application of carbene- and phosphine-precursors for incorporation into peptide frameworks that folds around a transition metal and forms relatively compact and stable globular structures with an enzyme like binding cavity for substrate binding and catalysis. The strategy is modular and well suited for a Split/Mix approach where a large number of catalysts may be generated in a single combinatorial synthesis. Backbone phosphinylated peptides were synthesized on polar PEGA supports and in solution and the catalytic activity was compared. The solid supported catalysts were very efficient and could be recycled at least 5 times without loss of activity. The palladium coordination of the phosphine could furthermore be combined with folding and complexation with other dedicated functional groups in the peptide. Backbone carbenes formed extremely stable palladium-peptido carbene complexes that did not loose any activity with time or use. These solid phase catalysts could be used in microwave assisted C-C and C-N couplings in water with good selectivities and quantitative yields.

Peptides in systems biology

S14-1 Inv

Profiling SH2 and tyrosine phosphatases target specificity

Tinti, Michele[1]; Palma, Anita[1]; Costa, Stefano[1]; Kiemer, Lars[1]; Miller, Martin[2]; Ferrari, Emanuela[1]; Schutkowski, Mike[3]; Mayer, Bruce[4]; Hooft, Rob[5]; Brunak, Soren[2]; Castagnoli, Luisa[1]; Cesareni, Gianni[1]

[1]University of Rome Tor Vergata, ITALY; [2]Center for Biological Sequence Analysis. Technical University of Denmark, DENMARK; [3]Jerini Peptide Technologies, GERMANY; [4]University of Connecticut Health Center, UNITED STATES; [5]Merck Serono International S.A, SWITZERLAND

Residues cycling between a phosphorylated and a non-phosphorylated form play a key role in the modulation of signal transduction. Over the past few years, we have designed and implemented a strategy to describe the network of interactions linking phosphorylated peptides to their binding domains and to the enzymes that control their phosphorylation levels. We will report the results of the characterization of the substrate specificity of the SH2 domain family and of the family of tyrosine phosphatases. We have cloned and expressed all the 120 human SH2 domains and for 72 of them we have been able to purify soluble GST fusions. Similarly we succeeded in expressing 34 of the 37 tyrosine phosphates in a mutant form (trapping mutants) that still binds to substrate peptides but does not carry the de-phosphorylation reaction through. We have then probed their recognition specificity by incubating the GST fusions with a glass chip containing approximately 6000 phospho-peptides covering most of the human phospho-proteome. The results have been used to train domain specific Neural Networks and to draw a global "naïve" phospho-tyrosine specific interaction network that only takes into account the ability to bind phospho-peptides in an in vitro system. Next we have used a context score that combines in a Bayesian approach orthogonal information, namely tissue co-expression, sub-cellular localization, target sequence conservation in evolution, vicinity in the protein interaction network etc , to rank interaction according to the probability of being functionally relevant.

S14-2

Peptide microarrays for detecting signal transduction-dependent changes in the pattern of molecular interactions

Stoevesandt, Oda[1]; Köhler, Karsten[1]; Sinzinger, Michael[2]; Wolf, Susann[1]; André, Thomas[1]; Brock, Roland[2]

[1]Interfaculty Institute for Cell Biology, University of Tübingen, GERMANY; [2]Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen Medical Centre, NETHERLANDS

Multiprotein complexes play an essential role in the propagation and integration of cellular signals. However, systems level analyses of signaling-dependent changes in the pattern of molecular interactions are still missing. Signaling in T lymphocytes is one prominent example, in which multiprotein complexes orchestrate signal transduction. We have implemented peptide microarrays comprising a set of interaction motifs of signaling proteins for network-based analyses of signaling-dependent changes in molecular interactions. Lysates of resting or stimulated cells are incubated on these arrays and the binding of signaling proteins is detected by immunofluorescence. Signaling-dependent changes in molecular interactions are reflected by changes of signals on the microarrays. Signal changes on the array upon complex formation may either result from a masking of a binding site for a peptide on the array, or from binding to a protein that indirectly mediates binding to a peptide on the array. Competition with peptides corresponding to interaction motifs provides detailed information on the architecture of signaling complexes; lack of individual signaling proteins reveals the functional interdependence of interactions in the network. In summary, for the analysis of signaling networks, peptide microarrays enable the parallel detection of changes in patterns of interactions which allow the identification of functional interdependencies with minimum a priori knowledge.

References:

1. Stoevesandt et al., Proteomics, 5 (2005) 2010-2017
2. Stoevesandt et al., Mol. Cell. Prot., 6 (2007) 503-513

S14-3

Artificial intelligence delivers superb antibiotics for superbugs

Jenssen, Håvard[1]; Hilpert, Kai[1]; Fjell, Christopher D.[1]; Waldbrook, Matt[1]; Mullaly, Sarah C.[1]; Volkmer-Engert, Rudolf[2]; Cherkasov, Artem[1]; Hancock, Robert E.W.[1]
[1]University, CANADA; [2]University, GERMANY

Increased multiple antibiotic resistance in the face of declining antibiotic discovery is one of society's most pressing health issues. Antimicrobial peptides represent a promising new class of antibiotics. Here we asked whether it is possible to make small broad spectrum peptides without employing prior assumptions, by capitalizing on already accumulated chemical and biological information. Using peptide array technology, two large semi-random 9-amino-acid peptide libraries were iteratively created using the amino acid composition of the most active peptides. The resultant data was used together with powerful machine learning techniques to create quantitative in silico models of antibiotic activity. Based on random testing these models proved remarkably effective in predicting the activity of 100,000 virtual peptides. The best peptides, representing the top quartile of predicted activities, were effective against a broad array of multi-resistant "Superbugs", more effective than the most advanced clinical candidate peptide, and protective against Staphylococcus aureus infections in animal models.

S14-4

The use of plasma proteomics to study disease pathogenesis

Hancock, William[1]; Hincapie, Marina[1]; Kullolli, Majlinda[1]; Plavina, Tatiana[2]
[1]Northeastern University, UNITED STATES; [2]Biogen Idec Inc., UNITED STATES

Mechanisms underlying disease pathogenesis are not well understood in the context of common etiological factors such as microbial infection, inflammation, malignancy or tissue breakdown. Such processes may be elucidated by identifying disease-related molecular markers, such as acute phase proteins, cytokines, cytoskeletal fragments and autoantigens. In an attempt to identify such markers, we used two proteomic methods to analyze plasma samples from patients and healthy donors in a series of studies ranging from cancer to autoimmune disease. The first method allowed to evaluate changes in concentration of glycoproteins, and to comprehensively survey the plasma proteome. The second method, intact peptidomics, was used to assess changes in endogenous proteolytic activity by analyzing the low molecular weight (LMW) component. The integrated proteomic and peptidomic analysis of plasma samples identified a number of cytoskeletal and Ca^{2+}-binding proteins and their proteolytic fragments in the disease samples. The measurements were compared to healthy donors and several of the observed differential quantitations were independently verified by ELISA. The identified changes in plasma proteome and peptidome, and the underlying altered endogenous protease activity may result in the generation of novel autoantigens. We have confirmed this hypothesis by the observation of autoantibodies in patients and upon extension of these studies to larger populations of patients; we may gain additional understanding of the role of etiological factors in different disease pathways and genomic penetrance.

Peptide biotechnology

S15-1

Phosphorylation-Dependent Protein Design: Design of Protein Kinase-Inducible Domains as Genetically Encodable Sensors of Protein Kinase Activity

Zondlo, Neal; Balakrishnan, Shalini; Gao, Feng
University of Delaware, UNITED STATES

Protein phosphorylation is a ubiquitous signaling mechanism important in cellular regulation. Misregulation of protein kinases and phosphatases is implicated in diverse human diseases, including cancer, Alzheimer's disease, and diabetes, as well as bacterial and viral pathogenicity. We have used protein design to develop novel protein motifs, termed protein kinase-inducible domains (pKIDs), whose structures are dependent on their phosphorylation state. The basis of the design is the use of phosphoserine, phosphothreonine, or phosphotyrosine as a mimic of a structurally important Glu residue in a protein. Protein kinase-inducible domains are 15-25 amino acid sequences that were designed based on EF-hand domains, replacing the conserved Glu at residue 12, which binds metal in a bidentate manner, with phosphoserine, phosphothreonine, or phosphotyrosine. Non-phosphorylated pKIDs do not bind terbium and exhibit weak or no fluorescence, whereas phosphorylated pKIDs bind terbium and exhibit strong terbium fluorescence. The structure and fluorescence of pKIDs are dependent on phosphorylation, and may exhibit essentially complete phosphorylation-dependent switching. We demonstrate the generality of this architecture with pKID peptides containing complex protein kinase recognition sequences for multiple protein kinases, including PKA, PKC, Erk, and Abl. Protein kinase-inducible domains may be used as in vitro fluorescent sensors of protein kinase activity and protein phosphatase activity. Protein kinase-inducible domains are compatible with complex solution environments, including cell extracts. Protein kinase-inducible domains may also be used for the fluorescent detection of protein kinase inhibitors.

S15-2

Phage selection of chemically constrained peptides

Heinis, Christian
LMB, UNITED KINGDOM

The discovery of synthetic molecules with high affinity and specificity for biological targets is a central problem in drug discovery. We have developed an efficient strategy for the generation of chemically constrained peptides with high affinity and specificity for biological targets. Molecules with desired specificities were isolated by phage display using repertoires of random linear peptides that were, before phage selection, reacted with a chemical scaffold to form molecules with constrained peptide loops. We linked peptides (17 amino acids) on phage via three cysteine side chains to a small molecule scaffold to obtain a single product isomer. Reaction conditions were chosen to selectively modify the peptides but spare the coat proteins of the phage particles. Highly specific binders with nanomolar affinities were obtained against the two serine proteases human plasma kallikrein and cathepsin G. An affinity matured inhibitor of human plasma kallikrein with an apparent Ki of 1.5 nM efficiently suppressed contact activation in human plasma.

S15-3

PNA zipper: inducing dimerization of the basic region of a bZip protein by a PNA duplex

Pensato, Soccorsa[1]; Saviano, Michele[1]; Renda, Mario[2]; Leccia, Felicia[2]; Pedone, Paolo V.[2]; Pedone, Carlo[3]; Romanelli, Alessandra[3]
[1]Institute of Biostructure and Bioimaging/CNR, ITALY; [2]Department of Environmental Sciences/Second University of Naples, ITALY; [3]University of Naples/ School of Biotechnological Sciences, ITALY

The sequence specific DNA binding of gene-regulatory proteins is often mediated by dimeric proteins, which recognize DNA as homodimers or heterodimers. The basic leucine zipper (bZip) DNA binding proteins are transcriptional regulatory proteins consisting of a coiled coil leucine zipper dimerization domain and a highly charged basic region responsible for the DNA binding. Several efforts have been devoted to the synthesis of minimized bZip analogues, able to bind DNA. The dimerization of the basic region (BR) has been achieved in several ways, for example by host-guest complexes of β-cyclodextrin and adamantane, by photoresponsive devices as azobenzene, by Fe(II) complexes, by disulfide bond between extra cysteines. In this work we present a new tool for the dimerization of the basic region of bZip proteins based on the use of a short PNA duplex. We used the PNA duplex as dimerization tool in consideration of its high thermal, chemical and enzymatic stability, which will reveal useful for its future use as a decoy. The PNA dimerizer was linked to the BR peptide by a native chemical ligation reaction. The peptide, corresponding to the basic region of the GCN4 protein, was obtained by solid phase synthesis with an extra Lys residue at the C-terminus. The Lys side chain was selectively deprotected on the resin and reacted with the monobenzylthioester of the succinic acid to give the C-terminus thioester peptide. The peptide was reacted with complementary PNA strands bearing a cysteine respectively at the C and the N terminus to give the PNA-peptide conjugate. Formation of the PNA duplex induces dimerization of the BR peptides, to give a molecule able to bind its target DNA. CD spectra of the PNA-peptide single strand and of the dimeric (PNA-peptide) show the pronounced helical tendency of the ligated molecules. After complexation with the target DNA sequence, the BR helical content increases. The ability of binding DNA was further confirmed by EMSA assays.

S15-4

Developing Peptide Antagonists for both UFH and LMWH

Vaynshteyn, Yekaterina; Liang, Jun F. (James)
Stevens institute of Technology, UNITED STATES

Low molecular weight heparin (LMWH), derived from native heparin by chemical or enzymatic method, is a new generation of anticoagulant in an attempt to abate the side-effects of heparin. LMWHs have high bioavailability after subcutaneous injection, long half-life, and fewer adverse effects compared with unfractionated heparin, in particular, a much lower risk of heparin-induced thrombocytopenia and osteoporosis. Despite the clinical benefits, LMWH suffers from shortcomings of its own. Unlike unfractionated heparin (UFH) can be reversed by protamine, protamine, a anti-heparin drug, appears to only neutralize approximately 50 % of the anti-Xa activity of LMWH. Therefore, even though it is approved as effective anticoagulant for the prevention and treatment of various thrombotic disorders, LMWH is restrained from being used in the patients at high risk of bleeding, or in whom rapid reversal of anticoagulation may be required such as in intensive care units, operating theatres, and for patients with renal impairment. So far, there is no proven method for LMWH neutralization, rendering the limitation and risk of LMWH drug. We have tried to identify the key heparin neutralization sequences in protamine and expected that small peptides with key sequences may neutralize LMWH completely. Through carefully designed enzyme digestion approaches and peptide isolation technology, new peptides with strong anti-heparin activity to both unfractionated heparin (UFH) and low molecular weight heparin (LMWH) have been found. Preclinical studies show that the new anti-heparin peptides have strong heparin and LMWH neutralization activity but with much less toxicity compared to the protamine on the market. Finding of antagonists with complete heparin and LMWH neutralization ability is a very exciting achievement of great significance. Now we are working with local pharmaceutical company to extend the preclinical trails on these peptides.

Analytical chemistry in peptide research

S16-1 Inv

Frontier and Perspectives – Specific and Sensitive Identification of Peptides Using Peptidomics and MALDI Imaging Mass Spectrometry

Andrén, Per
University of Uppsala, Dept. of Pharmaceutical Biosciences, Medical Mass Spectrometry, *SWEDEN*

Imaging MALDI mass spectrometry (IMS) and peptidomics (nanoLC ESI MS) are powerful tools utilized for thorough imaging and profiling of a large number of neuropeptides, small proteins and drugs. IMS is a relatively new technology and can be used to locate specific molecules from frozen tissue sections of a tissue. Using a raster of mass spectra over a given area of a section, images of samples are produced in specific m/z values. Each spot on the sample irradiated by the laser is approximately 50 microns in diameter and the resulting spectrum contains hundreds of individual signals. These m/z values can then be assembled from these mass spectra to produce selected m/z images. However, peptidomics and IMS analysis may be complicated by protein fragments that may be produced post-mortem during conventional sample handling. A novel sample preparation method and peptide identification tools for analysis of brain lysates and tissue sections were used to study changes in peptide expression in experimental Parkinson's disease (PD) and to identify novel, potentially biologically active neuropeptides. The brain tissue were either analyzed by nanoLC-ESI MSMS after homogenization of specific brain regions or cut by a cryomicrotome and studied by IMS. To optimize the identification process for endogenous peptides we used our in-house constructed SwePep database (www.swepep.org). The analysis demonstrated several differentially expressed peptides and small proteins in the experimental models of PD. Furthermore, in drug discovery and development the knowledge and the ability to determine properties such as absorption, distribution, metabolism, and elimination (ADME) of a drug candidate is essential in the drug development process. Preclinical methods that could improve thecharacterization of these features as early as possible in the drug development would help identifying potentially successful drug candidates and/or eliminate those that have adverse properties. The direct analysis of tissues using IMS allows both endogenous and exogenous compounds present in tissues to be detected with molecular specificity while maintaining their spatial orientation. In the present study we have applied IMS to characterize the distribution of a drug in lung of rats dosed by inhalation. The result showed that although the highest concentration of the drug was observed in the central lung compartment, it was rapidly transported from the central conducting airways to alveolar beds in the upper and lower parenchyma. The distribution pattern and the levels of the drug in consecutive lung tissue sections correlated very well when comparing the analysis performed in MS and MS/MS mode. It is concluded that IMS proved sensitive, specific, and highly useful to the image analysis of traditional small molecule drugs directly in tissue sections.

S16-2

CREDEX-MS: Molecular elucidation of carbohydrate recognition peptides in lectins and related proteins by proteolytic excision- mass spectrometry

Przybylski, Michael[1]; Moise, Adrian[1]; Siebert, Hans-Christian[2]; Gabius, Hans-Joachim[2]
[1]University of Konstanz, Department of Chemistry, Laboratory of Analytical Chemistry, Konstanz, GERMANY; [2]Ludwig-Maximilians-University of Munich, Department of Physiological Chemistry, Munich, GERMANY

The emerging relevance of glycan-encoded information in a plethora of biological events directs increasing attention to interaction structures between bioactive glycan determinants and their endogenous receptors, such as lectins (1). Structures of carbohydrate complexes with lectins and antibodies have been determined in a few cases by X-ray crystallography and NMR, however both methods are limited by large amounts and high purity of material required. We report here a new direct method for molecular mapping of peptide motifs in lectin carbohydrate domains (CRD) by the combination of proteolytic excision of protein-carbohydrate complexes and mass spectrometry (CREDEX: Carbohydrate-REcognition-Domain-EXcision). The CREDEX-MS method was applied to the identification of CRDs of human galectin-3 and galectin-1, for which X-ray crystal structures of their lactose complexes have been determined. Lactose was covalently coupled to epoxy-activated Sepharose, galectins added to the affinity matrix and the lectin complexes digested using trypsin. After removal of unbound fragments, competitive elution of remaining affinity-bound gal-peptides with lactose followed by MALDI-MS provided the identification of specific peptides, gal-3(152-162) and –(177-183), and gal-1(37-48) and (64-73), in complete agreement with CRDs from the crystal structures. Their specificity was confirmed by affinity-MS (2) of the synthetic CRD peptides, and by inhibition studies with gal-3 in human lymphoma cells. Most recent applications to CRD identifications of hitherto unknown lectin-carbohydrate complexes ascertain the CREDEX-MS approach as a powerful tool for the direct determination of CRDs in solution, suggesting a wide range of applications to define contact sites for lectin ligands of human sugar recetors in biological material.

References:

1. Gabius HJ, Crit. Rev. Immunol. 2006, 26: 43-80.
2. Macht M, Marquardt A, Deininger SO, Damoc E Kohlmann M, Przybylski M Anal. Bioanal. Chem. 2004, 378: 1102-1111.

S16-3

Cdc2 Kinase is Specific for Ser-*trans*-Pro Substrates: An LC-MSMS Method with Alkene Isosteres

Song, Zhao; Etzkorn, Felicia A.
Virginia Tech, UNITED STATES

Regulation of the cell cycle by Pin1 can only be accomplished if a pSer/Thr-*cis/trans*-Pro equilibrium is upset by outside action. We hypothesized that a kinase upstream of Cdc25C is conformation specific and causes just such an upset of the equilibrium by phosphorylating one isomer at a pSer-Pro site in Cdc25C. Conformationally constrained alkene isosteres of Ser-cis-Pro and Ser-trans-Pro were incorporated into Cdc25C peptides corresponding to a phosphorylation site for Cdc2 kinase. The peptide isosteres were phosphorylated by Cdc2 kinase with ATP. Product phosphopeptide isosteres were detected using SIMS in LC-MSMS. Control phosphopeptide isosteres were synthesized to optimize MS sensitivity. Cdc2 kinase phosphorylated only the Ser-*trans*-Pro isostere, and only at the specific site, not at three other possible sites in the peptide, as shown by MSMS peptide sequencing.

S16-4

Modifications in the chemical structure of Trojan carriers: impact on cargo delivery

Aussedat, Baptiste[1]; Aubry, Soline[1]; Dupont, Edmond[2]; Sagan, Sandrine[1]; Joliot, Alain[2]; Lavielle, Solange[1]; Chassaing, Gérard[1]; Burlina, Fabienne[1]
[1]UMR CNRS 7613, Université Paris 6, FRANCE; [2]UMR CNRS 8542, ENS, FRANCE

Cell-penetrating peptides (CPPs) are promising tools for delivering bioactive cargoes. One of the challenges now is to design CPPs with improved efficiencies of delivery and which can specifically target cargoes to the different cellular organelles. Using an original library of pseudo-peptide carriers, we have explored the impact on cargo delivery of changing the functional groups of the carrier (ammonium, guanidinium, myristyl) and the spatial distribution of these groups (Aussedat et al. 2008). These pseudo-peptide carriers were obtained by modular construction from the alpha, alpha-disubstituted amino acid “bis-ornithine”. This amino acid offers great flexibility in the design of polymers with linear, branched or dendrimeric structures. The quantification of the internalized cargo and the study of its intracellular degradation were performed by a method based on MALDI-TOF MS. Its intracellular localization was studied by confocal microscopy. Data show that the chemical structure of the carrier has a strong impact on the intracellular localization of the cargo. Aussedat et al., Chem. Commun, 2008, 12, 1398.

Medical peptide chemistry

S17-1

The type of bond between lipid chain and peptide determines the route of uptake of lipopeptides into antigen presenting cells: example of an encephalitogenic epitope.

Pfender, Nad ge[1]; Grosch, Sylvie[2]; Roussel, Guy[2]; Koch, Marc[3]; Greer, Judith[4]; Trifilieff, Elisabeth[1]
[1]UniversitŽ Louis Pasteur Strasbourg/CNRS, FRANCE; [2]INSERM U575 / Centre de Neurochimie, FRANCE; [3]UniversitŽ Louis Pasteur Strasbourg/IGBMC, FRANCE; [4]The University of Queensland / Neuroimmunology Research Unit, AUSTRALIA

Proteolipid protein (PLP) is the most abundant protein of central nervous system myelin, and is posttranslationally acylated at six cysteine residues by covalent attachment of palmitic acid via thioester linkage. PLP is a potent immunogen which induces experimental autoimmune encephalomyelitis (EAE), an animal model for multiple sclerosis. We were interested in the effects of palmitoylation on the encephalitogenic and immunogenic properties of PLP. We have shown that thiopalmitoylation of PLP encephalitogenic epitopes (S-palm peptide) increased the immune response as well as the development and chronicity of EAE. Our work also suggested that the lability of the thioester bond between the peptide and fatty acid was important for CD4+ T cells, as PLP lipopeptides synthesized with the fatty acid attached via an amide linkage at the N-terminus (N-palm peptide) induced a greater proportion of CD8+ T cells and were not encephalitogenic. Recently, we have determined the mechanisms responsible for these observations and have shown that: - the efficiency of uptake of both the S-palm and N-palm peptides by antigen presenting cells (APC) is much greater than for non palmitoylated peptide. - the type of bond between lipid chain and peptide determines the route of uptake of the lipopeptides by APC: (i) The S-palm peptide is taken up into APC via an endocytic route, the thioester bond is cleaved in the endosome, and the peptide is subsequently presented in the context of MHC class II (ii) The N-palm peptide rapidly enters into the cytoplasm of the APC, the amide bond between peptide and lipid is not cleaved, and the lipopeptide travels via the endoplasmic reticulum to complex with MHC class I These findings have potential application for the development of methods to specifically induce MHC class II-restricted responses and also for vaccine development.

References:

Greer JM, et al., J. Immunol., 2001, 166, 6907 Pfender NA, et al., J. Immunol., 2008, 180, 1398

S17-2

Identification of the determinant of hepatitis B virus liver tropism and its implications for hepatocyte-specific drug targeting

Schieck, Alexa; Mier, Walter; Urban, Stephan; Haberkorn, Uwe; Mueller, Thomas; Schulze, Andreas
Universitätsklinikum heidelberg, GERMANY

A hallmark of Hepatitis B Virus (HBV) infection are the extraordinary specificity and efficiency by which virions target hepatocytes in the liver. We have recently (Nat. Biotech., 2008) demonstrated that synthetic fragments encompassing the 47 N-terminal amino acids of viral surface proteins block HBV infection of primary human hepatocytes at already picomolar concentration. The efficient inhibition of hepatitis B virus infection by the acylated peptides is similar to the gp-41-derived T 20-peptides already approved for HIV-therapy. Approximately 40 derivatives of the HBVpreS lipopeptide were obtained by solid phase synthesis. The peptides were radioactively labeled and the organ distribution with respect to a specific targeting to the transplanted hepatocytes, as well as the peptide stability was investigated. We observed that the peptides exclusively target the liver, obviously encompassing a species-independent determinant of hepatotropism. Using the series of peptides carrying deletions, point mutations, D-amino acid exchanges, sequence permutations and lipid variations we found that (i) N-terminal acylation prevents renal secretion of the peptide and leads to systemic retention, (ii) a highly conserved 7 a.a. sequence motif is the pharmacophore required for liver-targeting (iii) peptides containing this sequence are taken up by hepatocytes and accumulate within the cells. This process is highly specific and differs from constitutive hepatic delivery via the blood, since single amino acid exchanges within the conserved motif resulted in a total loss of specificity. HBVpreS-mediated drug targeting opens a highly selective approach to deliver drugs to hepatocytes or hepatoma cells. Possible applications include the delivery of interferons, inhibitors of HCV or HBV replication, cell cycle inhibitors for HCC treatment, inhibitors of plasmodium falciparum, siRNAs or peptides for MHC-mediated antigen presenta

S17-3

A Myxobacterial Cyclic Peptide Metabolite is a Highly Selective Proteasome Inhibitor and Potent Antitumor Lead

Nickeleit, Irina[1]; Zender, Steffen[1]; Sasse, Florenz[2]; Geffers, Robert[3]; Brandes, Gudrun[4]; Sörensen, Inga[1]; Steinmetz, Heinrich[5]; Kubicka, Stefan[6]; Carlomagno, Theresa[7]; Menche, Dirk[8]; Buer, Jan[3]; Gossler, Achim[1]; Manns, Michael P.[6]; Kalesse, Markus[8]; Frank, Ronald[2]; Malek, Nisar P.[1]

[1]Institute for Molecular Biology, Medical Highschool Hannover, GERMANY; [2]Dept. Chemical Biology, Helmholtz Centre for Infection Research, GERMANY; [3]Dept. Cell Biology, Helmholtz Centre for Infection Research, GERMANY; [4]Institute for Cell Biology, Medical Highschool Hannover, GERMANY; [5]Res. Group Microbial Drugs, Helmholtz Centre for Infection Research, GERMANY; [6]Dept. of Gastroenterology, Hepatology and Endocrinology, Medical Highschool Hannover, GERMANY; [7]Dept. NMR Structural Biology, MPI for Biophysical Chemistry, GERMANY; [8]Dept. Medicinal Chemistry, Helmholtz Centre for Infection Research, GERMANY

The Helmholtz Centre for Infection Research has implemented a HTS infrastructure to enable the systematic search for novel antiinfective compounds. Its compound archive of currently around 90,000 samples includes a unique collection of natural products isolated from myxobacteria [Reichenbach H, Höfle G (1999) Myxobacteria as producers of secondary metabolites. In: Grabley S, Thieriecke R (eds) Drug discovery from nature. Springer Verlag, Berlin, pp 149-17]. This infrastructure is also open to other users and other applications through the German National ChemBioNet (www.chembionet.de).
In collaboration with the research group of N. Malek at the Medical Highschool Hannover, a cell-based fluorescence reporter assay was utilized to screen for compounds that are able to increase the intracellular level of cell cycle regulator p27. This screen revealed among other small organic molecules a unique cyclic heptapeptide structure (HZI150006) with unusual amino acid building blocks as a potent positive effector molecule. Mode of action studies confirmed a highly specific inhibition of all three catalytic activities of the eukaryotic proteasome, which results in a reduced degradation of p27. Intracellular increase of p27 effects growth arrest and apoptosis in many tumor cell lines. HZI150006 is reducing growth of solid tumours in in vivo mouse models at a much lower dose and toxicity compared to the approved proteasome inhibitor Bortezomib. We report also on the chemical synthesis of this cyclic peptide and first SAR studies.

S17-4

Development of peptides modulating the activity of prostatic proteases

Koistinen, Hannu[1]; Närvänen, Ale[2]; Pakkala, Miikka[2]; Hekim, Can[1]; Mattsson, Johanna[1]; Huhtala, Tuulia[2]; Zhu, Lei[1]; Laakkonen, Pirjo[3]; Stenman, Ulf-Håkan[1]

[1]University of Helsinki, Department of Clinical Chemistry, FINLAND; [2]University of Kuopio, Department of Biosciences and AIV Institute, FINLAND; [3]University of Helsinki, Molecular Cancer Biology Research Program and Institute of Biomedicine, FINLAND

Prostate produces several proteases, the most abundant ones being PSA (kallikrein-related peptidase 3, KLK3) and hK2 (KLK2). PSA expression is lower in malignant than in normal prostatic epithelium and it is further reduced in poorly differentiated tumors, in which the expression of hK2 is increased. PSA has been shown to inhibit angiogenesis, while hK2 may mediate tumor growth and invasion by participating in proteolytic cascades. Thus, it may be possible to control prostate cancer growth by modulating the proteolytic activities of PSA and hK2. We have used phage display technology to develop peptides, which very specifically stimulate the activity of PSA or inhibit that of hK2, making them potentially useful for tumor imaging, targeting and treatment. Using these peptides we have established peptide-based methods for determination of enzymatically active PSA. PSA-stimulating peptides enhance the antiangiogenic activity of PSA, supporting our hypothesis that enhancing the activity of PSA by our peptides could be used to reduce tumor angiogenesis and, thus, to reduce tumor growth. However, in a xenograft tumor model the effect of the peptides on tumor growth has been variable, perhaps due to rapid degradation and clearance of the first generation peptides. Currently we are modifying the peptides to make them suitable for in vivo applications. So far, we have been able to considerably improve the stability of hK2-binding peptides by backbone cyclization.

S17-5

Biomimetic Furan Oxidation: a 'Trojan Horse' Strategy for Crosslinking and Labeling of Peptides

Deceuninck, Annelies; Stevens, Kristof; Op de Beeck, Marieke; Madder, Annemieke
Ghent University, BELGIUM

Chemical cross-linking of protein-DNA complexes is a very powerful tool for studying protein-DNA interactions. The transformation of a non-covalent, transient complex into a covalent adduct renders characterization of the complex by classical techniques (mass spectrometry, HPLC, NMR, …) much easier. Recently, a new method for DNA cross-linking has been developed in our laboratory, which relies on the incorporation of a furan modified nucleoside building block into a DNA strand. This furan ring can be oxidized into a very reactive enal species, which reacts with the free amino functions of the complementary strand. The strategy is based on the known oxidative ring opening of furan under the influence of Cytochrome P450 and the reaction of the resulting cis-butene-1,4-dial with free amino and sulfhydryl functions of biomolecules.
The aim of the current research is to apply a similar strategy for peptide/protein - DNA cross-linking purposes by using furan modified peptides. Small peptides are synthesized on solid phase incorporating a furan moiety. These peptides will be tested for their DNA cross-linking ability and first results will be discussed.
In addition to cross-linking reactions, the peptides were used as a test system for selective labeling. On-bead oxidation and subsequent labeling with a fluorescent amine in the presence of sodium cyanoborohydride leads to fluorescently labelled peptides. Details of the new labeling technique will be given.

New approaches in peptide technology

S18-1

Synthesis of smart potential MRI contrast reagents that bind aggregated βamyloid for the diagnosis of Alzheimer's disease

Austen, Brian; Cheng, Elliott; Mohammed, Yeser
St George's University of London, UNITED KINGDOM

Several peptides that contain a retroinverso form of a βamyloid binding domain ffvlk, gadolinium chelated by DOTA, and a potential blood brain penetration moiety, have been synthesised and characterised by HPLC and mass spectrometry. Peptides containing a cell penetrating peptide with clustered cationic residues show rapid, reversible binding to immobilised aggregated βamyloid by SPR in a Biacore; wheras those containing aliphatic amines show much poorer binding. APP-transfected cells treated with lactalysin to allow accumulation of βamyloid, showed high localisation of the poly-cationic peptide, and weaker localisation of the aminated peptide. The aminated form, however, showed, better uptake into brain in vivo. The MRI T2 image obtained 2 hours after iv injection of 0.5mg of the aminated reagent gave hypointense regions in the hippocampal area in an APP/PS double transgenic mouse.

S18-2

A novel family of selective prolyl oligopeptidase (POP) inhibitors based on a cyclic dipeptide scaffold shows effective CNS-targeting and blood-brain barrier (BBB) transport

Teixidó, Meritxell; Tarrago, Teresa; Giralt, Ernest
Institute for Research in Biomedicine Barcelona, SPAIN

Several severe health disorders require treatment of the brain, including Parkinson's and Alzheimer's, but also schizophrenia (SZ), epilepsy, and bipolar disorder (BD). In recent years, the POP serine protease has gained importance as a target for the treatment of SZ and BD.(1,2) The search for new drugs for the treatment of CNS diseases involves an additional obstacle, the blood-brain barrier (BBB).
Our research interest focuses on finding a peptide that is a selective POP inhibitor and shows efficient CNS-targeting and BBB transport. Our design is based on two observations: i) Berberine, an alkaloid found in Rhizoma coptidis, has a pyridinium moiety and is a POP inhibitor ($i.c._{50}$ 135 μM)[2] and ii) some redox chemical delivery systems (3) are based on the transformation between pyridinium and dyhydropiridine moieties.
A first cyclic dipeptide (DKP) modified with a dihydropyridine moiety was prepared and evaluated. This DKP with the dihydropyridine moiety crossed the BBB by passive diffusion (PAMPA assay, Pe: 0.24 10^{-6} cm/s) but did not show inhibitory activity against POP. In contrast, the active DKP with a pyridinium moiety acted as a selective POP inhibitor ($I.C._{50}$: 101.2 μM), and will have restricted elimination because of its ionic nature. Using this candidate as a starting point, we synthesized and evaluated a family of modified cyclic dipeptides in order to improve both permeability across the BBB and POP inhibition. The modifications introduced were based on our results on BBB-transport of mono- and di-N-Me-DKPs.(4)

References:

1 Polgar, L. Curr. Med. Che.-CNS Agents 2002, 2, 251– 257.
2 Tarrago, T. et al. ChemMedChem 2007, 2, 354-359.
3 Prokai-Tatrai K. et al. Med. Chem., 2005, 1, 141-152.
4 Teixido M. et al. J.A.C.S. 2007, 129, 11802-11813

S18-3

Peptide antagonists of protein-protein interactions identified by screening protein fragments derived by enzyme degradation

Ruvo, Menotti[1]; Marasco, Daniela[1]; Viparelli, Francesca[1]; Tornatore, Laura[2]; Sandomenico, Annamaria[2]; Doti, Nunzianna[3]; Pedone, Carlo[2]; Benedetti, Ettore[2]; Monti, Simona Maria[1]
[1]Istituto di Biostrutture e Bioimmagini, CNR, ITALY; [2]Istituto di Biostrutture e Bioimmagini, CNR and Università di Napoli Federico II, ITALY; [3]Istituto di Biostrutture e Bioimmagini, CNR and Seconda Università di Napoli Federico I, ITALY

Protein tridimensional structure is the complex recapitulation of local and distant intramolecular forces that cooperatively contribute to maintain the global energetic equilibrium. In this scenario, secondary structure motifs and small protein domains can be seen as discrete building blocks that when isolated from the protein context can be studied to gain structural insights on the protein original structure or as structural modules useful to prevent interactions with external partners. In this context, peptides derived by protein digestion with proteases and separated by chromatographic techniques can be usefully utilized in screening assays to search for protein-protein interaction antagonists. This procedure has been successfully applied to several interacting systems, such as Bcl10 oligomers, Gadd45b dimers and to the PED-PLD1 interaction, identifying in some cases peptide inhibitors with nM efficacy.

S18-4

Chemo-enzymatic synthesis of C-terminal aryl amides of amino acids and peptides

Nuijens, Timo; Quaedflieg, Peter; Merkx, Remco; Cusan, Claudia
DSM Pharmaceutical Products - ASC&D, NETHERLANDS

C-terminal aryl amides of amino acids and peptides are very commonly used substrates for enzymatic assays and diagnostics. For instance, over 1000 peptide aryl amide substrates are known to determine enzymes of blood coagulation. Enzymatic hydrolysis of aryl amides is used for a variety of chromogenic (e.g. giving para-nitroaniline), fluorogenic (e.g. giving 7-amino-4-methylcoumarin) and amperogenic (e.g. giving 4-amino-2-chlorophenol) assays. In known methods, the synthesis of C-terminal aryl amides of N-protected amino acids and peptides is based on the use of highly reactive chemical condensing agents[1-3] causing (partial) racemisation. Furthermore, full protection of the amino acid side-chain functionalities is required. For the first time, we have demonstrated that these couplings can be performed chemo-enzymatically(4). Using commercial enzymes these conversions appeared feasible in very high yield, without detectable racemisation and no protection of the amino acid side-chain functionalities was required. This invention opens new possibilities for the production of C-terminal aryl amide substrates which could be used for assays and diagnostics. In this presentation several (industrial) proteases will be discussed which are able to couple a variety of aryl amines to the C-terminus of amino acids and peptides in very high yields.

References:

1. Zimmermann et al. Anal. Biochem. 1976, 70, 258 262.
2. Kato et al. Experientia 1978, 34, 319-320.
3. MacKenzie et al. Biochem. J. 1985, 226, 601 606.
4. Quaedflieg, PJLM, Nuijens, T, Cusan, C., WO Pat. Appl., filed Oct. 2007.

S18-5

Mimetibodies™, A New Platform Technology for the Development of Biologically Active Peptides that Prolongs the Half-Life

Bugelski, Peter[1]; Scallon, Bernie[1]; Nesspor, Tom[1]; Spinka-Doms, Tracy[1]; Makropoulos, Dorie[1]; Eirikis, Edward[1]; olk, Amy[1]; Shamberger, Kimberly[1]; James, Ian[1]; Fisher, Paul[1]; Pool, Chad[1]; Jang, Haishan[1]; Miller, Brandon[1]; Huang, ChiChi[1]; Knight, David[1]; Ghrayeb, John[1]; Heavner, George[1]
Centocor, Inc., UNITED STATES

In the past several decades, hundreds of peptides have been identified which have specific biological activity and are highly potent in in vitro assays but lack the prerequisite pharmacokinetics to become efficacious human therapeutics. We have developed a novel antibody-based platform technology that provides an improved pharmacokinetic profile for biologically active peptides, resulting in a long duration of action. One such Mimetibody™, CNTO 528, is a novel erythropoietin (EPO) receptor agonist. Although CNTO 528 bears no sequence homology to erythropoietin, it is a potent erythropoietin receptor agonist, rescuing EPO dependent cells from apoptosis in vitro and stimulating erythropoiesis in vivo. Studies were done in normal rats to explore the pharmacodynamics and pharmacokinetics of CNTO 528 in normal rats and to demonstrate its efficacy in rat models of anemia. In vitro, CNTO 528 was approximately 10 fold less potent than rhEPO in stimulating the growth of UT-7EPO cells. Despite this lower in vitro potency, when compared to rhEPO and darbepoietin in normal rats, a single subcutaneous dose of CNTO 528 resulted in a longer-lived reticulocytosis and longer-lived increase in hemoglobin. Also, CNTO 528 caused only minor changes in red cell distribution width (RDW) or mean cell volume (MCV) and led to the release of mature reticulocytes. We have also shown that CNTO 528 was efficacious in rat models of anemia and in a rat model of pure red cell aplasia. Taken together, our data show that CNTO 528 is a novel stimulant of erythropoiesis in rats. This platform has been applied to other biologically active peptides as well and has proven to be a robust platform for enhancing the pharmacokinetics of peptides that would otherwise be rapidly cleared.