O1. Abstract number: 499

Mimicry effect of the neo-epitope [Asn641(Glc)]FAN(635-655) with CSF114(Glc) detecting autoantibodies in Multiple Sclerosis

S.P. Shashank[1], E. Peroni[2], M.C. Alcaro[3], F. Rizzolo[1], F. Nuti[1], M. Chelli[1], S. Mata[4], P. Rovero[1], F. Lolli[4], A.M. Papini[2]

[1]Laboratory of Peptide & Protein Chemistry & Biology, University of Florence, Sesto Fiorentino (FI), Italy

[2]PeptLab-SOSCO, University of Florence and Cergy-Pontoise, Cergy-Pontoise, France

[3]Toscana Biomarkers, Siena, Italy

[4]Dept of Neurological Sciences & Azienda Ospedaliera Careggi, Firenze, Italy

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system possibly caused by autoimmune mechanisms. Several self-proteins have been investigated as potential targets for T and/or B cells but none has been unequivocally identified. [1,2,3] On the other hand we previously proposed a "chemical reverse approach"to select the synthetic glycosylated probe CSF114(Glc), able to detect specific and high affinity autoantibodies correlating with disease activity to characterize a Multiple Sclerosis disease form.[4]

In our present study, we wanted to look for mimicry effects of nervous system proteins with CSF114(Glc). We selected from SWISSPROT peptide fragments of proteins of the nervous system containing an N-glycosylation consensus site (Asn-Xaa-Ser/Thr). In particular [Asn^{641}]FAN(635-655) (FAN, Factor associated with neutral sphingomyelinase activation), [Asn^{192}]OMgp(186-204), (OMgp, Oligodendrocyte myelin glycoprotein), and [Asn^{179}]NogoR(173-191) (NogoR, Nogo receptor) displayed 8/21, 3/21, and 5/21 residues homologous to CSF114(Glc), respectively.

The unglycosylated sequences and corresponding glucosylated ones were synthesized and used as synthetic antigens in SP-ELISA on sera of clinically definite MS patients (60 MS patients and 5 normal blood donors). The aberrantly glucosylated peptide [Asn^{641}(Glc)]FAN(635-655) showed similar reactivity to CSF114(Glc) and appeared to be a preferred ligand in the solid phase conditions of ELISA, for MS autoantibody recognition.

Moreover, affinity purified anti-[Asn^{641}(Glc)]FAN(635-655) IgGs showed cross reactivity with CSF114(Glc) in SP-ELISA and the complex of the purified antibodies and [Asn^{641}(Glc)]FAN(635-655) could be inhibited by CSF114(Glc) in competitive ELISA. These results let us to hypothesize a mimicry effect of [Asn^{641}(Glc)]FAN(635-655) with CSF114(Glc).

Immunohistochemistry experiments demonstrated that anti-[Asn^{641}(Glc)]FAN(635-655) and anti-CSF114(Glc) IgGs both recognised CNS structures and spinal cord in situ. In particular, both IgGs were able to recognize neuronal cytoplasms over the cortex and whole gray matter with higher intensity on the motorneuron cytoplasms, sparing the white matter in CNS and spinal cord respectively.

1. Hohlfeld, R. Brain 1997, 120, 865-916.

2. Bauer, J., Rauschka, H. & Lassmann, H. Glia 2001, 36, 235-243.

3. Brosnan, C. F. & Raine, C. S. Brain Pathol. 1996, 6, 243-257.

4. F. Lolli, et al. Proc. Natl. Acad. Sci. USA 2005 102, 10273-10278.

O2. Abstract number: 133

Cyclic peptides with a diversely substituted guanidine bridge: synthesis, structural and biological evaluations of model peptides

J.-F. Hernandez[1], Y. Touati-Jallabe[2], E. Bojnik[3], L. Chiche[4], M.C. Averlant-Petit[5], A. Aumelas[4], N.N. Chung[6], P.W. Schiller[6], D. Berthomieu[7], A. Hamzé[8], V. Lisowski[2], S. Benyhe[3], J. Martinez[2]

[1]IBMM, Montpellier, France

[2]Institut des Biomolécules Max Mousseron, Montpellier, France

[3]Biological Research Center - Institute of Biochemistry, Szeged, Hungary

[4]Centre de Biochimie Structurale, Montpellier, France

[5]Laboratoire de Chimie Physique Macromoléculaire, Nancy, France

[6]Laboratory of Chemical Biology and Peptide Research, Montreal, Canada

[7]Institut Charles Gherardt - MACS, Montpellier, France

[8]Biocis - Laboratoire de Chimie Thérapeutique, Chatenay-Malabry, France

The structural and biological behaviour of cyclic peptides with a diversely substituted guanidine bridge has been evaluated. Their synthesis followed a convergent solid phase strategy involving the primary formation of a thiourea bridge between the side-chains of two diaminoacyl residues. Guanidinylation was then performed in two steps i.e. i) S-methylation and ii) reaction with NH_4AcO (non-substituted guanidine bridge) or primary (mono-substituted) or secondary (di-substituted) amines, just before cleavage. The influence of the degree of guanidine substitution (non-, mono-, di-) was assessed on two model peptides. A structural study was performed on a series of proline-containing RGD-related cyclic peptides varying by the ring size and the guanidine bridge substitution. 2D-NMR showed a significant influence of the substitution degree on the proline cis/trans ratio. In particular, an opposite ratio (90/10 vs 20/80) was observed in the shortest series (18 atoms, ring size) between a mono- and a di-substituted guanidine. To explain these data, a molecular modelling study, combined to quantum mechanics to define the electronic and geometric properties of the guanidine group, was performed. The first results indicated that the substitution degree, due to potential steric contacts, can influence the orientation (four theoretically possible orientations) of the planar guanidine group inside the cycle backbone and therefore the peptide conformation. In addition, curiously, each favoured orientation was preferentially associated to one of the cis or trans proline conformer. Taken together, the modelling results were consistent with NMR data. Two series of cyclic enkephalin analogues (22 and 15 atoms, ring size) were also prepared and evaluated in mu/delta opioid receptor binding and functional assays. All were found agonists and more or less selective for mu. More importantly, in the shortest series, a significant influence of the degree of guanidine substitution on the binding affinity and the selectivity for mu was evidenced. The mono-methylated analogue was found to be the best mu binder (Ki = 2 nM) as well as the most selective for this receptor (160/delta). 2D-NMR structural analysis of selected compounds has been undertaken to relate structure to activity and will be presented.

Taken together, these results show that this kind of cyclization can represent a new tool to easily modulate the conformation and the biological activity of a unique peptide sequence.

O3. Abstract number: 394

Photolabile protecting goups based on novel thiocoumarins and thioquinolones: synthesis and photorelease of a model amino acid conjugate

S. C. Fonseca, M.S.T. Gonçalves, S.P.G. Costa
University of Minho, Braga, Portugal

In recent years, a need for developing new light-sensitive protecting groups has evolved considering the importance of such tools in organic synthesis and cell biology, for example in molecular caging, towards the design of more efficient protecting groups that allow orthogonal deprotection/cleavage for application in biomolecules. To be useful in biological experiments, a photolabile (caging) group must undergo photolysis rapidly, in high yield, and at wavelengths that are not detrimental to the biological system [1].

Considering our research interests in the development of new fluorescent heterocyclic compounds and their application as photocleavable protecting groups [2], and having in mind previous work on the comparison of the performance of a series of substituted coumarins and quinolones as carboxyl protecting groups cleavable by light [3], we now extend this research to the synthesis of fluorescent conjugates based on novel thiocoumarins and thioquinolones and phenylalanine, as a model amino acid.

The synthesis of the thionated coumarin and quinolone derivatives was accomplished by reaction of the corresponding model phenylalanine conjugates with Lawesson's reagent. Full characterisation by the usual spectroscopic techniques, including UV-vis and fluorescence spectroscopy, was performed. The photocleavage of thiocoumarin and thioquinolone conjugates was studied at different wavelengths of irradiation, in a photochemical reactor equipped with lamps of 254, 300, 350 and 419 nm, monitored by HPLC/UV and 1H NMR. It was found that the thionated analogues cleaved significantly faster and at longer wavelengths than the parent oxocompounds and cleavage kinetics parameters were obtained.

Thanks are due to Foundation for Science and Technology (FCT-Portugal) for financial support through project PTDC/QUI/69607/2006 and a PhD grant to A. S. C. Fonseca (SFRH/BD/32664/2006). The NMR spectrometer Bruker Avance III 400 is part of the National NMR Network and was purchased in the framework of the National Program for Scientific Re-equipment, contract REDE/1517/RMN/2005 with funds from POCI 2010 (FEDER) and FCT.

[1] Mayer, G. et al. Angew. Chem. Int. Ed. 2006, 45, 4900.
[2] (a) Fonseca, A. S. C. et al. Tetrahedron 2007, 63, 1353. (b) Fernandes, M. J. G. et al. Tetrahedron 2008, 64, 3032. (c) Soares, A. M. S. et al. Amino Acids 2010, DOI 10.1007/s00726-009-0383-z.
[3] Fonseca, A. S. C. et al. Amino Acids, 2010, DOI 10.1007/s00726-010-0492-8.

O4. Abstract number: 483

Amino Acid Coupling Reactions In Aqueous Environment Using Microwave Assistance Heating

S. Galanis[1], F. Albericio[2], M. Grøtli[3]
[1]university Of Patras, Patras, Greece
[2]institute For Research In Biomedicine, Barcelona Science Par, Barcelona, Spain
[3]university Of Gothenburg, Gothenburg, Sweden

The solid-phase method is the principal method for peptide synthesis, but it requires a large amount of organic solvent. As the safe disposal of organic solvent waste is an important environmental issue, a method for peptide synthesis in water would be desirable. Thus, the aim of this project is to replace costly organic solvents for peptide synthesis with inexpensive, environmentally-benign solvents, such as water. Towards, we previously reported the solid phase peptide synthesis in aqueous environment using microwave heating, Boc-derivatives and the mixture of HONB/EDC as coupling reagents [1,2]. In subsequence, we herein present the studies of the twenty native amino acids coupling reaction using commercial available Boc-derivatives. A variety of common amino acid derivatives in combination to coupling reagents and solid supports, were evaluated by the use of microwave energy in order to optimize the coupling reactions of amino acids in aqueous solvent, on resin. Moreover, comparison among the microwave heating efficiency and the conventional thermal heating method is also reported. The combination of microwave energy and water can efficiently be used to conduct cost-effective and environmentally friendly solid phase peptide synthesis.

1. A. S. Galanis, F. Albericio, M. Grøtli. In "Peptides 2008"(Hilkka Lankinen Eds.), The Finish Peptide Society, Finland, 2008, pp. 164-165,
2. A. S. Galanis, F. Albericio, M. Grøtli. Org. Lett. 2009, 11, 4488-4491

O5. Abstract number: 414

Click Chemistry in the Synthesis of large Peptide Molecular Constructs and Peptide-based Polymers

R.M.J. Liskamp[1], C. Yim[1], R. Merkx[1], R. Elgersma[2], M. Van Dijk[1], A. Brouwer[1], O. Boerman[2], R. Van Nostrum[1], W. Hennink[1], R. Pieters[1], J. Kruijtzer[1], D. Rijkers[1]
[1]Utrecht University, Utrecht, The Netherlands
[2]Radboud University, Nijmegen, The Netherlands

For modulation of interaction and affinity of carbohydrate-protein, peptide-peptide and peptide-protein interactions, we use molecular scaffolds based on dendrimers as well as small CTV and TAC-based scaffolds. We have developed a versatile synthesis for (non-natural) amino acid based dendrimers. These dendrimers are now widely used in multivalent approaches to increase the (low)affinity of especially carbohydrate ligands. For this purpose we have also developed an efficient method for 'clicking' carbohydrates and peptides to these dendrimers using a microwave-assisted cycloaddition reaction for preparation of multivalent glycodendrimers and dendrimeric peptides. Dendrimeric (cyclic)peptides are applied for tumor and infection imaging and/or treatment.[1] Recently, we have developed 'sulfo-click' for ligation as well as for site-specific conjugation with peptides, fluorophores, and metal chelators.[2] In addition to using 'click' chemistry in chemoselective (bio)conjugation reactions of carbohydrates and peptides, we have expanded its applications towards the preparation of peptide-based polymers, which may open up possibilities for the synthesis of new biopolymers. Thus, biodegradable peptide-based polymers were synthesized by microwave-assisted click chemistry.[3,4] Furthermore, microwave-assisted click polymerization was used for the synthesis of Alzheimer Aβ(16-22) cyclic oligomers and their self-assembly into polymorphous aggregates including fibrils was studied.[5]

1. C.-B. Yim, O.C. Boerman, M. de Visser, M. de Jong, A.C. Dechesne, D.T.S. Rijkers, R.M.J. Liskamp, Bioconjugate Chem. 2009, 20, 1323.
2. D.T.S. Rijkers, R. Merkx, C.-B. Yim, A.J. Brouwer, R.M.J. Liskamp, J. Pept. Sci. 2010, 16, 1.
3. M. van Dijk, K. Mustafa, A.C. Dechesne, C.F. van Nostrum, W. E. Hennink, D.T.S. Rijkers, R.M.J. Liskamp, Biomacromolecules 2007, 8, 327.

4. M. van Dijk, M.L. Nollet, P.Weijers, A.C. Dechesne, C.F. van Nostrum, W.E. Hennink, D.T.S. Rijkers, R.M.J. Liskamp, Biomacromolecules 2008, 9, 2834.
5. R.C. Elgersma, M. van Dijk, A.C. Dechesne, C.F. van Nostrum, W.E. Hennink, D.T.S. Rijkers, R.M.J. Liskamp Org. Biomol. Chem., 2009, 7, 4517.

O6. Abstract number: 66

Bis-azobenzene photoswitchable a-amino acids for nanomaterials applications

A. Moretto[1], M. Crisma[2], P. Fatàs[3], G. Ballano[3], A.I. Jiménez[3], C. Cativiela[3], C. Toniolo[1]
[1]Department of Chemistry, University of Padova, Padova, Italy
[2]IBC, CNR, Department of Chemistry, University of Padova, Padova, Italy
[3]Department of Organic Chemistry, ICMA, University of Zaragoza, Zaragoza, Spain

The photoisomerization of an azobenzene moiety between the extended (*trans*) and compact (*cis*) conformations is reversibly triggered by light at two differing wavelengths. The resulting modifications in the molecular 3D-structures have been extensively exploited to photoswitch transformation in chemical species reversibly for various types of applications, *e.g.* in the construction of optoelectronic devices. Here, we describe synthesis and chemical characterization of two achiral, C^{α}-tetrasubstituted α-amino acids, each characterized by two azobenzene moieties covalently linked to their α-carbon atom, di[4-(phenylazo)benzyl]Gly and its 3-(phenylazo)benzyl analogue. These "albatros-like"compounds were characterized by UV-Vis spectroscopy and, one of them, by X-ray diffraction as well. We found that both compounds undergo multiple, reversible isomerization in a variety of solvents by irradiation with Vis light (to *trans*) or UV light (to *cis*). However, when the α-amino acids are dispersed in a paraffin medium, their photoswitch processes are blocked. Interestingly, using HPLC and NMR we were also able to unravel an intermediate state in the interconversion, namely the racemate of the *trans*/*cis* and *cis*/*trans* configurations. This phenomenon was further, albeit indirectly, demonstrated by photoisomerization of the chiral dipeptide of one azo-residue with H-L-Leu-OMe, which afforded two diastereomeric intermediates, as detected by HPLC and verified by NMR. We are currently extending this study to more complex molecular systems, where the two amino acids, appropriately N-functionalized, are bound to various type of nanoparticles.

O7. Abstract number: 360

Stabilization of a-conotoxin AuIB: influences of disulfide connectivity and backbone cyclization

N.L. Daly[1], E. Lovelace[1], S. Gunasekera[1], C. Alvarmo[1], R. Clark[1], S. Nevin[1], A. Grishin[1], D. Adams[2], D. Craik[1]
[1]The University of Queensland, Brisbane, Australia
[2]RMIT University, Melbourne, Australia

α-Conotoxins are peptides isolated from the venom ducts of cone snails that target nicotinic acetylcholine receptors (nAChRs). They are valuable pharmacological tools and have potential applications for treating a range of conditions in humans, including pain. However, like all peptides, conotoxins are susceptible to degradation, and to enhance their therapeutic potential it is important to elucidate the factors contributing to instability, and to develop approaches for improving stability. AuIB is a unique member of the α-conotoxin family because the non-native "ribbon"disulfide isomer exhibits enhanced activity at the nAChR in rat parasympathetic neurons compared with the native "globular"isomer. Here we show that the ribbon isomer of AuIB is also more resistant to disulfide scrambling, despite having a non-native connectivity and flexible structure. This resistance to disulfide scrambling does not correlate with overall stability in serum because the ribbon isomer is degraded in human serum more rapidly than the globular isomer. Cyclization via the joining of the N- and C- termini with peptide linkers of 4-7 amino acids prevented degradation of the ribbon isomer in serum and stabilized the globular isomers to disulfide scrambling. The linker length used for cyclization strongly affected the relative proportions of the disulfide isomers produced by oxidative folding. Overall the results of this study provide important insights into factors influencing the stability and oxidative folding of α-conotoxin AuIB and might be valuable in the design of more stable antagonists of the nAChR.

O8. Abstract number: 472

Stabilization of β?turn conformation in melanocortin like peptide by click reaction

C.T. Testa[1], S. Carganico[1], F. Nuti[1], M. Scrima[2], A.M. D'Ursi[2], N. Lubin Germain[1], M. Chorev[3], P. Rovero[2], A.M. Papini[1]
[1]PeptLab-SOSCO,Université de Cergy-Pontoise, Cergy-Pontoise Cedex, France
[2]Department of Pharmaceutical Science, Salerno, Italy
[3]Department of Medicine, Brigham and Women's Hospital, Boston, United States of America

Melanocortin receptors are involved in many physiological functions, including sexual function, feeding behavior, energy homeostasis and pigmentation, making them potential targets for drugs to treat obesity, sexual dysfunction, etc. Therefore understanding the conformational basis of the receptor-ligand interactions is crucial to the design of potent and selective ligands for these receptors. MT-II is a potent super-agonist of melanocortin receptors, characterized by lactam bridge between residues i and i+5 stabilizing a type-II βturn structure fundamental for the bioactivity.[1] The minimal active sequence is identified by the following tetrapeptide: His6-D-Phe7-Arg8-Trp9[2] .

In our previous work [3] we designed and studied a new intramolecular side chain-to-side chain [1,2,3]triazolyl modification bioisosteric to the lactam, based on the well studied i-to-i+4 side-chain to side-chain structure present in parathyroid hormone-related protein (PTHrP).

In current study we applied this strategy on MT-II sequence, stabilizing the β-turn conformation by the introduction of i-to-i+5 side chain-to-side chain cyclization via formation of a 1,4-disubstituted 1,2,3-triazolyl bridge. In this context we performed the synthesis of different azido and alkynyl amino acids [4,5], to introduce them in the sequence of MT-II. Nα-Fmoc-ω-azido-α-amino acids are obtained by diazo-transfer of the Nα-protected ω-amino-α-amino acid. Nα-Fmoc-ω-ynoic-α-amino acids were prepared by alkylation of Ni(II) complexes of the Schiff bases derived from glycine and a chiral inducer with alk-ω-ynyl bromides [6]. By click reaction we obtained 1,4-disubstituted 1,2,3- triazolyl-bridge corresponding to the ring size of MT-II, characterized by 5 methylenes. Our goal is to identify the permutations that mimic at the best the affinity to melanocortin receptors. Conformational NMR studies are in progress to evaluate the best type-ĮIβ-turn behavior required for biological activity.

[1] F. Al-Obeidi et al., J. Med Chem., 1989, 32, 2555-2561.
[2] J. Ying et al., Biopolymers, 2003, 71, 696-716.
[3] S.Cantel et al., J.Org.Chem, 2008, 73, 5663-5674.

[4] A. Le Chevalier Isaad et al., J. Pept. Sci., 2009, 15, 451-454.
[5] A. Le Chevalier Isaad et al., Eur.J. Org. Chem, 2008, 31, 5308-5314.
[6]M. Scrima et al., Eur. J. Org. Chem, 2010, 446-457.

O9. Abstract number: 404

Synthesis and Evaluation of Novel Small Proteins: GCN4 Leucine Zipper Directed Assembly of CA150 WW Domains

Z. Fidan, R. Volkmer
Institute for Med. Immunology, Berlin, Germany

The alpha-helical Coiled-Coil (CC) motif is a versatile protein-interaction-domain which is involved in many biological processes like transcription, scaffolding or signalling. Composed of at least two left-handed amphipathic alpha-helices that are coiled-up around each other (supercoil) Coiled-Coils are also characterized by the recurrence of a periodic heptad sequence and the ability to form multiple complexes of different orientation. Based on their unique self-associating properties we are currently aiming to use Coiled-Coil sequences as molecular building blocks for complex oligopeptide assembly.

In a model study several synthetic GCN4-leucine zipper variants (association segment) [1] were linked to various CA150-WW-domains (functionality segment) [2] by native chemical ligation (NCL) involving a C-terminal thioester. Furthermore, the structural features (self-association, self-recognition, folding) of these novel chimeric oligopeptides were investigated by CD and ITC.

[1] M. Portwich, S. Keller, H. M. Strauss, C. C. Mahrenholz, I. Kretzschmar, A. Kramer, R. Volkmer, Angewandte Chemie Int. Ed. Engl. 2007, 46, 1654.
[2] C. Sune, T. Hayashi, Y. Liu, W.S. Lane, R.A. Young, M.A. Garcia-Blanco, Mol. Cell Biol. 1997, 24(20), 9176.

O10. Abstract number: 242

Impact of ionic liquids on the conformation of peptides studied by HR-MAS NMR spectroscopy

A.R. Richardt, C. Mrestani-Klaus, F. Bordusa
Martin-Luther-University Halle-Wittenberg/Biochemistry and Biotechnology, Halle, Germany

In our research, we could successfully use ionic liquids (ILs) as reaction medium additives in the protease-mediated acylation and ligation of cleavage-sensitive and hydrophobic peptide and protein fragments without any hydrolytic side reactions.[1] In the attempts to explain these findings, the impact of ILs on the *cis/trans* ratio of Xaa-Pro peptide bonds was studied by solvent jump experiments, where a strong influence of ILs on the native *cis/trans* equilibrium depending on the nature of Xaa was found. In order to verify the obtained results, structural investigations of peptides upon dissolving in ILs were carried out using conventional solution NMR as well as HR-MAS (high-resolution magic angle spinning) NMR spectroscopy as a powerful technique.

The studies include extensive NMR measurements of short-chain model peptides of the type Suc-Ala-Xaa-Pro-Phe-pNA (Xaa = Gly, Lys)[2] and Ala-Xaa-Pro-Phe (Xaa = Ala, Glu, Gly, Lys, Phe) dissolved in aqueous solution (as a point of reference) and in three different imidazolium-based ILs. Highly resolved 1D and 2D HR-MAS spectra allowed complete proton resonance assignments of both the peptides as solutes and ILs as solvents. Chemical shift difference data have been utilized to derive implications on IL/peptide interactions.

Our results indicate that the observed proton chemical shift changes of peptide signals upon dissolving in ILs arise from a direct interaction between the IL and peptide and an IL-induced conformational change in certain peptide regions. Several factors have an influence on the strength, position and type of interactions between ILs and peptides, e.g. the nature of the amino acid in the peptide sequence as well as the nature and type of the IL anions. Strong interactions with the ILs along the peptide backbone indicate hydrogen bonding with the solvent anions. In the presence of proline and aromatic amino acids stacking effects with the IL imidazolium ring stabilize or destabilize the corresponding part of the peptide molecules. Furthermore, the interactions are significantly different in both *trans* and *cis* Xaa-Pro isomers, particularly when Xaa = Gly, pointing to a conformer-specific IL/peptide interaction.

1. N. Wehofsky et al., ChemBioChem. 2008, 9, 1493-1499.
2. C. Mrestani-Klaus et al., Green Chem. 2010, submitted.

This work was supported by a grant of the Deutsche Forschungsgemeinschaft (DFG) to F.B. and C.M.-K. within the DFG priority program SPP 1191.

O11. Abstract number: 99

Next generation peptide microarrays

V.S. Stadler[1], R. Bischoff[2], T. Felgenhauer[1], F. Breitling[3]
[1]PEPperPRINT GmbH, Heidelberg, Germany
[2]German Cancer Research Center, Heidelberg, Germany
[3]Karlsruhe Institute of Technology, Karlsruhe, Germany

In recent years, DNA chips have revolutionized genome research. A similar trend is predicted for peptide chips in proteome research. Their use ranges from the characterization of antibodies, sera and enzymes through the screening of new protein biomarkers up to the development of affinity tags, peptide-based drugs and vaccines. Due to complex manufacturing processes, however, peptide chips available fall short of the spot density and thus information content of corresponding DNA-Chips by far.

To eliminate these obstacles, we developed a new combinatorial technique with amino acids embedded in solid ”amino acid toner”particles. These toners are printed onto glass slides with micron resolution, using a custom 20-color laser printer. With all the different amino acid particles finally printed, they are melted at once to initiate coupling. Repeated printing and melting finally result in custom peptide microarrays with up to 156,000 spots per chip.

Thereby peptide quality is equivalent to standard synthesis from solution. The low toner need in turn considerably reduces material consumption and thus costs. That way, proteome research can be provided for the first time with a highly efficient tool that can play a similar role as DNA chips in genome research do today. Therefore we also present a number of case studies to demonstrate the usefulness and flexibility of this new peptide microarray generation.

O12. Abstract number: 141

CIS display, a DNA-based in vitro selection technology for therapeutic peptides

C. Ullman, N. Cooley, L. Frigotto, P. Mathonet, C. Stace, G. Ivanova, C. Baar, M. Writer
Isogenica Ltd., Little Chesterford, Essex, United Kingdom

CIS display is an in vitro display technology that is capable of displaying very large effective libraries of polypeptides (>10^13). The technology uses a DNA template that encodes a bacterial replication initiator protein (RepA) that binds back to the very same DNA template from which it was transcribed. Peptide-DNA fusions are generated with high efficiency using an in vitro transcription/translation mixture derived from lysed bacterial cells. Selection and

enrichment against the target of interest can be monitored by direct measurement of the DNA recovery and binding peptides can be determined by sequencing their attached DNA template. Highly specific, high affinity peptides to a wide range of targets have been isolated. Hits from these selections have been rapidly progressed through maturation for further enhancement to sub-nanomolar affinity and low nanomolar activity; examples will be provided. Adaptation of the technique has allowed both the selection of protease resistant and cell-penetrating peptides. These features enable the development of candidates for therapeutic and other in vivo applications.

O13. Abstract number: 137

Discovery and distribution of circular peptides in flowering plants

C.W. Gruber[1], D. Craik[2], B. Kopp[1]
[1]University of Vienna, Department of Pharmacognosy, Vienna, Austria
[2]The University of Queensland, Institute for Molecular Bioscience, Brisbane, Australia

Cyclotides are disulfide-rich miniproteins with the unique structural features of a circular backbone and knotted arrangement of three conserved disulfide bonds. These features make them exceptionally stable and they have applications as host defense (insecticidal) agents and stable drug frameworks. So far they have been found mainly in two plant families, including in every species of the violet family (Violaceae) so far examined, and in a few species of the coffee family (Rubiaceae). Rubiaceae is the fourth largest flowering plant family, comprising approximately 13,000 species, and is one of the largest and most important living biomasses due to their geographical distribution and economic importance.

We analyzed >200 Rubiaceae species and confirmed the presence of cyclotides in 22 species. Additionally, we analyzed >140 species in related plant families to Rubiaceae and Violaceae and for the first time have evidence for the occurrence of cyclotides in the Apocynaceae. On the basis of the phylogeny of cyclotide-bearing plants and the analysis of cyclotide precursor gene sequences, we hypothesize that cyclotide evolution occurred independently in various plant families after the divergence of Asterids and Rosids (~125 mya). This is strongly supported by recent findings on the biosynthesis of cyclotides, which involves ubiquitously present enzymes for folding and processing. In conclusion, we predict that the number of cyclotides within the Rubiaceae may exceed tens of thousands, potentially making cyclotides one of the largest protein families in the plant kingdom.

O14. Abstract number: 491

Imide-click reaction. Application to prodrug design and to diblock peptide synthesis

O.M. Melnyk[1], R. Mhidia[1], N. Bezières[1], A. Blanpain[2], N. Pommery[3]
[1]CNRS, Lille, France
[2]Pasteur Institute of Lille, Lille, France
[3]Université de Lille Nord de FranceL, Lille, France

The reaction of peptide thioacids with sulfonyl azides (1) is efficient and leads to a stable N-acyl sulfonamide bond. Alternately, the reaction of thioacids with azidocarbonyl derivatives, which results in the formation of an imide bond, was scarcely studied.(2) In this work, we disclose the interest of imide ligation, i.e. the reaction between peptide thioacids and azidocarbonyl derivatives, for access to complex peptide scaffolds.

The data presented show that imide ligation features most of the characteristics displayed by click reactions: the reaction is chemoselective, racemization free, gives good yields using simple reaction conditions in water and requires only readily available starting materials. Last but not least, nitrogen and elemental sulphur, the byproducts formed in this reaction, are non toxic.

Two different applications illustrate the usefulness of imide ligation. First, the reaction was exploited for the design of a novel prodrug strategy named click-unclick. An imide-click reaction allows the formation of an imide bond between a drug and a peptide carrier. The second unclick step is defined as a process leading to the elimination of the linker and to the release of the drug. Essential to the unclick concept is the participation of the imide bond formed by click chemistry in the disassembly mechanism. Typically, unclick process can be triggered by an enzyme (prostate specific antigen, PSA) specific for the target tissue (prostate cancer).

In the second application, imide ligation was exploited for diblock peptide synthesis. In a first step, reaction of a C-terminal peptide azide (fragment 1) with an N-terminal thioaspartyl peptide (fragment 2) leads a construct in which the side-chain of Asp and the last residue of fragment 1 are connected by an imide bond. Unmasking of the alpha-amino group of fragment 2 triggers acyl migration of fragment 1 from the side-chain to the amino group through a 6-membered intermediate, and thus to the formation of a native peptide bond with an Asn residue at the ligation site.

Overall, imide ligation complements the toolbox of powerful peptide chemical ligation techniques.

1. Merkx, R. et al. Org Lett 2005, 7, (6), 1125-8.
2. Shangguan, N. et al. J Am Chem Soc 2003, 125, (26), 7754-5.

O15. Abstract number: 372

Total Chemical Synthesis of a Trimeric Integral Membrane Enzym - Escherichia coli Diacylglycerol kinase

F.W. Becker[1], S. Lahiri[2], D. Olschewski[2], M. Brehs[1]
[1]Technische Universität München, GARCHING, Germany
[2]Max-Planck Institute of Molecular Physiology, DORTMUND, Germany

Escherichia coli diacylglycerol kinase (DAGK) is a small integral membrane enzyme that catalyzes the production of phosphatidic acid by transferring the γ-phosphate group of ATP to diacylglycerols[1;2]. Three monomers, comprising 121 amino acids each, assemble to form a functional homotrimer, which plays a vital role in the lipid metabolism of Gram-negative bacteria[3;4]. DAGK is a unique protein based on several features such as being the smallest kinase described up to date and by being unrelated to other members of the large family of phosphotransferases. Total chemical synthesis was used to generate milligram amounts of this integral membrane enzyme. Two consecutive native chemical ligation reactions were carried out to link three peptide segments prepared by Boc-based solid phase peptide synthesis, each comprising one of the three transmembrane domains of DAGK[5]. Folding of synthetic full length DAGK was achieved by direct reconstitution from HPCL elution buffer containing trifluoroethanol into detergent-containing aqueous buffer with high efficiency[2]. Reconstituted DAGK showed a typical α-helical CD spectrum and a trimeric oligomerization state. Enzymatic activity of synthetic DAGK was demonstrated by a coupled-enzyme assay measuring the consumption of ATP[6]. The obtained data indicate a similar activity of synthetic DAGK as measured for wild-type E. coli DAGK. To the best of our knowledge this is a first example for the total chemical synthesis of an integral membrane enzyme.

The ability to synthesize, reconstitute and characterize such integral membrane enzymes extends the still limited toolbox for the synthesis and semisynthesis of membrane proteins [7] and provides many possibilities for further investigations of the mechanism and folding of DAGK.

[1.] V. A. Lightner, R. M. Bell, P. Modrich, J.Biol Chem 1983, 258 10856-10861.
[2.] C. R. Loomis, J. P. Walsh, R. M. Bell, J.Biol Chem 1985, 260 4091-4097.
[3.] F. W. Lau, X. Chen, J. U. Bowie, Biochemistry 1999, 38 5521-5527.
[4.] W. D. Van Horn, H. J. Kim, C. D. Ellis, A. Hadziselimovic, E. S. Sulistijo, M. D. Karra, C. Tian, F. D. Sonnichsen, C. R. Sanders, Science 2009, 324, 1726-1729.
[5.] P. E. Dawson, T. W. Muir, I. Clark-Lewis, S. B. H. Kent, Science 1994, 266 776-779.
[6.] F. W. Lau, J. U. Bowie, Biochemistry 1997, 36 5884-5892.
[7.] D. Olschewski, C. F. Becker, Mol.Biosyst. 2008, 4 733-740.

O16. Abstract number: 74

Extending the repertoire of native chemical ligation

O. Seitz, F.S. Mende, C.H. Haase
Institute of Chemistry, Humboldt University Berlin, Berlin, Germany

The current methods for the construction of protein arrays rely on protein modules from recombinant sources. Chemical synthesis has still not reached the level of maturity that allows the on-chip synthesis of folded protein domains. Fragment ligation techniques may provide a solution to this problem, in particular the most powerful ligation method to date, the Native Chemical Ligation (NCL). However, NCL has limitations which constrict the applicability to high throughput formats.[1] We herein describe our recent achievements that may facilitate the chemical synthesis of protein interaction domains.

Native chemical ligation relies on the reactivity of peptide thioesters. However, solid phase synthesis of peptide thioesters often is plagued by the need for additional solution steps and time-consuming purification. We designed a method for Fmoc-based synthesis of peptide thioesters that avoids the need for HPLC purification.[2] Only the full-length peptide thioesters are selectively detached and directly used in native chemical ligation. We demonstrate the automation of the self-purifying synthesis of 20-40 aa long peptide thioesters. An improved method facilitated the synthesis of difficult peptides. We also show the application in a surface-based cysteine scan, which has been used to facilitate the identification of suitable ligation sites in SH3 domains.

Many protein domains do not contain a single cysteine residue. Different strategies have been developed to extend the repertoire of native chemical ligation. The ligation-desulfurization approach draws on beta-thiol amino acids wherein NCL followed by desulfurization. We show the use of penicillamine as a commercially available precursor to valine.[3] NCL at valine grants access to hydrophobic ligation sites which have not been available by the existing methods. We also show that sequence-internal cysteine residues can be used to accelerated peptide thioester based peptide couplings.[4]

1. C. Haase, O. Seitz, *Angew. Chem. Int. Ed.* **2008**, *47*, 1553.
2. F. Mende, O. Seitz, *Angew. Chem. Int. Ed.* **2007**, *46*, 4577.
3. C. Haase, H. Rhode, O. Seitz, *Angew. Chem. Int. Ed*. **2008**, *47*, 6807.
4. C. Haase, O. Seitz, *Eur. J. Org. Chem*. **2009**, 2096.

O17. Abstract number: 119

Synthesis of Artificial Membrane Peptides: Recognition, Assembly and Structural Studies at the Membrane/Water Interface

S.M. Muellar[1], P.E. Schneggenburger[1], G. Sicoli[2], C. Steinem[1], M. Bennati[2], U. Diederichsen[1]
[1]Georg-August-Universität Göttingen, Institut für Org. und Biomol. Chemie, GÖTTINGEN, Germany
[2]Max-Planck-Institut für biophysikalische Chemie, GÖTTINGEN, Germany

Recognition between membrane anchored protein species is crucial for a variety of cellular processes like transport, signal transduction, posttranslational modification and membrane insertion of nascent proteins. While some membrane proteins function as monomers others need to assemble into oligomeric structures to carry out their biological function.[1] Based on the structural requirements of a recently reported homodimeric peptide pore motif for membrane insertion, novel D,L-alternating double helical hairpins were designed in structural analogy to the natural antibiotic gramicidin A.[2,3] Our research focuses on the induced aggregation of artificial transmembrane domains (TMDs) within lipid bilayer complexes by nucleobase pairing mediated recognition at the membrane`s exterior. Therefore, the hairpin-TMD lead structure was equipped with analytical probes and functionalized with peptide nucleic acid (PNA) sequences serving as molecular recognition moieties, respectively. Peptide synthesis was carried out applying an orthogonal protecting group strategy. The functionalized TMDs were reconstituted in large unilamellar vesicles (LUVs). The in-membrane pore formation by adopting β5.6 double helices was investigated using CD spectroscopy. Site directed spin labeling led to the estimation of intramolecular constrains and allows for orientational as well as interaction analysis by Electron Paramagnetic Resonance (EPR) studies. Additionally, fluorescence probes were used to determine the dynamic aggregation process via Förster Resonance Energy Transfer (FRET). A dimerization of the peptide/PNA conjugates via off-membrane recognition and transmembrane assembly could be observed within lipid vesicles (DLPC) and was proven to be triggerable by the applied temperature.[4]

[1] Z. W. Hall, *Trends Cell. Biol.* **1992**, 2, 66-68.
[2] E. Alexopoulos, A. Küsel, G. M. Sheldrick, U. Diederichsen, I. Usón, *Acta Crystallogr., Sect. D* **2004**, 60, 1971-1980.
[3] A. Küsel, Z. Khattari, P. E. Schneggeburger, A. Banerjee, T. Salditt, U. Diederichsen, *ChemPhysChem* **2007**, 8, 2336-2343.
[4] P. E. Schneggenburger, S. Müllar, B. Worbs, C. Steinem, U. Diederichsen, *J. Am. Chem. Soc.*, submitted.

O18. Abstract number: 154

Profiling Of Membrane Structure Changes During The Adsorption, Destabilisation And Lysis Of Antimicrobial Peptides

M. Aguilar[1], T.H. Lee[1], M. Swann[2], C. Heng[1], J. Gehman[3], Y. Park[4], G. King[5], K.S. Hahm[4], F. Separovic[3]
[1]Monash University, Clayton, Australia
[2]Farfield Group Ltd, Crewe, United Kingdom
[3]University of Melbourne, Parkville, Australia
[4]Chosun University, Gwangju, South-Korea
[5]University of Queensland, St Lucia, Australia

The membrane interaction of antimicrobial peptides (AMPs) is a multiphase process which involves dynamic structural changes in both peptides and membrane lipids.

Continuous changes in membrane structure as a function of bound peptide has not yet been examined in real-time, but would provide new insight into this complex process and may open up new avenues for the design of potent specific antimicrobial peptides. In this work, the real-time multiphase changes of unilamellar supported planar lipid bilayer (Uni-SLB) composed of DMPC, DMPC/DMPG (4:1), DMPE/DMPG (4:1), DMPC/DMPG/Chl (16:4:5) and E coli lipids induced by the binding of AMPs are studied using dual polarisation interferometry [1]. With a collection of 10 natural and synthetic AMPs, the influence of peptide length, charge, hydrophobicity and amphipathicity on the membrane lipid ordering was studied by evaluating the rate of peptide binding verses the rate of membrane ordering changes. This novel approach provides a way to differentiate the multiphase AMPs-membrane interaction into a combination of different stages for each peptide. This new approach allows us to distinguish various mechanisms of AMPs function as (1) membrane surface bound without changes in membrane structure; (2) surface association with a small degree membrane change leading to membrane lysis at a threshold amount; (3) surface association with large degree membrane change with selective membrane lysis; (4) surface association followed by insertion and membrane lysis above a threshold amount; (5) insertion without membrane lysis and possible translocation above a threshold amount.

[1] Lee TH, Hall K, Swann MJ, Popplewell JF, Park YK, Hahm, KS and Aguilar MI, 'The Membrane Insertion of Helical Antimicrobial Peptides from the N-Terminus of Helicobacter pylori Ribosomal Protein L1', Biochim Biophys Acta (Biomembranes), 1798 (2010) 544-557.

O19. Abstract number: 562

Designed Hairpins Modulate the Amyloidogenesis of alpha-Synuclein: Oligomerization Inhibition and Diversion to Non-amyloid Aggregates

N.H. Andersen[1], K. Huggins[1], M. Bisaglia[2], L. Bubacco[2]
[1]University of Washington, Seattle, United States of America
[2]Department of Biology, University of Padova, Padova, Italy

Previous work, reported at the 30th EPS, established that analogs of a designed hairpin system (KKLTVS-IpGK-KITVSA, p = D-Pro) in which cross-strand pairs of Trp and Tyr residues were introduced reduced the yield of amyloid fibril when co-incubated with 8 microMolar human pancreatic amylin (hAM) in pH 7.2 buffer containing 2 vol-% HFIP (hexafluoro-isopropanol). The most potent analog (WW2, KKLTVW-IpGK-WITVSA, with a turn-flanking W/W interaction) was an effective inhibitor at equimolar concentrations and resulted in 70% reduction in fibril yield (as measured by thioflavin T, ThT, fluorescence) over a 12 hour period with a 6-fold increase in the "lagtime"to fibril formation. These results have now been confirmed by Congo red (CR) staining and TEM imaging.

In order to establish whether aryl-rich hairpins might represent a general strategy for disrupting amyloid formation pathways, the study has been extended to alpha-synuclein (a-syn, the largely unstructured 140-residue peptide that produces the Lewy body amyloid deposits associated with Parkinson's disease). Similar assay conditions (100uM a-syn, 1.5 vol-% HFIP) yield reproducible amyloid formation with the onset of beta-structuring (by CD and ThT fluorescence) occurring in 6 h and complete after 16 h. The effects of seventeen peptides on a-syn aggregation have been determined. While there were instances of lagtime increases, and hairpins with loop-flanking W/W interactions that had no effect on a-syn aggregation, the most common observation was the rapid diversion of a-syn to aggregates that failed to display any of the diagnostics of amyloid (CR staining with green birefringence, enhanced ThT fluorescence, fibril morphology by TEM). The effects are structure specific; for example, C_2H_5CO-W-IpGK-WTG-NH_2, a stable W/W-flanked turn species, accelerates hAM amyloid formation but retards a-syn amyloidogenesis. WW2 was one of the most effective agents at preventing a-syn amyloid formation by diversion to non-amyloid aggregates. The single-strand control for WW2, KKLTVWI, appears to inhibit the oligomerization step in a-syn amyloidogenesis and a helical state of a-syn is evident by CD in incubations including this peptide. Our structure activity results and current hypotheses regarding the mechanisms involved will be presented.

O20. Abstract number: 380

Ultra-long acting insulin degludec can be combined with rapid-acting insulin aspart in a soluble co-formulation

I. Jonassen, T. Hoeg-Jensen, S. Havelund, U. Ribel
Novo Nordisk, Maaloev, Denmark

Insulin analogues more closely mimic the mealtime and basal components of endogenous insulin secretion than regular human insulin preparations and are now an established part of diabetes management. Currently, premixed insulin analogue suspensions are widely used to avoid the need for separate injections of mealtime and basal insulin. It is anticipated that combining rapid-acting and long-acting insulin analogues in a soluble co-formulation could offer clinical advantages over premixed insulin suspensions. However, with currently marketed soluble insulin analogues, it is not possible to maintain the individual absorption profiles of rapid- and long-acting insulin analogues when they are co-formulated.

We have therefore developed a new generation ultra-long acting insulin (insulin degludec) in a novel soluble co-formulation with rapid-acting insulin aspart. Insulin degludec is desB30 human insulin derivatized at B29 lysine with hexadecanedioic acid via gamma-L-glutamic acid as a linker. The ultra-long effect of degludec is primarily due to the slow release of degludec monomers from soluble multi-hexamers that form after subcutaneous injection. Degludec provides a smooth and stable pharmacokinetic profile at steady state (t1/2 >24 hours) that gives rise to a longer duration of action than current basal insulin formulations.

Size-exclusion chromatography was conducted for candidate formulations intended for injection as well as under conditions mimicking the site of subcutaneous injection to identify the conditions required to retain distinct rapid- and long-acting insulin profiles. A co-formulation was identified where multi-hexamers composed distinctly of degludec are formed under physiological conditions, thus providing basal insulin coverage, while rapid-acting insulin aspart is predominantly present as monomers, allowing for fast absorption into the bloodstream.

O21. Abstract number: 130

Peptides Designed for Medical Applications

V.J. Hruby
University of Arizona, Tucson, United States of America

Though numerous peptides are utilized in clinical medicine, for many the development of peptides as drugs has lagged behind small molecule development, possibly due to misconceptions of peptides as potential pharmaceuticals by many chemists and biologists. This should change substantially in the future due to numerous developments

in novel peptide design and novel delivery systems. Here, we will discuss recent developments in design of bioactive peptides that are highly stable to proteolytic degradation, that cross the blood-brain-barrier, and that have good bioavailability for specific targets in vivo. Specific examples will include peptides that have promise for treating neuropathic pain, that can enhance cancer detection, and that can modulate energy utilization (feeding behavior). Emphasis will be placed on examples where in vivo studies have demonstrated stability, bioavailability, and little or no long term toxicity or tolerance.

Supported by grants from the U.S. Public Health Service, National Institutes of Health.

O22. Abstract number: 60

Selective Targeting of Extracellular Cyclophilins by Novel Cyclosporin A Derivatives.

M. Malesevic[1], J. Kühling[1], V. Kahlert[1], F. Erdmann[1], M.A. Balsley[2], M.I. Bukrinsky[2], S.L. Constant[2], G. Fischer[1]

[1]Max Planck Research Unit for Enzymology of Protein Folding, Halle, Germany

[2]The George Washington University, 2300 Eye St. Nw, Washington, United States of America

In modern drug design, selectivity gains ever-growing scientific attention. As a consequence of their selectivity, therapeutic agents could act at low concentrations with minimal or no side effects. In particular, selectivity could be achieved even for non-selective enzyme inhibitors by their targeting to a specific cell population or cell compartment. Cyclosporin A (CsA), a cyclic undecapeptide extracted from the fungus Tolypocladium inflatum, is such an indiscriminately tight binding inhibitor of Cyclophilins (Cyps), peptidyl-prolyl cis-trans isomerases. CypA/CsA binary complex inhibits the protein Calcineurin, a calcium-calmodulin-dependent serine/threonine phosphatase. It seems that the CsA immunosuppressive effect, used in transplantation medicine and autoimmune diseases, is mediated by this pathway. However, elevated levels of Cyps are also found in several pathological processes including viral infections, cardiovascular diseases, inflammatory diseases and cancer. Non-immunosuppressive CsA derivatives could be also used for treating these diseases.

Novel CsA derivatives for selective targeting of extracellular Cyps were designed and synthesized. A trifunctional template, obtained from trimesic acid, was functionalized with the fluorescent marker 5(6)-carboxytetramethylrhodamine and a side chain-extended [D-Ser8]-CsA analog. The remaining third position was occupied with a (D-Glu)6-Gly-OH moiety. The highly negatively charged (D-Glu)6-Gly-OH was used to improve the derivative solubilities at physiological conditions and to diminish their cell permeabilities.

The novel CsA derivates proved to be non-immunosuppressive but potent CypA and CypB inhibitiors. Moreover, in a chemotactic assay these derivates completely inhibit activated mouse CD4+ T cell migration in response to CypA and CypB.

M. Malešević, J. Kühling, F. Erdmann, M. A. Balsley, M. I. Bukrinsky, S. L. Constant, G. Fischer

Angew. Chem. Int. Ed. 2010, 49, 213-215. Angew. Chem. 2010, 122, 219-222.

O23. Abstract number: 224

Design Of Peptidyl-Inhibitors For Glutathione S-Transferase (Gst)

Useful In Targeted Cancer Chemotherapy

P. Cordopatis[1], I. Axarli[2], G. Kotzia[2], C. Petrou[3], N. Labrou[2], Y. Clonis[2]

[1]University of Patras, Patras, Greece

[2]Department of Agricultural Biotechnology, Agricultural University of Athens, ATHENS, Greece

[3]Department of Pharmacy, University of Patras, Patras, Greece

Glutathione-S-transferases (GSTs) are a family of cell detoxification enzymes that catalyse the conjugation of the tripeptide glutathione (GSH) to a wide variety of endogenous and exogenous electrophilic compounds, including certain chemotherapeutic drugs. GSTs have emerged as a promising therapeutic target because specific isozymes are overexpressed in a wide variety of tumours and may play a role in the aetiology of other diseases, including neurodegenerative diseases, multiple sclerosis, and asthma. A common problem encountered in cancer chemotherapy is the appearance of chemotherapeutic resistant tumour cells. A possible origin for the problem appears to be an increase in the expression of total GST activity. It is plausible that GSTs serve two distinct roles in the development of drug resistance via direct detoxification as well as acting as an inhibitor of the mitogen-activated protein (MAP) kinase pathway. In addition to glutathione conjugating activity, GSTs exhibit sulphonamidase activity and catalyze the GSH-mediated hydrolysis of sulphonamide bonds. Such reactions are of interest as potential tumour-directed pro-drug activation strategies. In the present work we report the design and synthesis of chimaeric sulphonamide-derivatives which can be activated by the model human isoenzyme GSTA1-1 (hGSTA1-1). These chimaeric molecules consist of (i) a bombesin-moiety (analogues [Leu^{13}]-bombesin, [Phe^{13}]-bombesin and [Ser^{3},Arg^{10}, Phe^{13}]-bombesin) as a structural element determining the drug selectively to tumour cells, able to recognise bombesin receptors present on the tumour cell surface, and (ii) an aromatic sulphonamide moiety. After hGSTA1-1-mediated cleavage of the sulphonamide bond of the chimaeric molecule, the released aromatic sulphonamide moiety is conjugated with GSH, thus leading to a S-aromatic-glutathionyl conjugate which is an inhibitor for GST. This inhibition was found to be of a competitive-type with respect to GSH (K_i = 5.1±0.8 μM) and of a non-competitive type with respect to the aromatic substrate CDNB (K_i = 8.6±0.7 μM). This inhibitory effect may reduce drug resistance of cancer cells.

O24. Abstract number: 347

New binders for proteins - versatile tools for drug development

L. Baltzer

Uppsala university, Biochemistry and Organic chemistry, Uppsala, Sweden

A novel concept for protein recognition is presented based on de novo designed synthetic peptides which can be turned into high-affinity recognition entities ”binders”by combing rational design and adaptive fitting protocols. The overall concept is based on helix-loop-helix motifs that can be site-specifically and sequentially modified with one or several small molecule ligands to form a binding site towards an epitope of a given target. These small molecule ligands can be any organic molecule (eg. peptide, sugar, lipid, oligonucleotide). In case the epitope of the target is known and when there exists an inhibitor, agonist and antagonist directed to this particular epitope the strategy is straight forward, cf the assay developed for detection of the enzyme Human Carbonic Anhydrase (HCA) using the inhibitor benzenesulphoneamide . In case the binding site is unknown it is necessary to adopt a combinatorial approach to screen for affinity binders. Most of the small

molecule ligands (eg. inhibitors) possess themselves a moderate affinity towards the target, typically in the mM-µM regime. However, by adopting the concept of rational design and adaptive fitting it is possible to boost the affinity to sub-nM affinities. This concept has been used for development of a binder for the C-reactive protein (CRP), a inflammatory marker, where the small molecule ligand is phophatidylcholine, PC, (Kd ~ 5 µM) is boosted to 0.1 nM upon proper attachment to the helix-loop-helix peptide motif. The concept enables early phase drug fragments with only moderate affinity to be turned into tight binders that can be sed for target validation and is thus of interest to the Pharma industry.

O25. Abstract number: 293

Exploring the HBV-envelope protein for liver-specific drug targeting

T.M. Müller, A. Schieck, B. Beijer, A. Meier, U. Haberkorn, S. Urban, W. Mier
University Hospital Heidelberg, Heidelberg, Germany

Many pharmaceuticals used today do not exhibit a specific targeting to their site of action. We have discovered a peptide derived from the large envelope protein of the hepatitis B virus which displays a very pronounced liver tropism. It consists of the 47 N-terminal amino acids of the large envelope protein of the hepatitis B virus and is stearoylated on the N-terminus. It could be shown in NMRI mice that the peptide is transported quickly to the liver. Already 10 minutes after intravenous injection, 88% of the injected peptide was found inside the liver. In in vitro experiments, it could be shown that the peptide binds specifically to various liver cell lines including primary human hepatocytes. Several drugs could be linked via different routes to the peptide, leaving the extraordinary liver specificity intact.

Different conjugation methods were chosen in order to link different drugs to the peptide. One approach was the formation of an intracellular cleavable disulfide bond. For this purpose, the peptide sequence was enlarged by an additional cysteine as the drug conjugation site. Furthermore, the sequence was enlarged by a tyrosine residue for radioactive labeling. The drug primaquine was derivatised by modifying its primary amine with a cysteine derivative. The derivative chosen was the commercially available Boc-Cys(NPyS)-OH. The 3-nitro-2-pyridinesulfenyl (NPyS) group reacts selectively with thiols by forming disulfide bonds. The conjugation reaction was carried out in aqueous solution at pH 8 and the product was purified by RP-HPLC and obtained in high purity. Other approaches include the coupling of a bromoaceto-derivative of penicillin to a cysteine residue, and the coupling of doxorubicin via an ester/amide linkage to an additional lysine residue.

O26. Abstract number: 426

Modified Cryptophycins - New Cytotoxic Cyclodepsipeptides

N. Sewald, C.H. Mast, S. Eißler, M. Nahrwold, B. Sammet, T. Bogner, C. Weiss
Bielefeld University, Bielefeld, Germany

Anticancer chemotherapeutics like paclitaxel interfere with microtubule dynamics and prevent microtubules from forming correct mitotic spindles, which causes cell-cycle arrest and apoptosis. Cryptophycins are a class of 16-membered highly cytotoxic macrocyclic depsipeptides isolated from cyanobacteria [1]. The biological activity is based on their ability to interact with tubulin. Strong antiproliferative activities with 100- to 1000-fold increased potency compared to paclitaxel and vinblastine have been observed. Cryptophycins are highly promising drug candidates, since their biological activity is not negatively affected by P-glycoprotein, a drug efflux system commonly found in multdrug resistant cancer cell lines and solid tumors [2]. Cryptophycin-52 had been investigated in phase II clinical trials, but failed because of its high neurotoxicity [3].

We have developed efficient strategies for the synthesis of cryptophycins and their analogues [2] taking specific emphasis on the synthetically most challenging unit A [4]. In addition, new interesting functionalities have been introduced in different positions for SAR studies [4,5].

The quasi-isosterism of 1,4-disubstituted 1*H*-1,2,3-triazoles and trans-amide bonds is still under debate. Therefore, we additionally synthesized an analogue of cryptophycin-52 where the trans-amide bond between units B and C is replaced by a 1,4-disubstituted 1*H*-1,2,3-triazole. The cytotoxic activity is largely retained for this ”clicktophycin”, generated by a [3+2] ”click”cycloaddition reaction. Consequently, this proves the bio-equivalence of 1,4-disubstituted 1*H*-1,2,3-triazoles and trans-amide bonds even in complex compounds [6].

[1] R. E. Schwartz et al., J. Ind. Microbiol. 1990, 5, 113-124.
[2] S. Eißler, A. Stoncius, M. Nahrwold, N. Sewald, Synthesis 2006, 22, 3747-3789.
[3] G. D’Agostino et al., Int. J. Gynecol. Cancer 2006, 16, 71-76.
[4] S. Eißler, M. Nahrwold, B. Neumann, H.-G. Stammler, N. Sewald, Org. Lett., 2007, 9, 817-819; S. Eißler, A. Stoncius, H.-G. Stammler, B. Neumann, N. Sewald, Synlett 2008, 2, 273-277; B. Sammet, H. Radzey, B. Neumann, H.-G. Stammler, N. Sewald, Synlett 2009, 3, 417-420.
[5] S. Eißler, T. Bogner, M. Nahrwold, N. Sewald, Chem. Eur. J. 2009, 15, 11273-11287.
[6] M. Nahrwold, T. Bogner, S. Eißler, S. Verma, N. Sewald, Org. Lett., 2010, 12, 1064-1067.

O27. Abstract number: 556

Biocompatible triazole ligations via 1,3-dipolar cycloadditions of peptidyl phosphoranes and azido peptides

J. Rademann, Ahsanullah
Leibniz Institute of Molecular Pharmacology (FMP) and Leipzig University, Berlin, Germany

Ligations of peptides under mild, metal-free conditions preferably conducted in aqueous buffer are of interest for diverse applications in medicinal chemistry and chemical biology. 1,2,3-Triazoles are attractive ligation products being thermodynamically and metabolically stable. Moreover, the integration of either 1,4- or 1,5-disubstituted triazoles in peptides enables the control of peptide conformations by locking specific positions in either the trans- or the cis-orientation. Unfortunately, the standard protocols for click chemistry rely on the use of heavy metal salts Cu(I) and Ru(II), limiting their use in many applications. We have developed a metal-free method for triazole ligation with full stereocontrol which is entirely integrated in the operations of Fmoc-based peptide synthesis. The method relies on peptidyl phosphoranes which react with various azides stereoselectively yielding 1,5-disubstituted triazoles only. The method was used to ligate short peptides. Stable conformations of resulting ”Clack Peptides”in DMSO were studied through NMR spectroscopy. Structural analysis revealed that 1,5-disubstitutd triazole ring introduce turn in the peptide.

[1] When peptidyl phosphoranes were terminated with an azido acid, the obtained azido-peptidyl phosphoranes were employed to deliver cis-triazolyl-cyclopeptides through

cyclative cleavage. The method completely avoided formation of soluble, non-cyclized, oligomeric by-products and is therefore superior to solution protocols with respect to synthetic efficiency, yields and purity.
[2] References1. Ahsanullah, P. Schmieder, R. Kühne, J. Rademann, Angew. Chem. 2009, 121, 5143-5147; Angew. Chem., Int. Ed. 2009, 48, 5042-5045.2. Ahsanullah, J. Rademann, Angew. Chem., Int. Ed. 2010, in press.

O28. Abstract number: 417
Semisynthesis of Glycosylated Human Interleukin 6
C. Unverzagt, S. Siebenhaar, A. Tröster, A. Reif, P. Velisetty
Bioorganische Chemie, Uni Bayreuth, Bayreuth, Germany
Since the interest in well-defined glycoproteins is steadily increasing, various methods for the synthesis of homogeneously glycosylated proteins were developed [1]. A very versatile basis is the sequential or one-pot native chemical ligation (NCL) developed by Kent et al [2].
As a model glycoprotein we chose the human interleukin 6 (IL-6), a cytokine with pleiotropic functions in cell proliferation, immune response or thrombopoiesis [3]. When used as a thrombopoietic reagent [4] recombinant, non-glycosylated IL-6 shows only a short half-life in vivo. Thus the native IL-6 glycoforms are of therapeutic interest.
For the semisynthesis of glycosylated IL-6 the protein was divided into three fragments. The N-terminal thioester fragment 1-42 and the C-terminal cysteine-fragment 49-183 were envisioned to be produced via expression of intein fusion proteins in E. coli. The N-terminal IL-6 segment 1-42 was inserted between two inteins. Expression gave inclusion bodies, which were refolded giving the native 1-42 thioester after optimized cleavage of both inteins. The cysteine fragment IL-6 49-183 was expressed as a single intein fusion protein also leading to inclusion bodies. Intein cleavage released IL-6 49-183 with an N-terminal cysteine, which was isolated after protection of the three thiols as mixed disulfides [5]. The glycosylated central IL-6 fragment 43-48 thioester was synthesized via a safety-catch linker [6] on a solid phase and contained an N-terminal thiazolidine [2]. After synthesis of the GlcNAcAsn + Met43Nle containing thioester 43-48 further trials were undertaken to obtain the native Met43 thioester. Additionally, the corresponding Met43Nle thioester 43-48 carrying an asparagine with complex type nona-saccharide was synthesized.
With this set of suitable protected building blocks in hand sequential ligations were carried out leading to the different full length IL-6 glycoproteins.
1. D. P. Gamblin, E. M. Scanlan, B. G. Davis, Chem. Rev. **2009**, *109*, 131-163.
2. D. Bang, S. B. H. Kent, Angew. Chem. Int. Ed. **2004**, *43*, 2534-2538.
3. J. Simpson, Protein Science **1997**, *6*, 929-955.
4. J. V. Castell, Eur. J. Biochem. **1988**, *177*, 357-361.
5. C. Piontek, P.Ring, O. Harjes, C. Heinlein, S. Mezzato, N. Lombana, C. Pöhner, M. Püttner, D. Varón Silva, A. Martin, F. X. Schmid, C. Unverzagt, Angew. Chem. Int. Ed. **2009**, *48*, 1936-1940.
6 . R. Ingenito, E. Bianchi, D. Fattori, A. Pessi, J. Am. Chem. Soc. **1999**, *121*, 11369-11374.

O29. Abstract number: 406
Recombinant protein hydrazides,versatile derivatives for site-specific labeling of proteins:Application to site-specific protein PEGylation.
G.J. Cotton, J. Roberts, J. McGregor, K. Woznica, J. Mysliwy, D. Anderson, A. Marques
Almac Sciences (Scotland), Gladsmuir, East Lothian, United Kingdom
The application of protein engineering technologies to protein therapeutics has opened up new opportunities for this increasingly important class of drugs. For example, the conjugation of cytotoxic agents to peptides and proteins, and in particular antibodies, is being successfully used for targeted drug delivery, and the attachment of large polymers such as PEG is routinely used to improve the pharmacokinetic properties of biotherapeutics. Thus, methods for the site-specific engineering of proteins are highly valued, particularly when compared to non-selective labeling technologies.
To this end, we have recently developed a novel protein ligation approach that harnesses the inherent properties of intein fusion proteins. In this procedure intein fusion proteins are chemically cleaved with aqueous hydrazine to generate the corresponding C-terminal hydrazide.This unique functionality within the primary sequence facilitates the site-specific introduction of labels into the protein through hydrazone bond forming ligation reactions. This versatile approach enables folded, disulphide bond containing, C-terminal hydrazide proteins to be generated and then site-specifically modified under aqueous conditions.
We have now developed this technology for protein PEGylation. By generating novel ‘pyruvoyl-containing’ derivatives of PEG, site-specific C-terminal PEGylation of proteins can be achieved in high yield (50 - 90%) through formation of the corresponding alpha-oxo hydrazone bond . Using this approach C-terminal PEGylated derivatives of IFNalpha-2b and IFNbeta-1b have been generated. The antiviral activity of C-terminal PEGylated IFNalpha-2b was circa two and half times higher than the approved non-selectively PEGylated IFNalpha-2b therapeutic (PEG-Intron) whilst C-terminal PEGylation of IFbeta-1b maintains the full antiviral activity of this protein. These data clearly demonstrate the utility and advantage of our approach in the PEGylation of therapeutic proteins.
Also, we have found this technology to be an excellent method for engineering single domain antibodies (sdAb). A C-terminal PEGylated derivative of a sdAb targeting prostate specific antigen was produced in high yield from cytosolic expression of the corresponding intein fusion protein. This C-terminal PEGylated sdAb maintained full PSA binding activity, demonstrating that this is a versatile platform for modifying this important class of proteins.

O30. Abstract number: 286
Running rings around proteins: protease-mediated biosynthesis of circular peptide and proteins
D.J. Craik[1], M. Colgrave[2], J. Mylne[1], B. Conlan[3], M. Anderson[3]
[1]The University of Queensland, Brisbane, Australia
[2]CSIRO, Livestock Industries, Brisbane, Australia
[3]La Trobe University, Bundoora, Australia
Ribosomally synthesized cyclic peptides ranging in size from 10-80 amino acids have been discovered in bacteria, plants and animals over the last decade [1]. Their head-to-tail cyclic peptide backbone, which means they are devoid of N- or C-termini, makes them resistant to exoprotease digestion and they are thus exceptionally stable. They have a wide range of biological activities but most are involved in host-defense. The cyclotides [2] are the largest family of plant-derived circular proteins, and occur in plants from the Violaceae (violet), Rubiaceae (coffee) and Cucurbitaceae (cucurbit) families. They have a diverse range of biological activities, including uterotonic, anti-HIV, antimicrobial, and insecticidal activities, the latter consistent with a natural

function in plant defense. Individual plants express suites of 10-100 cyclotides. Cyclotides contain ~30 amino acids, and as well as their head-to-tail cyclized backbone, they incorporate three disulfide bonds arranged in a cystine knot topology. 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. Their stability and compact structure makes them, and other cyclic peptides, attractive protein frameworks onto which bioactive peptide epitopes can be grafted to stabilize them, thus making them of interest in drug design applications [2]. Cyclotides are matured from larger precursor proteins via a series of processing events, including excision, and head-to-tail peptide ligation via asparaginyl endoproteinase enzyme activity. This mechanism of biosynthesis in which a protease effectively operates in reverse to make rather than break peptide bonds appears to be common to other known classes of ribosomally synthesized cyclic peptides, including sunflower trypsin inhibitor-1 (SFTI-1), a 14-amino acid peptide from sunflower seeds. This presentation will describe the discovery and characterization of the precursor proteins for cyclotides and describe in vitro studies on SFTI-1 which demonstrate the generality of protease-mediated cyclisation.

[1] Craik D J: Seamless proteins tie up their loose ends. Science, 2006, 311, 1561

[2] 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.

O31. Abstract number: 20

Advanced Chemical Tools to Study Ubiquitin Biology

A. Brik

Ben-Gurion University, Beer Sheva, Israel

The attachment of ubiquitin to a target protein is a widely utilized posttranslational modification in eukaryotes and is involved in various aspects of cellular function. In this process, three distinct enzymes, known as the E1-E3 system, collaborate to achieve a site-specific tagging of the lysine residue(s) in a target protein. This condensation step generates an isopeptide linkage between the ε-NH2 of the lysine residue and the activated C-terminal glycine of ubiquitin. The overwhelming majority of the studies in the field rely on the in vitro enzymatic reconstitution of this complex posttranslational modification for the protein of interest. However, this process is often challenged by the heterogeneity of the modified protein, the isolation of the specific ligase (E3), and obtaining reasonable quantities of the ubiquitylated protein. We have recently developed a highly efficient and chemoselective peptide and protein ubiquitylation method using 5-mercaptolysine (1). Protecting group variations of this amino acid allowed its incorporation in peptides using Boc- and Fmoc-SPPS as well as its use in sequential ligation (2). To allow for the synthesis of polyubiquitin chains we have developed the first total chemical synthesis of ubiquitin thioester (3). This battery of tools allowed the chemical synthesis of homogeneous ubiquitylated alpha-synuclein conjugate to support the ongoing efforts aiming at studying the effect of ubiquitylation in Parkinson's disease. Moreover, these tools facilitated the first total chemical synthesis of the polyubiquitin chains, which are known to be valuable reagents in the journey of unraveling the biology of ubiquitin.

1. K. S Ajish Kumar, M. Haj-Yahya, D. Olschewski, H. A. Lashuel, A. Brik Angewandte Chemie Int. Ed., 2009, 48, 8090. (Highlighted in Chemical & Engineering News 87/46, 2009).
2. M. Haj-Yahya, A. K. S. Kumar, L. A. Erlich, Biopolymer, In press.
3. L. A. Erlich, K. S. Ajish Kumar, M. Haj-Yahya, P. E. Dawson A. Brik, Organic & Biomolecular Chemistry, In press.

O32. Abstract number: 395

Collagen-like peptide supramolecules that regulate cell adhesion

T. Koide, C.M. Yamazaki

Waseda University, Tokyo, Japan

We developed a system for preparing totally synthetic collagen-like supramolecules with native-like collagen triple helices, through the self-assembly of synthetic collagenous peptides. The peptides are disulfide-linked trimers of 27- to 33-mer peptides comprised mostly of collagenous Gly-Pro-Hyp triplet repeats. Between the adjacent peptide strands, 12 to 21 residue staggers were introduced to create self-complementary cohesive ends. These self-complementary peptides are able to form elongated supramolecular architectures through spontaneous self-assembly. Upon cooling the peptide solutions, peptide supramolecules are formed by spontaneous intermolecular triple helix formation. The transition temperatures were found to be tunable by the design of the peptides.

We further aimed to add one of the specific cellular functions of native collagen to the collagen-like supramolecule. A similar self-assembling peptide containing the integrin-binding sequence Gly-Phe-Hyp-Gly-Glu-Arg was synthesized, and was found to exhibit significant binding activity to human dermal fibroblasts. The supramolecular structure was found to be essential for cell-attachment activity in the in vitro culture system. Cell adhesion was shown to be comparable to that of native collagen, and was further demonstrated to be mediated solely by integrin α2β1. Well-grown focal contacts and actin stress fibers were observed in cells spread on the supramolecular collagen-mimetic. This study successfully demonstrated that a defined collagen function can be elicited by the specific interaction between a cell surface receptor and an epitope displayed on a synthetic triple helical scaffold. Peptide-based artificial collagen would be useful in future studies to analyze and regulate individual cell functions normally mediated by native collagen. Using this supramolecular system, preparation of a multi-functional collagen-like material could be also expected. Such a pure and flexible strategy for synthetic biomaterial design will provide benefits for cell culture and cell engineering applications.

O33. Abstract number: 87

Peptide-amphiphile based polydiacetylenes: making nanofibers of defined dimensions

D.W.P.M. Lowik, M. Van den Heuvel, J. Van Hest

Radboud University Nijmegen, Nijmegen, The Netherlands

Nature provides many examples of proteins that form fibers and have important functionalities in the cell. Such fibers can be mimicked using smaller and synthetically more accessible peptide amphiphiles that organize themselves due to the interplay of hydrophobic and hydrogen bonding interactions and can be decorated with a variety of functionality.

One of the main limitations of such self assembled structures is that there is virtually no control over one of the dimensions, namely fiber length, since the build-up in this direction is a spontaneous uncontrolled process. Now, we take control over the organization of the architectures one

step further by creating assemblies which can be controlled in organization in all dimensions, including the length of the fiber. To reach this goal, we designed peptide amphiphile based nanofibers of which specific parts can be stabilized by polymerization while exploiting their propensity to align in a strong magnetic field, after which we are able to remove the non-stabilized parts.
For this purpose, peptide amphiphile fibers with a diacetylene moiety which only polymerize in the direction of the fiber were employed. We show that the rate of polymerization in our self-assembled, diacetylene containing peptide amphiphiles is dependent on the polarization of the incident light. Employing polarized light to polymerize a randomly oriented sample yielded a dichroic colored sample, thus introducing anisotropy in an otherwise isotropic sample using only polarization of light. Moreover, with an aligned sample and spatially addressed polarized light obtained via polarization holography, polymerization took place only at positions where the polarization of the incident light was parallel to the fiber orientation, yielding a pattern of polymer. The pitch of this pattern could easily be varied by changing the angle at which the two incident beams are combined. With this strategy the length of the fibers can be controlled.

O34. Abstract number: 326
Grafting of extracellular loops of the Y1 receptor, a human GPCR, onto a stable beta-barrel scaffold
O. Zerbe[1], R. Walser[1], J. Kleinschmidt[2]
[1]University of Zurich, Zurich, Switzerland
[2]Department of Biology, Konstanz, Germany
Structure-based drug discovery is currently considered to be one of the most reliable and efficient methods for the design of novel drugs. Therefore, much effort has been undertaken to determine the structure of new potential drug targets. In this context, the G-protein coupled receptor protein family (GPCRs) represents one of the most important class of drug-targets. Unfortunately, only a limited number of GPCRs structures has been solved up to now, exclusively using X-ray crystallography. Besides crystallography, NMR spectroscopy is a powerful alternative for determination of structure of membrane proteins.
Unfortunately, expression, purification and refolding of GPCRs for structural studies are hampered by a number of technical issues, and amounts of protein available for such studies are usually low. We have asked ourselves whether the binding epitopes of GPCRs that are targeted by peptide hormones, most likely presented by the extracellular loops, may not be grafted onto a more suitable scaffold. The latter should be expressed at high yields and be easily purified and reconstituted. To this end we have grafted all extracelullar loops of the human Y1 receptor onto a beta-barrel protein from E. coli, the outer membrane protein A (OmpA), itself a membrane protein. We show that such constructs can be expressed at 100-200mg/L culture. Using NMR and ^{15}N uniformly labeled peptide hormones we could demonstrate that the scaffold is recognized by some of these constructs. In addition, control peptides, which are shown to display much reduced affinity towards the wild-type receptors, are also binding the artificial receptors with much reduced affinity. These results indicate that the loops itself significantly contribute to the binding affinities of the GPCRs towards the ligands.

O35. Abstract number: 96
Peptide Fragmentomics
A. Zamyatnin
Universidad Tecnica Federico Santa Maria, Valparaiso, Chile
Natural fragmentation of biological molecules including peptides is well known. Fragmentary structural organization is characteristic of both the simplest and most complex biological molecules. Low molecular weight fragments of biological substances can be easily seen on metabolic maps. There are numerous examples showing that relatively small natural physiologically active substances are fragments of larger ones. They are a significant object of biochemical and physiological investigations. In this connection, the term ”fragmentomics”is grounded and defined, the bases and determination are given for the notion of the ”fragmentome”as a set of all fragments of a single substance, as well as for global fragmentome of all chemical components of living organisms [1, 2].
A steady increase in the number of publications dealing with protein fragment structure and function has been seen in recent years. For some proteins there are already hundreds of fragments that have been studied in detail, and it seems that concepts concerning functional importance of the totality of possible fragments of a single protein will be formed. Main ideas of fragmentomics and principles of their usage in studies of natural peptide structures are formulated in this work. For peptide structures, fragmentomics can be considered as a notion that combines proteomics and peptidomics.
It is considered how protein-peptide fragments are formed in nature, what experimental and theoretical methods are used for their investigation, as well as mathematical characteristics of fragmentomes. Individual fragmentomes of all subunits and of complete fragmentomes of some proteins are considered in detail. Structural and functional variety of their possible fragments was revealed by special computer analysis. Formation of an exogenous-endogenous pool of oligopeptides in an organism and correlation of these data with concepts of structure-functional continuum of regulatory molecules [3] was shown on an example of milk and meet protein fragments. Possible practical importance of the use of natural fragments in dietology, therapy, as well as in sanitary hygiene and cosmetics was noted.
[1] A.A.Zamyatnin. Fragmentomics of proteins and natural oligopeptides. Biophysics, 53, 329-335, 2008.
[2] A.A.Zamyatnin. Fragmentomics of natural peptide structures. Biochemistry (Moscow), 74, 1575-1585, 2009.
[3] I.P.Ashmarin, M.F.Obukhova. Regulatory peptides. A functional continuum. Biochemistry (Moscow), 51, 531-545, 1986.

O36. Abstract number: 127
Thiopalmitoylated peptides from the peripheral nervous system myelin P0 protein: synthesis, characterization and neuritogenic properties.
W. Beaino[1], E. Trifilieff[2]
[1]Institut de Physique Biologique, Strasbourg, France
[2]Université de Strasbourg, CNRS, Strasbourg, France
Thiopalmitoylation (i.e., the covalent attachment of palmitic acid via a thioester linkage to cysteine residues in the polypeptide backbone) is a common post-translational modification of proteins. In a previous study, we have shown that thiopalmitoylation of encephalitogenic T-cell epitopes of central nervous myelin proteolipid protein (PLP), as occur naturally in vivo, enhanced immune responses as well as the development and chronicity of experimental autoimmune encephalomyelitis (EAE), an animal model of the human inflammatory demyelinating disease, multiple sclerosis (MS) (1,2). These results

suggest that the immune response induced by endogenous thioacylated peptides that are released during myelin breakdown may play a role in the development and chronicity of autoimmune inflammatory demyelinating diseases. To confirm this hypothesis we have studied the effect of thiopalmitoylation on the immunogenic and neuritogenic properties of P0 protein, the major protein of peripheral nervous system (PNS) myelin. P0 is thiopalmitoylated at cysteine 153 and described as a candidate autoantigen in Guillain-Barré Syndrome (GBS), a human inflammatory demyelinating disease of the PNS.
For our study, we have synthesized the palmitoylated and non palmitoylated peptides P0(180-199) and P0(152-171) by solid phase peptide synthesis using Fmoc/tBu strategy. Palmitoylation was performed on-resin by using specific cysteine side chain protecting groups: Mmt (labile in diluted acid) and StBu (labile in the presence of tributylphosphine). Mmt was efficiently used for P0(180-199) thioacylation, but it was not suitable for thiopalmitoylation of P0(152-171) because of a premature deprotection of the Boc protecting group on the □-NH2 Lys in the presence of 2% TFA, leading to dipalmitoylation. Palmitoylated P0(152-171) was successfully obtained by using StBu as the thiol protecting group. We showed by circular dichroism that palmitoylation has no influence on the structuration of the peptide in solution, but palmitoylation increases the stability of the peptide in the presence of serum. We have used experimental autoimmune neuritis (EAN), the animal model of GBS, to compare the immunological properties of palm and non-palm P0 peptides and we have shown that thiopalmitoylation has indeed important effects on the immunogenicity and neuritogenicity of P0 antigens.
1. Greer J.M. et al, J. Immunol, 2001, 166, 6907-6913
2. Pfender N.A. et al, J. Immunol, 2008, 180, 1398-1404

O37. Abstract number: 204

Molecular knots as templates for protein engineering: the story of lasso peptides

S.F. Rebuffat, K.P. Yan, S. Zirah, Y. Li, C. Goulard, R. Ducasse, A. Blond, J. Peduzzi
Muséum National d'Histoire Naturelle, Paris, France

Mechanically-interlocked molecular architectures, such as molecular knots, arise from molecular connections that result from a specific topology instead of traditional bonds. Lasso peptides are bacterial ribosomally synthesized peptides sharing an unusual knotted structure. In this structure, a macrolactam ring between the Gly1/Cys1 N-terminus and the carboxyl side chain of an Asp/Glu at position 8/9 is penetrated by the C-terminal tail that is threaded and sterically maintained inside the ring. Lasso peptides have diverse properties, including antibacterial activities, as exemplified by microcin J25 (MccJ25) [1], capistruin [2] or lariatins [3]. Although their modes of action are diverse, lasso peptides generally bind proteins, acting as enzyme inhibitors or receptor antagonists. As such, the MccJ25 antibacterial activity has been shown to involve a recognition step by a siderophore receptor and to target RNA polymerase, the ring and the loop being involved in enzyme inhibition and in receptor recognition, respectively. Similar to other gene-encoded peptides, lasso peptides are formed from a precursor, which must undergo subsequent post-translational modification to acquire the typical rigid lasso structure, i.e. proteolysis and cyclization. Our comprehension of the biosynthesis of lasso peptides made a step forward when successful in vitro reconstitution of MccJ25 maturation was performed in our laboratory, demonstrating for the first time that two enzymes encoded by the genetic system (McjB and McjC) are sufficient for the maturation of MccJ254.
Recent advances on structure-activity relationships of lasso peptides and deeper insights into their mechanisms of maturation are presented here, as exemplified by microcin J25, pointing to the potential of the lasso structure for protein engineering.
[1] Rebuffat, S., Blond, A., Destoumieux-Garzón, D., Goulard, C., Peduzzi, J. (2004) Curr. Protein Pept. Sci. 5, 383-391.
[2] Knappe, T.A, Linne U., Rebuffat, S., Xie, X., Marahiel, M.A. (2008) J. Am. Chem. Soc. 130, 11446-11454.
[3] Iwatsuki, M, Uchida, R., Takakusagi, Y., Matsumoto, A., Jiang, C-L., Takahashi, Y., Arai, M., Kobayashi, S., Matsumoto, M., Inokoshi, J., Tomoda, H., Omura, S. J. Antibiot. (2007) 60, 357-363.
[4] Duquesne, S., Destoumieux-Garzón, D., Zirah, S., Goulard, C., Peduzzi, J., Rebuffat, S. (2007) Chem. Biol. 14, 793-803. 2186.

O38. Abstract number: 569

Insights into the uptake mechanism of NrTP, a cell-penetrating peptide exquisitely targeting the nucleolus of tumoral cells

D. Andreu, B. De la Torre, G. Rádis-Baptista
Pompeu Fabra University, Barcelona, Spain

Nucleolar targeting peptides (NrTPs) are 14-15 residue-long sequences with two Lys-Lys-Gly triads, designed by structural minimization of a snake toxin (J. Med. Chem. 2008, 50, 7041-7044). NrTPs such as NrTP1 (YKQCHKKGGKKGSG) and analogues are capable of penetrating human cervix epithelial carcinoma (HeLa) cells and homing into their nucleoli. Cellular uptake studies have showed that NrTP1 similarly penetrates and localizes in the nucleolus of tumor cells derived from human pancreatic (BxPC-3) and colorectal (CaCO2) adenocarcinomas and human ductal mammary gland carcinoma (BT-474). Drugs inhibiting receptor- and chlatrin-mediated endocytosis severely decrease the cell uptake of NrTP1. Live cell confocal microscopy imaging, in combination with flow cytometry analysis of NrTP1 uptake under conditions where HeLa cells were arrested to defined phases of their cycle confirmed that peptide uptake and nucleolar homing were independent of the cell cycle phase. These results are remarkable since NrTPs are remarkable among cell penetrating peptides for their ability to target preferentially an important, dynamic sub-nuclear structure such as the nucleolus.

O39. Abstract number: 409

Phylomer libraries as a structurally rich source of bioactive peptide which function inside and outside of cells

P.M. Watt
Phylogica Ltd, Perth, Australia

Phylomer libraries are a new source of peptides, derived from genomic fragments of biodiverse archael and bacterial species which encode structural motifs which have evolved within proteins. Phylomer peptides can exhibit superior functional hit-rates for blockade of protein interactions, when compared to randomly derived peptides, due to evolutionary selection for structure and stability. Phylomer peptides have been vadidated in 5 animal models of diseases, including multi-drug resistant gram negative infections, wound healing, stroke/TBI and acute respiratory distress syndrome.
Several Phylomer peptides have been identified which have picomolar target affinities, before any sequence maturation. Structurally related clusters of such Phylomer sequences suggest common families of folds, which are

ideally suited to binding to particular targets and direct lead optimization efforts. Phylomer libraries can also be used to identify new protein transduction domains for delivery of macromolecules into cells. Phylomers have been identified with potent activity against multiple intracellular targets in vivo since most Phylomers lack disulphide bonds and are stable in reducing environments such as the cytoplasm. We will also present ex vivo and in vivo studies of potent blockers of the extracellular target CD40Ligand (CD40L) for inflammatory diseases, including the results of intranasal administration approaches.
We have exploited the high functional hit rates from Phylomer libraries, to allow direct screening for particular phenotypes. For example a hit rate as high as 0.1% of unselected Phylomers, for specific blockade of the AP1 dependent signaling pathway has been observed, using the Phylomer itself for target identification using Mass Spectroscopy.
Watt PM (2006) Nature Biotechnology 24 (2):177-83
Watt, PM (2009) Future Medicinal Chemistry 1 (2)

O40. Abstract number: 392

Exploration of the Dipolar [2+2] Cycloaddition-Cycloreversion Reaction to Access Cyanobutadiene-scaffolded Peptides as Novel Chromophores‡

D.T.S. Rijkers[1], F De Prada Lopez[1], R Liskamp[1], F Diederich[2]
[1]Utrecht University, Medicinal Chemistry & Chemical Biology, UTRECHT, The Netherlands
[2]Laboratory of Organic Chemistry, ETH Zurich, Zurich, Switzerland

There is a growing interest in novel synthetic approaches for the preparation of highly functionalized peptides to obtain advanced peptide-based materials. Of particular interest to us are peptides that are functionalized with π-conjugated moieties since such peptides can be used as supramolecular building blocks, as light-induced molecular switches, as light-harvesting bionanomaterials, as well as intense chromophores for imaging purposes. To access a new class of π-conjugated peptidic donor-acceptor (D-π-A) chromophores, the recently developed reaction between electron-rich alkynes with electron-deficient ethylenes was applied.1 As a typical example, Nα-(4-ethynylphenyl)-Nα-(methyl)-glycyl-valine methyl ester (1) reacted smoothly with tetracyanoethylene (TCNE) to give in almost quantitative yield, the deeply colored (λmax 492 nm, ε 60,000 M-1cm-1) tetracyanobutadiene-scaffolded peptide 2. The conditions for this 'click-chemistry'-type reaction were found to be very mild, and allow several functional groups to be present in the peptide moiety. As an alternative for TCNE, and for increasing peptide diversity, Nα-((4-(2,2-dicyanovinyl))benzoyl)-alanine ethyl ester (3) was synthesized and reacted with methyl ester 1. This cycloaddition reaction was run in CH2Cl2 at 75ºC for 1 h under microwave irradiation to give densely functionalized butadiene-scaffolds with two different peptide sequences in a yield of >80%. The presented chemistry can be considered as a proof of principle for bioorthogonal modification of peptides (and proteins) with these new imaging chromophores. Herein, details of the syntheses, the variation of the Nα-(4-ethynylphenyl)-Nα-(methyl)-glycyl- and Nα-((4-(2,2-dicyanovinyl))benzoyl)- cores, and structural characterization by NMR and MS, and extensive UV-vis data of the cyanobutadienes will be presented.
1. T. Michinobu, C. Boudon, J.-P. Gisselbrecht, P. Seiler, B. Frank, N.N.P. Moonen, M. Gross, F. Diederich, Chem. Eur. J. 2006, 12, 1889; P.D. Jarowski, Y.-L. Wu, C. Boudon, J.-P. Gisselbrecht, M. Gross, W.B. Schweizer, F. Diederich, Org. Biomol. Chem. 2009, 7, 1312; Y.L. Wu, P.D. Jarowski, W.B. Schweizer, F. Diederich, Chem. Eur. J. 2010, 16, 202; S.-i. Kato, F. Diederich, Chem. Commun. 2010, 46, 1994. These investigations have been initiated by DTSR and FD at the ETHZ and continued by DTSR at the UU.

O41. Abstract number: 156

Structural Characterization of the Relaxin-3/INSL5 Chimera Peptide - A Selective RXFP3 and RXFP4 Agonist

M. Haugaard-Kedström[1], M.A. Hossain[2], N.L. Daly[3], R.A.D. Bathgate[2], J.D. Wade[2], D.J. Craik[3], K.J. Rosengren[1]
[1]Linneus University, Kalmar, Sweden
[2]Howard Florey Institut, The University of Melbourne, Melbourne, Australia
[3]Institute for Molecular Bioscience, The University of Queensland, Brisbane, Australia

The relaxin peptide hormone family has seven members, namely relaxin1-3 and INSL3-6, which all consist of two chains, A and B, that are held together by two inter-chain disulfide bonds and one intra A-chain disulfide bond. Each chain comprises of about 24 to 30 amino acids. The relaxin ligands bind to four known G-protein coupled receptors, namely relaxin family peptide receptor 1 to 4 (RXFP1-4). The relaxin peptides have various functions such as regulating reproduction, stress, appetite and wound healing, making them interesting pharmaceutical targets.
Relaxin-3 is the endogenous peptide ligand for the RXFP3 receptor and both relaxin-3 and RXFP3 are primarily expressed in the brain and have been linked to stress responses and appetite regulation. Relaxin-3 also has the ability to bind and activate RXFP1 and RXFP4 in addition to RXFP3, which makes pharmacological characterization of its receptor function complicated. In rats, the RXFP4 is a pseudo gene and thereby not expressed, however to understand the biological role of relaxin-3, a selective ligand for RXFP3 over RXFP1 is needed. Recently it was shown that by combining the B-chain of relaxin-3 and the A-chain of INSL5 such a selective ligand could be obtained. To get structural insights into this relaxin-3/INSL5 chimera peptide (R3/I5), and understand the consequences of combining two chain from different relaxins, R3/I5 was subjected to solution NMR. The solution structure of human R3/I5 displays a relaxin like fold and thus the INSL5 A-chain has the ability to structurally support a native relaxin-3 like B-chain conformation. These results indicates that the loss of activation of the RXFP1 is due to loss of a secondary binding sites in the relaxin-3 A-chain and rather not due to conformational changes in the primary binding site, which has been shown to be located in the relaxin-3 B-chain.

O42. Abstract number: 215

Cryptides: receptors and signaling mechanisms for novel neutrophil-activating peptides hidden in mitochondrial proteins

H Mukai[1], T Seki[2], Y Hokari[2], A Fukamizu[2], Y Kiso[1]
[1]Kyoto Pharmaceutical University, Kyoto, Japan
[2]Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki, Japan

The physiological roles of peptides that are simultaneously produced during maturation and degradation of peptidergic hormones and neurotransmitters have recently been of particular interest. Namely, bioactive peptides are matured by specific cleavages of their precursor proteins which have no biological functions, and then degraded by various proteases. During these processes, many fragmented peptides are also produced from the same precursor

proteins. Although physiological importance of these peptides has not been well elucidated, they may have various unexpected biological functions. Recently, we purified and identified novel neutrophil-activating peptides mitocryptide-1 and mitocryptide-2 that were cleaved from mitochondrial cytochrome *c* oxidase subunit VIII and cytochrome *b*, respectively [1,2]. We also found many neutrophil-activating peptides derived from various mitochondrial proteins [1-3]. Moreover, peptides produced from not only mitochondrial but also other proteins such as hemoglobin were shown to regulate a variety of biological functions including cells proliferation [1,3,4]. These lines of evidence proposed that such peptides play critical roles including triggering inflammatory responses and healing damaged tissue. We therefore named these functional 'cryptic' peptides that are hidden in protein structures as 'cryptides', and those cryptides that are derived from mitochondrial proteins as 'mitocryptides' [1-3]. Here, we report the discovery and comprehensive identification of various cryptides that activate neutrophils. The investigation on receptors and intracellular signaling mechanisms for these identified cryptides are also presented.

References: [1] Mukai, H., Hokari, Y., Seki, T., Takao, T., Kubota, M., Matsuo, Y., Tsukagoshi, H., Kato, M., Kimura, H., Shimonishi, Y., Kiso, Y., Nishi, Y., Wakamatsu, K., and Munekata, E. (2008) *J. Biol. Chem.*, **283**, 30596-30605. [2] Mukai, H., Seki, T., Nakano, H., Hokari, Y., Takao, T., Kawanami, M., Kiso, Y., Shimonishi, Y., Nishi, Y., and Munekata, E. (2009) *J. Immunol.*, **182**, 5072-5080. [3] Ueki, N., Someya, K., Matsuo, Y., Wakamatsu, K., and Mukai, H. (2007) *Biopolymers (Pep. Sci.)*, **88**, 190-198. [4] Gomes, I., Grushko, J.S., Golebiewska, U., Hoogendoorn, S., Gupta, A., Heimann, A.S., Ferro, E.S., Scarlata, S., Fricker, L.D., and Devi, L.A. (2009) *FASEB J.*, **23**, 3020-3029.

O43. Abstract number: 291

The cyclotheonamide scaffold: a privileged platform for the development of beta-tryptase inhibitors

N. Schaschke[1], D. Janke[1], C.P. Sommerhoff[2]
[1]Universität Bielefeld, Bielefeld, Germany
[2]Klinikum der LMU, München, Germany

beta-Tryptase is a serine protease with trypsin-like activity that is virtually exclusively expressed in mast cells. The enzyme is thought to play a pivotal role in the pathogenesis of allergic and inflammatory disorders, and thus inhibiting its proteolytic activity represents a promising approach for therapeutic intervention [1].

beta-Tryptase has an extended substrate specificity: beside the basic S1 ligand, which is essential for substrate recognition and processing, the protease shows an additional preference for basic residues in P3 position [2]. To take advantage of this feature for inhibitor design, cyclotheonamides provide good structural prerequisites. As shown by the X-ray structure of cyclotheonamide A in complex with trypsin, these cyclic pentapeptides adopt an extended conformation stabilized by macrolactamization, thus allowing to address the S1', S1, S2, and S3 pocket along the active-site cleft in a substrate-like manner [3]. The S1 ligand, (*S*)-3-amino-6-guanidino-2-oxo-hexanoic acid, interacts through its guanido function with Asp-189 at the bottom of the S1 pocket. In addition, the ketone covalently modifies the gamma-oxygen of Ser-195 through hemiketal formation.

As starting point for the development of selective beta-tryptase inhibitors we have focused on cyclotheonamide E4. The cyclic peptide scaffold was modified at two positions: (i) the S1 ligand was replaced by beta-homo amino acids derived from lysine and arginine to obtain chemically more stable and reversible binding protease inhibitors, and (ii) the alpha-amino function of (*S*)-2,3-diamino propionic acid was used as anchoring point for basic P3 ligands of different chain lengths to exploit interactions with the negatively charged Glu-217 of beta-tryptase. These analogs were synthesized by a combination of solid phase and solution phase chemistry [4]. Among them we have identified a potent and selective inhibitor of beta-tryptase [4].

[1] C. P. Sommerhoff, N. Schaschke, Curr. Pharm. Design 2007, 13, 313.
[2] J. L. Harris, A. Niles, K. Burdick, M. Maffitt, B. J. Backes, J. A. Ellman, I. Kuntz, M. Haak-Frendscho, C. S. Craik, J. Biol. Chem. 2001, 276, 34941.
[3] A. Y. Lee, M. Hagihara, R. Karmacharya, M. W. Albers, S. L. Schreiber, J. Clardy, J. Am. Chem. Soc. 1993, 115, 12619.
[4] N. Schaschke, C. P. Sommerhoff, ChemMedChem 2010, 5, 367.

O44. Abstract number: 256

Design of radiolabeled ghrelin agonists and inverse agonists for PET Imaging

C. Chollet[1], S. Els[1], R. Bergman[2], A.G. Beck-Sickinger[1]
[1]University of Leipzig, Leipzig, Germany
[2]Research center Dresden - Rossendor, Dresden, Germany

Gastro-intestinal peptide hormones mediate hunger and satiety, food intake, energy homeostasis and body weight.[1] Ghrelin is the only known orexigenic gastro-intestinal peptide. It has a central role in the short and long term regulation of hunger and energy homeostasis although its mode of action and way of signalling remain unclear.[2] Remarkably, the ghrelin receptor possesses a naturally high constitutive activity representing 50% of its maximal activity.[3] Therefore, ghrelin antagonists and inverse agonists have emerged as potential anti-obesity drugs.[4]

Positron Emission Tomography (PET) is a non-invasive imaging method allowing the localisation and quantification of a radiotracer in vivo. Therefore, MicroPET is a unique tool for pharmacokinetic studies of bioactive compounds in rodents.

In this context, ghrelin agonist and inverse agonist tracers, suitable for imaging, were developed to provide a better understanding of ghrelin mode of action and for further design of ghrelin inverse agonists.

The native ghrelin and short ghrelin inverse agonists were synthesised by solid-phase peptide synthesis. NODAGA,[5] a bifunctional chelator, was coupled at the N-terminus or at the N^{ε} of a Lysine of the resin-bound peptides. Then, the NODAGA-peptides were labeled in solution with ^{68}Ga or ^{64}Cu in mild condition, without any degradation and high radioactivity incorporation.

The activity of ^{69}Ga-NODAGA-peptides was evaluated in vitro by inositol phosphate turnover assay. Incorporation of the Ga-NODAGA complex at Lys^{16} of ghrelin doesn't modify its potency. Introduction of the Ga-NODAGA complex at the N-terminus of ghrelin inverse agonists decreases their potency from 1.5 to 6 fold. Nevertheless, pharmacokinetic profiles of these compounds were investigated by microPET on rodent.

1. Murphy, K. G.; Bloom, S. R., Nature, 2006, 444, 854-9.
2. Cummings, D. E., Physiol Behav, 2006, 89, 71-84.
3. Holst, B.; Cygankiewicz, A.; Jensen, T. H., et al., Mol Endocrinol, 2003, 17, 2201-10.
4. Chollet, C.; Meyer, K.; Beck-Sickinger, A. G., J Pept Sci, 2009, 15, 711-30.

5. Eisenwiener, K. P.; Prata, M. I.; Buschmann, I., et al., Bioconjug Chem, 2002, 13, 530-41.

O45. Abstract number: 259

Cdc42 Derived Peptides Conjugated To A New Cell Penetrating Carrier. Effect On The Rearrangement Of Actin And Weibel-Palade Bodies Secretion

V. Tsikaris[1], C. Papadopoulos[1], S. Zografu[2], A. Ntouhaniari[2], E. Papadopoulou[2], P. Kouklis[2], S. Christoforidis[2]
[1]University of Ioannina, Ioannina, Greece
[2]School of Medicine, University of Ioannina, Ioannina, Greece

Intracellular delivery of drugs and bioactive compounds in cells requires their conjugation to cell penetrating peptides (CPPs). Up to date the CPPs, used, are able to transport only one copy of cargo. In this study we present a new cell penetrating sequential carrier (CPSC), which is able to transport multiple copies of cargoes into the cell. CPSC is formed by the repetitive -Lys-Aib-Cys- moiety, which incorporates a cysteine residue for anchoring bioactive molecules through a thioether or disulfide bond (Papas et al. 2007). This CPSC was applied for defining the minimal conserved region of Cdc42 for association with its cytoplasmic partner N-WASP. This interaction is important for cellular functions such as the rearrangement of actin cytoskeleton, the secretion of vWF from Wiebel-Palade bodies in endothelial cells. Using circular dichroism spectroscopy (CD), affinity column and immunofluorescence techniques we concluded that: CPSC combining both the cationic and the conformational amphipathicity characteristics,is a very strong cargo transporter into cells (I), the Cdc42(181-187) region is the minimal conserved region that interacts with the Wiskott-Aldrich Syndrome Protein (WASP) (II), and the CPSC-Cdc42(181-187) conjugate induces the rearrangement of actin cytoskeleton and inhibits the secretion of vWF from Wiebel-Palade bodies on HMEC and HUVEC cell lines respectively. *References: Papas et al. (2007), J. Pept. Sci. 13, 662-671. Acknowledgements to the GSRT and EU (Pened 03EΔ629) for the financial support.*

O46. Abstract number: 490

MCD4-HS12 : closing and locking doors for HIV-1 entry

F. Baleux[1], P. Clayette[2], F. Arenzana[1], D. Bonnaffe[3], H. Lortat-Jacob[4]
[1]Institut Pasteur, PARIS, France
[2]SPIBio, Fontenay Aux Roses, France
[3]UPS, Orsay, France
[4]IBS, Grenoble, France

An original and new concept has been developed to inhibit attachment and entry of HIV-1 in cells: mCD4-polyanion conjugates. The proof of concept has been demonstrated by mCD4-HS12 conjugate, that inhibits PBMC infection by HIV-1 X4, R5 and dual-tropic X4R5 viral strains at low nanomolar range (ED90 between 3 to 11 nM)[1]. mCD4-HS12 simultaneously targets gp120's CD4, coreceptors and heparan sulphate binding sites. In order to simplify the conjugate structure and synthesis, HS12 (sulphated dodecasaccharide) has been replace by a shorter HS fragment and more interesting an acidic peptide. Even though less active, the ED90 are still in the nanomolar range. On going work will be presented, in particular, HS12 replacement by heparin mimic peptides.

1. Baleux, F.; Loureiro-Morais, L.; Hersant, Y.; Clayette, P.; Arenzana-Seisdedos, F.; Bonnaffe, D.; Lortat-Jacob, H. A synthetic CD4-heparan sulfate glycoconjugate inhibits CCR5 and CXCR4 HIV-1 attachment and entry. *Nat Chem Biol* 2009, 5, 743-8.

O47. Abstract number: 152

Turning nonspecific cytotoxic drugs into tumor-selective agents via conjugation to branched peptides.

C. Falciani[1], J. Brunetti[1], B. Lelli[1], C. Pagliuca[2], S. Menichetti[2], L. Vitellozzi[2], S. Scali[1], S. Bindi[1], A. Pini[1], L. Lozzi[1], L. Bencini[3], R. Moretti[3], L. Bracci[1]
[1]University of Siena, Siena, Italy
[2]University of Florence, Florence, Italy
[3]Careggi Regional and University Hospital, Florence, Italy

The selective targeting of tumor cells is the goal of modern cancer therapy aimed at overcoming the non-specific toxicity of most anticancer agents against normal cells.

We created new modular branched peptides, based on the sequence of human neurotensin conjugated, with different fluorophores and drugs, and tested their possible application for tumor-selective targeting in different human cancers.

The branched scaffold allows conjugating different functional units to the tetra-branched peptides, making them efficient target-selective carriers, either for in vitro or in vivo spotlighting of tumor cells or for killing them, simply by exchanging the functional moiety coupled to the conserved receptor-targeting core (1).

Branched peptides can retain or even increase, through multivalent binding, peptide biological activity and are very resistant to proteolysis, thus having a markedly higher in vivo activity in respect of correspondent monomeric peptides (2).

Fluorophore-conjugated peptides were used to measure tumor versus healthy tissue binding in human surgical samples, resulting in validation of the peptides as highly tumor-specific. Drug-armed branched peptides were synthesized with different conjugation methods resulting in uncleavable adducts or drug-releasing molecules (3-4).

The drug-release modality together with cytotoxicity were studied in different human cancer cell lines. Human cell lines from colon (HT-29), pancreas (PANC-1) or prostate (PC-3) carcinoma were challenged with branched NT conjugated with 6-mercaptopurin, combretastatin A-4, monastrol and 5-fluoro-deoxyuridine. Results indicated branched NT conjugated with combretastatin A-4 and 5-fluoro-deoxyuridine as the most active agents on HT-29 (EC50 1.1e-007 M) and PANC-1 (EC50 5.0e-007 M) respectively.

Tetra-branched NT armed with 5-FdU was used for in vivo experiments in HT-29 xenografted mice and produced a 50% reduction in tumor growth with respect to animals treated with the free drug. An unrelated branched peptide carrying the same drug was completely ineffective.

Results reported in this presentation indicate drug-armed branched peptides as very promising pharmacodelivery options.

1. Falciani C et al Mol Cancer Ther (2007), 6, 2441.
2. Bracci L.et al J Biol Chem (2003), 278, 46590.
3. Falciani C et al ChemMedChem (in press).
4. Falciani et al CCDT (in press).

O48. Abstract number: 407

Use of ester-containing peptides toward understanding the functions of amyloid beta peptide and human insulin

Y. Sohma[1], S.B.H. Kent[2], Y. Kiso[1]
[1]Kyoto Pharmaceutical University, Kyoto, Japan
[2]The University of Chicago, Chicago, United States of America

Replacing the native amide (*N*-acyl moiety) by an ester (*O*-acyl moiety) at beta-hydroxyl group of Ser or Thr residue (designated "*O*-acyl isopeptide") significantly changes the

secondary structure of the native peptide. Additionally, due to the presence of an additional amino group, the *O*-acyl isopeptide is generally hydrophilic. The target peptide is then generated from the corresponding *O*-acyl isopeptide via an *O*-to-*N* intramolecular acyl migration [1]. Based on the *O*-acyl isopeptide method, we are establishing an *in situ* system in which monomer amyloid beta peptide (Abeta) 1-42 is quickly produced from the water-soluble *O*-acyl isopeptide possessing an ester bond at the Gly^{25}-Ser^{26} sequence [2]. The intense and uncontrollable self-assembling nature of Abeta1-42 has led to difficulties in preparing monomer Abeta1-42. The difficulties have brought irreproducible or discrepant experimental results. Thus, the *O*-acyl isopeptide system has been used in Abeta-related Alzheimer's disease research to more clearly explain the functions of Abeta.

On the other hand, we have recently performed total chemical syntheses of insulin-related proteins [3]. As a minimal surrogate for proinsulin we designed and synthesized an 'ester insulin' precursor in which the A- and B-chains of insulin are covalently connected via an ester bond between the beta-hydroxyl group of Thr^{B30} and the gamma-carboxyl group of Glu^{A4}. The ester linkage made the precursor molecule as favorable for folding/disulfide formation as does the 35 amino acid residue C-peptide in proinsulin. Folded ester insulin was readily saponified to give insulin with full biological activity. The ester-linked insulin strategy provides a simple and effective approach to the total synthesis of insulin and chemical analogues with optimized properties.

Chemical synthesis of peptides/proteins provides an efficient and versatile tool for elucidating in unique ways the molecular basis of function. This presentation represents new insights into the utility of ester-containing peptides toward understanding the functions of bioactive peptides/proteins.

[1] Y. Sohma, Y. Kiso et al. *Chem. Commun.* 124-125 (2004). [2] A. Taniguchi, Y. Kiso et al. *ChemBioChem* 10, 710-715 (2009). *Angew. Chem. Int. Ed.* Spotlights, 48, 2444 (2009). [3] Y. Sohma, S. B. H. Kent et al. *Angew. Chem. Int. Ed.* 47, 1102-1106 (2008); *J. Am. Chem. Soc.* 131, 16313-16318 (2009); *Angew. Chem. Int. Ed.* (2010) in press.

O49. Abstract number: 89

Imidazoquines: proline-mimetic derivatives of primaquine with antimalarial activity

P. Gomes[1], N. Vale[1], J. Matos[1], J. Gut[2], M. Collins[3], M. Prudêncio[4], M. Mota[4], M. Cushion[3], P. Rosenthal[2], R. Moreira[5]

[1]Universidade do Porto - Faculdade de Ciências, Porto, Portugal

[2]University of California@San Francisco - San Francisco General Hospital, San Francisco, CA, United States of America

[3]University of Cincinnati - Department of Internal Medicine, Cincinnati, OH, United States of America

[4]IMM - Institute of Molecular Medicine, University of Lisbon, Lisbon, Portugal

[5]IMED.UL - Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal

Primaquine (PQ, **1**) is active against all exoerythrocytic forms of *Plasmodia* parasites, which have not developed significant resistance against that drug. PQ's gametocytocidal activity is a key feature enabling blockage of transmission from humans to mosquitoes. PQ is also active against against hypnozoites, the latent liver forms responsible for relapse of vivax and ovale malarias. However, PQ has a low therapeutic index, due to both its hematotoxicity and its fast and extensive conversion into an inactive metabolite, carboxyprimaquine (**2**), by oxidative deamination during 1st pass metabolism [1]. Following the work by Portela *et al.* on *N*-aminoacyl derivatives of primaquine (**3**) that displayed some gametocytocidal activity *per se* [2], we have further modified these structures by introduction of an imidazolidin-4-one moiety (**4**,**5**) which acts as a proline surrogate while conferring higher resistance towards enzymatic degradation [3-10]. Compounds **4** and **5** are highly stable in buffer and in human plasma at physiological *p*H and *T* and not susceptible to oxidative deamination, while displaying an overall bioactivity pattern comparable to that of PQ, with particular emphasis on their ability to block the transmission of *P. berghei* malaria between Balb/C mice and *Anopheles stephensi* mosquitoes [4,7-10].

[1] Vale, N. et al. Eur. J. Med. Chem. 2009, 44, 937; [2] Portela, MJ et al. Pharm. Res. 1999, 16, 949; [3] Gomes, P et al. Tetrahedron 2004, 60, 5551; [4] Araújo, MJ et al. J. Med. Chem. 2005, 48, 888. [5] Chambel, P et al. Tetrahedron 2006, 62, 9883.[6] Ferraz, R et al. J. Org. Chem. 2007, 72, 4189. [7] Vale, N et al. Bioorg. Med. Chem. Lett., 2008, 18, 485; [8] Vale, N et al. Bioorg. Med. Chem. Lett. 2008, 18, 4150; [9] Vale, N et al. Tetrahedron 2008, 64, 11144; [10] Vale, N et al. J. Med. Chem. 2009, 52, 7800.

Acknowledgments. The authors thank Fundação para a Ciência e Tecnologia for funding through project PTDC/QUI/64142/2006 and post-doctoral research grant SFRH/BPD/48345/2008 (NV).

O50. Abstract number: 334

Chemical synthesis and structure-function studies of marinostatin, a potent serine protease inhibitor

M.T. Taichi[1], T. Yamazaki[2], K. Kawahara[3], D. Motooka[3], S. Nakamura[4], T. Teshima[1], T. Ohkubo[3], Y. Kobayashi[5], Y. Nishiuchi[1]

[1]Peptide Institute, Inc., Ibaraki, Japan

[2]Protein Research Unit, National Institute of Agrobiological Science, Tsukuba, Japan

[3]Graduate School of Pharmaceutical Science, Osaka University, Suita, Japan

[4]Research Institute for Microbial Diseases, Osaka University, Suita, Japan

[5]Osaka University of Pharmaceutical Science, Takatsuki, Japan

Marinostatin (MST: F^1ATMRYPSDSDE^{12}) isolated from a marine organism, pseudoalteromonas sgamiensis, is a potent serine protease inhibitor consisting of 12 amino acids with two internal ester linkages that are formed between the β-hydroxyl and β-carboxyl groups, Thr^3-Asp^9 and Ser^8-Asp^{11} [1]. MST was synthesized by a regioselective esterification employing two sets of orthogonally removable side-chain protecting groups [2]. To elucidate the structure-activity relationship (SAR) of MST, a series of its analogs were synthesized by applying the same synthetic strategy as that for MST [3]. This indicated that the ester linkage with Thr^3-Asp^9, the *cis*-conformation at Pro^7, and *N*-terminal Phe-Ala may play a critical role in expressing the inhibitory activity. Particularly, the *cis*-Pro^7 is essential for promoting the hydrogen bond between the NH proton of Arg^5 and the carbonyl oxygen atom of the ester linkage with Thr^3-Asp^9 to fix the scissile bond of Met^4-Arg^5. This was demonstrated by the NMR analysis of MST[P7A], in which Ala^7 takes a trans-conformation, resulting in loss of the internal hydrogen bond and the inhibitory activity. These findings could be also interpreted from the crystal structure of MST in complex with subtilisin, clearly indicating that the *N*-

terminal Phe[1]-Ala[2] forms an anti-parallel β-sheet with protease and that Tyr[6]-Pro[7] takes a rigid turn structure. Furthermore, we demonstrated that the structure motif of MST is a useful tool for rational design of inhibitors by changing the MST serine-protease specificities from subtilisin into trypsin, or chymotrypsin.

1. (a) Kanaori, K., Kamei, K., Taniguchi, M., Koyama, T., Yasui, T., Takano, R., Imada, C., Tajima, K., Hara, S. *Biochemistry*, **2005**, *44*, 2462-2468; (b) Taniguchi, M., Kamei, K., Kanaori, K., Koyama, T., Yasui, T., Takano, R., Harada, S., Tajima, K., Imada, C., Hara, S. *J. Pept. Res*. **2005**, *66*, 49-58.
2. Taichi, M., Yamazaki, T., Kimura, T., Nishiuchi, Y. *Tetrahedron Lett*., **2009**, *50*, 2377-2380.
3. Taichi, M., Yamazaki, T., Kawahara, K., Nakamura, S., Harada, S., Teshima, T., Ohkubo, T., Kobayashi, Y., Nishiuchi, Y. *J. Pept. Sci*., *in press*.

O51. Abstract number: 123

Synthetic CXCR4 agonists with SDF1-like chemotactic activity

E. Escher
Department of Pharmacology, Université de Sherbrooke, Sherbrooke QC, Canada

The chemokine receptor CXCR4 and his endogenous ligand SDF1 are of paramount importance during embryogenesis, foetal development and many normal physiological roles, among them bone-marrow homing of haematopoietic stem cells. CXCR4 is also a co-receptor for HIV entry for the development of AIDS. CXCR4 is therefore an important drug target with very challenging properties since its normal household functions need to be maintained throughout an eventual therapy. All synthetic ligands for CXCR4 are however of antagonistic nature and, besides SDF1, none are available that are of agonistic, chemotactic nature.

Photolabelling studies on CXCR4 with analogues of T140, a cyclic tetradeca peptide of strongly cationic nature, together with homology modelling have indicated binding modes of T140 in CXCR4 (Biochem Pharmacol 2009,78, 1382). This, combined with our results on ligand binding modes of AT1, a class A GPCR (JBC 2009, 284, 26603 and J Med Chem 2010, 53, 2063.) similar to CXCR4, led to in silico docking experiments of SDF1 to CXCR4 where the N-terminal octapeptide of SDF1 penetrates the receptor core towards the cytoplasmic side. Since short N-terminal SDF1 peptides are of μM affinity and the only known chemotactic agonists (besides SDF1 with nM affinity), we hypothesized that synthetic peptide chimera of T140 together with the SDF1 N-terminal oligopeptides could act as agonists on CXCR4. We have prepared a series of such chimeras and have found peptide analogues that have chemotactic activities identical to SDF1 and are thus the first CXCR4 agonists with nanomolar affinities. Signalling pathway analysis of those agonists on CXCR4 bearing cells showed no recruitment of arrestin to CXCR4, contrary to SDF1 stimulation, but rather inhibited SDF-1 induced arrestin recruitment. Not only are T140-SDF1 chimeras the first synthetic agonists of CXCR4 for chemotaxis but they also show that chemotaxis is not arrestin dependent and that ligand based signalling is possible on CXCR4.

This research was supported by funds from the Canadian Institutes of Health Research.

O52. Abstract number: 520

Proteolytic intermediates in the oligomerisation-aggregation pathway of alpha-synuclein revealed by ion mobility mass spectrometry

M.P. Przybylski[1], C. Vlad[1], K. Lindner[1], T. Ciossek[2], B. Hengerer[2], M. Leist[1]
[1]University of Konstanz, Konstanz, Germany
[2]Boehringer Ingelheim Pharma, Biberach, Germany

A variety of diseases, previously thought to be unrelated, such as cancer and neurodegenerative diseases, are characterised by the formation of ”misfolded”protein aggregates. While ”soft-ionisation”mass spectrometry (MS), particularly electrospray-MS (ESI-MS), has substantially contributed to peptide analysis and proteomics, ESI-MS is not suitable to direct ”in-situ”analysis of conformational states and intermediates. Recently, ion mobility mass spectrometry (IM-MS) is emerging as a new tool to probe protein structures and interactions due to its potential for separation polypeptides by conformational states, shape and topology. We report here first applications of IM-MS to the characterization of reaction intermediates in the in vitro oligomerisation and aggregation of alpha-synuclein (αSyn), a key polypeptide in Parkinson’s disease. IM-MS of the in vitro aggregation of wt-αSyn enabled the structure elucidation of several hitherto unknown N- and C-terminal products, and a proteolytic fragment at V71-T72 in the aggregation domain (C-VT72; 7.2 kDa), which appears to be a key intermediate in the aggregation pathway; in vitro studies of this fragment prepared by chemical synthesis and bacterial expression showed a dramatically enhanced rate of aggregation. Most recently, IM-MS was also successfully applied to the direct analysis of affinity-captured αSyn from biological samples, such as brain homogenate, indicating this method as a powerful new tool to the molecular characterization of conformation-dependant intermediates of protein aggregation.