POSTER SESSION I ABSTRACTS

P 120 AROMATIC VERSUS HYDROPHOBIC CONTRIBUTIONS TO AMYLOID PEPTIDE SELF-ASSEMBLY

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Peptide self-assembly processes are central to the etiology of amyloid diseases. Much effort has been devoted to characterizing amyloid structure and the mechanisms of peptide self-assembly leading to amyloid. It has been proposed that aromatic side-chain interactions play a central role in early self-assembly recognition events, but this contention remains somewhat controversial. Recent studies have indicated that in some amyloid peptides, including the islet amyloid polypeptide (IAPP), aromatic residues can be exchanged for other hydrophobic residues and these non-aromatic variant peptides still retain competency to form amyloid, although with attenuated kinetics. In an effort to understand the relative contributions of aromatic versus generic hydrophobic interactions, studies to quantify the self-assembly properties of amyloid peptides as a function of increasing hydrophobicity and altered aromatic character have been undertaken. Model peptides have been chosen in which at least one aromatic side-chain is present: IAPP(20–29), A-beta(16–22), and the amphipathic (FKFE)2 peptide. The aromatic residues have been systematically replaced with natural and nonnatural residues with varying hydrophobicity and aromaticity and the self-assembly properties have been characterized. These studies confirm that aromatic interactions are not strictly required for amyloid formation.

P 121 REPLACEMENT OF ALA BY AIB IMPROVES STRUCTURATION AND BIOLOGICAL STABILITY IN THYMINE-BASED NUCLEOPEPTIDES

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Sequential nucleopeptides containing alanyl-thymine nucleoamino acid residues (AlaT) at *i,i+3* positions have been studied in view of applications as nucleic acid modulators. Indeed, if such nucleopeptides adopt a 310-helical conformation, the resulting alignment of the nucleobases along the helical axis might favor interactions with complementary strands. Three water-soluble sequential nucleo-heptapeptides containing two tripeptide units Xxx-AlaT-Xxx and a C-terminal lysine amide have been synthesized: the first (A) contains four Ala residues, the second (B) three Ala and one Aib (alpha-aminoisobutyric acid) residue (in the middle of its sequence), and the third (C) four Aib residues. A conformational investigation on the three nucleopeptides has been performed by 2D NMR in DMSO solution and by CD in phosphate buffer. Based on the NMR data, all nucleopeptides are folded in DMSO, although only the Aib-rich nucleopeptide (C) adopts a 310-helical structure. The same peptide appears to maintain its helical structure in aqueous solution (CD analysis), whereas only some degree of folding is observed for nucleopeptide (B) and none for nucleopeptide (A). Enzymatic stability tests in mouse serum have shown that the Ala-based nucleopeptide (A) is rapidly degraded. Nucleopeptide (B), containing one Aib residue, is much more resistant and the Aib-rich nucleopeptide (C) is stable even after 48 hours.

P 122 CONFORMATION AND STABILITY OF THE HELIX-LOOP-HELIX DOMAIN OF THE ID PROTEIN FAMILY

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The Id proteins (Id1-4) are helix-loop-helix (HLH) transcription factors that play a key role during development and tumorigenesis, mainly by inducing cell proliferation while inhibiting cell differentiation [1]. These proteins act as negative regulators of DNA transcription by sequestering positive basic-HLH factors into an inactive heterodimeric form that is devoid of the ability to form ternary complexes with the DNA [1]. As the highly conserved Id HLH motif is essential for protein sequestration, we are interested in the characterization and modulation of its folding and dimerization. By using synthetic peptides reproducing the native Id HLH sequences, we have shown that the HLH region of these proteins is a self-associating, helix-rich domain, with the C-terminal helix being much better defined than the N-terminal one [2,3]. Helix packing and orientation during self-association have been examined by performing thioldisulfide exchange experiments on appropriately modified Id HLH peptides. Further, based on the conformational properties of a series of Id HLH analogs displaying loop modifications (including O-acyl isodipeptide units) [4] and/or retro-sequences, we suggest that the junction between the loop and the C-terminal helix and the C-terminal helix itself are structural determinants of the Id-protein HLH fold.

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P 123 CATION-π INTERACTION STUDIES IN MODEL HELICAL PEPTIDES

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The cation-π interaction, the interaction between the quadrupole moment of an aromatic ring and a positively charged residue, has been demonstrated to play an important role in protein stabilization. The objective of this research is to gain a better understanding of the stabilizing non-covalent cation-π interaction through the use of monomeric model helical peptides. To the best of our knowledge, the stabilization energies of all six natural aromatic-cationic pairs of residues have not yet been determined using the same monomeric helical peptide scaffold. In this study, circular dichroism spectroscopy was used to determine the stability of 18-mer peptides that incorporate natural and unnatural amino acids in positions (i, i+4) and (i, i+5). The pair Cha/Arg was most stable followed by Cha/Lys, Tyr/Arg, Trp/Arg, Tyr/Lys, Phe/Lys, Trp/Lys and Phe/Arg. In addition, a statistical survey on aromatic/cationic pairs of residues in a subset of protein structures (PDBSELECT, 2003, 25% threshhold) that takes into account secondary structure, residue spacing and residue orientation has been performed. No simple correlation was observed between experimental stabilities obtained for small helical peptides and their frequency of occurrence in protein structures. Furthermore, TOCSY/NOESY NMR data for the Tyr/Arg - containing peptide will also be presented.

P 124 STRUCTURAL STUDIES OF MUCIN GLYCOPROTEIN GLYCO- PEPTIDE MOTIFS

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Mucin glycoproteins have sequences rich in S and T, loci for clus-tered sites of glycosylation starting with an α-O-GalNAc residue. A number of cell surface proteins posses mucin domains in their ex-tracellular portions. The glycoprotein domains mediate cellular inter-actions and responses, and can undergo changes with disease, par-ticularly cancer, making them useful biomarkers. As found in nature, these glycoproteins occur with high molecular weight and a degree of microheterogeneous glycosylation, precluding their use in quantita-tive structural studies at atomic detail needed for understanding their molecular recognition, and regulation of post-translational process-ing. Glycopeptide synthesis is a powerful approach for overcoming these limitations, providing adequate amounts of well-defined homo-geneous segments. A series of glycopeptides based on a MUC2 gly-coprotein sequence segment, PTTTPLK, have been synthesized, systematically varying the location and density of the α-O-GalNAc modified Ts. These constructs have allowed NMR studies revealing factors in the organization of the mucin glycopeptide scaffold. These and other glycopeptides bearing the S/T-α-O-GalNAc residue, the Tn antigen, have been printed on a microarray and probed with anti-Tn mAbs. With the NMR results we can decipher the details of the epi-topes recognized by the mAbs, and establish the importance of the larger glycoconjugate environment, beyond just the glycosylated amino acid, in glycoprotein recognition.

P 125 SYNTHETIC CHIMERAS: NEW TOOLS TO ENGINEER α-HELICAL INTERFACES

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The application of unnatural biopolymers for the selective inhibition of helical protein-protein interactions is an interesting strategy for drug discovery. Peptides composed of homologous amino acids at present are among the most widely studied biopolymers and their enormous potential for secondary [1] and quaternary [2] structure is already known. Theoretical studies (*ab initio* MO theory) have shown that hybrid peptides composed of alternating β- and γ-amino acids are well suited to mimic α-helical conformation [3], and our results support experimentally this idea [4]. To generate the chimera B3β2γ, the two central turns of the helix in a basic model α-peptide (Base-pp) were replaced by a pentad of alternating β- and γ-amino acids. Heteromeric coiled-coil formation between B3β2γ and the α-peptide (Acid-pp) was proven by a combination of analytical methods. Our data provide strong evidence for the specific contribution of β- and γ- amino acid side chains to the stability of the chimeric helical folding motif.

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P 126 DISCOVERY OF A CHEMICAL THAT INHIBITS THE AGE-RAGE INTERACTION

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In diabetic patients, the excess of advanced glycation endproducts (AGEs) is produced by complex reactions of reducing groups of sugar molecules, such as glucose, with amino groups of protein molecules. This is known as the Maillard reaction. AGEs tend to bind to their receptors (RAGE) on the vascular cells and to trigger intracellular signaling. This has been related to pathogenesis of diabetic vascular complications. We sought competitors for AGEs to prevent signaling by interfering with this binding. First, we determined the structure of the AGE-binding domain of RAGE by NMR. Then we investigated the mechanism of AGE-RAGE interaction. We are carrying out virtual screening based on this information to find compounds with sufficient binding affinities to RAGE. Assay of the inhibitory activity of these candidates will be done to select useful inhibitors.

P 127 EXPLORING THE COMPUTATIONAL DESIGN OF PROTEIN INTERACTION SITES

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The significant work that has been invested toward understanding protein-protein interaction has not translated into significant advances in structure-based predictions. In particular redesigning protein surfaces to bind to unrelated receptors remains a challenge, partly due to receptor flexibility, which is often neglected in these efforts. In this work, we computationally graft the binding epitope of various small proteins obtained from the RCSB database to bind to barnase, lysozyme, and trypsin using a previously derived and validated algorithm. In an effort to probe the protein complexes in a realistic environment, all native and designer complexes were subjected to a total of nearly 400 ns of explicit-solvent molecular dynamics (MD) simulation. The MD data led to an unexpected observation: some of the designer complexes were highly unstable and decomposed during the trajectories. In contrast, the native and a number of designer complexes remained consistently stable. The unstable conformers provided us with a unique opportunity to define the structural and energetic factors that lead to unproductive protein-protein complexes. To that end we used free energy calculations following the MM-PBSA approach to determine the role of non-polar effects, electrostatics and entropy in binding. Remarkably, we found that a majority of unstable complexes exhibited more favorable electrostatics than native or stable designer complexes, suggesting that favorable electrostatic interactions are not prerequisite for complex formation between proteins. However, non-polar effects remained consistently more favorable in native and stable designer complexes reinforcing the importance of hydrophobic effects in protein-protein binding. While entropy systematically opposed binding in all cases, there was no observed trend in the entropy difference between native and designer complexes. A series of alanine scanning mutations of hot-spot residues at the interface of native and designer complexes showed less than optimal contacts of hot-spot residues with their surroundings in the unstable conformers, resulting in more favorable entropy for these complexes. Finally, disorder predictions revealed that secondary structures at the interface of unstable complexes exhibited greater disorder than the stable complexes.

P 128 NMR SOLUTION STRUCTURE ANALYSIS OF THE C-TERMINAL LINEAR AND CYCLIC PEPTIDES OF PHEROMONE BIOSYNTHESIS- ACTIVATING NEUROPEPTIDE (PBAN) FROM THE SILKMOTH *BOMBYX MORI*

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In most moths, the sex pheromone production is regulated by pheromone biosynthesis-activating neuropeptide (PBAN), a 33-34 amino acid neuropeptide. PBAN exerts its pheromonotropic effects by binding to PBANR, a member of G protein-coupled receptors (GPCR), predominantly expressed in the pheromone-producing cells of the female pheromone gland. The shortest peptide with pheromonotropic activity is the C-terminal pentapeptide-amide, PBAN(29-33)-NH2 (FSPRL-NH2), and that the C-terminal amide group is required for the activity of PBAN. In this study, we have analyzed the solution structures of the C-terminal decapeptides derived from PBAN with an amidated and a free C-termini, and a cyclic active peptide by two-dimensional NMR, and compared their structures to reveal the structural requirements for PBAN activity. The NMR data indicated that these peptides did not adopt specific conformations in a buffer at pH 6.0, but they adopted specific conformations in the presence of dodecylphosphocholine-*d*38 micelles or 2,2,2-trifluoroethanol.

P 129 ENZYME-LINKED ENZYME BINDING ASSAY FOR SCREENING OF PIN1 WW DOMAIN LIGANDS

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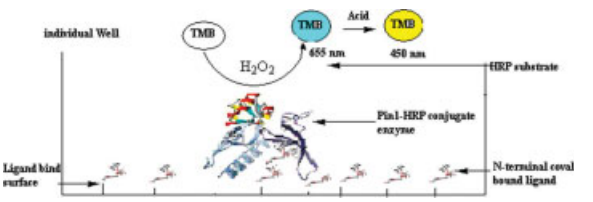
Several methods have been reported for the measurement of protein-ligand complexes formation such as; Nuclear Magnetic Resonance (NMR), Isothermal Titration Calorimetry (ITC), and Surface Plasmon Resonance (SPR). We describe an alternative assay for the identification of ligands that bind at Pin1 WW domain. The Enzyme-Linked Enzyme Binding Assay (ELEBA) offers an alternative method for the rapid screening of small molecules from compound libraries that bind to the group-IV WW domain. The assay proves to be advantageous over existing ELISA methods because no antibodies are required, and the 96-well plate with covalently attached ligand can be re-used after



appropriated washing steps and proper storage. Our competitor ligand Ac-VPPpTPV-NH2 was found to have a *K*d of 203 μM for full-length Pin1 chemically linked to a Horse Radish Peroxidase reporter enzyme.

P 130 SEMISYNTHETIC PHOSPHORYLATED NUCLEOSOMES EXHIBIT ALTERED STRUCTURES

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The nucleosome, 147 bp of DNA wrapped around an octamer core of histone proteins, is the basic unit of chromatin. Post-translational modifications of histone proteins have the potential to dramatically alter chromatin structure and dynamics. One such modification is phosphorylation of H3-T118, located at the histone-DNA interface in the dyad region of the nucleosome. We prepared semi-synthetic H3-pT118 by expressed protein ligation using a native Cys ligation site, and reconstituted specifically modified nucleosomes with several DNA sequences. We present biochemical and biophysical evidence that phosphorylation of H3-T118 results in formation of a highermass structure in addition to mononucleosomes. DNA site accessibility is altered throughout these structures. Our data suggest a role for phosphorylation of H3-T118 in regulation of chromatin structure.

P 131 CHEMICALLY MODIFIED HISTONES TO STUDY MODIFICATIONS IN THE NUCLEOSOME CORE

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Post-translational modifications of histone proteins are critical for many cellular processes, such as the regulation of transcription and DNA repair. More than 100 histone modifications have been identified, including over 30 in the nucleosome core, yet the molecular functions of these modifications are not well-understood. Modifications buried at the DNA-histone interface of the nucleosome are likely to perturb the interactions between the histone octamer and DNA. Here, we generate semisynthetic nucleosomes acetylated or phosphorylated at precise locations in the dyad and DNA entry-exit regions using a variety of peptide and protein ligation approaches. We find that lysine acetylation in the nucleosome dyad reduces the free energy of binding of the histone octamer to DNA, increases thermal nucleosome repositioning, and facilitates histone octamer dissociation from DNA. Phosphorylation in the dyad region results in formation of both mononucleosomes and a higher-mass protein-DNA complex with the potential to dramatically alter chromatin structure. Mutations mimicking these modifications do not, in general, replicate these effects. These results demonstrate the necessity of peptide and protein chemistry for determining the biological impact of histone modifications.

P 132 A PHOTOLABILE THIOL PROTECTING GROUP FOR CELLULAR STUDIES

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Caging groups that allow thiol groups to be revealed upon photolysis have significant utility in studying a plethora of biological processes. Here we describe the preparation of peptides and peptidomimetics that include cysteine residues that have been masked with a photoremovable BHQ (brominated hydroxyquinoline) group. The protecting group can be installed in peptides either by post-synthetic modification or via standard Fmoc SPPS. Kinetic analysis reveals that BHQ uncaging of thiols is significantly more rapid compared to nitrobenzyl-based groups although BHQ photolysis results in other nonproductive photoproducts that decreases the overall efficiency. A peptidomimetic farnesyltransferase inhibitor (FTI) has been prepared that contains a BHQ protected cysteine residue that attenuates its activity in inhibiting ras protein processing. Photolysis of that FTIBHQ conjugate in the presence of living cells results in farnesyltransferase inhibition and a concomitant decrease in ras processing. The BHQ protecting group can also be removed from the FTI by two-photon excitation with visible light suggesting that it should be possible to perform thiol uncaging in tissues at depths not accessible by one-photon processes.

P 133 LIGHT ACTIVATABLE, FLUORESCENCE REPORTING CAMP DEPENDENT PROTEIN KINASE

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Our group has constructed signaling proteins that can be switched on using short bursts of light. In this work, we successfully prepared a new generation of caged protein kinases, where both the ACTIVITY and the FLUORESCENCE are simultaneously unleashed upon photoirradiation. This design allows confirmation of protein activation and can potentially report the intracellular location of the activated protein via fluorescence. A second generation caged cAMPdependent Protein Kinase (PKA) was prepared by selectively modifying Cys343 of PKA with a designed peptide that contains a (1) thiol reacting group, (2) a fluorophore, (3) a photolabile linker, (4) a fluorescence quencher, (5) a PEG tether, and (6) a PKA inhibitor. This final conjugated caged PKA has minimal fluorescence and activity. Photolysis generates a nearly 25 fold enhancement in catalytic activity and a 10 fold enhancement in fluorescence. These large fluorescence and activity changes provide the opportunity to correlate activity as a function of location with cell behavior.

P 134 STRUCTURAL AND FUNCTIONAL STUDIES OF MukB

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MukB is required for chromosomal condensation in γ-proteobacteria. This protein shares structural homology with the ubiquitous Structural Maintenance of Chromsome (SMC) proteins whose members are required for chromosomal housekeeping functions. These proteins share common domain connectivity with globular N- and C- terminal domains that pack against one another to form an ABC-like ATPase domain. These termini are connected by a long antiparallel coiled coil domain which folds at a centralized dimerization domain.
We present a structural characterization of the coiled coil domain and subsequent studies into MukB function. Our structural analysis has determined several points of inter-strand interaction through the length of the coiled coil. This has led to a refined model for this domain where at least five segments of continuous coiled coil are joined by a conserved structural motif that may confer flexibility within this domain. Using this structural knowledge we have been able to design MukB truncation mutants that allowed us to identify a functional relationship between MukB and Topoisomerase IV and to achieve an atomic resolution structure of the dimerization domain.

P 135 USE OF OBOC COMBINATORIAL CHEMISTRY TO IDENTIFY PEPTIDE SUBSTRATE FOR TYROSYLPROTEIN SULFOTRANSFERASE

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Protein tyrosine sulfation is an ubiquitous post-translational modification catalyzed by a Golgi isoenzyme tyrosylprotein sulfotransferase (TPST) 1 and 2 in eukaryotic cells. Up to 1% of tyrosine in eukaryotic cell is sulfated. However, only about sixty proteins have been confirmed to be sulfated and some of these sulfo-tyrosines have been demonstrated to be important in protein-protein interaction. Compared to other post-translational modifications such as protein phosphorylation, both the TPST enzymes and tyrosine sulfated proteins have not been well studied. Here, by screening a one-bead-one-compound (OBOC) combinatorial peptide library with anti-sulfotyrosine antibody, we have identified specific peptide substrates for the TPST1 and TPST2. Identified TPST2 peptide substrates show a consensus aspartate at the -1 position and TPST1 substrates also show many acidic residues in the sequence. This result is comparable to previous sequencing on sulfated protein that showed acidic residue preference near the tyrosine sulfation site. Analysis of discrete sulfated peptide identified from the OBOC library may enable us to discover potential native protein substrates and reveal the function of protein tyrosine sulfation.

P 136 ASSESSMENT OF A TAT-DERIVED PEPTIDE AS A VECTOR FOR HORMONAL TRANSPORT

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Absorption through the small intestine is critical for successful oral-based drug delivery. Peptides are inherently impermeable to cellular membranes and thus display poor transport across the small intestines. Cationic peptides such as YGRKKRRQRRR, derived from the HIV Tat protein, display the ability to penetrate cells, and have been successfully used to deliver a wide degree of cargo molecules, including peptides into various cell types. We hypothesized that this peptide could function as a vector for the transcellular delivery of a cargo peptide across a cell monolayer, in particular the human epithelial cell layer that composes the small intestines. The covalent attachment of the Tat-peptide to the C-terminus of GLP-1 was determined to have little effect on GLP-1 bioactivity. However, the Tat-derived peptide did not convey transcellular transport of the GLP-1 cargo across CaCo-2 cells, as all GLP-1/Tat fusion peptides and the Tat-derived peptide alone were determined not to transport from the apical domain to the basolateral domain in differentiated CaCo-2 cells. Further study demonstrated that the Tat-peptide was of variable utility in facilitating the internalization of the GLP-1 cargo in CaCo-2 cells. The GLP-1/Tat fusion peptide with native GLP-1 sequence, the fusion peptide with a scrambled GLP-1 sequence, and the Tat-derived peptide alone displayed considerable uptake above 5 uM, whereas two GLP-1/Tat fusion peptides with a single amino acid substitution in the GLP-1 sequence displayed insignificant cellular uptake. Of particular importance was the observation that cellular uptake of GLP-1 agonists induced a cytotoxic effect on these cells at concentrations that correlated with cellular uptake. We conclude that this Tat-derived peptide when used as a transport peptide with GLP agonists demonstrated no apparent transcellular transport, a variable level of cellular uptake, and the potential for cytotoxic effects.

P 137 PEGYLATION – A KEY TECHNOLOGY TO IMPROVE SOLUBILITY AND PHARMACOKINETIC OF PEPTIDES, PROTEINS AND OTHER BIOPHARMACEUTICALS FOR SUPERIOR DRUG DELIVERY

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In 2006 the market of modern biopharmaceuticals has reached a volume of over $40.3 billion in USA and over $45 billion world wide (IMS Health, Inc.). It is projected to grow to an annual value of some $100 billion within the next 5 years. The big advantage of proteins, antibodies, siRNA, and other natural products in their usage as drugs is their high specifity in combination with their low side effects. They normally interact with the dedicated target only, and thus do not have activities at any other place in the body. A current focus is the study of modern drug carrier systems where polyethylene glycol linkers are connecting a recognition part with a drug-active part. Such conjugations can reach the size of a nanoparticle. The recognition part can be a peptide or hormone, which binds specifically to the surface of a certain cell. After internalization of the whole nanoparticle the active part (DNA or siRNA, for example) is released. Inhibition or activation of certain enzymes or the nucleus follows with the consequence to repair the sick cell, to shut it down by initiating apoptosis or other mechanisms. In conjugation with hydrophobic compounds forming amphiphilc and biodegradable block-copolymers like PEG-PLA (polylactic acid) and PEG-PLGA (copolylactic acid-glycolic acid) sophisticated micelles are formed, where drug molecules can be masked and protected against attacks of the immune system.

Modern biopharmaceuticals are ideal drugs, however, their significant drawback is their low stability under physiological conditions. Due to the fact that they are similar to biological components, they are also easily attacked by the immune system of the body, i.e. by antibodies and proteolytic degradation enzymes. Many efforts have been made by highly sophisticated formulation techniques, special application methods (depots) and chemical modification to improve their pharmacokinetic properties. One recent approach, which shows much better results than other methods tried in the past, is PEGylation, i.e. attaching Poly-Ethylene Glycol chains (PEG) to the active component. The simplest possibility of PEGylation is attaching a monofunctional PEG chain to a protein, antibody or small drug molecule. Using bifunctional PEGs a link between two compounds can be formed, in order to build dimers or more complex conjugates. Many highly sophisticated compositions are under development and already published.

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P 138 APPLICATION OF POROUS-RIGID METHACRYLIC SYNBEADS TO SOLID PHASE PEPTIDE SYNTHESIS

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Over the last twenty years peptide therapeutics (API Peptides) have found large application as pharmaceuticals. Despite many peptide drugs are now produced in multi-tons scale on solid support [1], inefficient large-scale manufacturing processes remain as problem as well. Here we demonstrate that Synbeads, a newly conceived methacrylic and highly porous polymer [2], can be efficiently applied in peptides synthesis, giving significant benefits in term of solvents, reagents and costs. To demonstrate the efficiency of the optimized Synbeads, some peptides of pharmaceutical interest were synthesized, comparing different synthetic routes and linkers (i.e. Ramage, Rink and HMPA linker). Data show that Synbeads can be easily applied in numerous different reaction conditions allowing peptide production with high yields and product purity. Efficiency of Synbeads was also compared to commercially available traditional swelling supports showing that it is possible to reduce solvent consuming up to 50%. Moreover HR-MAS NMR experiments highlight the presence of highly accessible flexible linkers, characterised by high motility, covalently anchored to the characteristic rigid matrix of Synbeads.

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P 139 HPLc PURIFICATION OF SYNTHETIC GLP-1 BY USING DAISOGEL

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The selection of stationary phase is the most important consideration in developing a reversed-phase chromatography process. First, we optimized conditions using standard stationary phase. Second, screening stationary phases under the condition obtained at first step. Usually, we need only small modification to re-optimize conditions from that we got at the first step. This study shows some comparison with our stationary phases and other commercially available phases in developing **GLP-1** purification.

P 140 ALCOHOL HAS ADVANTAGES FOR THE REVERSE PHASE ANALYSIS OF PEPTIDE PHARMACEUTICALS

C. Brook and D. Studelska
Covidien

Extant analytical methods were all release methods of marginal use for in-process analysis. The peptide contains cysteines, and is synthesized in a totally reduced form. The active peptide is folded into a compact form via the formation of –S-S- bridges, thus the "linear" reduced peptide must be oxidized to produce the correct product. The first two columns employed were not able to resolve the reduced from the oxidized form. A release method column could resolve these species, but the gradient was not optimal, and we altered mobile phase composition and the gradient to maximize resolution, and to mitigate a very noisy base-

line. With particles smaller than 5 microns (3.5 or 3 μ), the baseline noise with acetonitrile was particularly bad where the product eluted. To address this, we developed a method employing alcohol/water gradients instead of the acetonitrile/water. We employed Cadenza CD-C18 reverse-phase columns; 3 μ columns notable for high resolution and low backpressure. The low backpressure, combined with the greater eluent strength of alcohol, compensated for the increased viscosity of alcohol. With a 4.6 x 150 mm column we were able to run at 1 ml/min and stay within the recommended pressure limits of the column. More importantly, we obtained excellent separation of reduced and oxidized peptide. In view of the current shortage and high expense of acetonitrile, we think this approach deserves consideration.

P 141 BIOSYNTHESIS OF FOLDED CYCLOTIDES INSIDE LIVING BACTERIAL CELLS. A CONVENIENT ROUTE FOR GENERATION OF GENETICALLY-ENCODED CYCLOTIDE-BASED LIBRARIES

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The cyclotide MCoTI-II is a powerful trypsin inhibitor recently isolated from the seeds of *Momordica cochinchinensis*, a plant member of *cucurbitaceae* family. We report for the first time the *in vivo* biosynthesis of natively-folded MCoTI-II inside live *E. coli* cells. Our biomimetic approach involves the intracellular backbone cyclization of a linear cylotide-intein fusion precursor mediated by a modified protein splicing domain. The cyclized peptide then spontaneously folds into its native conformation. The use of genetically engineered *E. coli* cells containing mutations in the glutathione and thioredoxin reductase genes considerably improves the production of folded MCoTI-II *in vivo*. Biochemical and structural characterization of the recombinant MCoTI-II confirmed its identity. Biosynthetic access to correctly-folded cyclotides allows the possibility of generating cell-based combinatorial libraries that can be screened inside living cells for their ability to modulate or inhibit cellular processes.

P 142 STUDY ON THE AFFINITY LIGAND OF HEART-TYPE FATTY ACID- BINDING PROTEIN WITH PHAGE-DISPLAY 12-MER PEPTIDE LIBRARY

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In this study we obtained the specific sequence of a f f ini ty ligand of H-FABP with Phage Display 12-mer Peptide Library. With three rounds of panning and sequences comparison, we obtained one ELISA positive sequence of W-P-N-H-H-M-L-H-K-R-W-P. The target peptide was then synthesized by manual way. After reverse phase HPLC (high pressure liquid chromatography) and mass spectrometry, the target peptide coupled with pyrene as a fluorescent probe was detected with the samples of AMI patients. The experiment indicates that the samples of AMI patients are all positive. Kd value determinations of the peptide with AMI samples are from 37.9μM to 88.5μM. The result reflects that the target ligand peptide we got has good effect on clinical application for AMI. The study makes a new way to detect AMI earlier.

P 143 IDENTIFICATION OF INHIBITORS OF PROTEIN-PROTEIN INTERACTIONS THROUGH THE SCREENING OF SIMPLIFIED COMBINATORIAL PEPTIDE LIBRARIES

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In the field of drug discovery through the combinatorial preparation and HTS of arrays of compounds, our novel approach is based on the use of "simplified" peptide libraries [1] composed by a minimum number of non redundant amino acids for the assembly of short peptides. PED/PEA15 (Phosphoprotein Enriched in Diabetes/in Astrocytes) is over-expressed in several tissues of a consistent population of individuals affected by type 2 diabetes. It has been observed that increasing PED/PEA15 abundance in human cell lines, favours its interaction with PLD1 (phospholipase D) and alters a signalling that controls glucose transport. In order to find new antagonists for this interaction, we have prepared two simplified peptide libraries of general formula: (K or E)-G-G-X-X-X-X. The two libraries, generated in the Positional Scanning format using 12 different residues, have been screened using an ELISA-based competition assay with the two recombinant proteins identifying novel and effective antagonists.

1. Marasco D., *et al*.. Curr Protein Pept Sci.(2008), 9 (5):447.

P 144 SOLID PHASE SYNTHESIS OF AZA-PEPTIDES BY ALKYLATION OF AZA-GLY RESIDUES

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Aza-peptides possess one or more aza-amino acid residues in which the α-carbon is substituted for a nitrogen atom. A variety of methods have been developed for the introduction of aza-amino acids into peptides by solution-phase synthesis. Few strategies have, however, been reported for preparing aza-peptides on solid support and only recently have aza-peptide libraries been synthesized. Currently, most synthetic routes to modified peptides incorporate the individually prepared aza- amino acid residue by conventional solid phase peptide synthesis (SPPS). A more direct and flexible method for the preparation of aza-peptides would be to build the new side-chains onto the growing peptide strand during the peptide synthesis. In our approach, aza-glycine residues have been incorporated into the peptide chain by treating arylhydrazones with a phosgene equivalent such as *p*-nitrophenyl chloroformate and acylation of support-bound peptides and amino acids to form the respective *N*-terminal semicarbazones. Alkylation of the semicarbazone may then be achieved regio-selectively to introduce varying side-chain groups. Semicarbazone deprotection, acylation with natural amino acids and termination of the SPPS cycle completes the synthesis of the aza-peptides. This process for direct construction of aza-amino acid residues on resin surmounts issues of hydrazine synthesis in solution and opens up routes to aza-peptides with greater structural diversity.

P 145 APPLICATION OF OXIME-BASED POST SOLID-PHASE DIVERSIFICATION TO OPTIMIZATION OF POLO BOX DOMAIN- BINDING PEPTIDES

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Peptides modeled on consensus recognition sequences provide valuable starting points for the development of protein–protein binding inhibitors. We recently have incorporated aminooxy handles into consensus recognition sequences and used these for post-solid phase construction of libraries bearing tethered components. These libraries can be easily assembled and directly evaluated. We have previously reported our successful application to the discovery of TSG101- directed HIV-1 budding antagonists. The serine/threonine polo-like kinase 1 (Plk1) overexpression is tightly associated with oncogenesis in several human cancers. Interference with Plk1 function induces apoptosis in tumor cells but not in normal cells. So, Plk1 is a potentially attractive anti–cancer chemotherapeutic target. Plk1 possesses a unique phosphopeptide–binding "polo box domain" (PBD) that is essential for intracellular localization and substrate recognition. Because PBDs are unique to Plks, they are ideal targets for selectively inhibiting Plk1 functions. By examining various PBDbinding phosphopeptides, a small phosphopeptide (5–mer) that specifically interacts with the Plk1 PBD with a high affinity, but not with the two closely-related Plk2 and Plk3, has been identified. We will present our application of the oxime peptide library approach to optimize binding interactions at one proline position that has led to a significant enhancement in the Plk1 PBD-binding affinity. Our methodology is notable in that library construction was achieved with maintenance of the parent proline pyrrolidine ring system.

P 146 MACROCYCLIC PEPTIDE-PEPTOID HYBRIDS DESIGNED AS PPII HELIX MIMETICS

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Secondary structure provides critical recognition elements for peptide and protein interactions. Recently, left handed polyproline II (PPII) helixes have been attracting significant attention due to their dominant role in cellular signal transduction involving proline – rich motifs (PRMs). In contrast to alpha – helixes, which typically have 3.6 residues per turn and a system of internal $i + 4$ to i hydrogen bonds, PPII helixes are more open and contain three residues per turn with no internal hydrogen bonding. Stabilization of alpha – helixes by ring closing metathesis (RCM) – based hydrocarbon "stapling" has proven to be a promising means of increasing affinity and bioavailability of peptide ligands. In the HIV-1 life cycle, the ubiquitin E2 variant (UEV) domain of the human tumor susceptibility gene 101 (Tsg101) protein is recruited by major structural proteins of HIV-1 to facilitate viral budding. This recruitment involves the direct interaction of the Tsg101 UEV domain with a proline – rich motif in the viral Gag – p6 protein. NMR solution studies of the interaction between the p6 – derived 9 – mer peptide, P1E2P3T4A5P6P7E8E9 and Tsg101, have indicated that a key A5P6 di – peptide region binds to Tsg101 in a PPII helix – like conformation. To examine the potential utility of RCM – based hydrocarbon stapling to PPII helix stabilization in the p6 – derived 9 – mer peptide, we prepared more than 50 macrocyclic peptide – peptoid hybrids. We showed that this approach can stabilize PPII helix conformations to yield more bioavailable ligands.

P 147 SUBSTRATE PROFILING OF NOONAN SYNDROME MUTANTS OF PROTEIN TYROSINE PHOSPHATASE SHP-2

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The Ohio State University

Protein-tyrosine phosphatases (PTP's) remove the phosphoryl group from phosphotyrosyl proteins producing tyrosine and inorganic phosphate. SHP-2 is a protein-tyrosine phosphatase ubiquitously expressed in mammalian cells and is involved in numerous signaling pathways. Mutations in the catalytic domain of SHP-2 cause human diseases and conditions such as Noonan syndrome as well as LEOPARD syndrome. Some of the mutations (e.g. Q506P, N308D, and T507K) apparently alter the substrate specificity of SHP-2. In this work, the mutants were screened against a phosphotyrosyl peptide library to systematically determine their sequence specificities and compare with that of the wild-type enzyme.

P 148 SEQUENCE SPECIFICITY OF THE SRC FAMILY KINASE SH2 DOMAINS AS REVEALED BY A COMBINATORIAL LIBRARY APPROACH

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The Src family kinases (SFK) regulate signal transduction. The eight SFKs have overlapping function, and their Src homology 2 (SH2) domains often compete for binding to the same pY proteins. Determining the specificity of the SH2 domain will help identify cellular targets proteins and will help understand the molecular basis for their cellular function. The SFK SH2 domains were screened against a phosphotyrosyl (pY) peptide library and positive beads were sequenced by partial Edman degradation and mass spectrometry. The results revealed that the Src kinase family SH2 domains selected a single class of pY peptides containing hydrophilic residues at the pY+1 position and hydrophobic residues at the pY+3 position. Individual peptides were synthesized and tested for binding against these SH2s proteins. The dissociation constants revealed that some of the SH2 domains exhibited high selectivity while others did not. The protein database is searched to identify potential targets.

P 149 TETRAMIC ACID PEPTIDE DERIVATIVES AS CYTOTOXIC COMPOUNDS

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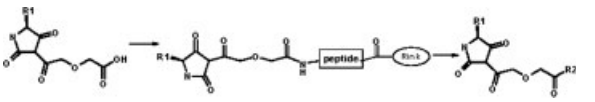
C-3 Acyl-tetramic acids are key structural motifs in many natural products. They exhibit a wide range of biological activities including antibiotic, antiviral, antifungal, cytotoxic and enzyme inhibitory activities. Thus, the synthesis of such compounds represents a worthwhile and challenging goal for the organic chemist. We have recently presented an improved method for the acylation of tetramic acid derivatives in solution using microwave allowing the access to a variety of acyl-tetramic derivatives. On this base we have designed and synthesized a novel acyl-tetramic building block that can be introduced into peptides using classical coupling methods. These building blocks were introduced into positional peptide libraries that were tested for cytotoxicity in PC-3 cell lines. The best ranked amino –acids for each position were combined in single peptides and their cytotoxicity was validated using the same cell lines. Results demonstrate that introduction of acyl-tetramic acid building block into peptides is an attractive approach for discovering novel selective cytotoxic compounds for treating cancer.



P 150 STUDY ON THE AFFINITY LIGAND OF HEART-TYPE FATTY ACID- BINDING PROTEIN WITH PHAGE-DISPLAY 12-MER PEPTIDE LIBRARY

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In this study we obtained the specific sequence of affinity ligand of H-FABP with Phage Display 12-mer Peptide Library. With three rounds of panning and sequences comparison, we obtained one ELISA positive sequence of W-P-N-H-H-M-L-H-K-R-W-P. The target peptide was then synthesized by manual way. After reverse phase HPLC (high pressure liquid chromatography) and mass spectrometry, the target peptide coupled with pyrene as a fluorescent probe was detected with the samples of AMI patients. The experiment indicates that the samples of AMI patients are all positive. Kd value determinations of the peptide with AMI samples are from 37.9μM to 88.5μM. The result reflects that the target ligand peptide we got has good effect on clinical application for AMI. The study makes a new way to detect AMI earlier.

P 151 THE RAPID CREATION AND SCREENING OF PEPTIDIC MACROCYCLIC LIBRARIES AGAINST PROTEINPROTEIN INTERACTION TARGETS

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Conformation-restricted macrocyclic structures can present functionally diverse chemical groups over a relatively large and distributed surface. This class of molecules is well suited to bind to the extended binding surfaces typical of protein-protein interactions that define key therapeutically-relevant pathways. DNA-programmed-chemistry is applied to generate libraries totaling more than 100,000 members. The libraries are applied to modified *in vitro* selection methodologies to screen for families of compounds that selectively bind targets of interest, where active molecules are identified by PCR amplification and sequencing of the coding DNA. Screening and analysis of the libraries are under way against a number of targets relevant to the oncology, inflammation, and anti-viral therapeutic areas.

P 152 SUBSTRATE PROFILING OF METHIONINE AMINOPEPTIDASES

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Methionine aminopeptidases (MetAPs) remove the N-terminal methionine from newly synthesized proteins. It is also a target for the development of antibacterial and antitumor agents. Despite the intense effort to solve the catalytic mechanism, no systematic study has been performed to determine their substrate specificity due to the limit of available methods. In this work, a new method has been developed to profile the substrate specificities of MetAPs. A onebead-one-compound peptide librariy (~1.8 million peptides) containing five random positions next to the N-terminal methionine was made on PEGA resin. The library was screened against EcMetAP and Human MetAPs. After treatment with enzyme, the peptides were labeled with DABCYL and treated with CNBr. This resulted the cleavage of DABCYL from beads that retained the Nterminal Met (poor MetAPs substrates) but not from peptides that have undergone MetAPs reaction. The results from the peptide sequencing indicated that the penultimate position was crucial in the initiator methionine removal and the other positions also contribute to the nascent protein modification. Mostly interestingly, this study revealed that MetAP form *E. coli* and that from human cells have distinct substrate specificities. The indentification of MetAPs substrate specificity provided a very useful profile for future MetAPs-related drug research and development.

P 153 PEPTIDE AMPHIPHILE POLYMER NANOFIBERS OF DEFINED DIMENSIONS

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Radboud University, Nijmegen

Nature shows 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 buildup 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.

P 154 PREPARATION OF STIMULI-RESPONSIVE HYDROGELS USING SELF-ASSEMBLING β-HAIRPIN PEPTIDES

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Kansai University

Hydrogels, major biologically relevant stimuli-responsive materials that respond to changes in pH and temperature, have been designed as potential tissue engineering scaffolds and DDS carriers. Hydrogel materials that expand or contract with changes in pH and temperature are pH and thermally responsive. Self-assembly of β-hairpin peptides is a new strategy that provides precise control in constructing new hydrogel materials that can respond to temperature.

In this study, pH responsive β-hairpin peptides were designed using hydrophilic and hydrophobic amino acids. These peptides have two extended sequences contain alternating hydrophilic and hydrophobic residues at the *N*- and *C*-terminal of a VDPPT tetrapeptide. We analyzed the stability of these peptides in several solvents and evaluated the effects of thermal and pH responses on the molecular interaction by circular dichroism(CD) spectroscopy.

These peptides self assemble into β-strand-turn-β-strand structures under physiological conditions, resulting in gel formation. Such peptides may find use as hydrogel materials *in vivo* tissue engineering scaffolds and DDS carriers.

P 155 FABRICATION OF EFFICIENT DYE-SENSITIZED SOLAR CELLS BY USING BULKY PEPTIDES

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Okayama University

The performance of dye-sensitized solar cells (DSSCs) was improved when dyes were adsorbed on an electrode through "bulky" peptides containing oligo(ethylene glycol). First, we fabricated DSSCs using new dyes connected to various chain length of oligo(ethylene glycol)s by solid-phase peptide synthesis. When the oligo(ethylene glycol)s were longer, the density of the dye-modified oligo(ethylene glycol)s on electrodes was lower due to steric hindrance between oligo(ethylene glycol)s. However, the dyes can avoid aggregations because of the steric hindrance. And conversion yield from photon to electron per the dye molecule was ascended by virtue of avoidance of the aggregation.

P 156 PEPTIDE NANOTUBE-ASSISTED ASSEMBLY AND PATTERNING OF CARBON NANOTUBES

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Single-walled arbon nanotubes (SWNTs) have potential as building blocks in diverse electrical and mechanical nanoscale devices. However, SWNTs are difficult to solubilize and organize into architectures necessary for many applications. In this study, we used diphenylalanine peptide nanotubes (PNTs) to encapsulate SWNTs and to control the assembly of the PNT-wrapped carbon nanotubes into macromolecular structures through peptide-peptide interactions between adjacent peptide-wrapped nanotubes. The PNTs exhibited selective encapsulation for carbon nanotubes as assayed by Raman spectrometry (Fig. 1). The SWNTs wrapped by PNTs can be assembled into hierarchical structures using templates. These nanotube structures are quite interesting for electronic applications such as field-effect transistors, microprobes, microarrays, and biosensing devices.

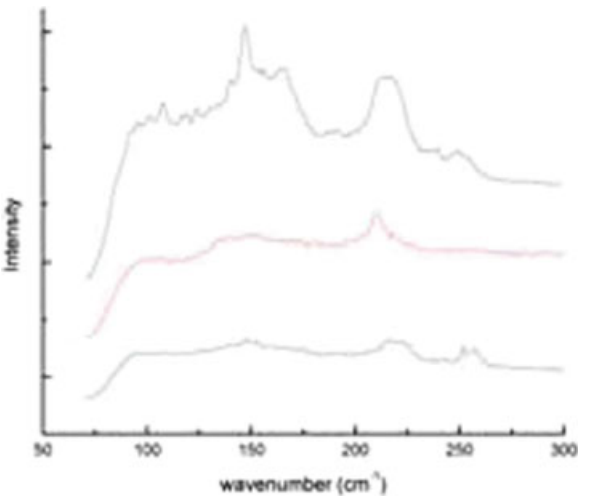

FIGURE 1 Raman spectra of SWNTs (top), PNT-wrapped SWNTs (middle) and unwapped SWNTs.

P 157 ANTIBACTERIAL PEPTIDE HYDROGELS: DESIGNING GELS ACTIVE AGAINST METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA)

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University Delaware

Hydrogel materials are finding use in tissue regenerative applications as implantable extracellular matrix substitutes. These materials aid the healing of chronic and traumatic wounds by providing a hydrated environment suitable for host cell function. However, among several important considerations for implantation of a biomaterial, a main concern is the introduction of infection. We have designed a peptidebased hydrogel from the self-assembling peptide, MAX1 whose surface exhibits inherent antibacterial activity. The gel surface exhibits broad spectrum antibacterial activity against gram positive (*Staphylococcus epidermidis, Staphylococcus aureus*, and *Streptococcus pyogenes*) and gram

negative (*Klebsiella pneumoniae* and *Escherichia coli*) bacteria, all prevalent in hospital settings. Although potent against drug-suspectible strains, MAX1 gels have limited activity against increased colony forming units of drugresistant strains. Starting with the sequence of MAX1, a new peptide (MARG1) hydrogel was designed that can kill Methicillin-*resistant Staphylococcus aureus* (MRSA) on contact. MARG1 gels display shear-thin/recovery rheological behavior. Thus gels can be easily syringe-delivered to either treat contaminated surfaces such as wound sites or applied as coatings to inhibit MRSA infection. The design, biophysical, mechanical and antibacterial properties of these peptidebased hydrogels will be discussed.

P 158 SITE-SPECIFIC DELIVERY OF BMP-2 PROTEIN VIA CLICK CHEMISTRY DERIVED BIFUNCTIONAL PEPTIDES

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Growth factors are potent signaling molecules initiating essential cellular programs for differentiation, proliferation and survival. Recently these macromolecules have been deployed on medical devices to enhance the efficacy and specificity of medical therapies. Affinergy, Inc has developed bifunctional peptides that help promote biology at the critical interface between a biomaterial (collagen) or synthetic device materials (polymer, metals) and a biological material (growth factors, cells). Conjugating a peptide designed to bind a growth factor to another peptide designed to bind a medical device offer a simple and target-specific therapeutic strategy. Click chemistry was attempted as one such modular approach towards linking peptides in order to produce a combinatorial array of potential bifunctional peptides.
In the present study, we demonstrate that bifunctional peptide linkers synthesized using click chemistry improved the retention of BMP onto a collagen matrix. We describe the design, synthesis and biological evaluation of a panel of "*collagen: BMP*" peptide linkers synthesized using this [3+2] cycloaddition reaction. We optimized the reaction conditions and developed methods to facilitate the coupling of large (>15 mer) peptides in aqueous solution in a selective manner without altering the properties of the individual peptides and their resulting biological effects.

P 159 SELF-ASSEMBLY OF COLLAGEN PEPTIDES INTO MICROFLORETTES VIA METAL COORDINATION

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The self-assembly of synthetic biomaterials can be harnessed for a range of biomedical applications. We report a ligand-modified, collagen triple helical peptide that rapidly and reversibly assembles in the presence of metal ions to form microspheres of reproducible size and shape. Folding intermediates are observed at low temperature and short incubation times that are composed of curved layered sheets. We demonstrate that unsatisfied metal/ligands exist on the surface and within the micropheres, and that these may be easily modified with His-tag functionalized molecules. These unprecedented microscopic structures offer opportunities in many areas, including tissue engineering and regeneration.

P 160 DESIGNING ANTIMICROBIAL PEPTIDE SWITCHES

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The emergence of bacteria that cause hardly treatable infections is being reported with an increasing frequency. This stimulates an extensive search for new models of antimicrobial intervention. Elucidating selection preferences in molecular mechanisms that can differentiate between cellular forms becomes an immediate priority in this regard. Biological membranes is one dimension at which such mechanisms are expressed, with preferential attention being given to selective bactericidal and bacteriostatic effects. Moreover, to those with both functions tuned at whim; that is designed.
Herein we report a novel approach of employing mimetics of host-defense peptides to selectively discriminate eukaryotic from bacterial membranes. The designed peptides have strong tendency for reversible amphiphilic folding, which proves to be remarkably instumental in controlling the growth of bacteria. It is possible to activate, switch on", and deactivate, switch off", the inhibition of the bacteria growth specifically by the designed peptides. Therefore, the designed system can be applied as an antimicrobial switch" and holds particular promise as a synthetic platfrom for new antibiotics and bacteria-sensing molecular probes.

P 161 INSULIN ANALOGUES WITH MODIFICATIONS AT POSITION B26: DIVERGENCE OF BINDING AFFINITY AND BIOLOGICAL ACTIVITY

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We prepared several shortened and full-length insulin analogues with substitutions at position B26. We compared the binding affinities of the analogues for rat adipose membranes with their ability to lower the plasma glucose level in nondiabetic Wistar rats *in vivo* after *s.c.* administration, and also with their ability to stimulate lipogenesis *in vitro*. We found that [NMeHisB26]-DTI-NH2 and [NMeAlaB26]-DTI-NH2 were very potent insulin analogues with respect to their binding affinities (214 and 465%, respectively, compared to that of human insulin), but they were significantly less potent than human insulin *in vivo*. Their full-length counterparts, [NMeHisB26]-insulin and [NMeAlaB26]-insulin, were less effective than human insulin with respect to binding affinity (10 and 21%, respectively) and *in vivo* activity, while [HisB26]-insulin exhibited properties similar to those of human insulin in all of the tests we carried out. The ability of selected analogues to stimulate lipogenesis in adipocytes was correlated with their biological potency *in vivo*. Our data suggest that the B26 residue and residues B26-B30 have ambiguous roles in binding affinity and *in vivo* activity. We hypothesize that our shortened analogues, [NMeHisB26]-DTI-NH2 and [NMeAlaB26]-DTI-NH2, have different modes of interaction with the insulin receptor compared with natural insulin and that these different modes of interaction result in a less effective metabolic response of the receptor, despite the high binding potency of these analogues.

P 162 SOLUTION NMR STUDIES OF DOUBLE-TM FRAGMENTS OF GPCRS

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We present our structural studies of two-transmembrane (2-TM) helix fragments of G-protein coupled receptors from two systems: the Ste2p receptor, a yeast GPCR produced by the labs of Naider and Becker. The second system comprises constructs derived from the Y4 receptor, a GPCR targeted by peptides from the NPY family. In case of Ste2p, a 80 aa construct comprising TM1-TM2 and a few adjacent residues was investigated [1]. We could demonstrate that the TM segments are structurally well-defined, and that contacts between the two helices are present in phospholipid detergent. In case of the Y4 receptor, data from a 116 residue fragment comprising the entire Nterminal domain as well as TM1-TM2 and the E1 loop are shown [2]. In addition, first data from a fragment comprising TM4-TM5 are presented. The polypeptides have been expressed in isotopically labelled form in *E. coli*. The challenging work needed optimization of procedures in all aspects of the work, e.g. in labeling procedures as well as in the spectroscopic work. In particular sample conditions and the detergent had to be extensively optimized. Progress and problems of the spectroscopy of large GPCR fragments in membrane mimetics will be reviewed. In addition, it will be discussed, in which way structures possibly differ from those of the entire receptors.
(1) Neumoin et al., *Biophys. J.*, in press.
(2) Zou et al., *J. Biomol. NMR* (2008), 42, 257–269.

P 163 CHEMICAL SYNTHESIS, ISOLATION AND ASSEMBLY OF C- SUBUNITS OF HUMAN FOF1-ATP SYNTHASE

T. Hara, A. Tainosho, T. Sato, K. Nakamura, T. Kawakami, H. Akutsu, S. Aimoto
Osaka University, Japan

The c-subunit of FoF1-ATP synthase is a 7–9 kDa protein composed of two transmembrane helices, and multimeric c-subunits assemble into ring architecture in the membranes that rotates as a rotary proton-channel of the Fo-proton motor. We have previously reported for the synthesis of a c-subunit of *E. coli* F1F0-ATP synthase by means of the thioester method.1 However, little is know about the structural basis of c-subunit of ***human*** F1F0-ATP synthase due to the inherent difficulties in producing, handling and purifying it. Here we described our study on preparation of a c-subunit of *human* F1F0-ATP synthase for elucidating the structure-function relationships of the ATP synthase using solution and solid-state NMR techniques. The peptide was synthesized by Fmoc solid-phase synthesis. Arg5-tag was then added to the N-terminus of the target peptide on solid phase via the newly developed photocleavable-linker, 5-(3-aminopropyloxy)-2-nitrobenzyl oxycarbonyl group, to prevent self-aggregation and enhance water solubility of the peptide. Following RP-HPLC isolation with taking the advantage of Arg5-tag, the tag and linker was simultaneously removed from the peptide by photolysis, yielding the full-length unmodified human c-subunit(1-75). Examining c-subunits folding and assembly will also be described. Reference: 1) Sato, T. et al., *J. Pept. Sci*. 2002, *8*, 172–180.

P 164 HYDROPHOBIC PEPTIDES AS PROBES OF MEMBRANE PROTEIN FOLDING

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Migration on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) that does not correlate with formula MWs, termed gel shifting, appears to be common for membrane proteins but has yet to be conclusively explained. In the present work, we investigate the anomalous gel mobility of helical membrane proteins using a library of hydrophobic peptide constructs of wild-type and mutant sequences ('mini-membrane proteins') derived from transmembrane segments 3 and 4 (residues 194-241) of the human cystic fibrosis transmembrane conductance regulator (CFTR), the chloride channel protein that becomes dysfunctional in CF disease. We find that these peptides migrate at rates of -10% to $+30\%$ vs. their actual formula weights on SDS-PAGE and load detergent at ratios ranging from 3.4-10 g SDS/g protein [Rath *et al., PNAS USA*, 106, 1760−1765 (2009]. We additionally demonstrate that mutant gel shifts strongly correlate with changes in peptide SDS loading capacity, indicating that gel shift behavior originates in altered detergent binding, *i.e.,* the higher the amount of detergent bound, the slower the migration on SDS-PAGE. Mutants loading high amounts of SDS tend to be the most hydrophobic and generally display high helicity. We suggest that this differential solvation by SDS may result from replacing protein-detergent contacts with strong protein-protein contacts that can maintain folded structures. The observed interdependence between peptide migration, SDS binding, and conformation suggests that detergent binding may provide a rapid screen for identifying membrane proteins with robust tertiary and/or quaternary structures.

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P 165 FUNCTIONAL STUDIES OF AT-I ANTAGONIST SII ANALOGUES REVEAL SELECTIVE CELL SIGNALING AND INHIBITION OF AT-II MEDIATED NHE3 ACTIVATION

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Angiotensin-II (AT-II) regulates NaCl reabsorption through both direct acting on the proximal tubule, and indirect acting on aldosterone secretion. In isolated proximal tubule cells and also brush-border membrane vesicle preps, AT-II stimulates Na+ absorption by increasing the activity of NHE3. One previous study have shown that increased NHE3 function contributes to hypertension observed in rat hypertension model induced by AT-II, and AT-II stimulates NHE3 activity through increasing NHE3 gene expression levels. These observations suggest the possible role of NHE3 in the pathophysiology of hypertension. The current study is designed to test group of synthetic peptides with functional selectivity, antagonist of Angiotensin II but activator of β-arrestin in the cellular level, which are homologues to AT-II block the effect of A-II on NHE3 expression.

Supported by Grants from the U.S. Public Health, Service, National Institutes of Health, DK 17420 and DA 06284

P 166 SHORT MEMBRANE-ACTIVE PEPTIDES STUDIED BY 19F- SOLID STATE NMR AND ORIENTED CIRCULAR DICHROISM SPECTROSCOPY

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Short membrane-active peptides can be broadly divided into three distinct categories. These include antimicrobial peptides, cell penetrating peptides and fusogenic peptides. Antimicrobial peptides kill bacteria by selectively targeting their cell membranes and act against a microbial infection. Cell penetrating peptides cross the membrane bilayer without causing any permanent damage to the membrane and are therefore used as delivery agents. Fusogenic peptides are short peptides which are active in cell-cell fusion as evidenced during viral infection and fertilization events. Antimicrobial and cell penetrating peptides are short, cationic and amphiphilic in nature where as fusiogenic peptides are usually hydrophobic sequences with flexible structures. Solid state NMR and oriented circular dichorism (OCD) have been successfully used to characterize the orientation and conformation of membrane active peptides. We have used solid state 19F-NMR and OCD spectroscopy to investigate these peptides in model membranes to understand their structure, orientation, realignment, oligomerization and aggregation as function of peptide concentration, temperature, and lipid composition. Further, we have tested the ability of antimicrobial and cell penetrating peptides to accomplish membrane fusion. These results are compiled to obtain initial design principles for optimizing sequences with specific biological functions.

P 167 STUDIES OF ENERGY BALANCE AND CANCER

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The anti-inflammatory activities of the neuroimmunomodulator α-melanocyte stimulating hormone (α-MSH) has been traced to a 'message sequence' contained in the C-terminal : the tripeptide amino acid Lys-Pro-Val (KPV; α-MSH 11-13). Inflammatory processes, in particular the induction of NF-κB by tumor necrosis factor(TNF- α) have been shown to be inhibited by α-MSH through inhibition of TNF- α. Cachexia is a clinical syndrome of wasting that accompanies many chronic diseases including cancer, renal failure, and heart failure. This condition is marked by an increase in energy expenditure and preferential loss of lean body mass, creating a striking catabolic state. Few treatments have proved to be of significant benefit to patients suffering from cachexia. One new treatment that shows promise is pharmacological blockade of the central melanocortin system. The importance of this system is in maintaining normal body weight. In humans it is highlighted by the finding that disordered melanocortin signaling results in early-onset morbid obesity and dramatic increases in lean body mass in humans. Emerging evidence suggests that blocking this system via pharmacological antagonists of the type 4 melanocortin receptor (MC4R) may restore appetite and lean body mass in subjects with cachexia caused by a variety of underlying disorders. Evidence from animal models suggests a compelling link between cachexia and inflammation, and a variety of pro-inflammatory cytokines such as TNF- α play an integral role in wasting syndrome. Thus, bifunctional ligands containing a TNF- α inhibitor using the KPV sequence and a MC4R inhibitor using known HMC4 antagonists MBP10 or SHU9119 have been designed and synthesized. A study using different amino acid

linkers was performed to determine if the type of linker used affected the KPV pharmacophore's influence on the HMC4 antagonist pharmacore activity. Bioassay results show no significant difference in the pharmacophores' influence over each other in each linker type studied. However, one compound does show promising agonist activity at the MC1R.

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P 168 PHOTOACTIVEISOPRENOID-CONTAININGPEPTIDES

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Protein prenylation is a common post-translational modification. The process consists of initial addition of a C15 or C20 isoprenoid to a tetrapeptide "CAAX box" sequence located at the C-terminus of a protein followed by proteolysis of the three C-terminal residues and subsequent methylation of the newly formed C-terminal prenylcysteine. Since many proteins involved in signal transduction pathways including ras proteins contain this modification, inhibition of protein prenylation has attracted significant attention as a possible target for cancer therapy. The initial prenylating enzymes have been studied in considerable depth, in part due to the fact that they are soluble enzymes. In contrast the CAAX protease and methyltransferase are membrane bound rendering them considerably more challenging to characterize. To facilitate structural analysis, we have prepared "CAAX box" peptides that incorporate photoactive isoprenoids in place of the naturally occurring farnesyl or geranylgeranyl groups normally processed by the enzymes and have demonstrated that these modified molecules are substrates for the "CAAX box" protease rce1. Experiments to label the enzyme and identify the site of labeling are currently in progress.

P 169 GT(340-350) ANALOGS BIND OPSIN SIMILAR TO X-RAY CRYSTAL STRUCTURE

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Understanding how rhodopsin interacts with transducin offers insight into the visual signal-transduction process as well as valuable information regarding GPCR (G-protein coupled receptor)/G-protein complex formation and activation. Recently, the X-ray crystal structure of opsin bound to Gt_{α}(340-350), the C-terminal region of the α subunit of transducin, the G-protein of the eye, was solved, giving insight into the rhodopsin/transducin interaction. The X-ray crystal structure of Gt_{α}(340-350) bound to opsin was very similar to the TrNOE structure solved nearly a decade ago by Kisselev et al. in our lab. In this study, we used several docking programs to dock TrNOE bound-structures of Gt_{α}(340-350) analogs. All analogs bound at the same site (Loop-III) and some in a orientation very similar to the Gt_{α}(340-350)K341L X-ray crystal structure of the opsin complex.

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P 170 MODULATION OF FUNCTION IN HEME BINDING MEMBRANE PROTEIN

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We previously reported successful design of functional membrane protein, by designing heme-binding site in Glycophorin A. ME1 binds Fe (III) Protoporphyrin IX with submicromolar affinity, has a redox potential of –128 mV, and displays nascent peroxidase activity. G25F mutant was explored to replicate the aromatic Π-stacking and T-stacking interactions observed between aromatic side chains and the heme in the majority of hemoprotein structures reported to date. Binding of hemin to G25F was assessed by spectrophotometric titrations (Kd, app, 6.49 x 10-8 M). Thus, introducing a single aromatic residue (G25F) results in tighter binding compared to ME1. The electrochemical reduction midpoint potential (*E1/2*) of G25F-Hemin complex, determined by UV-vis monitored potentiometric titration, is -280 mV vs. SHE. The most efficient catalyst is ME1, with a *kcat/* KM of 5.05 x104 M-1s-1, compared to 1.1 x104 M-1s-1 for G25F.The trend in our data shows a strong correlation between binding affinity for the ferric porphyrin, midpoint potential, and catalytic activity: as the binding affinity for the ferric heme increases, the midpoint potential becomes more negative, and the catalytic activity decreases.

P 171 CHOLESTEROL INFLUENCE ON THE INTERACTION OF THE HIV GP41 MEMBRANE PROXIMAL REGION WITH LIPID BILAYERS – A BIOPHYSICAL STUDY

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A small sequence (LWYIK) inside the HIV-1 gp41 ectodomain membrane proximal region (MPR) is commonly referred to as a cholesterol (Chol) binding domain. To further study this unique and peculiar property we have used fluorescence spectroscopy techniques to unravel the membrane interaction properties of three MPR-derived synthetic peptides, which are MPRP-C, corresponding to the complete MPR, MPRP-S, that corresponds to the last five MPR amino acid residues (corresponding to the putative Chol binding domain) and MPRP-I, which corresponds to the MPRP-C peptide without the MPRP-S sequence. MPRP-C and MPRP-I membrane interaction is largely independent of the membrane phase. Membrane interaction of MPRP-S occurs for fluid phase membranes but not in gel phase membranes or Chol-containing bilayers. The gp41 ectodomain MPR may have a very specific function in viral fusion through the concerted and combined action of Chol-binding and non-Chol binding domains (i.e. domains corresponding to MPRP-S and MPRP-I, respectively).

P 172 SYNTHESIS OF N-URETHANE-PROTECTED THIOUREIDO LINKED PEPTIDOMIMETICS AND NEOGLYCOCONJUGATES EMPLOYING NOVEL N-PROTECTED-β-AMINO ALKYL ISOTHIOCYANATES

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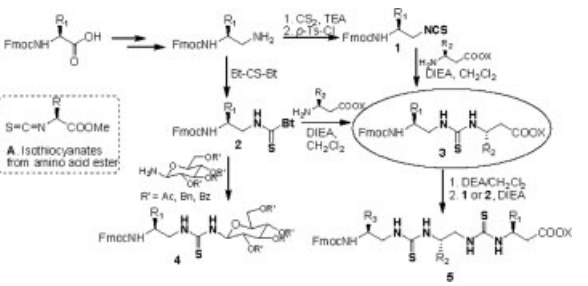
Isothiocyanate and its equivalents are the important precursors for thiourea synthesis, which have wide range of applications. In peptide chemistry, isothiocyanates derived from α-amino acid esters are known (Fig. A). We have synthesized and isolated a new class of Nprotected amino acid derived isothiocyanates 1. Alternatively, benzotriazole derived isothiocyante equivalent 2 has also been synthesized using thioacylating agent- Bt-CS-Bt. Their application for the synthesis of thioureido peptides 3, 5 and thiourea-linked glycoconjugates 4 has been demonstrated.



P 173 CHIRAL *N*-Fmoc-b-AMINO ALKYL ISONITRILES: A JOURNEY FROM *N*a/*N*b- FMOC AMINO ACIDS

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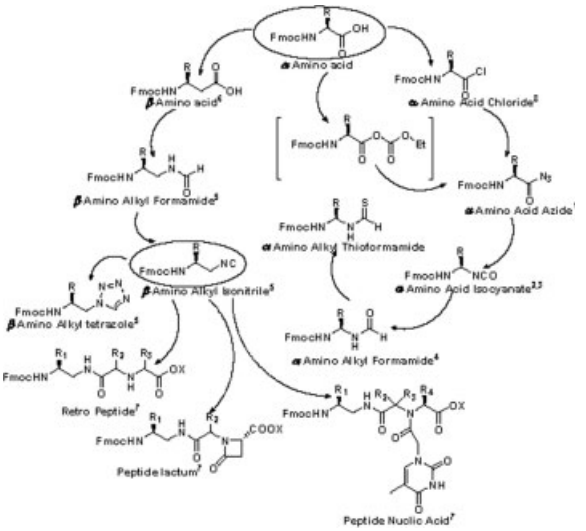
The merits offered by the Fmoc group have been explored by us to describe several *N*-Fmoc-amino/peptide acid derivatives, employable in peptidomimetics synthesis. Recently, we have synthesized *hitherto* unreported class of *N*Fmoc protected chiral amino alkyl isonitriles. Their applications in MCRs leading to peptide lactams, PNAs *etc*., are being explored.

Reference: Sureshbabu *et al*., (1) J. Chem. Soc., Perkin Trans. 1, 2000, 4328. (2) J. Org. Chem., 2003, *68*, 7274. (3) J. Org. Chem., 2006, *71*, 7697. (4) J. Org. Chem., 2007, *72*, 9360. (5) J. Org. Chem.,



2008 (DOI: 10.1021/jo801527d). (6) J. Chem. Soc., Perkin Trans. 1 2002, 2087. (7) manuscript under preparation (8) J. Org. Chem., 2007, *72,* 9804.

P 174 UNEXPECTED NEW RESULTS IN THE RGD FIELD

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For more than two decades the RGD sequence is known to be recognized by several integrins. Numerous peptides and non-peptidic ligands were developed as potent inhibitors. All of them contain a carboxyl group which binds to the metal in the MIDAS region of the integrin β-subunit. Any attempts to substitute this COOH by mimics failed so far. We found that hydroxamic acids can be used for this purpose and peptide mimetics with high activity and selectivity could be developed.
Another exciting new topic was the discovery that Asn in the retrosequence NGR is used as switching element in the ECM protein fibronectin by rearrangement into *iso*-Asp. We synthesized a library of cyclic peptides containing the sequence *iso*-Asp-Gly-Arg and discovered some peptides with low nanomolar activity.
Activities and selectivities between the integrins $\alpha v\beta 3$ and $\alpha 5\beta 1$ are investigated. In an ongoing work we use receptor modelling to understand these results.
Good selectivities and activities for $\alpha v\beta 3$ and $\alpha 5\beta 1$ have been obtained recently in our group using two different peptidomimetic scaffolds (tyrosine based and diacylhydrazine based) using a homology model of the integrin $\alpha 5\beta 1$.

P 175 DhHP-6 EXTEND *CAENORHABDITIS ELEGANS* LIFESPAN IN A SIR2.1 DEPENDENT MANNER

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Aging is an important biological phenomenon and a major contributor to human disease and disability, but till now, no drugs have been demonstrated to delay human aging. DhHP-6 is a novel peptide mimic of peroxidases designed and synthesized in our laboratory. It has been reported to increase lifespan by 20 percent of wild type nematode *Caenorhabditis elegan*s. Here we show that this lifespan extension by DhHP-6 requires histone deacetylase SIR2.1 in *C.elegans*, a member of the sirtuin family of NAD+- dependent deacetylases, because DhHP-6 don't extend the mean lifespan significantly in knock out mutant of *sir-2.1 (ok434) IV* and increase the expression of *sir-2.1* gene significantly in wild type nematode. Unlike other sirtuin activators, DhHP-6 can further extend lifespan of *C.elegans* when nutrients are restricted. In summary, these data indicate that DhHP-6 extend *C.elegans* mean lifespan in a SIR2.1 dependent manner but may not be related to caloric restriction.

P 176 REACTIONS OF ALPHA/BETA PROLINE ENOLATES FOR THE SYNTHESIS OF CONSTRAINED PEPTIDOMIMETICS

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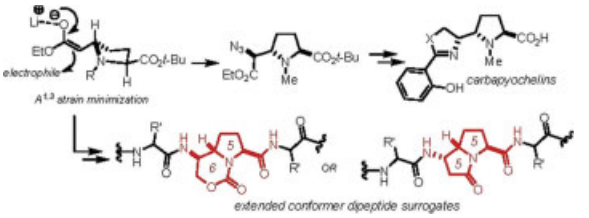
We recently described the synthesis of carbapyochelins (confuguration-ally stable analogs of the microbial siderophore pyochelin) based on the regio- and stereoselective electrophilic azidation of a 5-(ethoxycarbonyl)methylproline derivative. In this study, we explore the enolization of related alpha/beta bis-proline derivatives and their reactions with various electrophiles to yield novel chimeric prolines. The key intermediates have been elaborated into new dipeptide surrogates designed to mimic an extended peptide backbone conformation. The synthesis of these proline-based scaffolds will be presented in addition to preliminary structural studies.



P 177 PEROXIDASE MIMETIC DhHP-6 PROTECT CARDIOMYOCYTE AGAINST OXIDATIVE INJURY

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DhHP-6 is a novel peptide mimic of peroxidases designed and synthesized in our laboratory, which has been demonstrated to exert significant cardioprotective effects against myocardial ischemia reperfusion injury in rats previously. In this study, we investigate the protective effects of DhHP-6 on cardiomyocytes against oxidative injury induced by hydrogen peroxide and hypoxia/reoxygenation. Compared with the model injury cells ($P<0.01$), in both H2O2-induced rat neonatal cardiomyocytes and hypoxia/reoxygenation-induced H9c2 cardiomyocytes injury, pretreatment with DhHP-6 significantly increased SOD activity, decreased the MDA level and LDH release, and improved Ca2+-ATPase activity. And DhHP-6 also significantly increased the viability of hypoxia/reoxygenation-induced H9c2 cardiomyocytes by 20%, and the viability of H2O2-induced rat neonatal cardiomyocytes by 45%. Furthermore, DhHP-6 significantly reduced apoptotic cells, caspase-3 activity, the mRNA expression levels of Hsp70, P21, and Heme oxygenase, but unchanged the catalase mRNA level by real time-PCR in H2O2-induced rat neonatal cardiomyocyte. In summary, DhHP-6 exerts significant protective effects on cardiomyocytes against oxidative injury induced by H2O2 and hypoxia/reoxygenation, and these effects may be related to its antiapoptotic properties.

P 178 ROLE OF THE GUANIDINE GROUP IN POSITION 11 OF PTH(111) ANALOGUES

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The N-terminal 1-34 fragment of parathyroid hormone (PTH) is fully active *in vitro* and *in vivo* and it can reproduce all biological responses

characteristic of the native intact PTH. Recent studies have demonstrated that helicity-enhancing substitutions yielded potent analogues of PTH(1-11) and PTH(1-14). The role of α-helicity on biological potency is well known. In the context of searching the pharmacological properties of PTH(1-11) analogues, we studied the role of a positive charge at the C-terminal position, which was identified to play an essential role in bioactivity and binding [1]. A series of modified peptide analogues of PTH(1-11) was synthesized and characterized. As a reference, we synthesized an analogue containing Lys in position 11. This analogue exhibited an activity similar to that of [$Ala^{1,3}$, Gln^{10}, Har^{11}]PTH(1-11)NH_2, reference peptide. The peptide series was synthesised by SPPS employing Fmoc protected amino acids and a small library of Fmoc-protected glutamic acid amides of variable chain length previously prepared in solution. A circular dichroism study of the synthesized peptides was performed to compare their conformation with biological activity. We found a reduced activity for modified analogues which could be justified by the role of the C-terminal amide group. New syntheses of analogues containing mimetics are underway to establish the correlation between the guanidine function and bio-activity of PTH(1-11) analogues.

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P 179 DESIGN AND SYNTHESIS OF ALPHA-HELIX MIMETICS BASED ON A TRIS-BENZAMIDE SCAFFOLD TO TARGET BCL PROTEINS

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Apoptosis is utmost important for cell homeostasis and any impairment in this process may lead to a disease like cancer. One example is an over-expression of Bcl, an anti-apoptotic protein that regulates cell death process by making protein complexes with proapoptotic proteins like Bak. Thus, a small molecule that has a capability to break these protein complexes (e.g., Bcl/Bak) would be of high value in treating cancers. Pro-apoptotic proteins, such as Bak, Bad, Bax, Bid and Bik, interact with Bcl proteins through a BH3 domain that was found to adopt an α-helical structure determined by NMR. This finding gave us a rationale for the design of α-helix mimetics to represent BH3 domains in pro-apoptotic proteins for targeting anti-apoptotic Bcl proteins. The resulting peptidomimetics of BH3 domains are expected to disrupt the protein complex formation between anti- and pro-apoptotic proteins, which would ultimately lead to cell death. For the design of BH3 mimetics, a rigid scaffold, tris-benzamide was used to place three functional groups found at the *i*, *i+4*, and *i+7* positions of BH3 helices. A series of α-helix mimetics were synthesized to emulate BH3 domains in Bak and Bad proteins and led to the discovery of several compounds with high cytotoxicity to cancer cells.

P 180 THE DESIGN AND SYNTHESIS OF SMALL SUBSTANCE P (1-7) MIMETICS BASED ON SUBSTITUTED HETEROAROMATIC SCAFFOLDS

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Substance P (SP), which is a neurotransmitter and neuromodulator at the G-protein coupled NK1 receptor, plays a well known role in pain transmission [1]. The N-terminal fragment of SP, the heptapeptide SP1-7 (H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-OH), is a bioactive metabolite that has been shown to oppose several effects of SP [2]. Specific binding sites for SP1-7 that differ from the NK1 receptor have been identified in both mouse and rat [3, 4]. In the aspiration to characterise the binding site for SP1-7 and for studies aiming to reveal the role of the heptapeptide in complex animal models, drug-like low molecular weight SP1-7 mimetics as research tools are highly desirable. Recently, we reported the discovery of small peptides as potent ligands of the specific binding site for SP1-7 [5].

In this poster, the work of transforming the small peptides in to compounds of less peptide character by backbone modifications is presented. Different substituted heterocycles has been synthesized and incorporated as scaffolds with the aim of mimicking amino acid residues in the peptide. A sub goal is to develop a fast and easy method to couple different heterocycles and amino acids without racemisation.

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P 181 DESIGN AND SYNTHESIS OF NOVEL AMPHIPHILIC ALPHA- HELIX MIMETICS BASED ON A NEW TRIS-BENZAMIDE SCAFFOLD

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An α-helix is one of the most common structural motifs in proteins and has a significant role in mediating protein-protein interactions. Design and synthesis of new α-helix mimetics using rigid and preorganized scaffolds with low molecular weight and high synthetic efficiency is currently an active field of research. Most of α-helix mimetics reported until now have focused only on one face of a helix organized by side chains found at the *i*, *i+3/i+4* and *i+7* positions. Compared to the one-sided α-helix mimetics, engineering a molecule to represent both helical faces is still a formidable task. Amphiphilic α-helix mimetics are expected to have higher affinity and biological activity due to stronger interaction with target proteins by superior α-helix mimicry. Herein we report a novel amphiphilic tris-benzamide scaffold that places five side chain functionalities at the *i*, *i+3/i+4*, *i+7* positions on one face of a helix and at the *i+2*, *i+5* positions on the opposite side. Side chain functional groups at the *i*, *i+3/i+4* and *i+7* positions were introduced by *O*-alkylations of phenolic moieties, whereas functional groups at the *i+2* and *i+5* positions were installed though Pd/Cu-catalyzed Sonogashira cross-coupling reactions followed by hydrogenation. Following this approach, we introduced a variety of functional groups including hydroxyl, amino, alkyl, aryl and carboxylic groups. To evaluate these tris-benzamides as amphiphilic α-helix mimetics, we have targeted a helical peptide hormone, glucagon-like peptide-1 since its analogues are found to have high therapeutic promise for type 2 diabetes.

P 182 SYNTHESIS OF BIS-BENZAMIDES AS NEW AMPHIPHILIC ALPHA-HELIX MIMETICS

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α-Helices are one of the most commonly found secondary structures in proteins and bioactive peptides, and play an important role in regulating protein-protein and protein-nucleotide interactions. However, when taken out of proteins helical peptide segments are often damaged by entropy effect and fall out to random coil or other structures. Thus, many approaches have been developed to stabilize α-helices in solution, such as a lactam, salt, or disulfide bridge formation between the *i* and *i+4* positions of an α-helix. Another alternative approach is to mimic α-helices by using rigid and preorganized scaffolds through rational design. Most of previously reported α-helix mimetics focused on one face of a helix by presenting side chains at the *i*, *i+4* and *i+7* positions, and α-helix mimetics that have a capability to represent both helical faces are not known yet. Thus, we report a novel amphiphilic bis-benzamide scaffold that properly places side chain functional groups found at the *i*, *i+7* positions on one face of a helix and at the *i+2*, *i+5* positions on the opposite side. Through two hydrogen bonds between amide proton and two adjacent phenolic ethers, the amphiphilic bisbenzamide adopts a stable and rigid conformation for superior α- helix mimicry. Four functional groups were installed on the bisbenzamide scaffold by *O*-alkylation reactions. For the evaluation of bisbenzamides, we have synthesized amphiphilic α-helix mimetics to emulate α-helical regions found in a peptide hormone, glucagonlike pepide-1.

P 183 DEVELOPMENT OF CALPASTATIN MIMETIC USING PHAGE DISPLAY

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Calpains are ubiquitous calcium-dependent cysteine proteases whose over-activity is thought to contribute to cell death in a variety of diseases or conditions such Alzheimer's disease, traumatic brain injury,

muscle protein loss, cataract formation and others. In addition to calcium and redox state, calpain is regulated by its endogenous inhibitor, calpastatin. Recent crystallographic studies have confirmed the interaction of specific calpastatin sub-domains with both the active site and calcium-binding domains of calpain. Based on this and evidence suggesting that calpastatin acts as a suicide-substrate we sought to identify novel small peptide binding molecules that target the calpain-calpastatin interaction. Using a 12-mer phage-display library we screened over 2 billion random sequences to identify the optimal amino acid sequence that bound to domains within calpain that undergo calcium-mediated conformational changes. Using purified calpain we performed four iterative rounds of phage display using a calcium-chelator, EGTA, to elute phage. From these studies we identified a small number of candidate sequences with the pentamer LSEAL being the major representative. This sequence shows high homology to domains within calpastatin known to interact with calpains. Both in vitro and in situ studies have confirmed LSEAL inhibitory action towards calpain.

P 184 SYNTHESIS OF NOVEL GLUTAMINE MIMICS AND INCORPORATION INTO PEPTIDOMIMETIC INHIBITORS OF SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION 3 LEADS TO LOW NM AFFINITIES AND INHIBITION OF TUMOR CELL GROWTH

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Signal transducer and activator of transcription 3 (Stat3) transmits cell survival signals from IL-6 family cytokines, EGF, Src, etc., and is constitutively activated in several human cancers. Since sustained Stat3 activation promotes cell survival and cancer progression this protein is a target for drug design. Stat3 binds to cytokine and growth factor receptors via its SH2 domain, which recognizes pTyr-Xxx-Xxx-Gln sequences. Because of the importance of glutamine at position pY+3, we have synthesized two libraries of Gln mimics and incorporated them at the C-termini of peptidomimetics derived from a lead peptide, Ac-pTyr-Leu-Pro-Gln-Thr-Val-NH_2. In the first, Fmoc-D-amino acids were converted to aldehydes and these were extended with Wittig vinylogation. Reduction of the olefin produced new glutamine analogues. In the second library, reduction of the carboxyl group of Fmoc-Glu(tBu)-OH to an alcohol followed by iodination formed a substrate a series of 4-*R*-methyl-4-aminobutyramides. To convert the lead peptide to a non-peptide, we replaced the pTyr with 4-phosphoryloxycinnamate (pCinn) and the central dipeptide with either Haic (5-amino-1,2,4,5,6,7-hexahydro-4-oxo-azepino[3,2,1-hi]indole-2-carboxylic acid) or Nle-*cis*-3,4-methanoproline. Incorporation of the new Gln led to inhibitors with IC_{50} values as low as 35 nM in fluorescence polarization assays. Conversion to phosphatase-stable, cell-permeable prodrugs led to complete inhibition of Stat3 phosphorylation between 0.5 and 1 μM and inhibition of the growth of lung tumor lines at 1 – 5 μM. Thus these peptidomimetics have potential as cancer chemotherapeutics.

P 185 ANGIOGENESIS INHIBITION USING VEGF RECEPTOR BLOCKAGE APPROACH

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Angiogenesis is the process of new blood vessel formation from existent vascular network and it is tightly regulated by a balance between pro- and anti-angiogenic factors1. Vascular endothelium growth factor (VEGF) is a glycoprotein consisting of an anti-parallel homodimer structure with inter and intra molecular disulfide bonds characteristic ofPDFG cystine-knot family. In this study we evaluated VEGF activated pathway and a approach to target angiogenesis, the blockage of VEGF and VEGFR-2 receptor interaction using peptides designed to mimic the VEGF binding site. We utilized an angiogenesis model cell line, HUVEC that respond to VEGF activation in several in vitro assays: proliferation, matrigel tube formation and VEGFR-2 phosphorylation and observed the ability of VEGF mimic peptides to block the interaction between VEGF and VEGFR-2. We also evaluated the ability of the retro-inverso peptide to demonstrate anti-angiogenic effects. We tested two different animal models, where tumor growth is dependent (RIP1-TAG2) or accelerated (VEGF+/-/Neu 2-5+/-) upon VEGF expression.

P 186 PHOSPHATASE-STABLE, CELL-PERMEABLE PEPTIDOMIMETIC PRODRUGS TARGETING THE SH2 DOMAIN OF SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION 3 INHIBIT TUMOR CELL PROLIFERATION IN VITRO

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Signal transducer and activator of transcription 3 (Stat3) transmits signals from IL-6 family cytokines, EGF, Src etc., is constitutively activated in head and neck, breast, prostate, and other cancers, and is a target for cancer drug design. To disrupt Stat3 activity we are targeting the SH2 domain with phosphopeptide-based inhibitors. Molecular modeling of a lead peptide, Ac-pTyr-Leu-Pro-Gln-NHBn, docked to the Stat3 SH2 domain suggested that a methyl group on the β carbon could interact with side chain methylenes of Glu638 to enhance affinity. A series of peptidomimetics incorporating 4-phosphoryloxy-β-methylcinnamate for pTyr exhibited IC_{50} values ranging from 35 – 120 nM in a fluorescence polarization assay, 2-3 fold higher affinities compared to those containing the simpler 4-phosphoryloxycinnamate. Several were converted to phosphatase-stable, cell-permeable prodrugs by replacing the phosphate with the phosphonodifluoromethyl group and capping the phosphonate oxygens with pivaloyloxymethyl (POM) groups. Of these, PM-72G completely inhibited phosphorylation of Stat3 (Tyr^{705}) at 500 nM in breast tumor cells and inhibited proliferation of NSCLC lines in culture at 1-5 μM, with negligible effects on normal human lung fibroblast cells. Inhibition was dose-dependent and independent of EGFR-mutational status. Furthermore, PM-72G inhibited tube formation in Human Umblical Vein Endothelial Cells (HUVEC) suggesting potential angiogenesis inhibition. We conclude that inhibiting Stat3 activity is a promising modality for chemotherapeutic treatment of lung cancer.

P 187 DhHP-6 EXTEND *CAENORHABDITIS ELEGANS* LIFESPAN IN A SIR2.1 DEPENDENT MANNER

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Aging is an important biological phenomenon and a major contributor to human disease and disability, but till now, no drugs have been demonstrated to delay human aging. DhHP-6 is a novel peptide mimic of peroxidases designed and synthesized in our laboratory. It has been reported to increase lifespan by 20 percent of wild type nematode *Caenorhabditis elegan*s. Here we show that this lifespan extension by DhHP-6 requires histone deacetylase SIR2.1 in *C.elegans*, a member of the sirtuin family of NAD+- dependent deacetylases, because DhHP-6 don't extend the mean lifespan significantly in knock out mutant of *sir-2.1 (ok434) IV* and increase the expression of *sir-2.1* gene significantly in wild type nematode. Unlike other sirtuin activators, DhHP-6 can further extend lifespan of *C. elegans* when nutrients are restricted. In summary, these data indicate that DhHP-6 extend *C.elegans* mean lifespan in a SIR2.1 dependent manner but may not be related to caloric restriction.

P 188 *N*-ALKYLAMINOOXY AMINES FOR THE SYNTHESIS OF GLYCOPEPTOIDS FOR THERAPEUTIC AND BIOMATERIALS APPLICATIONS

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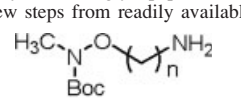
The chemoselective glycosylation of *N*-alkylaminooxy side chain moieties with unprotected reducing sugars has proven useful for the synthesis of glycopeptides and glycoproteins. We now extend the *N*alkylaminooxy strategy to the synthesis of glycopeptoids. Amines 1 and 2 were made efficiently in few steps from readily available starting materials



1 n = 1
2 n = 2

and incorporated into peptoids using standard submonomer synthesis protocols. The *N*-alkylaminooxy side chains react selectively with reducing sugars in mildly acidic aqueous buffers (pH 4) to furnish glycopeptoids. Here we present details of the synthesis of the amines and their use in glycopeptoids of therapeutic and biomaterial interest.

P 189 FREE RADICAL SCAVENGING ACTIVITY OF HYDROXYCINNAMOYL AMINO ACID AMIDES

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In the recent years the physiological functionality of hydroxycinnamic acids and their analogues have attracted much attention and have been studied by many research groups. The compounds are known to have antibacterial, antiviral, anti-inflammatory, neuroprotective and etc. These properties are associated with either their properties as antioxidants. In this study, cinnamoyl-, feruloyl-, sinapoyl- and caffeoyl amides of C-protected cysteine and phenylalanine have been synthesized. The synthesized amides have been characterized by UV, ^{1}H-NMR and EI-MS. The study of radical scavenging activity of the synthesized esters is in progress.

P 190 DESIGN, SYNTHESIS AND STRUCTURE OF PEPTIDOMIMETIC INHIBITORS OF EUKARYOTIC RIBONUCLEOTIDE REDUCTASE: A TARGET FOR CANCER CHEMOTHERAPY

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Eukaryotic ribonucleotide reductase (RR) catalyzes nucleoside diphosphate conversion to deoxynucleoside diphosphate. Crucial for rapidly dividing cells, RR is a target for cancer therapy. RR activity requires formation of a complex between subunits R1 and R2 in which the R2 C-terminal peptide binds to R1. Mammalian RR (mRR) can be inhibited by competitive binding at the mR1 subunit by a heptapeptide, NAcFTLDADF (P7), corresponding to the C-terminus of the mR2 subunit and its peptidomimetic, Fmoc(Me) PhgLDChaDF, denoted P6. Recently, we reported crystal structures of heterocomplexes containing P7 or P6 bound to Saccharomyces cerevisiae R1 (ScR1) at a locus consisting of residues that are highly conserved between yeast, mouse, and human R1s (Xu et al., J. Med. Chem. 2008, 51, 4653–4659). Molecular modeling based on these structures led to the design of [1,2,3]-triazolyl containing cyclic peptides with the potential for very tight binding to R1. Synthesis of these compounds proceeded via Cu (I) catalyzed i, i+3 intramolecular azide-alkyne 1,3-dipolar cycloaddition. The solid-phase assembly of linear precursors, on-resin diazo transformation of Lys or Orn into Nle(ε-N3) or Nva(ε-N3), and cyclization by condensation with propargylglycine (Pra), were achieved on TGA resin. The crystal structures of these compounds as bound to ScR1 will be presented.

P 191 PLASMEPSIN 9 AS A NEW TARGET FOR ANTIMALARIAL DRUG DISCOVERY

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We have produced recombinant plasmepsin 9 from *P. falciparum* and have explored the active site specificity to provide information for drug design. Our results led to the preparation of a peptidomimetic with strong and selective binding to the enzyme, as compared to other plasmepsins and enzymes in the aspartic proteinase family. Data showing the effect of inhibitors on growth of the parasite in culture with and without over-expression of plasmepsin 9 show that inhibitors act by directly blocking enzymatic function.

P 192 METAL FREE TRIAZOLE LIGATIONS DELIVER CONFORMA- TIONALLY LOCKED "CLACK PEPTIDES"

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Ligation reactions have earned great fame in the recent decade enabling the facile synthesis of large biopolymers.[1] Triazole ligations are especially suitable for peptides because triazole ring is found to be a bioisoster of amide bonds. Unfortunately, published methods for regioselective triazole synthesis rely on heavy metal catalysts. We introduce a method avoiding heavy metal catalysts but retaining regioselectivity. Peptides were constructed on resin via C-acylation of a phosphorane linker.[2] The obtained polymer supported peptidyl phosphoranes act as dipolarophiles and reacted with azides in 1,3 dipolar cycloaddition reactions.[3] Reactions with electron poor azides occurred smoothly at room temperature in apolar solvents. Electron rich azides, however, required polar solvents and slightly elevated temperature. The reaction delivered exclusively 1,5-disubstituted triazoles as the sole product. The method was used as a metal free and chemoselective ligation approach to 1,5-disubstituted peptidyl-triazolyl-peptides. As the proucts were suspected being isosteres of cis-peptide bonds, their conformations were studied in DMSO via NMR spectroscopy and molecular dynamics simulations. Structural analysis revealed that the 1,5-disubstituted triazole ring introduce a hairpin-turn within the peptide. Being metal-free the method has a wide range of applications, especially in chemical biology and protein ligand design. Moreover, it is superior to earlier methods for the preparation of triazolyl peptides, being fully integrated into Fmoc-based peptide synthesis.

P 193 TEMPORAL ANALYSIS OF PHOSPHOPEPTIDE INDUCED BY NOCODAZOLE TREATMENT

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Protein phosphorylation is thought to play an important role in a wide variety of the biological activities of a drug. The phosphorylation status in the pathway potentially determines a distinct biological output. Nocodazole, known as a microtubule-interacting agent, induces the activation of stress response pathways, M-phase cell cycle arrest, and the induction of apoptosis. Protein phosphorylation has been shown to affect mitotic events such as mitotic checkpoints, spindle formation, and the anaphase-promoting complex. However, detailed molecular understanding of protein phosphorylation is limited. Previously, we performed phosphoproteomic analysis in the presence or absence of nocodazole, known as a microtubule-interacting agent, of three distinct tumor cell lines (HeLa, HCT-116, and NCI-H460) and found that 14 proteins were phosphorylated in all 3 tumor cell lines only as a result of treatment with nocodazole. To gain more insight into the phosphorylation, we performed phosphoproteomic analysis of HCT-116 cells treated with nocodazole for 0, 6, 10, 14, and 24 h containing $<$10%, $\sim$30%, $\sim$50%, $\sim$80%, and $>$90% M-phase cells, respectively. Temporal changes of phosphorylation induced by nocodazole varied among molecules. Some changes were induced after 6 h and sustained until 24 h, whereas other changes were observed after 14 h or more when the majority of the cells were arrested in the M-phase. These results imply that phosphorylation may be involved in the induction of the M-phase arrest by nocodazole or may be induced as a result of M-phase arrest.

P 194 A NEWLY DEVELOPED HYDROPHILIC POLYMER-BASED ION EXCHANGE CHROMATOGRAPHY COLUMN FOR SEPARATION OF VARIOUS BIOLOGICAL MOLECULES

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Ion Exchange Chromatography (IEX) is widely used for analysis and purification of biomolecules.We have developed a new polymer-based IEX column, named YMC-BioPro, specially designed for separation of proteins, peptides and nucleic acids. YMC-BioPro IEX columns are based on 5 micron porous and non-porous hydrophilic polymer beads with low nonspecific adsorption, and they show higher binding capacity and higher recovery of biomolecules compared to conventional IEX columns. The completely spherical and monodispersed beads, combined with optimal packing technology, provide high theoretical plate number and symmetrical peak shape. Excellent resolution is achieved from the high column efficiency coupled with the excellent selectivity of QA and SP ion exchangers.
In this poster, we will show benefits of YMC-BioPro IEX columns and superior separation of important biomolecules, such as monoclonal antibodies and DNA. We also show comparing and combining of IEX and Size Exclusion Chromatography (SEC) and Reversed Phase (RP) Chromatography. We also show PREP BioPro data for large scale industrial purification comparing with competitors bulk material.

P 195 VENOMICS, THE VENOMOUS SYSTEMS GENOME PROJECT

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The Venomics initiative is an ambitious project that was initiated among IST members back in 2003. It aims to characterize the unique genes and gene products of venomous animals for a better understanding and exploitation of the venomous system. The project consists in charting the genomes of a few selected animals and, in parallel, in analyzing their peptide and protein venom components and the mRNAs that encode these in venomous glands. The resulting data will offer a powerful body of information to interpret genome sequencing data and in particular to determine the genes associated with the venomous system, to uncover their organization along a genome sequence, and hence to illuminate both fundamental and practical aspects associated with venomous function. Furthermore, the great diversity of compounds, *e.g.* toxins, produced in the glands of venomous animals and acting on key physiological systems of vertebrates and/or invertebrates provide a great potential for the development of new drugs. Sponsored by the European Commission, the Venomics project "CONCO - The cone snail genome project for health" will be presented. CONCO involves 18 European laboratories, the J.Craig Venter Institute (USA) and the non-for-profit Toxinomics Foundation.
CONCO is an integrated project funded by the European Commission, Proposal N° 037592, FP6-2005-LIFESCIHEALTH-6.
Reference: Ménez A, Stöcklin R and Mebs D, 2006. 'Venomics' or: The venomous systems genome project. *Toxicon*, p. 255–259. See http://www.toxinomics.org and http://www.conco.eu.

P 196 PEPTIDOMICS AND PROTEOMICS OF *CONUS CONSORS* CONE SNAIL VENOM

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With the aim at identifying and characterizing all detectable peptides and proteins from a cone snail venom, an extensive study was performed using a combination of sampling techniques. The venom of several specimens of the piscivorous cone snail *Conus consors* was obtained, either by milking or by dissection. After a first step of chromatography, all collected fractions from both venoms were submitted to ESI mass spectrometry investigation. Data analysis revealed only ~50% of molecular mass overlap between both samples with 150 and 1078 masses detected in the milked and dissected venoms, respectively. Interestingly, several known conotoxins were found in the dissected, but not in the milked venom. Alternatively, milked and dissected venom were separated using two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) to analyse the components of the venom having molecular masses higher than 10 kDa. We noticed, similarly as in the case of the low molecular venom components, that the milked venom contained less proteins than the dissected venom and, more importantly, that their compositions were significantly different. With a view to obtain a broader knowledge of *Conus consors* venom, these results underline the important complementarities of the venom sampling techniques.

Financial Support: EC Integrated Project CONCO LSHB-CT-2007-037592, Contract Number 037592 (http://www.conco.eu) and a grant P1-0207 from the Slovenian Research Agency. We are grateful to the government of New Caledonia, to the French Navy and to the French Institut de Recherche pour le Développement (IRD) for their support.

P 197 AN AUTOMATED SCREENING PLATFORM FOR THE DISCOVERY OF ION CHANNEL MODULATORS FROM NATURAL PRODUCT LIBRARIES USING SNAKE VENOM AS A MODEL

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Efficient screening of natural compounds on ion channel function requires the capacity to perform functional tests on large series of molecules. Here we present new developments that facilitate functional screening of natural or synthetic libraries and identify a novel structural class of nAChR ligand from the venom of the Australian snake of the species *Pseudonaja textilis*. In contrast to conventional large-scale random screening strategies, our first step was to use a unique set of databases to assist in the selection of crude venom from our proprietary Melusine collections for targeted screening (see www.melusine.com). This *in silico* step reduced cost and time by identifying likely candidate venoms. The second step employed fully automated liquid handling and sample preparation with state-of-the-art analytical methods (nanoLC-ESI-MS/MS and MALDI-TOF/TOF-MS/MS). Crude venom fractions were distributed in microplates and lyophilised to facilitate storage and transportation. The third step consisted of a fully automated electrophysiological screening of fractions on ion channel targets expressed in *Xenopus* oocytes (see www.hiqscreen.com). Fractions showing activity were submitted to further sub-fractionation and re-tested. Purity and content of active sub-fractions were determined by mass spectrometry profiling and the peptide sequence was determined by automated Edman sequencing. The final step involved the synthesis and detailed pharmacological characterization of active peptides to confirm the identified hits. The combination of these automated approaches streamlines and facilitates the discovery of active molecules from natural libraries.

P 198 *IN VIVO* PHARMACOKINETIC AND METABOLIC STUDIES OF PEPTIDE DRUGS BY MS AND MS/MS

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There is an increasing need for appropriate tools for *in vivo* metabolic, tissue distribution and PK-PD studies for endogenous and exogenous peptides and proteins. Such an investigation is prerequisite in the development and validation of drugs for human health. Furthermore, the detection and characterization of stable degradation intermediate products that have very short half-life allow the detection and tracing of drug administration on a much longer period than existing methods. We first introduce analytical methods for fast, precise and sensitive measurements of peptide hormone levels in body fluids and tissues using LC-MS and LC MS/MS strategies through the example of lutheinizing hormone-releasing hormone (LHRH) and analogues. Following a simple pre-analytical extraction protocol, addition or not of an internal standard, an on-line LC-MS/MS analysis allows to reach a sub-pM lower limit of detection. A second approach, using stable isotope labelled analogues and named isotope dilution assay (IDA), will be illustrated with insulin. IDA provides accurate and sensitive measurements of protein levels with direct positive identification of the target protein or peptide by ESI-MS, MS/MS or MALDI-TOF-MS. The importance of pre-analytical procedures, as well as of method development and validation according to FDA guidelines will be stressed.

P 199 PEPTIDOMICS TECHNOLOGIES AND THEIR APPLICATION FOR BIOMARKER DISCOVERY AND DRUG PROFILING

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While most proteomic methods include an enzymatic cleavage of the proteins into smaller well-defined fragments, small proteins and peptides can usually be directly submitted to separation and mass spectrometric detection. As a result, the analyzed sample is much less complex, which considerably facilitates the subsequent treatment of the

data. Furthermore, while conventional proteomics uses approaches based on a systematic identification and attribution of each detected proteolytic fragments, peptidomics is looking only at *m*/*z* signals with no need for database matching. We use bioinformatics and statistical analyses only to identify signals that significantly vary between two sets of samples (*i.e.* increase or decrease between healthy *versus* pathological). Only those signals of interest are then further investigated for identification structural determination. Furthermore, the information if a peptide was formed naturally in the body, *e.g.* through normal proteolytic activity or a pathological process, is kept. This is of major importance to identify degradation fragments or other metabolites, which would not be possible with conventional proteomic studies. For a reliable interpretation of the results, powerful proprietary biocomputing tools for the processing of the chromatographic and mass spectrometric data, such as automatic peak detection, alignment, and integration followed by statistical analysis to determine significant hits, have been developed in house and optimized, which we also like to use for drug profiling.

P 200 SYNTHESIS AND ANALYSIS OF ANALOGS OF HIAPP (1-19), A PEPTIDE INVOLVED IN MEMBRANE DISRUPTION

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Eastern Michigan University

Human islet amyloid polypeptide (hIAPP) is co-secreted with insulin in the β-cells of the pancreas. Capable of forming amyloid fibers and causing membrance disruption, this 37 amino acid peptide is cytotoxic to β-cells in type II diabetic patients. The N-terminus of hIAPP (residues 1-19) has been shown to bind to membranes and induce damage despite lacking the amyloidogenc (20-29) region. In this study, we show the activity of hIAPP (1-19) to be similar to that of full length hIAPP in causing disruption of lipid vesicles in a model membrane system. These results indicate that the N-terminal (1-19) region of hIAPP can be used as a model in drug target studies, in place of the expensive and synthetically challenging full length hIAPP. In addition, it has been proposed that aggregation is mediated by pi-stacking interactions between phenylalanine residues of hIAPP. The hIAPP (1-19) sequence was therefore modified by replacing phenylalanine and tested against differently charged lipids. The activity of the modified hIAPP sequences was then compared with that of hIAPP (1-19), as well as hIAPP.

P 201 DEVELOPMENT OF INSULIN-BASED INHIBITORS OF HUMAN ISLET AMYLOID POLYPEPTIDE

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Human islet amyloid polypeptide protein (hIAPP) is secreted in the pancreas along with insulin and is assumed to play a role in pathological development of type II diabetes. Amyloid is formed due to misfolding of the protein, which is cytotoxic to beta cells. Since insulin has been reported to interact with hIAPP and block amyloid formation, small fragments of insulin which contain the hIAPP binding site were synthesized, and the inhibitory effects were studied against an hIAPP analog in the presence of phospholipid vesicles. Preliminary results indicate that these sequences actually enhance membrane damage, as measured by the increased leakage of carboxyfluroscein dye from the vesicles.

P 202 CELL MEMBRANE DAMAGE BY THE TYPE 2 DIABETES ASSOCIATED PEPTIDE AMYLIN IN THE PRESENCE OF INSULIN

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Amylin (Islet Amyloid Polypeptide) and insulin are both stored and secreted from the β-cells of the pancreas. In Type 2 diabetics, insulin resistance leads to an increase in both insulin and amylin release. This excess amylin is known to cause damage to the β-cells of the pancreas, and plaques of the peptide are found in these patients postmortem. The purpose of this study was to determine if insulin, which has been shown to limit amylin fiber formation, would protect against its damaging effects on cell membranes. Carboxyfluorescein-encapsulating vesicles composed of different lipids were created as model membranes. Different concentrations of amylin and insulin were added alone and together, and the percent leakage of fluorescent dye from the vesicles was calculated. Results indicate that amylin causes damage to the model membranes in a concentration dependent manner as expected, and insulin does not induce damage on its own. Moreover, insulin failed to prevent amylin from causing membrane damage in the assay, and in some cases actually increased it. This implies that the destructive effects of amylin are mediated by more than merely fiber formation.

POSTER SESSION II ABSTRACTS

P 203 SUB-FEMTOMOLAR ACTIVITY OF A NEW CELL MIGRATION INHIBITOR

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UPARANT is a new peptide inhibitor of cell migration and invasion designed on the uPAR chemotactic sequence (SRSRY). Its mechanism of action is unprecedented: it binds with extremely high affinity ($IC_{50} \approx 10^{-18}$ M) to FPR and with very high affinity ($IC_{50} \approx 10^{-13}$ M) to the av chain of integrins. Several different human and mouse tumor cells exposed to UPARANT reacts within few minutes by reducing cell migration in a typical Boyden chamber experiment.

UPARANT is an easy to synthesize and purify low Mw modified peptide (620 amu). It adopts a turned structure in solution as foreseen in the design, and as confirmed by NMR spectroscopy. It is stable to chymotrypsin and trypsin digestion, and to several tissue protein extract for at least 4h.

UPARANT blocks the growth of primary tumor and metastasis by potently inhibiting neo-angiogenesis. Intravenous injections of 3 mg/kg UPARANT inhibits vascularization of subcutaneous implantation of matrigel in mice. UPARANT is also quite effectives in rabbit corneal pocket assay of VEGF-induced angiogenesis.

UPARANT blocks metastasis spread. Intravenous injection of highly invasive GFP-expressing HT1080 cells in the presence of 3 mg/kg UPARANT causes a 3- to 5-fold reduction of lung metastasis size in nude mice. The 30 months *in vivo* effect of UPARANT on six dogs with spontaneous breast carcinomas will also be presented.

Due to its low toxicity and efficacy UPARANT may represent a promising drug for anti-metastatic therapy and for chemoprevention.

P 204 PEPTIDES AS GENERIC ANTIDOTES TO COUNTER THE ACTION OF RICIN AND SHIGA TOXINS

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The bacterial protein Shiga-like toxin 1 (SLT-1) produced by pathogenic *E.coli* strains and the plant toxin ricin are well known examples of potent ribosome-inactivating proteins (RIPs) that block protein synthesis in eukaryotic cells. Their cytotoxic A domains share sequence homologies and catalyze the same cellular event, namely the depurination of a single adenine base in 28S rRNA. Yet, these toxins target different cell types. Since RIPs and in particular Shiga toxins are frequently found in worldwide outbreaks of contaminated food and water supplies, our group has been interested in finding generic approaches to block the action of all RIPs irrelevantly of their cellular targets. We have recently discovered by tandem mass spectrometry that the A chain of SLT-1 docks on ribosomes by first binding to the ribosomal proteins P0, P1, and P2. Moreover, the removal of the common last 17 amino acids of P1 and P2 abolishes their interaction with the SLT-1 A chain. When a synthetic peptide representing this 17-amino acid sequence was added to either the ricin A chain or SLT-1, these toxins were unable to block protein synthesis *in vitro*. Similarly, adding this peptide to cells exposed to SLT-1 led to their survival and proliferation. This peptide thus represents a starting chemical scaffold for designing generic antidotes to RIPs.

Supported by CIHR

P 205 MODULATING CONFORMATIONAL CHANGES IN SECRETED CHAPERONES: FROM IN SILICO DESIGN TO PRECLINICAL PROOF OF CONCEPT

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Compugen

Blocking conformational changes in proteins is a challenging task. Inspired by the susceptibility of viral entry to inhibition by synthetic peptides that block the formation of helix-helix interactions in viral envelope proteins, we developed a computational approach for predicting interacting helices. Using this approach, which combines correlated mutations analysis and Fourier transform, we designed peptides that target gp96 and clusterin, two secreted chaperones known to shift between inactive and active conformations. The gp96-derived peptide inhibited the production of inflammatory cytokines in stimulated human blood mononuclear cells and reduced circulating levels of endotoxin-induced TNF_, IL-6 and IFN_ in mice. The clusterinderived peptide arrested proliferation of several neoplastic cell lines, and significantly enhanced the cytostatic activity of Taxol *in vitro* and in a xenograft model of lung cancer. Furthermore, the predicted mode of action of both active peptides was experimentally verified. These results demonstrate a novel approach for rational design of protein antagonists.

P 206 ACTIVITY-DEPENDANT NEUROPROTECTIVE PROTEIN DERIVED PEPTIDE NAP: EVALUATING PROTECTION AGAINST HYPOBARIC HYPOXIA INDUCED OXIDATIVE STRESS IN RAT BRAIN

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Hypobaric hypoxia exerts a differential impact on brain affecting cognitive, memory and behavior functions. Areas of brain often affected include the cortex, the hippocampus, the basal ganglia, and the cerebellum. Focus of this study is on investigating the neuroprotective efficacy of the NAP peptide (NAPVSIPQ = Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln) derived from activity-dependant neuroprotective protein (ADNP) for acute hypobaric hypoxia mediated oxidative stress. Male Sprauge-Dawley rats (200 ± 10 g body weight) were exposed to laboratory simulated hypobaric hypoxia (25,000 ft, 250C) in the following groups (n = 6): (1) 3 days hypoxia group, (2) 7 days hypoxia group, (3) 14 days hypoxia group and (4) 21 days hypoxia group alongside intranasal administration of NAP peptide (2 ug/Kg body weight). For each exposure respective control groups were administered with PBS (Phosphate buffer saline) only. The brain samples were collected and assayed for antioxidant status of brain as well as parameters like SOD, Catalase, GST and GPx. The amounts of NAP peptide were also determined in different brain regions by HPLC. Furthermore, activation of nitric oxide pathway was also studied by activation of cGMP by ELISA. Currently studies are in progress to understand the molecular mechanism of NAP mediated protection against hypoxia-mediated oxidative injury and downstream mechanisms of action.

P 207 TMZ-BIOSHUTTLE, AN EXEMPLARY DRUG REFORMULATION BY INVERSE DIELS ALDER CLICK CHEMISTRY

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The syntheses as well as the characteristic physico-chemical traits of the inverse Diels Alder Reactions (DARinv) are described (*1–3*).

The chemical characteristics documented in (*4*) predestines the DARinv as an appropriate technology for intravital ligation of components. Hi gh local concentrations of diagn ostics will be so ught for molecular imaging and for s pecific therapeutics des igned as follows:Peptide Nucleic Acid (PNA) is documented as a powerful tool for biochemical applications. The PNA monomer is a kind of DNA which is dr amatically modified.(*5*) To enhance the application spectrum the development of suitable reactants is e ssential. Our favourite is th echemical synthesis of the Reppe anhydride-PNA monomer. (RE-PNA). This monomer differs from the commonly used PNA by a substitution with the Reppe anhydride compound allowing coupling to carrier molecules. This dienophile realizes th e fu rther functionalization by DARinv inside of living cells like liga tion of the drug temozolomide (TMZ) diene compound modified.(6) This reformulated TMZ-BioShuttle chould be considered as an example for prospective s ynthesis strategies of active m olecules b y the cl assic solid phase chemistry and for ligation chemistry.

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P 208 SYNTHESIS AND CONFORMATIONAL ANALYSIS OF A NATURAL PEPTIDE INHIBITOR OF HIV-1 INTEGRASE

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Integramide A is a 16-mer long, Iva (isovaline)/Aib-rich, effective peptide inhibitor of the enzyme HIV-1 integrase, an important target for anti-HIV therapy. Here, we describe the strategy and details of the peptide synthesis in solution of the natural compound and its DIva14-L-Iva15 diastereomer and the results of their chromatographic/mass spectrometric characterizations. We also report on our in-depth conformational analysis of the two compounds and some of their synthetic intermediates of different main-chain length in the crystal state (by X-ray diffraction) and in solvents of different polarities (using CD, FT-IR absorption, and 2D NMR techniques).

P 209 FUNCTIONAL RECONSTRUCTION OF STRUCTURALLY COMPLEX EPITOPES USING CLIPS™ TECHNOLOGY

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The functional mimicry of folded protein surfaces using peptides is a scientifically challenging problem. We recently developed CLIPS™ (Chemical LInkage of Peptides onto Scaffolds, (http://www.pepscan.com), a generic technology for structural fixation of linear peptides.[1] This paper summarizes an illustrative set of data from our research on the reconstruction of structurally complex protein surfaces, *i.e.* those that fail to be mimicked properly by linear peptides. In the past 5 years, our technology has proven an extremely valuable tool for 1) binding site mapping of therapeutically relevant mAbs, 2) generating hyperimmune sera via immunization with CLIPS peptides,[2] and 3) generation of monoclonal antibodies (mAbs) via the use of hybridoma technology. We currently have data available for more than 50 therapeutic targets. The examples described in this paper illustrate the potential of this powerful new technology.

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P 210 PEPTIDE VASODILATORS AS ANTI-TUMOUR DRUG DELIVERY VEHICLES

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Bradykinin (BK) is a physiologically and pharmacologically active vasodilating nonapeptide that has been found to be a crucial and potent factor in the growth of tumour cells. BK has been linked to the stimulation of growth in tumour cells causing them to migrate and invade normal cells.

This report presents extensions of the BK sequence to include sulfonamides and/or cyclodextrin (CD). Solid phase peptide synthesis techniques have been employed to allow sulfonamides to be generated at either end of the BK sequence. In addition, CD has been functionalised to allow the attachment of two distinct peptides in addition to performing all/part of this with CD on Rink resin. This three-way scenario is envisaged to allow a fourth option - using the CD cavity to carry added anti-cancer potential. Preparation of such species employing solid phase peptide synthesis appears non-existent.

Overall this study aims to investigate the use of vasodilators to invade and deliver anticancer agents into tumours. This proposal will combine the vasodilator potential of BK with cyclodextrin and/or sulfonamide chemistries in the quest for new cancer therapies. In addition to a tumour targeted drug-delivery system, the results of this project should lead to a better understanding of targeting and specific drug delivery to not only cancer sites, but also sites of other afflictions as well.

P 211 CRYPTIDES: DISCOVERY OF FUNCTIONAL CRYPTIC PEPTIDES HIDDEN IN PROTEIN STRUCTURES AND IDENTIFICATION OF THEIR SIGNALING MECHANISMS

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Peptidergic hormones and neurotransmitters are produced from their inactive precursor proteins by specific proteolytic cleavages. Recently, we purified and identified mitocryptide-1, a novel neutrophil-activating peptide that was produced from mitochondrial cytochrome c oxidase subunit VIII [1,2]. We named these functional peptides that were hidden in protein structures as "cryptides" [2,3]. We also found that many cryptides activated other cells such as mast cells [4–6]. Here, we report the discovery of mitocryptide-2 (MCT-2), another type of neutrophil-activating cryptide from porcine heart. MCT-2 activated not only HL-60 cells, which differentiated into neutrophilic/granulocytic cells, but also purified neutrophils from human peripheral blood. In this presentation, the signaling mechanisms induced by cryptides are also discussed.

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P 212 PEGYLATION OF PEPTIDES TARGETING FcRn: SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS

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The neonatal Fc receptor, FcRn, controls the half-life of IgG antibodies, therefore FcRn represents a possible drug target for the treatment of IgG-mediated autoimmune diseases.[1] High-affinity dimeric peptide binders[2] of human FcRn were derivatized with linear and branched PEGs of various size (5-40 kDa) and at various positions on the peptide to study the effect of these modifications on the pharmaceutical properties of the peptide. This presentation will discuss the synthetic strategy and structure-activity relationships of PEGylating anti-FcRn peptides correlating both *in vitro* activity with *in vivo* efficacy in model rodent studies.

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P 213 EFFECT OF NEW LEPTIN FRAGMENTS ON FOOD INTAKE AND BODY WEIGHT OF RATS

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Leptin plays an important role in the regulation of a variety of physiological functions, including food intake, body temperature and body weight maintenance. Tertiary structure of the leptin molecule reveals the existence of a four-helix bundle that is characteristic of the short-helix cytokines. In order to identify regions of the leptin molecule responsible for its bioactivity, a new series of decapeptides encompassing the region of fragments 98–122 [1] were synthesized and their effects on body weight and food intake were assessed when administered into the lateral cerbroventricle of normal rats. Peptides were synthesized by SPPS, purified by RP-HPLC and characterized by LC/ESI-MS. We also performed a conformational study of the peptides by circular dichroism in order to correlate the biological activity and secondary structure of the leptin fragments. Among the fragments tested, we found that Ac-hLEP110-119-NH2 was able to induce a significantly reduction in both body weight (>10%) and food intake (>39%). Interestingly, with fragment Ac-hLEP113-122-NH2 we observed a significative increased in the food consumption (>25%) but without any change in the body weight in comparison to the control. The use of synthetic leptin-derivate fragments may offer the basis for the development of compounds with potential application in human obesity or to its related metabolic dysfunctions. *Supported by FAPESP, CNPq, CAPES and UNIFESP/FADA.*

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P 214 A TETRAMERIC PEPTIDE-DRUG CONJUGATE TARGETING THE INTEGRIN $\alpha_V\beta_6$-POSITIVE NON-SMALL CELL LUNG CANCER

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Most chemotherapeutics exert their effects on tumor cells as well as their healthy counterparts, resulting in dose limiting side effects. The selective delivery of therapeutic agents to receptors over-expressed in cancer cells without harming the rest of the body is a major challenge in clinical oncology today. We have previously identified a peptide, named H2009.1 (sequence: RGDLATLRQL), from a phage displayed peptide library which binds to a large number of human lung adenocarcinoma cell lines. The cellular receptor for this peptide has been identified as the integrin $\alpha_v\beta_6$. Paclitaxel (Taxol) is a potent antitumor agent commonly used in the treatment of ovarian, breast and lung cancers, yet patients have to suffer some side effects caused by its normal tissue toxicity and aqueous insolubility. Here we report the design and synthesis of Taxol conjugated with H2009.1 tetrameric peptide via an ester linkage. The appropriate control-scrambled peptide conjugate has been synthesized as well. Characterization of tetrameric peptides and their conjugates has been determined by NMR, RP-HPLC and MALDI-MASS. H2009.1 tetrameric peptide-Taxol conjugate is more cytotoxic towards H2009 cells than scrambled peptide conjugate indicating that cellular uptake is mediated by H2009.1 peptide. Importantly, H2009.1 tetrameric peptide-Taxol conjugate is more cytotoxic towards a targeted cell than a cell line that does not express $\alpha_v\beta_6$ integrin. Finally, the peptide conjugate is highly water soluble which is a great advantage considering the severe hypersensitivity reactions experienced by patients treated with free Taxol in a Cremophore/ethanol emulsion.

P 215 USE OF PEPTOID-PEPTIDE HYBRIDS IN THE DEVELOPMENT OF Shc SH2 DOMAIN –BINDING INHIBITORS

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Src Homology 2 (SH2) domains participate in oncogenic cell signaling by recognizing and binding to specific phophorylated tyrosine sequences. Recently, using solid-phase derived peptides containing fluorescein isothiocyanate (FITC), we developed a fluorescence anisotropy (FA) competition - based binding assay for the Shc SH2 domain. We then employed this assay to examine several open - chain bis-alkenylamide containing peptides that were originally prepared as precursors for the planned synthesis of ringclosing metathesis (RCM) derived macrocycles. Unexpectedly, high binding affinities were observed for some of these open-chain peptides in which the original Gly was replaced with *N*a-substituted Gly (NSG) "peptoid" residues. Certain of these "peptoid-peptide hybrids" of the form, Ac-pY-Q-[NSG]-L-amide showed up to 40-fold higher Shc SH2 domain-binding affinity than the parent Glycontaining peptide. This presents the first application of peptoidpeptide hybrids to the design of SH2 domain-binding antagonists. Work is currently in progress to examine structural modifications that will lead to further enhancement of binding affinity.

P 216 THE USE OF PARAMAGNETIC AND FLUORESCENT-QUENCHING AMINO ACID TOAC FOR EVALUATING OF ANGIOTENSIN I- CONVERTING ACTIVITY

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Advantageously to other fluorescent quenching probes, the TOAC (2,2,6,6-tetramethylpiperidine-1-oxyl-4-amino-4-carboxylic acid), introduced earlier by us in chemistry [*J. Am. Chem. Soc.* (1993) 115, 11042] can be inserted at any position of an enzyme substrate. This work examined the specificity of angiotensin I-converting enzyme (ACE) that cleaves the angiotensin I (AI, DRVYIHPFHL) to produce the angiotensin II and inactivates bradykinin (BK, RPPGFSPFR). TOAC-attaching AI analogues at positions 0, 1, 3, 5, 8, 9 and 10 indicated that the first four analogues are substrates for ACE with k_{cat}/k_m values of 11.9, 9.2, 3.2 and 2.0 $\mu M^{-1}.min^{-1}$, respectively, in comparison with 15.4 $\mu M^{-1}.min^{-1}$ of the native AI. These results confirm that greater the proximity of the unnatural probe to the cleavage site (8-9), the smaller is the substrate specificity of analogues. Greater decrease in the substrate activity occurred with BK, where $TOAC^0$-BK and $TOAC^3$-BK presented k_{cat}/k_m of 20.9 and 38.9 $\mu M^{-1}.min^{-1}$, respectively (against 202 $\mu M^{-1}.min^{-1}$ for BK). Other analogues were devoid of substrate activity. EPR spectra indicated greater mobility for those analogues that were ACE substrates. A clear quenching property of TOAC affecting the Tyr^4 and $Phe^{5;\,8}$ residues in AI and BK, respectively was detected. The fluorescence of labeled substrates decreased with increasing distance between both residues, thus suggesting them extended structures. Differences between EPR spectra of TOAC-containing AI and BK substrates and cleavage products allowed the monitoring of ACE enzymatic activity.

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P 217 DESIGN, SYNTHESIS AND BIOLOGICAL ACTIVITIES OF NEW UROTENSIN II-RELATED PEPTIDES (URP)

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Urotensin-II (UII), a disulfide bridged peptide, is currently considered as one of the most potent mammalian vasoconstrictor identified. Recently, a UII paralogue, named urotensin II-related peptide (URP), was discovered and it was suggested that URP rather than UII may be the biologically active peptide in the regulation of autonomic, cardiovascular and reproductive functions. Both peptides exert their action through the activation of a specific membrane-bound UT receptor. The multiple effects of U-II and the broad expression pattern of its receptor indicate that U-II may be involved in physiopathological processes. In this study, we have designed and synthesized new URP analogs in which Trp-4 was replaced with natural, unnatural, constrained and charged amino acids in order to determine important physicochemical features for receptor binding and activation. Using different pharmacological models, we assessed the impact of these modifications on binding affinity and Ca^{2+} mobilization using UT-transfected cells, as well as in a contraction of aortic ring assay. Preliminary binding results demonstrated that analogs bearing an aliphatic side chain ([Cha^4]URP) or a turn inducing residue ([Tiq^4]URP) retained good binding affinity whereas other modification (Deg, Tic or Sar) led to inactive analogs. These data will give us new insights regarding the biological conformation of URP and will be used for the rational design of drug candidates potentially useful for the treatment of cardiovascular, endocrine and/or neurodegenerative disorders.

P 218 FUSION OF BIOACTIVE PEPTIDES TO ANTIBODY FRAGMENTS

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The attachment of bioactive peptides to immunoglobulin G fragment crystallizable (IgG Fc) can have several desirable effects which can aid in biochemical studies or the development of therapeutics. These effects include increasing the avidity of binding of bioactive peptides through dimeric presentation by the Fc region, linking antibody effector functions to binding of peptides to cellular targets, and increasing the *in vivo* circulatory half-life of small bioactive peptides. Investigations into the production and study of bioactive peptide-Fc fusion proteins using both recombinant and chemical methods will be presented. In this research yeast expression systems have been utilized to produce antibody Fc regions with controlled glycosylation for study of antibody effector functions. Examples of HIV entry inhibitor, integrin antagonist, and diabetes related peptides fused to IgG Fc will be discussed.

P 219 STRUCTURAL CHARACTERIZATION OF A NOVEL GLP-1 ANALOG TASPOGLUTIDE BY NMR SPECTROSCOPY

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Taspoglutide ([Aib8,35]hGLP-1(7–36)-NH2) is a human GLP-1 analog which is currently in phase 3 clinical development for the treatment of type 2 diabetes. With the α-aminoisobutyric acid (Aib) substitutions at positions 8 and 35, the peptide is highly resistant to enzymatic degradation at both N- and C-termini. In this study, Taspoglutide was structurally characterized using CD and high resolution NMR spectroscopy in the presence of a zwitterionic lipid to mimic the membrane environment. The results of the study reveal two α-helical domains located at the N- and C-termini which are connected via a flexible region around Gly22. Unlike the native GLP-1, a well defined C-terminal helix extends to the very end of the peptide. This extension of α-helix is clearly due to the presence of Aib35 substitution and is consistent with the higher potency observed in the biological studies.

P 220 DEVELOPMENT OF XEN2174: NON-CLINICAL STUDIES AND AN OPEN-LABEL I/IIa STUDY OF INTRATHECAL USE IN ONCOLOGY PATIENTS WITH MODERATE TO SEVERE PAIN

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Xen2174, a synthetic 13-amino acid peptide developed from χ-conopeptide MrIA, non-competitively inhibits the norepinephrine transporter. Analoguing revealed critical binding determinants were located in the larger of its two cysteine-stabilised loops. From these studies, Xen2174 was selected as a product candidate for the treatment of moderate to severe pain. Nonclinical studies demonstrated that Xen2174 provided a high level of neuropathic pain relief as well as long-lasting antinociception in a post-surgical pain model. Preclinical pharmacokinetic (PK) studies showed plasma Xen2174 to be rapidly cleared by the kidney. Toxicology data and a Phase I intravenous study supported the investigation of bolus intrathecal (IT) doses of = 40 mg in humans. A single bolus IT injection in a mixed oncology patients (N=37) with chronic pain demonstrated that Xen2174 was safe and tolerated at doses ranging from 0.025 to 30 mg. The majority of adverse events (AEs) reported were mild or moderate in severity and no consistent trends in AEs were identified. PK analysis revealed that both Cmax and AUC0–inf values increased dose-dependently. Clinical data over 4 days posttreatment suggested a possible association between dose and reduction in patient pain, especially in patients with a baseline VAS pain score of = 40 out of 100. The favorable clinical safety profile of single IT doses of Xen2174 up to 30 mg, and an association with pain reduction in this open-label study, support the continued clinical investigation of the usefulness of Xen2174 in pain management.

P 221 DIPEPTIDYL-QUINOLONE DERIVATIVES INHIBIT HYPOXIA INDUCIBLE FACTOR-1α PROLYL HYDROXYLASES-1, -2, AND - 3 WITH ALTERED SELECTIVITY

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Intracellular levels of the hypoxia-inducible transcription factor (HIF) are regulated under normoxic conditions via posttranslational oxidation of residues Pro402 and Pro564 in the HIF-1a subunit by prolyl hydroxylases (PHD-1,-2, and-3) and subsequent proteosomal degradation. Treatment of cells with PHD inhibitors stabilizes HIF-1a, eliciting an artificial hypoxic response that includes the transcription of genes involved in erythropoiesis, angiogenesis, and glycolysis. The different *in vivo* roles of the three PHD isoforms are not yet known, making a PHD-selective inhibitor useful as a biological tool. We hypothesized that elaboration at the 6-position of a newly identified quinolone scaffold with a short peptide could provide inhibitors with altered selectivity toward PHD-1, -2, and -3. Pd-catalyzed reductive carbonylation of the 6-iodoquinolone derivative was used to form the aldehyde directly, which was then attached to the solid support via reductive amination. Dipeptidylquinolone derivatives were prepared, and an initial screen of the compounds revealed retention of PHD inhibitory activity but an altered PHD-1, -2, and -3 selectivity profile.

P 222 PEGYLATION OF THE ANOREXIGENIC PEPTIDE NEUROMEDIN U YIELDS A PROMISING CANDIDATE FOR THE TREATMENT OF OBESITY

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Neuromedin U (NMU) is a highly conserved endogenous peptide, whose role in the regulation of feeding and energy homeostasis is supported by both pharmacologic and genetic data, including the report that central administration of NMU in mice produces inhibition of food intake and increase in energy expenditure, that NMUdeficient mice are hyperphagic and obese, while transgenic mice overexpressing NMU are lean and hypophagic. Two high affinity NMU receptors have been identified: NMUR1, predominantly expressed in the periphery, and NMUR2, primarily expressed in the brain. In spite of this literature suggesting NMU receptors as targets for the development of anti-obesity agents, reports of small molecule agonist leads are limited, and the very short half-life of native NMU hampers its direct use as therapeutic.
Here we describe the development of a metabolically stable analog of human NMU, based on conjugation of the native peptide to poly(ethylene) glycol (PEG). PEG-NMU was optimized by varying PEG size, site of attachment, conjugation chemistry, and length of the peptide moiety. As for other PEGylated agents, PEG-NMU is significantly less potent *in vitro* relative to NMU. However unlike the parent peptide, it is stable in plasma and has excellent pharmacokinetics in mice. More importantly, in diet-induced obese (DIO) mice a single subcutaneous administration of PEG-NMU induces robust and long-lasting food intake suppression and body weight loss; and this effect is sustained upon chronic treatment. In light of these data, PEGylated derivatives of NMU represent promising candidates for the treatment of obesity.

P 223 ORAL DELIVERY OF PEPTIDES: CLINICAL DEVELOPMENT OF A CALCITONIN TABLET

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Peptides such as salmon calcitonin (sCT) have poor oral bioavailability due to their relatively large size and the presence of labile peptide

bonds. A solid dosage tablet for oral delivery of peptides has been developed and tested in animals and humans. This enteric-coated tablet contains excipients that modulate intestinal proteolytic activity and enhance peptide transport. The enteric coating permits passage of the tablet through the stomach into the small intestine. A novel coated organic acid in the formulation inhibits pancreatic and brush border proteases, which have a neutral to alkaline pH optimum. Optionally, an acyl carnitine is used as an absorption enhancer. As part of the development of oral recombinant sCT (rsCT), 18 postmenopausal women were given a single tablet and plasma levels of rsCT and CTx-1 were measured at various times up to 24 hours following dosing. An acute decrease in CTx-1 levels (>75%) was observed in all 18 subjects with a nadir at approximately 6 hours post-dosing. A second dose selection study was then carried out in 24 postmenopausal women. Based on blood levels and CTX-1 measurements, an oral dose of 200 μg was selected to move forward to the pivotal study. The design, dosing regimen and end-points of this 12 month pivotal study will be presented. Stability studies indicate that the rsCT tablet is stable at room temperature for at least 9 months. The availability of this oral rsCT formulation will result in a safe and patient-friendly therapy for the treatment of osteoporosis.

P 224 NOVEL BH3 STAPLED HELICAL PEPTIDES FOR CANCER THERAPY

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Intracellular protein-protein interactions frequently involve helical peptide molecular recognition to modulate signal transduction, proliferation, survival, metabolism, transcription, translatioin and other regulatory pathways. Amongst a plethora of potential therapeutic targets and pathways involving such helical peptide molecular recognition are the Bcl2 family of apoptotic proteins. In particular, the BH3-only subfamily has provided the inspiration for the first in vivo effective synthetic peptides incorporating macrocyclic hydrocarbon 'stapling' (J. Amer. Chem. Soc. [2000]) to stablize helical conformation as well as key biological properties, including metabolic stability, cell-penetration, and good pharmacokinetic properties (Science [2000]). In this presentation, the design, structureactivity and in vivo efficacy of a second-generation of promising BH3 stapled helical peptides will be described. These studies are ultimately aimed at identifying a clinical candidate for the treatment of Bcl2-dysregulated cancers.

P 225 RETRO-ENANTIO N-METHYLATED PEPTIDES AS β-AMYLOID AGGREGATION INHIBITORS

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Current treatments for Alzheimer's disease (AD) offer only symptomatic benefits to patients. Therefore, the development of new treatments to actually slow, stop or cure the disease is urgently needed. One emerging attractive target for treating AD is to inhibit aggregation of β-amyloid protein (Aβ). Here we present a new peptide, inrD, designed using the retro-enantio approach in tandem with the use of N-methylated amino acids and show that disrupts β-amyloid aggregation and decreases βA cytotoxicity while proteolytically stable. In vitro and in silico studies have revealed that our strategy could be very promising not only for designing molecules to treat Alzheimer's disease, but also for designing ligands for a protein surface having a β-sheet surface-patch.

In conclusion, inrD is a very promising lead compound to treat AD, owing to its ability to interfere with βA aggregation and decrease βA cytotoxicity, while being stable to proteases. Docking and molecular dynamics calculations indicate that although inrD does not maintain the native sidechain pairing observed A molecules within the fibril, it can establish an effective hydrogen-bonding pattern with the fibril. Our results with inrD are testament of the value that the retro-enantio approach can have for obtaining peptides able to interact with β-sheet proteins. This observation may have major implications for the design of bioactive peptides for myriad therapeutic indications, including those targeted at disrupting protein-protein interactions.

P 226 PEROXIDASE MIMETIC DhHP-6 Protect Cardiomyocyte against Oxidative Injury

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DhHP-6 is a novel peptide mimic of peroxidases designed and synthesized in our laboratory, which has been demonstrated to exert significant cardioprotective effects against myocardial ischemia reperfusion injury in rats previously. In this study, we investigate the protective effects of DhHP-6 on cardiomyocytes against oxidative injury induced by hydrogen peroxide and hypoxia/reoxygenation. Compared with the model injury cells ($P<0.01$), in both H2O2-induced rat neonatal cardiomyocytes and hypoxia/reoxygenation-induced H9c2 cardiomyocytes injury, pretreatment with DhHP-6 significantly increased SOD activity, decreased the MDA level and LDH release, and improved Ca2+-ATPase activity. And DhHP-6 also significantly increased the viability of hypoxia/reoxygenation-induced H9c2 cardiomyocytes by 20%, and the viability of H2O2-induced rat neonatal cardiomyocytes by 45%. Furthermore, DhHP-6 significantly reduced apoptotic cells, caspase-3 activity, the mRNA expression levels of Hsp70, P21, and Heme oxygenase, but unchanged the catalase mRNA level by real time-PCR in H2O2-induced rat neonatal cardiomyocyte. In summary, DhHP-6 exerts significant protective effects on cardiomyocytes against oxidative injury induced by H2O2 and hypoxia/reoxygenation, and these effects may be related to its antiapoptotic properties.

P 227 COMPETITIVE IMMUNOASSAY STRATEGY FOR RAPID ESTABLISHMENT OF BIOANALYTICAL METHODS TO SUPPORT PHARMACOKINETIC ASSESSMENTS IN LEAD OPTIMIZATION STUDIES OF PEGYLATED PEPTIDE THERAPEUTICS

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Selection of candidate PEGylated versions of peptide therapeutics in early phase drug development relies heavily on pharmacokinetic (PK) assessments as a major component of the ADME characterization. However evaluation of the PK behavior of different PEGylated analogues is a non-trivial task that's complicated by numerous bioanalytical issues. To overcome these challenges we devised a versatile competitive assay strategy for rapid development of sensitive RIAs to expedite PK evaluations. This strategy involves 4 phases: 1) generation of an immunogen and subsequent generation of anti-peptide polyclonal antisera, 2) peptide radioiodination, 3) PAb characterization, and 4) development of a prototype RIA. This strategy is insensitive to the PEG moiety, as antisera are raised against a non-PEGylated version of a peptide therapeutic. Moreover RIA utilization only requires a crude polyclonal antiserum and obviates the need for Ab purification. We have used this strategy successfully a number of times for *de novo* establishment of RIAs for several PEGylated peptides in typical timeframes of only 4-5 months. Despite the trend of increased use of ELISAs for bioanalysis, we concluded that competitive RIAs represent an attractive alternative for rapid establishment of bioanalytical methodology to support PK assessments in Lead Optimization of PEGylated peptide therapeutics.

P 228 ROLE OF ALZHEIMER'S AMYLOID-BETA PEPTIDE AS A PUTATIVE TRANSCRIPTION FACTOR

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The amyloid beta-peptide (Aβ) containing neuritic plaque is one of the major hallmarks of AD. An exact physiological role for Aβ is presently unknown. Intracellular Aβ42 was shown to activate the p53 gene promoter resulting in p53-dependent apoptosis (Ohyagi et al, 2005). Our objective is to study DNA–protein interaction between Aβ and specific promoter sequences of different genes involved in AD, such as the larger Aβ precursor protein (APP), the beta–site APP–cleaving enzyme (BACE1) and apolipoprotein E (APOE). We have determined i)

whether the regulatory regions of APOE, APP, and BACE1 bind to Aβ; ii) what the target DNA motif and binding domain within Aβ are; and iii) whether APP single–nucleotide polymorphisms (C↔T at -3829 and T↔C at -1023, +1 transcription start, Lahiri et al., 2005) correspond to a change in Aβ binding capacity. Thirteen double–stranded oligonucleotides were designed, based upon 80% similarity to a p53 gene promoter decamer. Three promoter regions from APP, 4 from APOE and 3 from BACE were tested. The p53 oligomer was used as positive control. All oligomers were radiolabeled with ^{32}P, incubated with Aβ species and subjected to gel shift assay. Our gel shift binding results suggest that seven of the 13 oligomers showed DNA–protein interaction with Aβ in addition to positive control. Oligomers that showed DNA–protein interaction were incubated with various subsections of Aβ. Analysis of Aβ subunit binding with oligomers showed that residues 35–40 of Aβ were necessary for DNA–protein binding. Notably, the recently characterized APP promoter single–nucleotide polymorphism at -3829 had correspondence between loss of Aβ binding and loss of function in reporter gene assay. Thus, Aβ binds in a specific manner to a G–rich decamer. In addition some of the functional sites contain potential methylation sites. Disruption of a single critical base within the decamer eliminates binding, and this disruption corresponds to a loss of promoter function in a portion of the APP gene. These results suggest a physiological role of Aβ by its possible participation in gene regulation and the Aβ peptide as a therapeutic target.

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P 229 THE ApoE MIMETIC PEPTIDE, Ac-hE18A-NH2 RECYCLES IN THP-1 MONOCYTE-DERIVED MACROPHAGES

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The apoE mimetic peptide, Ac-hE18A-NH2 is composed of the receptor-binding domain LRKLRKRLLR covalently linked to the lipid associating peptide, 2F. Administration of 2F to dyslipidemic mice does not alter plasma cholesterol levels, while Ac-hE18A-NH2 dramatically reduces plasma cholesterol in apoE null mice and Watanabe Heritable Hyperlipidemic rabbits.

To determine if the addition of LRKLRKRLLR to 2F alters its effect on cellular function.

Pulse chase studies using [125I]Ac-hE18A-NH2 in THP-1 monocyte derived macrophages showed greater amounts of intact peptide in the cells than in the medium at later time points suggesting that the peptide is recycled in macrophages like apoE. However, this was not observed with [125I]Ac-18A-NH2. This difference was also reflected in the ability of Ac-hE18A-NH2 to facilitate the prolonged secretion of preβ-HDL particles for 3 days in the medium of HepG2 cells while Ac-18A-NH2 –mediated preβ-HDL secretion did not last beyond 1.5 days. In addition, Ac-hE18A-NH2 enhanced the secretion of paraoxanase-1 in Hep G2 cells. Both peptides were able to efflux cholesterol from macrophages. However, unlike Ac-18A-NH2, Ac-hE18A-NH2 –mediated efflux was not ABCA1 dependent. Both peptides enhanced the secretion of apoE from macrophages.

These results suggest that Ac-hE18A-NH2 is more like apoE in its action than apoA-I. The covalent linkage of LRKLRKRLLR to 2F confers apoE-like properties to an apoA-I mimetic. The ability of Ac-hE18A-NH2 to recycle in macrophages would prolong its potential therapeutic effect.

P 230 SELECTIVE VPAC-2 LIGANDS AS POTENTIAL ANTI-DIABETIC AGENTS

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Pituitary adenylate cyclase activating peptide (PACAP) and vasoactive intestinal peptide (VIP) share a common signaling pathway through two receptors: VPAC-1 which mediates increased hepatic glucose output and VPAC-2 which independently stimulates insulin secretion. Hypothetically, the selective activation of the VPAC-2 pathway may offer the benefit of enhanced glucose-dependent insulin secretion in the absence of increased glucose output. Our research program which was aimed at attaining a selective VPAC-2 agonist involved alignment and analysis of PACAP, VIP, helodermin and exendin sequences, and optimization at key sites to achieve maximal potency and selectivity. Several chimeric analogs with subnanomolar EC50 potencies at the VPAC-2 receptor and greater-than 100fold margins of selectivity with respect to VPAC-1 and PAC-1 receptors were produced. Selected analogs were further modified for enhanced proteolytic stability and time action. Validation in several in vivo models confirmed a favorable pharmacokinetic and pharmacodynamic profile and suggested that these agents may be useful for the treatment of Type II diabetes.

P 231 DESIGN AND SYNTHESIS OF POTENT INHIBITORS OF PHEX PART 1: MERCAPTOACYL DIPEPTIDES

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The PHEX gene (formerly PEX; Phosphate-regulating gene with homologies to endopeptidases on the X chromosome) was identified by a positional cloning approach as the candidate gene for X-linked hypophosphatemia (XLH). XLH is a dominant disorder of phosphate homeostasis characterized by growth retardation, rachitic and osteomalacic bone disease, hypophosphatemia, and renal defects in phosphate re-absorption and vitamin D metabolism. PHEX mRNA has been detected in bones and various studies have suggested that PHEX plays an important role in the control of bone metabolism. In order to improve our understanding of PHEX structure-function relationships and provide an insight into how bone mineralization may be modulated, we initiated a search for PHEX inhibitors.

Here we report on the design, synthesis, and *in vitro* biological activity of mercaptoacyl dipeptide-based inhibitors of PHEX. A parallel solid phase peptide synthesis approach was used for producing focused compound libraries resulting in single digit nanomolar PHEX inhibitors. Structure activity relationships studies revealed that the P1' aspartic acid residue is critical and its deletion or modification lead to a large decrease in activity. We explored multiple sites of diversity around the central aspartic acid and these results are also reported. In assessing selectivity for PHEX versus NEP, all the derivatives tested were highly selective for PHEX. Such compounds may have potential usage in regulating bone mineralization and/or as osteogenic agents.

P 232 DEVELOPMENT OF A NOVEL CLASS OF SYNTHETIC PEPTIDES TARGETING MITOCHONDRIA FOR CLINICAL APPLICATIONS

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As the cell's power house, mitochondria have been implicated in many human diseases. Enormous efforts have been putting in the quest for effective mitochondria targeted therapies, however, none have yet been made available. Here, we will share the discovery, research and development of novel mitochondria targeted peptides to prevent and treat mitochondria dysfunction related diseases. SS peptide, is a novel class of mitochondria-targeted peptides that can selectively partition to the inner mitochondrial membrane and protect the mitochondria. In vivo studies have demonstrated SS peptides' efficacies in many disease models, such as Parkinson's diseases, amyotrophic lateral sclerosis, insulin resistance, stroke, renal fibrosis and cardiac ischemia reperfusion injuries (1-2), just to name a few.

In addition to their pharmacology efficacy, SS peptides possess superior pharmacological properties that are rarely seen in peptide therapeutics. Pharmacokinetic studies demonstrated desirable absorption, distribution, metabolism and excretion (ADME) profile of SS peptides in vivo with an average of $t_{1/2} > 30$ mins in rodents and non-human primate. The toxicity study showed that there were no negative effects on biological systems associated with administering SS peptide at a concentration up to100 fold more than the doses used for in vivo efficacy studies. Moreover, the ease of manufacture and formulation of SS peptides have further proven them as desirable therapeutic candidates.

Based on the above, one of the SS peptides is currently being developed as therapeutic candidates for clinical indications related to ischemia reperfusion injury. The first-in-human trial will be initiated in second quarter of 2009.

1. Szeto HH. *Ann N Y Acad Sci*. 2008, 1147, 112–21. Review.
2. Szeto HH. *Antioxid Redox Signal*. 2008, 10, 601–19. Review.

P 233 DESIGN AND SYNTHESIS OF POTENT INHIBITORS OF PHEX PART 2: SUCCINIC ACID HYDROXAMATES

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In order to further study the inhibition of PHEX and potentially increase our understanding of its physiological role, we considered a different class of inhibitors other than the mercaptoacyl-based design reported in Part 1. The hydroxamic acid-based MMP inhibitors have been studied extensively and it is well established that the hydroxamate group is an efficient zinc binding moiety. In Part 2, we report on the design, synthesis, and *in vitro* biological activity of a series of PHEX inhibitors based on the use of a hydroxamate zinc binding group. Specifically, the focus is on hydroxamates derived from 2-methylcarboxyl-succinic acid. As in Part 1, SAR studies confirmed the importance of the carboxylic acid P1' side chain. One derivative with a 17 nM binding activity showed osteogenic properties in a rat model with mandibular defect.

P 234 PEPTIDES INHIBITORS OF F11R/JAM-A ADHESION MOLECULES AND POTENT ANTI-ATHEROSCLEROSIS DRUGS

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The F11 receptor (F11R) was characterized as an adhesion protein, aka JAM-A, which under normal physiological conditions is expressed constitutively on the surface of platelets and localized within tight junctions of endothelial cells. The utilization of specific F11R/JAM-A peptide antagonists and recombinant proteins demonstrated a role for F11R/JAM-A in the process of platelets adhesion to inflamed endothelial cells which initiates plaque formation leading to inflammatory thrombosis and atherosclerosis, where the platelets have a critical influence in the progression and development of cardiovascular disease (Babinska et al.,Thomb.Haemost.88:43–50, 2002). Thus, the development of new drugs antagonizing the F11R/JAM-A function could evolve as an effective new strategy for the treatment of atherosclerosis, heart attacks and stroke (Babinska et al., Thromb.Haemost.97:272–81, 2007). We present one of the first trials toward development of peptide-based inhibitors of F11R/JAM-A function. Among many trials, the peptide D-Lys-Ser-Val-Ser-D-Arg-Glu-Asp-Thr-Gly-Thr-Tyr-Thr-Cys-CONH2 proved to be a potent inhibitor of human platelets aggregation in vitro. Further molecular docking experiments showed that this peptide makes favorable hydrophobic and electrostatic interactions within the proposed binding site of JAM-1 (the X-Ray structure 1nbq.pdb was used as a template).

P 235 STRUCTURAL OPTIMIZATION OF GLUCAGON ANTAGONISTS

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Glucagon is a key counter-regulatory hormone that opposes insulin action. The structure-activity relationship of glucagon has been studied appreciably with the emphasis on the identification of peptide antagonists. The seminal advancements have been the discovery that N-terminal truncation and select substitution at amino acid nine provide potent and selective glucagon antagonism. In this report, we investigated structure-activity relationships of glucagon analogs using a highly sensitive measure of cAMP accumulation in HEK293 cell membranes that over express the human glucagon receptor. Two well studied glucagon antagonists [desHis^1, Glu^9]glucagon amide and [desHis^1, desPhe^6, Glu^9]glucagon amide were observed to display partial weak agonism and less than full antagonism in this cellular assay. The molecular basis for this residual weak agonism was explored through a set of N-terminally shortened [Glu^9], glucagon C-terminal amide analogs. It was observed that the stepwise deletion of both Ser^2 and Gln^3 were of little consequence to receptor binding, but eliminated any sign of glucagon agonism. Further shortening of the N-terminus demonstrated that Gly^4 and Thr^5 could also be removed without consequence to receptor binding or activation. Removal of Phe^6 significantly reduced the affinity of the respective glucagon for its receptor and rendered it a poor antagonist. The nature of amino acid six was explored through synthesis and characterization of various phenylalanine analogs, using [Glu^9]glucagon (6-29) amide as the peptide backbone. The full set was observed to vary in the potency of antagonism but in each instance the peptide analogs were observed to be full antagonists without any sign of residual weal agonism. These observations highlight the importance of a highly sensitive biochemical assay and a minimal peptide sequence length to minimize the prospect of residual agonism in subsequent *in vivo* studies.

P 236 STRUCTURE-ACTIVITY RELATIONSHIP OF INSULIN AT POSITION A^{19}

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Insulin A19 is an extremely conserved site among all insulin and most insulin-related peptides that have been sequenced. It has been determined by biophysical structural studies to reside at the purported active site of the hormone. Its location is in close proximity to other keys residues that reside at the N-terminal end of the A-chain and the C-terminal end of the B-chain. Despite its apparent importance to biological function few structural modifications have been introduced at this site. This is partially a function of the poor potency observed in the limited set of insulin analogs where the native tyrosine was replaced with other aromatic amino acids, such as phenylalanine and tryptophan. A series of insulin analogs with different amino acids at position A^{19} has been chemically synthesized and biochemically characterized. It was observed that there was little tolerance for chemical modification relative to native tyrosine where the specific positioning of the aromatic hydroxyl group was observed to be of appreciable importance to achieving full activity. Additionally, functionalization of the phenyl ring of tyrosine was also observed to lower potency. Nonetheless, two non-tyrosine mimetics were identified that demonstrated virtually full potency at the insulin receptor in binding and activation, as well as comparable specificity as native insulin at the related IGF-1 receptor. The structure-activity relationship provides insight in the design of insulin analogs that might provide full potency and improved therapeutic index.

P 237 DYNORPHIN A AS A NOVEL LIGAND AT BRADYKININ RECEPTORS

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The endogenous opioid peptide Dynorphin A has long been known to have effects that are not typical of endogenous opioids. In primary neuronal cultures and cell lines des-tyrosyl Dynorphin A activates intracellular calcium signaling by an unknown mechanism that is independent of opioid receptors (Lai et al., *Nature Neurosci*, 9: 1534–40 (2006)). In experimental models of abnormal pain, dynorphin A levels in the spinal cord are significantly elevated and neutralizing spinal dynorphin levels reverses abnormal pain. Recently, our lab showed that the Dynorphin A-induced elevation of intracellular calcium levels could be blocked by the Bradykinin 2 receptor antagonist HOE 140. HOE 140 also blocked intrathecal dynorphin-induced hyperalgesia. Furthermore, unlike control mice, Bradykinin 2 receptor knockout mice do not display dynorphin-induced hyperalgesia. We hypothesize that Dynorphin A promotes chronic pain ultimately via the Bradykinin receptor.

We are examining the minimum sequence of dynorphin A required to develop a direct radioligand binding assay of dynorphin A at the bradykinin receptor and to evaluate the structure-activity relationship of Dynorphin A. Several fragments of Dynorphin A and fragments of Dynorphin A where the N-terminus and/or C-terminus were altered have been synthesized. Competition of [^{3}H]Kallidin binding by Dynorphin A fragments in rat brain membranes where non-specific binding is

defined by 10 μM bradykinin produced Ki values for the compounds Ac-DynA(2-7)-NH2, NH2-DynA(2-7)-NH2, Ac-DynA(2-9)-NH2 and Ac-DynA(3-10)-NH2 of 7.1, 1.3, 0.96 and 1.7 μM, respectively. These results indicate that these fragments have similar Ki values as Dynorphin A.

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P 238 PEPTIDE ANTAGONISTS OF HUMAN BAFF/BAFF RECEPTOR BINDING

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BAFF (B cell Activating Factor of the Tumor Necrosis Factor family), a soluble plasma membrane cleaved homotrimer, is a key regulator of B lymphocyte maturation. The application of Phage Display and Affymax Peptides on Plasmids technologies has identified a series of peptide sequences which bind specifically to human and murine BAFF. Furthermore, novel peptides with sequence features similar to these selected sequences have also been shown to antagonize BAFF binding to the BAFF-R in an ELISA assay. Importantly, these peptides were also shown to inhibit the proliferation of primary B cells when cultured in the presence of human BAFF. The structure activity relationships of the lead sequence, including an Ala scan, various architectures, and different sites and sizes of PEGylations, have been explored to enhance antagonist activity in the proliferation assay. A selected lead peptide, that shows equal potency to a BAFF antagonist currently in the clinic, was shown to significantly inhibit B cell proliferative responses in Balb/c mice stimulated with BAFF.

P 239 LEPTIN RECEPTOR ANTAGONIST PEPTIDES ACTIVE IN MOUSE MODELS OF CACHEXIA AND BREAST CANCER

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Leptin protein is a subject of many clinical trials against metabolic diseases but pharmacology issues pose recurrent problems. In particular, the therapeutic protein is unable to reach the hypothalamus where it can stimulate its receptor (ObR). As both leptin and ObR are overexpressed in cancer tissues, drug development also focuses on leptin mutants as antagonists. Nevertheless, peptide fragments rarely exhibit a consistent pharmacological profile in the presence and absence of leptin protein. We identified a modified 9-amino acid fragment of the ObR- binding leptin domain III that showed antagonist activities to cells expressing ObR at 50 nM when leptin was present, but without leptin protein the peptide analog became a 3 nM agonist and indeed induced minor weight loss in normal mice. After further chemical modifications we were able to identify full antagonists with IC50 and IC100 values of 5 and 50 nM, respectively. When administered intraperitoneally, subcutaneously or even orally at 0.1-1 mg/kg/day in normal mice, the peptides increased appetite and induced weight gain by up to 5% of total body weight yet showed no acute toxicity at 30 mg/kg. In a breast cancer xenograft model, these ObR antagonists significantly reduced tumor size. With their excellent serum stability and tolerability these modified peptides are prime candidates against some tumor forms and cachexia.

P 240 DISCOVERY OF PEPTIDES AS GRANULOCYTE COLONY- STIMULATING FACTOR RECEPTOR AGONISTS (SERIES I)

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Granulocyte colony-stimulating factor (G-CSF) is the major physiological regulator of granulopoiesis. Recombinant G-CSF has become a valuable supportive agent in the clinic that permits the administration of high dose chemotherapy by stimulating the production of granulocytes, and as a result, shortens the neutropenia associated with chemotherapy. Screening against Affymax headpiece dimer mutagenesis libraries yielded a range of binding peptide families that were subjected to a chemistry based optimization program. More importantly, these peptide sequences have shown no homology to either the natural G-CSF ligand or the receptor. One hit series containing three cysteines, after being allowed to dimerize via disulfide bond formation, acted as an antagonist with a single digit nM activity. Creating a tetramer version of this peptide (i.e., a dimmer of dimers) has been shown to behave as a full agonist in a cell proliferation assay with similar activity to recombinant G-CSF. In the more relevant CFU-G colony assay, however, the activity of the best hits was not equivalent to G-CSF. Further optimization of a lead peptide including selected amino acid mutations, architecture modifications, as well as various PEGylation strategies will be discussed.

P 241 DISCOVERY OF PEPTIDES AS GRANULOCYTE COLONY- STIMULATING FACTOR RECEPTOR AGONISTS (SERIES II)

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Granulocyte colony-stimulating factor (G-CSF) is the major physiological regulator of granulopoiesis. Recombinant G-CSF has become a valuable supportive agent in the clinic that permits the administration of high dose chemotherapy by stimulating the production of granulocytes, and as a result, shortens the neutropenia associated with chemotherapy. A novel synthetic peptide, which has shown no homology to either the natural G-CSF ligand or the receptor, was discovered using proprietary Affymax mutagenesis libraries. This peptide, which contains a cyclized disulfide bond through its two cysteines, bound to the G-CSF receptor with an IC50 of 5 μM. Upon dimerization with a chemical crosslinker, the peptide was an agonist in the cell proliferation assay with an EC50 of $\sim$ 100 nM. Optimization of this initial hit peptide, including mutations, different chemical linkers, and position of dimerization, has resulted in a significant enhancement of both activity and efficacy in the cell proliferation assay. A new series of peptides was discovered using a 2nd mutagenesis library based on this initial hit. After further optimization, several lead families of peptides were shown to have comparable activity to recombinant G-CSF with full efficacy in a cell proliferation assay. Selected lead PEGylated peptides were also shown to induce granulopoiesis in normal mice and several mouse models of neutropenia.

P 242 DESIGN & SYNTHESIS OF CALCITONIN-GENE RELATED PEPTIDE (CGRP) ANTAGONISTS

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Calcitonin gene related peptide (CGRP) is a 37 amino acid peptide and is one of the most potent endogenous vasodilators (*in vitro* EC_{50}=0.06 nM). CGRP is the major peptide transmitter within the trigeminal nociceptors and it is involved in cranial blood vessel dilation as well as peripheral neurogenic inflammation. Studies indicate that peripheral sensitization of the trigeminovascular system is involved in the initiation of migraine attacks.[1, 2] CGRP levels within the jugular vein are increased during migraine attacks and cessation of migraine pain is correlated with reduced CGRP concentrations.[3] Truncation of residues 1-7 results in a potent antagonist of the CGRP receptor (*in vitro* K_b=3.0 nM) but is rapidly degraded in human plasma (t½ = 20 min). We recently investigated CGRP(8-37)-NH_2 SAR in an effort to identify a prolonged in vivo circulating CGRP peptide antagonist. We initially found that multiple residues could be substituted to increase CGRP1 receptor affinity >50-fold, generating antagonist with a K_i of 0.06 nM.[4] Using complimentary in vitro and in vivo metabolic studies, we iteratively identified degradation sites and prepared analogs with significantly improved plasma stability and high affinity to the CGRP1 receptor. These studies enabled the generation of peptide antagonist of the CGRP receptor with >100-fold increased stability over CGRP(8-37)-NH_2 and with cynomolgus monkey and human in vitro plasma half-lifes of 68 h and 38 h, respectively. These metabolically stable and high affinity CGRP peptide antagonists are useful leads for the identification of a prolonged in vivo circulating antagonist to the CGRP1 receptor.

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P 243 HIGHLY CONSTRAINED GLUCAGON-LIKE PEPTIDE-1 ANALOGUES WITH IMPROVED BIOLOGICAL ACTIVITY AND ENZYME STABILITY

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GLP-1 offers a promising approach as a therapeutic agent for the treatment of type 2 diabetes due to its glucose-dependent insulinotropic effect. However, its rapid proteolysis by DPP-IV and aminopeptidase enzymes upon administration limits its clinical application. Thus in an effort to address this drawback and simultaneously to increase the potency of GLP-1, we synthesized a series of GLP-1 analogues containing multiple lactam bridges. Lactam bridges have been used to decrease conformational flexibility in peptide chains and often resulted in improved biological activity as well as enzyme stability. In our previous study, we employed lactam bridges between Lys*i*/Glu*i+4* to induce and stabilize α-helical conformation in GLP-1, which successfully led to the identification of GLP-1 analogues with increased potency. Based on the findings from our previous work, we have designed and synthesized a series of GLP-1 analogues containing multiple lactam bridges between Lys*i*/Glu*i+4* or Glu*i*/Lys*i+4*, using standard Fmoc/tBu chemistry. Lactam bridges in the highly constrained GLP-1 analogues were placed at both N- and C- terminal regions of GLP-1. The synthesized GLP-1 analogues were examined for their capability to activate the GLP-1 receptor and several showed up to 10-fold increase in potency. In addition, these highly constrained GLP-1 analogues were found to be quite stable to enzymes that are known to degrade GLP-1, including DPP-IV and NEP.

P 244 SOLID STATE ISOTOPIC EXCHANGE OF HYDROGEN IN PEPTIDES AND PROTEINS

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We summarize here recent data on the theoretical and experimental study of high-temperature solid phase catalytic isotope exchange (HSCIE) reaction that proceeds in a solid mixture consisting of organic substances applied onto an inorganic support [1].
The HSCIE reaction preliminarily applied onto an inert inorganic support occurs on the acidic catalytic centers formed in the solid mixture under the action of the spillover hydrogen. An important peculiarity of this reaction is that HSCIE proceeds at 150–200°C with a high degree of chirality retention in amino acids and peptides. The virtually complete absence of racemization at HSCIE makes this reaction a valuable preparative method.
The HSCIE reaction can be applied to obtain tritium labeled proteins (hemoglobin, insulin, and interferon) with high molar radioactivity. The HSCIE reaction enables to identify the area of contact of protein subunits and the sites of protein globule that participate in the interaction with other macromolecules.
The evenly tritium-labeled biologically active peptides with a molar radioactivity of 50-150 Ci/mmol were obtained by the HSCIE reaction. The distribution of the isotope label among all amino acid residues of these peptides allows the simultaneous determination of practically all possible products of their enzymatic hydrolysis.
The method, based on the use of evenly tritium-labeled peptides, allows the determination of peptide concentrations and the activity of enzymes involved in their degradation in biological samples both in vitro and in vivo (for example, the pharmacokinetics of Selank, an anxiolytic peptide, was studied in brain tissues using the intranasal in vivo administration).
The HSCIE reaction makes it possible to produce uniformly deuterium labelled amino acids, peptides, containing even non-standard amino acids and glycosylated fragments that can be used for quantitative MS analysis. Introduction of specified amounts of these deuterium labelled standards to biological objects prior to isolation, separation and trypsinolysis will make it possible to collect, with the help of chromato-mass spectrometry, quantitative information about the presence of proteins and polypeptides under study in the tissues.
The HSCIE reaction can be used also for the production of deuterium labelled pharmaceuticals (melatonine, histamine and dizocilpine with average incorporation of deuterium atoms exceeding 70%).

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P 245 A FAST AND ACCURATE EMPIRICAL EXPRESSION FOR PREDICTING PROTEIN-PROTEIN AND PROTEIN-PEPTIDE INTERACTIONS

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There is interest in developing protein and peptide based drugs. Hence, there is a need for accurate and efficient computational methods to predict protein and peptide interactions. Hence, we have developed an empirical expression for predicting protein and peptide binding free energies. The function was used to blindly and accurately predict binding affinities for a diverse test set of 31 protein interactions (R2 4 0.8, rmsd 4 1.0 kcal). The function was also used to calculate binding free energy surfaces for 21 protein-protein interactions, with a 90% success rate. In a third round of testing, the function was used to accurately predict the binding stability changes associated with interface alanine mutations. We recently used the function to dock tripeptides to the OPPA bacterial transporter protein. The function can be used to calculate binding affinities in seconds. The function is physics-based and provides a sound framework for rationalizing results. The data suggests the function can be used to efficiently predict therapeutic protein and peptide interactions. Finally, the function was recently integrated into our proprietary virtual combinatorial peptide screening (VCPS) platform. In a first round of testing the VCPS platform was used to reproduce the consensus sequence for peptide ligands of the Grb2 domain.

P 246 STRUCTURE-PROPERTY RELATIONSHIPS OF PEGYLATED PEPTIDES IN NORMAL AND RENAL INSUFFICIENT RAT MODELS

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PEGylation is an important approach for prolonging the *in vivo* action of therapeutic peptides and proteins, since the peptide is protected from renal clearance and proteolytic digestion by the polymer. However, while PEGylation is advantageous for improving the pharmacokinetic properties of the peptide, some loss in potency is typically observed.
We explored PEGylation as a technique to extend the effective duration of peptide activity for therapeutic peptides and proteins. Aspects of PEGylation chemistry which have been explored include polymer length (5–40 kDa), site of attachment, linkage chemistry (disulfide, amide, alkane and thioether) and spacers of different length and rigidity. As expected, 40 kDa PEG conjugates presented the most attractive PK profile in rats, with an *in vivo* half life of >10 hours. In conjunction with the extended plasma exposure of these PEG adducts, losses in potency were observed correlating with polymer length, but independent of the structure of the PEGylated moiety.
In disease indications where patients have renal insufficiency, PEG adducts of smaller size may be sufficient to achieve prolonged drug exposure. In a rat model of renal insufficiency, 5 kDa and 20 kDa PEGylated analogs of selected peptides showed a similar *in vivo* half life of 6 hours, suggesting that low molecule weight PEG offers the same level of protection as high molecule weight PEG in the absence of renal clearance. Low molecule weight PEG (2 kDa and 5 kDa) may be expected to have minimal effect on the intrinsic potency of the peptide, but can potentially contribute to the stability of the peptide in the absence of renal clearance. These studies may help the design of PEGylated peptides for optimal application in disease indications in which different clearance pathways dominate.

P 247 NMR-DETERMINED STRUCTURAL CHANGES THAT ENABLE α- MELANOCYTE STIMULATING HORMONE-DERIVED CYCLIC PEPTIDE TO PARTITION INTO MEMBRANE

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Obesity is a problem in the food consumption controlling system and the metabolic balance of the body from the pharmacological perspective. The melanocortin pathway plays a key role in controlling these systems. BL3020-1 (c(Phe1(C2)-D-Phe2-Arg3-Trp4(N2)-Gly5-NH2)) is a backbone cyclic peptide based on the naturally-occurring neuro-peptide alpha-melanocyte stimulating hormone that preferentially activates the human melanocortine receptor four. Oral bioavailability requires the compound to cross the gastrointestinal tract without succumbing to metabolic processes. NMR-determined structures of BL3020-1 and a numberof analogs were determined in solutions that imitate the vicinity of the lipid membrane (TFE/water) and the lipid environment (DPC micelles) to investigate the mechanism of trans-membrane permeation. Significant structural changes were induced inBL3020-1 in the membrane-mimetic environment where its backbone adopted a beta II turn-like motif, stabilized by a hydrogen bond that was not present in an inactive analog or in the non-lipidic solution. The structures also showed that in DPC the bulky aromatic rings shielded the hydrophilic residues thereby creating a hydrophobic shielding effect. The combination of the internal hydrogen bond and the hydrophobic shielding of polar residues may enable BL3020-1 to partition into a hydrophobic environment and penetrate the membrane.

P 248 SYNTHESIS AND BIOLOGICAL ACTIVITY OF NEW ESTERS OF ACYCLOVIR WITH BILLE ACIDS

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Acyclovir, 9-[(2-hydroxyethoxy)methyl] guanine (ACV) is an acyclic guanine nucleoside analogue that is widely used clinically as an antiherpetic agent. Its limited absorption (15%-20%) in humans after oral administration prompted the search for prodrugs possessing higher bioavailability. The L-valyl ester of aciclovir (valaciclovir) with bioavailability of 60% is obtained in this manner. The aim of this study was to design and to synthesize of new esters of acyclovir with bille acid (cholic, chenodeoxycholic and deoxycholic acids) and to explore their activity on the HSV-1, HSV-2 and Epstein-Barr virus (EBV). Ester of acyclovir with chenodeoxycholic acid showed index toxicity:activity the highest among the tested compounds and is as high as the acyclovir is.

P 249 pSK — A NOVEL AMPHIBIAN SKIN BOWMAN-BIRK-LIKE TRYPSIN AND TMPRSS2 INHIBITOR

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Here we describe a novel C-terminally amidated heptadecapeptide, isolated from the skin secretion of the frog of the genus *Rana pipiens*,{SAPRGCWTK SYPPKPCK-amide (pSK)} that contains a disulphide loop between Cys6 and Cys16. pSK shares a high degree of homology with the core inhibitory motif found in Bowman–Birk type protease inhibitors (BBI) such as sunflower seed trypsin inhibitor (aSFTI). A synthetic replicate of pSK, was found to be a potent inhibitor of trypsin with a Ki just slightly less than 96 nM, a value falling between those determined for aSFTI (2.2 nM) and the core inhibitory loop of BBI (119.0 nM). pSK also exhibited inhibitory activity (Ki = 6.85 μM) against TMPRSS2 (type II transmembrane-bound serine protease), a trypsin-like serine protease involved in tumor proliferation. This compares very favorably with the degree of inhibition observed with aSFTI (Ki = 6.97 μM) and BBI (Ki = 19.55 μM). pSK one of the first examples of a naturally occurring inhibitor of TMPRSS2 and could prove useful in delineating the role of this protease in tumour proliferation and invasion.

P 250 SYNTHESIS AND CHARACTERIZATION OF PEPTIDES WITH GLUCAGON ANTAGONISM AND GLP-1 AGONISM

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Adult-onset diabetes (type 2) is characterized by the overproduction of glucose by the liver and reduced insulin secretion from the pancreas. Glucagon stimulates gluconeogenesis and glycogenolysis. The pursuit of selective glucagon antagonism constitutes an attractive target for new drug development. Glucagon-like peptide 1 (GLP-1) induces glucose-dependent insulin secretion. Exendin-4 is a GLP-1 homology that has proven efficacy in the treatment of type 2 diabetes. It seems plausible that glucagon antagonism and GLP-1 agonism would be additive in glucose lowering. The identification of dual acting peptides constitutes a challenge as glucagon and GLP-1 receptors possess high homology and the molecular basis for antagonism at one and agonism at the other is not known.

A peptide of mixed glucagon antagonism and GLP-1 agonism has been reported[1]. These peptides are structurally surprising as they possess the N-terminal histidine and this residue has been deleted in virtually all high quality glucagon and GLP-1 antagonists. Starting with glucagon antagonists of high potency, specificity and without an N-terminal histidine we have engineered the addition of GLP-1 agonism to such peptides. These peptides were biochemically demonstrated to be pure glucagon antagonists and full GLP-1 agonists. In the course of characterizing these peptides we were unable to substantiate the glucagon antagonism purported for the historical dual acting peptide[1]. Biophysical analysis of these dual acting peptides by CD analysis demonstrated that enhanced α-helical structure benefited recognition at the two receptors. To investigate the relationship between the secondary structure and the dual function of these peptides, crystallization and NMR studies have been initiated.

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P 251 IMMUNOGLOBULIN Fc-BASED PEPTIDE FUSION PROTEINS AS A BASIS FOR OPTIMIZING IN VIVO PHARMACOLOGY

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Immunoglobulin G (IgG) is the most abundant antibody class found circulating in human blood. Peptide fusions to the Fc portion of IgG are being explored for multiple research purposes and as a means to optimize pharmacology of certain medicinal agents. When fused to biologically active peptides, the Fc can improve pharmacological action by multiple mechanisms. Peptide Fc-based chimeric proteins increase the duration of action by decreasing in vivo clearance. Since the peptide is presented as a dimmer, the Fc-fusion protein can also serve to increase inherent potency through an increase in the avidity of interaction with a target peptidyl-receptor. Lastly, the Fc-domain can stabilize peptides during biological-based synthesis and facilitate purification to dramatically increase the total yield. We have conducted studies directed at the production of peptide Fc-fusions in engineered yeast cells. Yeast expression, unlike bacteria, provides glycosylation, and yet it is faster and less costly than biosynthesis with mammalian or insect cells. Furthermore yeast expression is less expensive than chemical synthesis and when produced as an Fc-fusion does not require additional chemical modification to extend duration of action. We have produced a fusion of a glucagon-like peptide 1 (GLP-1) agonist to a specific IgG Fc as an example of this approach to medicinal peptide chemistry. Expression, purification and in vitro biochemical characterization of the GLP-1-Fc fusion protein produced in *Pichia Pastoris* will be presented.

P 252 COORDINATED INTERACTION OF THE INSULIN B-CHAIN HELICAL DOMAIN WITH THE AROMATIC ACTIVE SITE

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Insulin constitutes a hormone of central importance in physiology and a vital element in glucose management. Its use in diabetes care has been

of seminal significance for nearly a century. The advent of chemical biotechnology provides a new venue for optimizing insulin pharmacology through the use of synthetic chemistries that otherwise would be prohibitively expensive for commercialization. Based on previous work, amino acid 24 of the insulin B-chain has proven highly restrictive to structural alteration where subtle changes significantly destroy bioactivity. DKP Insulin has three modifications (B10Asp, B28Lys, and B29Pro) in the B chain which prevent the formation of non-covalent self-associated higher-order multimers. Our current report focuses on the synthesis and characterization of DKP insulin analogues at the 24th position of B-chain. We have observed that $AspB^{10}$ was specifically capable of restoring the insulin receptor binding affinity of low potency B^{24} insulin analogs to a variable degree that ranged from two to fifty-fold. This single B^{10} Asp modification is well recognized to enhance native insulin bioactivity to a modest degree and the NMR-based solution structure of DKP-insulin has been reported. The uniqueness of the current finding is that poorly potent insulin agonists were restored to virtually full potency through the simultaneous mutation at B^{10}. The results demonstrate that the local interactions at the C-terminal aromatic active site region are more heavily influenced by the central helical domain of the B-chain than previously appreciated and establish a basis for exploring further enhancements in agonism through the simultaneous changes in both regions of the hormone.

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P 253 SYNTHESIS AND ANALYTICAL ASSESSMENT OF A BICINE-BASED APPROACH TO PEPTIDE PRODRUGS

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Bioactive peptides constitute a rich source of new drug candidates. They typically display unique pharmacology, appreciable potency and molecular specificity. The most notable limitations are the parenteral nature of most peptide-based drugs and their relatively short duration of action, as a function of susceptibility to protease degradation and rapid renal clearance [1]. Employment of prodrug chemistry is an attractive approach to minimize the undesirable pharmaceutical properties [2]. While medicinal prodrug chemistry is a well developed field its application has been largely directed at conventional small molecule drugs and approaches to enhancing oral bioavailability. Our work focuses on the development of prodrug chemistry suitable for peptides and proteins with a specific emphasis on pharmacokinetics. A prodrug method divorced from secondary elements such as protease-cleavage is deemed most desirable as a means to maximize reproducible inter- and intra-patient pharmacology. We describe here the use of a bicine-based prodrug strategy to reversibly inactive peptide hormones at active site amines through site specific formation of reversible amides. The peptide synthesis of such bicine-based prodrugs is suitable to conventional solid-phase chemical methodology. A set of model peptides were synthesized and their intramolecular degradation to the parent peptide was studied by HPLC and MS methods under physiological conditions. The speed of reaction was observed to be a function of two chemical cleavages where the first is controlled by structure of a terminal dipeptide. The observed results with model peptides provide a basis for application to bioactive peptides to study the rate of cleavage in biologically relevant solvents such as plasma in *ex vivo* and *in vivo* settings.

P 254 EFFECT OF SIDE-CHAIN CHIRALITY ON PEPTIDE HELIX TYPE AND SCREW SENSE

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The role of side-chain chirality in directing type and handedness of peptide helices is still an unsettled issue. We have synthesized on solid-phase, purified, and fully characterized two Na-acylated hexapeptide amides each containing two helicogenic Aib residues and either four L-Ile or four L-alloIle residues. A detailed conformational analysis was performed by use of FT-IR absorption, 2D-NMR, and electronic and vibrational CD spectroscopies in CDCl3, MeOH and TFE solutions. The two peptides are largely helical despite the presence of 66% of non-helicogenic, β-sheet former (Ile, alloIle) residues. In these peptides based on amino acids characterized by asymmetric α- and β-carbons, the helical screw sense is largely dictated by the chirality at the α-position. The nature (whether 310 or a) of the helix formed seems to be poorly influenced by the side-chain chirality.

P 255 A PEPTIDO[2]ROTAXANE MOLECULAR MACHINE

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Peptido[2]rotaxanes based on an achiral, aromatic tetramide macrocyclic wheel locked onto various chiral Gly-L-Xxx dipeptide axles are known compounds.

Here, we synthesized and examined a non-symmetrical, three-station molecular shuttle peptido[2]rotaxane, powered by light and heat, based on a rigid, 310-helical, 9-peptide axle and a tetramide macrocycle. A significant part of the stabilization energy of this supramoleuclar structure arises from C=OH-N H-bonds. The axle, -D-Leu-(Gly)2-(Aib)6-O-(CH2)2-NH-FUM-NH-L-Leu- (where FUM is fumaric acid), is characterized by twelve amide and one ester groups. Photons were used as stimuli to shift the fumaric↔maleic equilibrium.

By use of NMR we were able to identify all of the three stations of this novel class of peptido[2]rotaxanes and to switch the wheel from one station to the next. This is the first example of a [2]rotaxane where the wheel makes a journey to one of the stations by wrapping up around a helical peptide axle. Interestingly, we also characterized simplified versions of this supramolecular system by X-ray diffraction.

P 256 A CONFORMATIONALLY CONSTRAINED, BENZOPHENONE CONTAINING, α-AMINO ACID PHOTOPHORE

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Photoreactive amino acids with benzophenone side chains, the prototype of which is Bpa (4-benzoyl phenylalanine), have found numerous applications as photoprobes for covalent modifications of enzymes and receptors. Here, we designed a new "constrained Bpa" amino acids, BpAib, belonging to the sub-class of the Cia↔Cia cyclized, Ca-tetra-substituted α-amino acids. First, the chemical synthesis and optical resolution of Boc-BpAib-OH will be reported. Then, an X-ray diffraction study of one of the two Z- BpAib-L-Phe-NHChx diastereomers allowed us to assign the absolute configuration of the BpAib enantiomers and demonstrated the high propensity for β-turn formation characterizing this amino acid. Intermolecular photo-cross-linking of Boc-(*S*)-BpAib-OMe with a simple Met dipeptide followed by an HPLC/MS/NMR analysis of the two equimolar and isomeric adducts, highlighted the photoreactivity of BpAib and the regioselective reaction of the benzophenone carbonyl carbon with the –SCH3 methyl function of the Met residue.

P 257 CHARACTERIZATION OF OCTAPEPTIDE GAP AS A PRECISE CARRIER FOR AN IMAGING OR CANCER THERAPEUTIC AGENT

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Glutamic Acid Polypeptide (GAP) has been employed as a targeted carrier, which conjugate to an anti-cancer agent via peptide or ester linkage. Moreover, its acid residue could chelate to radiometallic isotopes for imaging as well as radiotherapeutic application. Despite commercially available GAP, various ranges of peptide chain (MW 750-50,000) leads to occasionally difficult explanation of experiment results when it exerts action though glutamate receptors. Herein, we

rationally designed a new GAP to avoid complicated debates by limiting peptide size to eight glutamic acids. The octapeptide GAP was synthesized and well characterized to serve for an assessment of precise carrier.

P 258 DESIGN OF A NOVEL FRET SUBSTRATE WITH A LONG WAVELENGTH FLUOROPHORE FOR DETECTION OF MATRIX METALLOPROTEINASES

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Matrix metalloproteinases (MMPs) belong to a family of secreted or membrane-associated zinc endopeptidases involved in both normal and disease related tissue remodeling. They are capable of degrading extracellular matrix (ECM) proteins and processing a number of bioactive molecules. MMPs are key players in normal and pathological processes, including embryogenesis, wound healing, inflammation, arthritis, and cancer.

The use of FRET technology has facilitated MMP assay development. Previously described FRET substrates include Mca/Dnp, EDANS/DABCYL donor acceptor pairs. The introduction of the longer wavelength 5-FAM/QXL™ 520 FRET based MMP substrates increased assay sensitivity and signal/background ratio. To further improve MMP FRET assays, i.e. minimizing autofluorescence from reaction components, we developed a new series of MMP substrates containing the 5-TAMRA/QXL™ 570 FRET pair. In these FRET peptides, the fluorescence of 5-TAMRA is quenched by QXL™ 570 and recovered upon cleavage of the peptide by active MMP enzyme. The resulting fluorescence is monitored at excitation/emission wavelengths = 540 nm/575 nm. After screening several 5-TAMRA/QXL™ 570 substrates, one sequence was identified to be cleaved by most of the MMPs. This substrate was chosen for the development of the SensoLyte™ 570 Generic MMP Assay Kit. This kit provides high sensitivity and accuracy and can detect MMP-1, 2, 7, 8, 9, 10, 13, and 14. It is ideal for detecting of enzyme activity in the samples containing multiple MMPs or for high throughput screening of MMP inhibitors using purified enzymes.

P 259 CONFORMATIONS OF END-CAPPED MELANOCORTIN AGONISTS RCO-X-ZArg-Trp-NH2 BY 2D-NMR, CD AND COMPUTATIONS

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LK-184 Ph(CH2)3CO-HfRW-NH2 is a sub-nanomolar agonist at hMC1R [EC50 (nM): hMCR1/3/4 0.009 ± 0.004 / 4.7 ± 1.2 / 4.6 ± 2.8; *BMCL* 2003, 2647; 2004, 3997]. In order to understand origin of its activity, conformations of the core melanocortin tetrapeptide Ac-HfRW-NH2 (1), Ph(CH2)3CO-HfRW-NH2 (LK-184, 2), its D- and L-b-Phe analogs (3, 4),Ph(CH2)3CO-PfRW-NH2(5),Ph(CH2)3CO-(1-aminocyclopropanecarboxamide)fRW-NH2 (6), and the truncated analog LK-394 Ph(CH2)3CO-HfR-NH2 (7) were studied by CD and 2D-NMR. Contrary to the general belief that a b-turn is essential for melanocortin activity, the most potent (2) existed in a b-turn conformation only in MeOH, while low potent 1 showed the maximum percentage of b-turn in the whole set both in MeOH and water. Introduction of b-turn stabilizing amino acids in X (5, 6) or Z (3, 4) positions of XZRW did not result in a substantial increase in b-turn conformation and rendered compounds inactive. These data show that the phenylbutyric tail provides high MC1R agonism in 2 despite distorting the b-turn conformation of the HfRW backbone.Conformational analysis (Macromodel, OPLS_2005 in water, MCMM) confirms that 1 exists exclusively in a b-turn conformation (5 conformations within 10 kcal/mol!), 2 is much more flexible (122 conformations within 5 kcal/mol) but well aligned and 7 lacks any structure. Results of NMR constrained MD (Insight II, CVFF force field) will be discussed. Supported by Skin Cancer Foundation Henry W. Menn Memorial Award (JJK) and grant RO1CA114095 (Z.A-M).

P 260 EFFECTS OF CONTROLLED GAMMA IRRADIATION UPON STRUCTURES OF BRADYKININ AND ANGIOTENSIN II

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The reaction products and mechanisms involved in the radiolysis of macromolecules of biological relevance are object of several studies. These factors (mainly the oxidation induced by produced free radicals) are known to be involved in many pathological disorders such as diabetes, cancer and Alzheimer's diseases. In order to investigate the modifications that gamma radiation may cause upon structure of peptides, the present report selected the vasoactive bradykinin (RPPGFSPFR, BK) and angiotensin II (DRVYIHPF, AngII) as models for investigation. Purified BK and AngII were submitted to 1 to 15 kGy gamma irradiation doses as preliminarily reported [Nardi et al., *Int. J. Radiat. Biol.* (2008) 84: 937–944]. The most abundant components of the irradiated peptides solutions were purified for further characterization. The most proeminent BK analogues produced were Tyr^8-BK and m-Tyr^8-BK. Noteworthy, the gamma radiation only affected the Phe residue at position 8 and not at 5, thus depicting a clear sequence-dependent effect. In the case of AngII, the produced analogues were Tyr^8-AngII, m-Tyr^8-AngII and Lys^6-AngII. These findings allowed concluding that gamma radiation effect is clearly residue and sequence-dependent. Current studies are underway involving other physiologically relevant peptides aiming at elucidating the potential of this innovative approach for generating unusual peptide derivatives, in some cases, not easily achieved through the conventional peptide synthesis methodology.

Supported by Fapesp and CNPq.

P 261 REPLACEMENT OF THE Tyr1 HYDROXYL GROUP OF THE DELTA OPIOID ANTAGONIST TIPP WITH THE N-((4'-PHENYL)- PHENETHYL) CARBOXAMIDO GROUP RESULTS IN A POTENT AND SELECTIVE DELTA OPIOID AGONIST

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The novel phenylalanine analogues 4'-[N-((4'-phenyl)-phenethyl)carboxamido] phenylalanine (Bcp) and 2',6'-dimethyl-4'-[N-((4'-phenyl)-phenethyl)carboxamido] phenylalanine (Dbcp) were synthesized and substituted for Tyr1 in the d opioid antagonist TIPP (H-Tyr-Tic-Phe-Phe-OH; Tic = tetrahydroisoquinoline-3-carboxylic acid). Unexpectedly, [Bcp1]TIPP turned out to be a potent and selective d opioid agonist in the mouse vas deferens (MVD) assay with subnanomolar d receptor binding affinity, whereas [Dbcp1]TIPP was a potent antagonist with similarly high d receptor affinity. Docking studies using Mosberg's d opioid receptor models indicated that the two peptides had overall similar binding modes, except for the large, hydrophobic biphenylethyl moiety which occupied distinct receptor subsites with the agonist and the antagonist docked to the active and the inactive form of the receptor, respectively. The different positioning of the biphenylethyl group in the agonist and in the antagonist is due to the restricted side chain rotational freedom of Dbcp, caused by the 2',6'-dimethyl groups, as compared to Bcp. The C-terminally truncated analogues Bcp-Tic-Phe-OH and Bcp-Tic-OH were d antagonists with a weak d partial agonist component, whereas the dipeptide Dbcp-Tic-OH was a potent and highly selective d antagonist. Because of their highly hydrophobic nature, these d opioid agonists and antagonists are of interest as pharmacological tools with possible blood-brain barrier crossing ability.

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P 262 SYNTHESIS AND BIOLOGICAL CHARACTERIZATION OF NOVEL FLUORESCENT ANALOGS OF VASOPRESSIN

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Fluorescent ligands are useful tools for studying G protein-coupled receptors intracellular trafficking. We report here the synthesis and biological activities of fluorescent analogs of Lys8 vasopressin (VP) and des-amino1 VP. Peptides were synthesized using Fmoc solid-phase

chemistry. Linker amino acids of varying lengths, both hydrophobic or hydrophilic, were coupled to the side-chain amine of Lys8 in other wise fully protected peptide on the resin, and 5 or 6-carboxy tetramethylrhodamine (TAMRA) was coupled to amino group of these linkers. After cleavage and deprotection, analogs were cyclized to form a disulfide linkage between residues 1 and 6. After purification by HPLC and confirmation by mass spectrometry, these analogs were characterized for receptor affinity and cAMP production. [3H]-VP binding assay suggests that presence of a primary amine at the N-terminus of ligand increases their affinity for VP receptor type 2 (V2R). From the nine peptides studied, VP-Lys8-PEG-Suc-TAMRA (VPTMR) exhibited a half maximal inhibitory concentration (IC50) of 195 nM that is around 39 times higher than that of the native VP (5 nM). Live cell imaging revealed that within seconds of perfusion of VPTMR to LLC-pk1 cells expressing an N-terminally tagged GFP vasopressin receptor (V2R-GFP), the ligand co-localized with the receptor at the cell surface. At later time after washout (60 min), VPTMR was absent from the cell surface and redistributed with V2R-GFP in perinuclear areas where V2R are degraded. These data show that VPTMR is a promising new tool to study the V2R trafficking in live cells.

P 263 SEMI-RIGID MC1 SELECTIVE AGONISTS BASED ON N-CAPPED His-D-Phe-Arg-NH2 TRIPEPTIDE CORE

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Based on our superpotent hMC1R selective agonist LK-184 Ph(CH2)3CO-HfRW-NH2 and its less potent truncated analog LK-394 Ph(CH2)3CO-HfR-NH2 [*BMCL* 2003, 2647; 2004, 3997], a new series of 30 N-capped HfR derivatives was designed and tested on mMC1R, MC3R-MC5R. In contrast to hMCRs, LK-184 is more potent at mMC4R and MC5R, while LK-394 shows mMC1R selectivity albeit with 590-fold loss in potency. All synthesized tripeptides have very low binding at mMC3R-MC5R. Several derivatives of 3- or 4-substituted cinnamic acids were more potent than LK-394 with *trans*-4-HOC6H4CH=CHCO-HfR-NH2 being the most active [EC50 (nM): MC1R 83.3 ± 13.7, MC3R 20500 ± 12600, MC4R 18130 ± 3258 and MC5R 935 ± 310; selectivity 1:246:217:11]. Shifting position of the OH, O-methylation, CH=CH reduction or inclusion in a cyclic system leads to a 3-7 fold drop in potency. Elongation of the C2 spacer or addition of polarizable non-hydrogen bonding substituents into *m*- or *p*-position to the cinnamic moiety elicit a weak binding at mMC3R. The observed biological activity correlates with increased conformational rigidity of the HfR core stabilized by the semi-rigid cinnamoyl tail (modeled with Macromodel, OPLS_2005 in water, MCMM in Schrodinger Suite 2008). Structure-activity relationship sand melonocortin receptor structure will be discussed.

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P 264 ANTI-ANGIOGENIC PROPERTIES OF A RHEGNYLOGICALLY- ORGANISED CELL PENETRATING PEPTIDE DERIVED FROM ENDOTHELIAL NITRIC OXIDE SYNTHASE

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Cell penetrating peptides (CPP) are now widely utilised as biologically inert vectors to affect the highly efficient intracellular delivery of bioactive cargoes. Commonly, delivery is achieved by employing a sychnologically-organised tandem combination of a CPP and an additional bioactive moiety. More recently, a QSAR-based algorithm has been developed to identify cryptic CPP motifs within the primary sequences of proteins. Our studies have, therefore, focussed upon the identification of rhegnylogic CPPs in which the multiple pharmacophores for cellular penetration and other biological activities are discontinuously organised within a single peptide. As an example of this approach, we have studied a cryptic CPP derived from nitric oxide synthase, eNOS492-507, located within a helical domain known to tightly bind calmodulin. As a highly efficient CPP, eNOS492-507 inhibits the proliferation, migration and tube-forming capacity of primary endothelial cells and displays potent anti-angiogenic properties *in vivo*.

P 265 TARGETING THE NUCLEAR PORE COMPLEX WITH CELL PENETRATING PEPTIDES

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The past decade has witnessed an escalating interest in the therapeutic applications of CPP. We have employed a strategy for the modulation of intracellular biology using CPP that possess signal transduction modulatory properties. These proteomimetic CPP possess the dual function of modulating intracellular events whilst being intrinsically cell penetrant. One such peptide Cyt*c*77-101, derived from cytochrome *c*, demonstrates a strong propensity for cellular penetration and moderate intrinsic apoptogenic efficacy in astrocytoma. We therefore evaluated a range of chimeric constructs combining apoptogenic CPP and additional proteomimetic sequences. The most significant enhancement in cytotoxic potency was obtained using a chimeric construct *Ac*-Nup153980-987(Ahx)Cyt*c*77-101 composed of Cyt*c*77-101 and a target mimetic of FG nucleoporins (Nups), which demonstrated an LD50 of 0.73 μM, a concentration achievable *in vivo*. When translocated into cells by M918 as a disuphide-linked cargo, fluorescein-conjugated Nup153980-987 (*H*-CNFKGLSSK(Ne-5,6-carboxyfluorescein)-*NH2*) assumed a nuclear envelope distribution. We conclude that *Ac*-Nup153980-987(Ahx)Cyt*c*77-101 demonstrates potential as a potent inducer of apoptosis and propounds the nuclear pore complex as a therapeutic target.

P 266 SYNTHESIS OF ANTIBACTERIAL CICADAPEPTINS: A TOOL TO INTRODUCE UNDERGRADUATE STUDENTS TO RESEARCH

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Cicadapeptins are short sequences of seven amino acids isolated From the Entomopathogenic Fungus *Cordyceps heteropoda*. Among the unique amino acids present in Cicadapeptins are two aminoisobutyric acids and two hydroxyproline amino acids. Preliminary studies have shown that the Cicadapeptins posses antibacterial activity. To further explore their antibacterial properties their synthesis was started using solid-supported organic synthesis. A polystyrene-based resin with 2-chlorotrityl chloride as linker was utilized. The synthesis uses a convergent approach to increase the participation of undergraduate students new to research. The students were assigned one of the three fragments and were given full responsibility for the synthesis of their fragment. Our progress towards Cicadapeptin I will be reported along with the benefits the students obtained.

P 267 MIMICKING ENDOGENOUS PEPTIDE SECRETION BY INHALATION

A, Leone-Bay
MannKind Corporation

The lung is an excellent portal for the systemic delivery of peptide drugs because it provides direct access to the arterial circulation and bypasses degradation in peripheral tissue. Coupling this attribute with the very rapid drug absorption provided by Technosphere® technology gives a peptide delivery system with pharmacokinetic (PK) profiles that mimic normal physiology. Clinically, insulin inhaled as a Technosphere formulation (Afresa™) demonstrates a unique PK profile that mimics the body's natural response to a meal. The onset of insulin action occurs within minutes (C_{max} ~15minutes). This unique PK profile has the potential to provide improved postprandial glucose control, lower risk of hypoglycemia, and no weight gain. Clinically, GLP-1 inhaled as a Technosphere formulation is also characterized by a physiologic absorption profile without the nausea and vomiting characteristic of injected GLP-1.

Oxyntomodulin (OXM) and PYY administered to rats as Technosphere formulations are also absorbed very rapidly. Pharmacodynamic studies demonstrated that OXM and PYY administered as Technosphere powders provided greater reductions in food consumption than injected OXM and PYY despite the fact that drug exposure in the pulmonary groups was lower than that in the injected groups. Overall, Techno-

sphere technology is a versatile drug delivery platform that mimics intra-arterial injection characterized by very rapid drug absorption. This physiologic delivery profile provides advantages over the same drugs administered by injection.

P 268 AN INTEGRATED STRATEGY FOR IMPROVING PLASMA HALF-LIFE OF THERAPEUTIC PEPTIDES

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Peptides constitute a group of unique pharmaceutical medicines, and are considered as viable alternatives to biopharmaceuticals because of their advantages over small molecules (increased specificity) and antibodies (small size). However, their low oral bioavailability and vulnerability to proteolysis usually limit their clinical applications. One of the most attractive methods to improve peptide pharmacokinetics is to couple therapeutic peptides to human serum albumin (HSA) or a HSA-associated polypeptide, as HSA has a long circulation half-life ($t_{1/2} \sim$ 19 days). Such conjugation usually results in reducing the biological activity of peptides due to irreversible modification. Here we use glucagon like peptide-1 (GLP-1) as a model to demonstrate an integrative strategy for sustainedly releasing bioactive peptide from HSA-associated fusion polypeptides through plasma proteases. GLP-1 is derived from the translation product of the proglucagon gene and possesses several physiological properties that make it a subject of intensive investigation as a potential treatment of Type-2 diabetes. The circulation half-life ($t_{1/2}$) of GLP-1 is less than 5 min *in vivo*. Through our current strategy, unmodified GLP-1 can be sustainedly released from fusion polypeptides which associate with HSA by plasma proteases, while the $t_{1/2}$ of GLP-1 in fusion forms can be significantly increased in animal model. In addition to releasing GLP-1 effectively, this strategy could be also applied to other potent peptide therapeutics as well as small molecules, providing an alternative means of improving the pharmacokinetics of potent drug leads.

P 269 HIGH PENETRATION COMPOSITION (HPC) AND ITS APPLICATION IN TRANSDERMAL DELIVERY OF PEPTIDES

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We have developed a platform of high penetration composition with a design based on the structures of skin and cell membranes, and have successfully applied this platform to transdermally deliver nonsteroidal anti-inflammatory and anti-cancer drugs. This platform is revolutionary, as it may be applied to transdermally deliver peptides (up to 20 amino acids) without hurting the skin (no micro-needle or implant is required). One good example of such a use is with enterostatin (Val-Pro-Gly-Pro-Arg). A mechanism for the skin and membrane penetration of the high penetration composition of these peptides has been proposed. The penetration rate of the high penetration composition of Val-Pro-Gly-Pro-Arg through human skin (360-400 μm thick, from the anterior thigh area) is 305 ± 65 $\mu g/cm^2/h$. Anti-obese activity of the composition was determined. 40 obese female DB/DB mice (SLAC/DB/DB) with type II diabetes (16 weeks old, 50-60g) were divided into 4 groups. In group A, 0.1 ml of water was administered to the back of the mice (n=10) twice per day for 30 days. In Groups B, C, and D, 10 mg/kg, 2 mg/kg, and 0.5mg/kg of the pro-drug of the composition in 0.1 ml of water was administered transdermally to the back of mice (n=10) twice per day for 90 days. The results are shown in table 1.

Table 1. Anti-obese activity of the composition of Val-Pro-Gly-Pro-Arg in obese mice (SLAC/DB/DB) with Type II diabetes

Group	Weight (g) (Day 1)	Blood Glucose Levels (day 1) (mg/dL, no fasting)	Weight (g) (Day 90)	Blood Glucose Levels (day 90) (mg/dL, no fasting)
A (0 mg/kg)	55.5 ± 2.2	165.4 ± 31.2	71.5 ± 4.1	258.4 ± 38.1
B (10 mg/kg)	56.1 ± 1.8	160.4 ± 21.4	35.1 ± 2.4	122.4 ± 15.2
C (2 mg/kg)	57.8 ± 2.5	171.4 ± 23.1	41.3 ± 1.9	142.4 ± 18.7
B (0.5 mg/kg)	58.2 ± 2.3	168.9 ± 26.2	48.7 ± 2.1	151.4 ± 21.4

P 270 NEW PLATFORM TECHNOLOGY FOR PREPARATION OF ORALLY ACTIVE SMALL PEPTIDE DERIVATIVES: THE USE OF DIKETOPIPERAZINES AS THE UNIVERRSAL SCAFFOLD

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Oral delivery of peptide pharmaceuticals has long been an important challenge in the pharmaceutical industry. We have developed a platform for creating orally available biologically active compounds of low molecular mass (from 300 to 1000 Daltons), preferably composed of peptide derivatives, but not limited to peptides or peptidomimetics. These compounds consist of a diketopiperazine bio-carrier with built-in functionally active peptide fragments and bioactive molecules covalently attached via different linkers. Using our new platform, we have synthesized several libraries of compounds that affect the immune system and the central nervous system; they exhibit immunostimulating, immunosuppressing, adjuvant and analgesic activity. The platform technology we have developed allows taking a small peptide with a particular biological activity and transforming it into an orally available compound that possesses the same activity.

P 271 CHEMICAL TOOLS TO TARGET OBESITY: NOVEL PATHWAYS OF THE ADIPONECTIN-RECEPTOR SIGNALLING

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Adiponectin is an adipokine with anti-atherogenic, anti-diabetic and insulin sensitizing properties. Its effects on energy homeostasis, glucose and lipid metabolism are mediated by two ubiquitously expressed seven-transmembrane receptors, AdipoR1 and -R2. To incorporate the protective potential of adiponectin in drug development it is essential to understand the adiponectin signaling cascades in detail. First, we cloned the receptors from human tissue and expressed them as GFP-fusion proteins in mammalian cells. By using biophysical and protein biochemistry methods we could identify receptor dimers that are modulated in a ligand dependant manner. A yeast two-hybrid approach employing AdipoR1s cytoplasmatic N-terminus led to the identification of the regulatory subunit of protein kinase CK2. We confirmed the interaction in co-immunoprecipitation, ELISA experiments and co-localization analysis in mammalian cells. Next, we synthesized overlapping peptide segments that covered the full N-terminal segment of AdipoR1, as well as all intracellular receptor loops. We could localize the interaction site in an N-terminal basic region close to the transmembrane domain. Accordingly, we identified the regulatory subunit of protein kinase CK2 as a novel intracellular partner of AdipoR1 and have strong evidences of CK2 as an effector molecule in adiponectin signaling.

P 272 ROLE OF THE STRONGLY HELICOGENIC Aib RESIDUES ON THE PROPERTIES OF THE LIPOPEPTAIBOL TRICHOGIN GA IV

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Trichogin GA IV is a 10-mer member of the class of short lipopeptaibols that are linear, membrane-active, peptide antibiotics of fungal origin, characterized by the presence of three strongly helicogenic Aib residues, a fatty acyl moiety at the N-terminus, and a 1,2-aminoalcohol at the C-terminus. Trichogin GA IV and some of its analogues exhibit a strong activity against Gram positive bacteria and methicillin-resistant S. aureus with low hemolytic effect and a remarkable resistance to proteolytic degradation. In this work, we prepared by SPPS and fully characterized a set of trichogin GA IV analogues where the three Aib residues at positions 1, 4, and 8 are replaced by one, two, or three Leu residues. Leu is still an helix-supporting residue, but less effective than the non-coded Aib. Also, the Aib hydrophobicity is even enhanced in Leu.

A CD and FT-IR absorption preliminary conformational study, accompanied by membrane leakage experiments, was carried out to investigate the role of the Aib→Leu replacements.

P 273 SOLUTION AND SOLID-PHASE SYNTHESES AND PRELIMINARY CONFORMATIONAL ANALYSIS OF A NOVEL MEDIUM-LENGTH PEPTAIBIOTIC

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Ten years ago, a novel 14-mer peptaibol (tylopeptin B) was extracted from the fruiting body of the mushroom *Tylopilus neofelleus* and sequenced. Its primary structure, Ac-Trp1-Val-(Aib)2-Ala-Gln-Ala-Aib-Ser-Aib-Ala-Leu-Aib-Gln14-Lol (where Lol is the 1,2-aminoalcohol leucinol), is characterized by an N-terminal Trp (an excellent intrinsic chromophoric probe) and a central Ser (which can be easily functionalized in the side chain), both residues rarely occurring in peptaibiotics, and by two Gln (one in the center and one near the Cterminus) and five helicogenic Aib residues. This peptaibiotic is an interesting target in that it is selectively active against Gram-positive bacteria, but it is inactive against pathogenic fungi and Gramnegative bacteria. It is also almost unique among medium-length (14-15 amino acid) peptaibiotics in that it does not contain any Aib-Pro(Hyp) sequence. We synthesized in solution and in the solidphase as well, and fully characterized, tylopeptin B. A preliminary spectroscopic conformational analysis was performed on the full-length peptaibiotic and selected, protected, synthetic segments thereof.

P 274 OPTIMIZATION OF PROLINE-RICH ANTIBACTERIAL PEPTIDES FOR IN-VIVO APPLICATIONS

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The rapid emergence of drug-resistant bacterial strains, demands new antimicrobial compounds with novel modes of action. Here, we describe the design of novel peptide derivatives with (i) superior antimicrobial activities against Gram-negative bacteria (E. coli, K. pneumoniae, P. aeruginosa), (ii) high serum stabilities, (iii) no hemolytic activity, (iv) very low eukaryotic cell toxicity, and (v) bacteriocidal activity to overcome potential resistance mechanisms. The best peptide derivatives had minimal inhibitory concentrations (MIC) in the ng/mL range independent of antibiotic resistances. Additionally, the compounds killed the bacteria very fast in a nonlytic process by targeting intracellular bacterial targets. We could even extend the activity spectrum to some Gram-positive bacteria, by specific peptide modifications or more generally by addition of a short transporter peptide. First in-vivo studies show a low acute toxicity in mice and confirm the activity in a systemic infection model.

P 275 NOVEL ANTIMICROBIAL PEPTIDES FROM THE VENOM OF EUSOCIAL BEE HALICTUS SEXCINCTUS (HYMENOPTERA: HALICTIDAE)

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Two novel peptides named halictines were isolated and characterized from the venom of the eusocial bee *Halictus sexcinctus*. By Edman degradation and ESI-QTOF mass spectrometry, the primary sequences of halictines (Hal-1, Hal-2) were established as GMWSKILGHLIR-NH2 and GKWMSLLKHILK-NH2 respectively. Synthetic halictines exhibit potent antimicrobial activity against both Gram-positive and –negative bacteria and moderate hemolytic activity against rat erythrocytes. Halictines also have a potency to kill different cancer cells. CD spectra of Hal-1and Hal-2 measured in the presence of trifluoroethanol and SDS indicate that Hal-2 has a better tendency to adopt an amphipathic α-helical secondary structure in the anisotropic environment such as bacterial cell membrane than Hal-1. Also, NMR study showed that halictines in water solution behave as random coil peptides while in 30% trifluoroethanol solution the formation of α-helix (covering residues 4 to 11) was detected. The NMR secondary structure indicators confirmed higher population of α-helical conformation in Hal-2 compared to Hal-1.We prepared series of Hal-1 and Hal-2 analogs to study the effect of cationicity, hydrophobicity, amphipathicity and α-helicity on antimicrobial and hemolytic activities.

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P 276 EFFICIENT TOTAL SYNTHESIS OF DIPEPTIDIC ANTIBIOTICS (+)- NEGAMYCIN AND ITS DERIVATIVES

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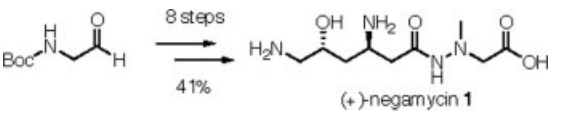
(+)-Negamycin 1, a dipeptidic antibiotic containing a hydrazine peptide bond is thought to be a potential therapeutic agent for genetic diseases such as Duchenne muscular dystrophy (DMD) with a nonsense mutation in the dystrophin gene. Negamycin has attracted a great deal of synthetic interest. Several syntheses of negamycin in both racemic and optically active forms have been accomplished over three decades after its discovery. However, an efficient shortened synthetic route of (+)-1 and its derivatives appears significant to develop promising new therapeutic candidates for DMD and other diseases caused by nonsense mutations. Here, we report a new synthetic route for (+)-1 from commercially available achiral Boc-Gly-H using modern organic chemistry techniques, including asymmetric allylboration, microwave-assisted ruthenium catalyzed cross-metathesis and asymmetric Michael addition to afford (+)-1 with an overall yield of 41% in 8 steps. Current efforts with this synthetic approach are expanding into Medicinal Chemistry to discover new drug candidates with potent read-through activity for chemotherapy of Duchenne muscular dystrophy.



P 277 SOLID-PHASE SYNTHESIS OF NON-RIBOSOMAL PEPTIDES AS POTENTIAL ANTI-CANCER AND ANTI-MICROBIAL AGENTS

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Two significant concerns of pharmacotherapy are the lack of effective anti-cancer agents and infection by multi-drug resistant pathogenic micro-organisms. Current therapeutic options for cancer are limited to a few well-established agents which are broadly toxic and of limited efficacy. Bacteria such as MRSA are exceptionally difficult to eradicate from the clinical setting, often demonstrating multiple resistance mechanisms to even last-resort anti-bacterials. The high probability of mortality in therapeutic failure necessitates the development of new classes of agents with novel modes of action.

Secondary metabolites from micro-organisms often have significant potential as chemotherapeutics as their biosynthesis has evolved as a defence mechanism against other micro-organisms. One class of natural products exemplifying this are the non-ribosomal peptides (NRPs), including daptomycin, triostin A and the mixirins. NRPs are typically structurally complex, hampering their exploitation as therapeutics since availability of material is dependent on laborious extraction/purification after fermentation. Chemical synthesis offers more rapid access to structurally-diverse analogues, with which to establish more well-defined SAR and to develop novel potent agents.

We have developed solid-phase methodology for the total synthesis of these complex natural products, following solution-phase synthesis of the appropriate constituent non-proteinogenic amino acids.

P 278 STRUCTURE-ACTIVITY RELATIONSHIP STUDIES OF SINGLE-POINT D-SUBSTITUTED GOMESIN ANALOGUES

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Gomesin (*Gm*) is a potent antimicrobial octadecapeptide isolated from hemocytes of the *Acanthoscurria gomesiana* spider It contains two

intramolecular disulfide bridges (Cys2,15 and Cys6,11) (pGlu-CRRLCYKQRCVT YCRGR-NH2). NMR studies showed that *Gm* is folded in a two-stranded antiparallel beta-sheet connected by a noncanonical beta-turn. Here we describe the synthesis of Gm analogues where the L-amino acid residues were individually replaced by its corresponding D-isomer. Antimicrobial activities were evaluated in a liquid growth inhibition assay against *S. aureus*, *E. coli* and *C. albicans*. CD studies were performed in different environments and revealed that the analogues exhibited the same conformational behavior than the observed with Gm. Bioassays results showed that the replacement of the amino acid residues Leu5, Tyr7, Gln9, Val12 and Thr13 by its corresponding D-isomer caused a significative reduction in their antimicrobial activities. Interestingly, [D-Arg3]-*Gm* showed to be 2-fold more potent than *Gm*. All together our results suggest that the hydrophobic residues and the turn region are important for the peptide interaction with the microorganism membranes. No obvious correlation could be found between the biological activity and the secondary structural features inferred from the CD studies. In addition, hemolytic activities determined at increasing peptides concentration showed that there is a direct correlation between antimicrobial and hemolytic activities of all peptide tested.

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P 279 NOVEL COMBINED DELIVERY OF ANTIMICROBIAL PEPTIDES AND ANTI-INFLAMMATORY AGENTS FOR TREATMENT OF *CLOSTRIDIUM DIFFICILE* INFECTION AND DISEASE

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Clostridium difficile is the main cause of nosocomial diarrhoea in developed countries. The incidence of disease due to this bacterium has been increasing in recent years, with several outbreaks, including from strains such 027 associated with increased severity and mortality.[1] The number of antibiotics for clinical treatment of *Clostridium difficile* infection is limited.[2] Antimicrobial peptides offer promise as novel anti-infective agents to which the development of resistance by microbes may be restricted.[3] We have developed a novel approach to combined delivery of antimicrobial peptides and agents that protect host epithelia for treatment of *Clostridium difficile* infection and disease.

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P 280 CYCLIC LIPODEPSOPEPTIDES AS PROMISING LEAD STRUCTURES FOR NEW ANTIBIOTICS DISCOVERY

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Natural products are important source of pharmacologically active compounds or lead structures for the development of novel synthetically derived drugs. Among them cyclic lipodepsipeptides represent very attractive class of compounds for the development of new therapeutics since they exhibit a diverse spectrum of biological activities. In particular, they have shown the greatest therapeutic potential as antimicrobial agents. Cyclic lipodepsipeptide daptomycin (Cubicin®) was approved in 2003 for the treatment of skin infections caused by Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus*. However, cyclic lipodepsipeptide exploitation as lead compounds for the development of new antimicrobial therapeutics strongly depends on gaining unlimited synthetic access to this class of natural products and their analogs.

Katanosin B, plusbacin A and fusaricidin A are interesting members of cyclic lipodepsipeptide family that exhibit promising antimicrobial activity against various kinds of fungi and Grampositive bacteria. In order to obtain larger quantities of these two antibiotics and their analogs, we have developed a rapid and efficient solid-phase synthesis fully compatible with the standard Fmocchemistry. Our synthetic strategy comprises resin attachment of the first amino acid *via* side chain, stepwise Fmoc solid-phase synthesis of a linear precursor peptide, lipid tail attachment followed by the last amino acid coupling *via* ester bond and on resin head to tail macrolactamization. Synthesized cyclic lipodepsipeptides showed promising activity against Gram-positive pathogens including methicillin-resistant *S. aureus* and vancomicyn-resistant *E. faecium*. In addition, Ala-scan analogs of these cyclic lipodepsipeptides revealed importance of the lipid tail, ester bond and aminoacids side chains for their antibacterial activity.

P 281 TOTAL Fmoc SOLID-PHASE SYNTHESIS OF NATURALLY OCCURRING DEPSIPEPTIDE ANTIBIOTIC KATANOSIN B

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Katanosin B exhibits strong activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus faecium* (VRE). However, lack of commercial sources of katanosin B producing organism PBJ-5356 and, particularly, unlimited access to its synthetic analogs hampered katanosin B utilization as a lead compound for development of new antibiotics. Therefore, a total solid-phase synthesis of this natural product represents the first step toward complete exploitation of its antibacterial potentials. Katanosin B is a 11-residue depsipeptide cyclized by a lactone bridge between the L-*threo*-β-phenylserine and the C-terminal L-serine. Our strategy for katanosin B total SPPS include amide resin attachment of L-HyAsp *via* side chain, use of combination of four quasi-orthogonal removable protecting groups, stepwise Fmoc solid-phase synthesis of a linear precursor peptide, followed by the last amino acid coupling *via* ester bond and on-resin head-to-tail macrolactamization. Key building blocks, unusual amino acids phenyl serine (PhSer) and *threo*-3-hydroxyasparagine (HyAsn), were prepared starting from commercially available racemic mixtures.

P 282 LYSINE PERMETHYLATION, A TOOL FOR IMPROVING THE THERAPEUTIC INDEX OF ANTIMICROBIAL PEPTIDES

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Despite their interest as alternatives to conventional antibiotics in the face of an alarming worldwide antibiotic resistance crisis, antimicrobial peptides (AMPs) are still to gain a foothold in therapeutics, largely due to the limitations intrinsic to their peptide nature. A number of structural modifications tending to enhance AMP biological lifetimes have been proposed with various results. Lys permethylation, despite its predicament in epigenetic studies, has surprisingly received little attention from peptide medicinal chemists. We have examined the effect of Lys permethylation using a well-known AMP platform, the cecropin A-melittin hybrid CA(1-7)M(2-9), KWKLFKKIGAVLKVL-amide. Permethylation of all five Lys residues caused a drastic loss of antimicrobial potency but, interestingly, an even more pronounced reduction in cytotoxiciy (measured as hemolysis). Singly and doubly Lys-permethylated analogues were next evaluated, with encouraging results: most analogues were non-cytotoxic up to 60 μM but retained antimicrobial activities close to 1 μM that amounted to considerable enhancements in therapeutic index. The solution structures of the analogues by 2D NMR revealed significant differences with the parent non-permethylated CA(1-7)M(2-9), and thus provide some basis for explaining the improved therapeutic profile of the Lyspermethylated analogues.

P 283 SHORTER ARGININE HOMOLOGUES TO STABILIZE PEPTIDES TOWARDS TRYPTIC DIGESTION

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Trypsin digests peptides at the position of arginine. Because shorter homologues of arginine with appropriate protecting groups for conven-

tional Fmoc/tBu peptide synthesis are now available, three model peptides containing arginine and two shorter homologues of arginine were synthesized. They were incubated with trypsin in order to explore how stable the corresponding peptides are towards enzymatic degradation. It could be demonstrated that a peptide gains significant stability if arginine is being exchanged by a homologue containing one methylene group less. If arginine is substituted by a homologue with two methylene groups less the model peptide was almost fully stable over 24h towards enzymatic degradation.

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P 284 SIDE-CHAIN ASSISTED LIGATION IN PROTEIN SYNTHESIS

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Chemical ligation methods for the assembly of functional proteins continue to advance our basic understanding of protein structure and function. In this seminar, we will present our progress towards the full synthesis of HIV-1 Tat through utilizing our newly developed ligation method; side-chain-assisted ligation (1). The HIV-1 Tat was assembled from three fragments wherein the two thioester peptides were synthesized efficiently using the side-chain anchoring strategy following Fmoc-SPPS. The side-chain assisted ligation step was efficient and provided the ligation product in good yield. Following this step, native chemical ligation was used to fully assemble the HIV-1 Tat protein. Together, side-chain-assisted ligation provides more flexibility in the synthetic scheme and when combined with other ligation strategies e.g. native chemical ligation this could lead to the synthesis of various protein structures.

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P 285 AN IMPROVED PROCEDURE FOR CHEMICAL SYNTHESIS OF HUMAN HEPCIDIN

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Hepcidin is a peptide produced by the liver in response to increased iron stores. Its function is to inhibit iron uptake and redistribution. It is also released in response to inflammation and has recently been implicated as a controlling element in the sequestration of iron stores in the anemia of chronic disease and cancer related anemia. Over-expression of hepcidin leads to iron deficiency anemia and low levels of hepcidin cause iron overload disorders. Hepcidin regulation may be relevant to many disease states and treatments. Access to hepcidin in sufficient quantities for comprehensive studies is a challenge. Although hepcidin is only 25 residue in length, it has eight cysteines involved in four disulfide bridges. Here we report optimized synthetic procedures for the solid-phase synthesis & folding of hepcidin which provide significantly increased yields.

P 286 PEPTIDE THIOESTER FORMATION AND LIGATION USING A CYSTEINYL PROLYL ESTER (CPE) AUTOACTIVATING UNIT

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Peptide ligation chemistry has been developed based on the use of peptide thioester as a building block in the thioester method [1] and native chemical ligation [2]. We previously found that a cysteine-containing peptide is converted to the corresponding *S*-peptide (peptide thioester) by an *N* to *S* acyl shift reaction [3], and designed a cysteinyl prolyl ester (CPE) autoactivating unit for peptide ligation [4,5]. A peptide containing a CPE moiety at the C-terminus (CPE peptide) is spontaneously transformed into a Ca-diketopiperazine thioester *via* an intramolecular *N-S* acyl shift reaction, followed by diketopiperazine formation. The CPE peptide can be ligated with a Cys-peptide in one pot procedure. The peptide diketopiperazine thioester is also transformed into a peptide thioester by an intermolecular thiol-thioester exchange reaction with thiol compounds. Since CPE peptides can be prepared by standard Fmoc solid phase synthesis, it is a versatile alternative to the peptide thioester, providing a flexible ligation strategy that promises to be useful in polypeptide synthesis.

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P 287 NOVEL TOTAL CHEMICAL SYNTHESES OF INSULIN-RELATED PROTEINS

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Chemical protein synthesis provides an efficient and versatile tool for elucidating in unique ways the molecular basis of protein function. We have recently performed a new generation of structure-activity studies of insulin-like growth factor 1 (IGF-1) made possible by total chemical synthesis. Our convergent synthetic strategy for IGF-1 was based on native chemical ligation of three unprotected peptide segments, and a key step made use of an improved aryl thiol catalyst for high yield ligation at a hindered Val-Cys site. We have also developed a novel convergent synthesis of human insulin, in which we introduced a temporary 'chemical tether' to link the A- and B-chain. After a convergent synthesis using a combination of oxime-forming and native chemical ligations, the tether enabled us to fold/form disulfides with high efficiency. Subsequently, we cleaved out the temporary chemical tether to give native folded insulin with full biological activity.

P 288 ROBOTIC PARALLEL SYNTHESIS OF PEPTIDE-THIOESTERS

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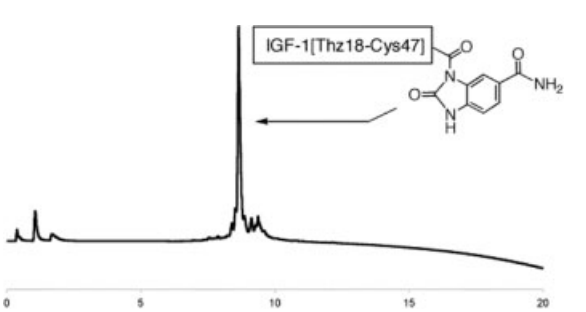
In the first stage of prototyping high throughput chemical protein synthesis, we have used x,y,z robotics and laboratory automation to develop efficient Fmoc chemistry SPPS protocols for the simultaneous parallel syn-



Automated robotic Fmoc SPPS preparation of peptide-thioesters.

thesis of the key peptide-thioester building blocks needed for chemical protein synthesis. This made use of a recently reported novel resin linker [Blanco-Canosa JB, Dawson PE: *Angew Chem Int Ed Engl.* 2008, 47:6851]. Typical data are shown in the Figure. We expect that this approach to parallel peptide synthesis will be satisfactory for the simultaneous production of ~100 peptide-thioester segments, each containing 30 to 40 amino acid residues. Ready preparation of peptide-thioesters enables the straightforward total chemical synthesis of proteins by native chemical ligation. Proof-of- concept total chemical syntheses of several predicted proteins from microbial genomes will be presented.

P 289 SOLID-PHASE-BASED SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL CHROMOPHORE ANALOGUES OF THE BIS- INTERCALATOR TANDEM

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Triostin A N-Demethylated (TANDEM) is a synthetic analogue of triostin A, a naturally occurring octadepsipeptide originally isolated from Streptomyces aureus S-2-210. This class of compounds interacts with duplex DNA by a mechanism of bisintercalation, thus conferring antibacterial and anti-tumour activity on these agents.

This work details the synthesis of twelve novel chromophore analogues of TANDEM by a solid-phase-based methodology. The flexibility of this approach has allowed access for the first time to compounds where the natural symmetry has been removed, by the installation of two different chromophores.

Biological evaluation of these compounds by footprinting assay demonstrates how minor alterations of the chromophore, or a removal of symmetry, can lead to dramatic changes in binding strength, specificity and biological activity.

P 290 SCALE-UP OF AN ANTIVIRAL POLYAMIDE COMPRISING PYRROLE AND IMIDAZOLE AMINO ACIDS WITH ANTIVIRAL ACTIVITY.

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Polyamides made up of N-methylpyrrole (Py) and N-methylimidazole (Im) can bind to DNA in a sequence-specific manner. By testing in human keratinocyte monolayers, we have discovered antiviral activity for certain Py/Im polyamides of the hairpin type. In order to further characterize the properties of active compounds, we needed to scale-up from typical manual or automated solid phase synthesis reactions that are done using up to 1 g of solid support. Here we report the manual scale-up of an antiviral compound that decreases the viral DNA load of human papillomavirus type 16 (HPV16) in human cell culture. tBoc methods were employed for protection of the amines, and these are compared with related reactions done using Fmoc methodology. The reactions were monitored by LC/MS analyses. The overall yield of the tBoc chemistry synthesis (16 cycles) was about 17%. Of particular issue with this chemistry is the poor nucleophilicity of the aromatic amines that are used for amide bond formation and are derived from pyrrole and imidazole-based amino acid building blocks. Prolonged coupling time and/or double coupling are often necessary when the synthesis is near completion. The Fmoc reagents became unstable during prolonged reaction times in the presence of DIEA.

P 291 SYNTHESIS OF FLUORESCENTLY LABELED O-MANNOSYLATED GLYCOPEPTIDES AS POMGnT1 SUBSTRATES TO INCREASE DETECTION SENSITIVITY

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Glycoproteins with *O*-Man glycans are important in muscle and brain development. Defects in these glycans are associated with neuromuscular disease. The POMGnT1 enzyme has a critical role linking a GlcNAc residue to the Man, and mutations in the enzyme give rise to glycosylation defects resulting in muscle-eye-brain disease, a form of muscular dystrophy. To explore the enzyme specificity of POMGnT1 and develop better assay substrates for diagnosis of disease, we have synthesized five mannosylated glycopeptides, FITC-PT(α-D-Man)AV, FITC-EPT(α-D-Man)AV, FITC-VEPT(α-D-Man)AV, FITC-YVEPT (α-D-Man)AV, and FAM-YVEPT(α-D-Man)AV, through incorporating 5(6)-fluorescein isothiocyanate (FITC) or 5(6)-carboxyfluorescein (FAM) on *N*-termini, based on the sequence from glycoprotein α-dystroglycan. Mannosylated glycopeptides were assembled starting from Fmoc-PAL-PEG-PS resin, using Fmoc-Thr-(Ac_4-α-D-Man)-OH as a building block. After resin cleavage, incorporation of FITC in aqueous $NaHCO_3$/acetone was compared before or after removal of acetyl groups on mannose residue. Alternatively, FAM was coupled to a resin-bound glycopeptide before cleavage and deprotection. The fluorescence of the fluorescently labeled *O*-mannosylated glycopeptides has an excitation maximum about 484 nm and emission maximum about 516 nm. Those glycopeptides with and without fluorescent probes are active substrates for POMGnT1.

P 292 SYNTHETIC STUDY OF VOLTAGE-GATED PROTON CHANNEL (VSOP/Hv)

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Voltage-gated proton channel (VSOP/Hv) has four transmembrane (TM) regions and permeates protons through a cell membrane by sensing membrane potential.1,2 To elucidate the voltage-sensing mechanism of VSOP, we started on chemical synthesis of VSOP(78-222), which sufficiently functions compatible with the full length VSOP.3 In this presentation, we describe the synthesis toward for VSOP(78-222). VSOP(158-222) containing two TM domains was prepared by Fmoc SPPS. *C*a-Peptide thioester of VSOP(121-157) and VSOP(78-120), each containing a single TM domain, were prepared based on Boc SPPS. These peptide segments are ligated in sequence from *C*- to *N*-terminus by the thioester method to provide VSOP(78-222). The detail of the segment preparation and ligation reaction will be reported.

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P 293 SELECTIVE ENZYMATIC MODIFICATION OF C-TERMINAL ESTERS OF PEPTIDES

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Peptide drugs may comprise different C-terminal functionalized groups. Besides the free acid and primary amide the Cterminal of a peptides may contain other functional groups, *e.g.* substituted amides and (thio)-esters. Several chemical methods have been developed for the C-terminal modification of peptides. However, each chemical method has its own limitations, often related to side reactions like racemization or side-chain rearrangement. Due to these limitations, new and mild selective methods are needed to obtain C-terminally functionalized peptides. Starting from different C-terminal peptide esters the serine protease subtilisin was used for the hydrolysis towards free acids, amidation towards (substituted) amides or transesterification in solution-phase synthesis. Firstly, a new process was developed for selective hydrolysis of C-terminal *tert*-butyl esters of peptide substrates [1]. Through medium engineering, conditions were defined to enhance the ratio of esterase *vs.* endopeptidase activity. Secondly, a highly efficient method for the conversion of C-terminal esters of peptides into (substituted) amide functions in dry organic media was developed [2]. Finally, a method was developed for the racemization-free transesterification of C-terminal peptide esters based on various alcohols.

The methodologies proved to be generally applicable to a broad range of peptides varying in sequence, length and C-terminal amino acid,

including non-natural amino acids like D-Ala. All reactions were highly chemo- and regioselective. High, up to quantitative yields were obtained depending on the actual C-terminal amino acid. Examples of all methods developed will be presented.

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P 294 CLONING, EXPRESSION AND PURIFICATION OF LARGE FRAGMENTS OF A GPCR

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G protein-coupled receptors (GPCRs) are a class of proteins, involved in cellular signaling cascades, which are composed of seven transmembrane (TM) domains connected by intra and extracellular loops (IL and EL, respectively). Structural information regarding these proteins is extremely valuable, but characterization of these proteins is extremely difficult due to their hydrophobicity, flexibility and large size. Smaller fragments of GPCRs are useful for studying membrane protein folding and NMR structural analysis. In this research two fragments of the *S. cerevisiae* GPCR, Ste2p, were prepared for biophysical investigations. A 3TM fragment containing 130 residues of Ste2p, G31-R161, including 19 residues from the Nterminal domain, the first TM through the third TM with connecting loops and five residues of the second IL was cloned downstream of the TrpΔLE fusion protein. Similarly, a 5TM fragment containing 212 residues of Ste2p, I128-L340, including 13 residues of the first EL, the third TM through the seventh TM with connecting loops, and 40 residues of the C-terminal domain was cloned into the same plasmid. Expression was optimized in *E. coli* strains in rich and minimal media. CNBr and Thrombin cleavages and purification of the target peptides from the fusion tag were attempted. The 3TM peptide has been isolated, characterized by MS and biophysical analysis has begun. The 5TM fusion protein has been proven to be more challenging and attempts at purification are in progress.

P 295 NEW CHEMISTRY AND NEW TOPOLOGIES FOR PEPTIDE-BASED APIs

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Over the last years, the pharmaceutical industry as shown a rekindled interest in peptides due to current and novel technological accomplishments, strategy developments, and advances in the areas of formulation and enhanced drug delivery of peptides. The manufacturing of new peptides involves longer amino acid sequences and more sophisticated building blocks and thus requires the development of new chemistry. Herein, we will discuss the new chemistry developed in our laboratory to fulfill the new challenges that peptide-based science is facing nowadays.

Topics to be addressed include: (i) the use of COMU and PyOCP, new uronium and phosphonium salts that do not contain explosive HOBt derivatives and provide a high yield and reduced racemisation; (ii) the use of acetonitrile as a solvent for the coupling of hindered amino acids and also as a good alternative for solid-phase peptide synthesis in the absence of DMF; (iii) the totally PEG-based, ChemMatrix®, which allows the synthesis of peptides containing up to 30-40 amino acids with a pharmaceutical grade of purity; (iv) the use of MIS, a new Arg side-chain protecting group that is more acid labile than the commercially available. Finally, new topological cyclic peptides with restricted conformation and better pharmacokinetic properties will be presented. These are *N*-Meaza peptides, *Siamese* bicyclic peptides, and *Dr. Jekyll & Mr. Hyde* peptides, which comprise a peptide part as well as a heterocyclic one.

P 296 N-METHYLATION AS SYNTHETIC RESOURCE FOR INCREASING STABILITY IN DEPSIPEPTIDES

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Many depsipeptides of natural origin display high biological activities. However, only a few of these compounds have entered clinical trials because of problems of bioavailability as well as low stability in plasma favoured by the presence of the ester bonds. Another important feature of natural peptides and depsipeptides is the presence of N-methylated residues in their sequence. Cyclosporin, for example bears six N-methyl amino acids. Since the presence of N-methyl groups confers resistance to proteolytic cleavage, the introduction of these groups has been widely used to prevent enzymatic degradation. Nevertheless, up to now, N-methylation has been limited mostly to the backbone but it has not been broadly studied as the replacement of the ester bond into a depsipeptide neither in side-chain to side-chain cyclic peptides. We describe herein the application of this strategy to three quite distinct depsipeptides of marine origin: (i) thiocoraline, a bicyclic thiodepsipeptide which acts as bisintercalator to the DNA; (ii) kahalahide F, which is now in preclinical stage; and (iii) IB-01212, a symmetrical octapeptide featuring a six-membered cyclic core. Bridged-NMe amides allow conservation of the hydrogen bonding map of the natural product. This approach could be used to enhance stability in other depsipeptides and side-chain to side-chain cyclic peptides with similar problems.

P 297 SOLID-PHASE PEPTIDE SYNTHESIS (SPPS) OF PROTECTED AND UNPROTECTED BRADYKININ AND BOMBESIN ANALOGUES

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Side-chain protected peptides are often required for down-stream synthetic manipulations.[1,2] Often these peptides are required in large amounts and high purity. Protected peptides are generally synthesized on highly acid labile resins and upon incubation with low acid concentrations the peptide is released with side-chain protecting groups covalently attached to reactive sites. We have evaluated several methods for the preparation of protected and unprotected bradykinin and bombesin analogues and here we report the results.

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P 298 LOW-SCALE AUTOMATED SYNTHESIS OF A PNA-PEPTIDE CONJUGATE ON THE PRELUDETM

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Peptide nucleic acid (PNA) is a DNA mimic in which the DNA backbone is replaced with a neutral pseudopeptide backbone. Its structure makes it a highly effective probe, but also results in low water solubility and poor cell membrane permeability. Peptides can be linked to PNA probes in order to overcome these disadvantages [1]. Because PNA syntheses are usually performed either manually or on a modified DNA synthesizer, the synthesis of a PNA-peptide conjugate usually requires two separate syntheses on different synthesizers. In this poster, a model PNA-peptide conjugate was synthesized in a single synthesis on a *Prelude* peptide synthesizer. The *Prelude's Single-Shot* delivery feature was used to deliver the expensive PNA monomers, and the *Prelude's* extremely low consumptions and frugal solvent usage were demonstrated.

[1]. Martijn C. de Koning, Gijs A. van der Marel and Mark Overhand, *Current Opinion in Chemical Biology* 7, 734–740 (2003).

P 299 FURTHER INVESTIGATIONS INTO MICROWAVE ASSISTED SOLID PHASE PEPTIDE SYNTHESIS: SYNTHESIS OF MODIFIED PEPTIDES

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The application of microwave energy for solid phase peptide synthesis (SPPS) represents a major breakthrough for overcoming incomplete

and slow reactions typical of conventional SPPS. Microwave energy has been applied successfully in a manual and automated approach for enhancing synthesis of peptides and peptidomimetics. We have recently demonstrated common side reactions such as racemization and aspartimide formation are easily controllable with optimized methods that can be applied routinely.[1] Our latest research has focused on the microwave assisted synthesis of modified peptides. Such modifications include *N*- and *C*-terminal modifications, cyclizations, and the incorporation of unnatural amino acids. These peptides were synthesized in a fraction of the time compared to conventional peptide synthesis without the need for unusual or expensive reagents and in a fully automated fashion to give peptides in high yield and purity.

1. Palasek, S. A.; Cox, Z. J.; Collins, J. M. *J. Pept. Sci.* 2007, *13*, 143–148.

P 300 FAST CONVENTIONAL SYNTHESIS OF A POLY-ALANINE PEPTIDE ON THE SYMPHONY®

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Protein Technologies, Inc. 4675 S. Coach Dr. Tucson, AZ 85714

Poly-alanine peptides have been associated with several human diseases and malformations [1] and have been used to form model beta sheet systems for studying Alzheimer's disease [2,3]. Due to their high propensity to aggregate, these sequences are extremely difficult to synthesize by conventional Fmoc solid phase peptide synthesis (SPPS). (Ala)10Lys was synthesized using conventional Fmoc SPPS in 17 hours. The effects of different resins, deprotection reagents and activators on the synthesis of the peptide were compared. It was found that adding 2% DBU to the deprotection solution resulted in a higher purity crude peptide, and allowed the synthesis time to be significantly reduced. A small portion of crude peptide was purified using an *RP*-HPLC column and the mass of the final product was confirmed with MALDI-TOF mass spectrometry.

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P 301 SYNTHESIS OF OPTICALLY ACTIVE ANTI-BETA-SUBSTITUTED GAMMA, DELTA-UNSATURATED AMINO ACIDS VIA ASYMMETRIC THIO-CLAISEN REARRANGEMENT

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Beta-substituted gamma, delta-unsaturated amino acids are important unnatural amino acids. The versatile reactivity of the terminal double bond and the ability to functionalize at the β-position make them extremely useful in peptide sciences. Among the methods to prepare these amino acids, the Claisen rearrangement turns out to be a very efficient strategy due to it high asymmetric selectivity. Chelation-Claisen rearrangement has already proved to be good in producing syn-beta-substituted-gamma, delta-unsaturated amino acids, but the corresponding anti amino acids were not readily available until our recent novel Eschenmoser-Claisen rearrangement came out. Following this success, we envisioned that the thio-Claisen could be a complimentary method towards these amino acid preparations: the selectivity of forming (Z)-N,S-ketene acetal and the pseudochairlike conformations during the rearrangement could provide good asymmetric introduction. We here report this novel synthesis of anti-beta-substituted gamma, delta-unsaturated-amino acids, which give excellent diastereoselectivities and enantioselectivies with good yields.

Supported by grants from the USPHS and NIDA.

P 302 A [(2+2+2+1)+2] SEGMENT CONDENSATION APPROACH TO HEMOPRESSIN SYNTHESIS

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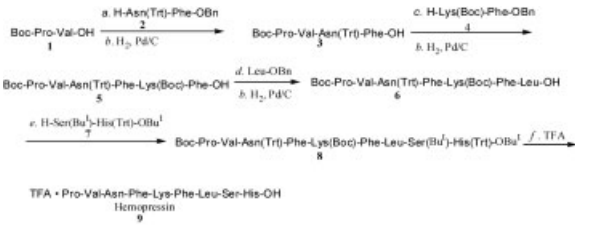
Hemopressin (HP) is a naturally occurring nonapeptide (Pro-Val-Asn-Phe-Lys-Phe-Leu-Ser-His-OH) derived from α-chain of hemoglobin. Interestingly HP has recently been identified as a peptide ligand that selectively binds CB1 cannabinoid receptors. Towards the synthesis and evaluation of a combination of HP peptide analogs, we developed a fragment condensation approach that generates dipeptide, tetrapeptide, hexapeptide, heptapeptide and nonapeptide. The synthetic details as outlined below will be presented.



P 303 INCORPORATION OF REVERSE TURN MIMETIC INTO CHIMERIC MELANOCORTIN-AGRP PEPTIDE TEMPLATE: STRUCTURE AND FUNCTION STUDIES

A. Singh,[1] J. Holder,[1] A. Wilczynski,[1] R. Witek,[1] M. Dirain,[1] A. Edison,[2] C. Haskell-Luevano[1]
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Based upon the studies that bioactive conformation of agonist at melanocortin receptor (MCR) involves _-turn containing His-Phe-Arg-Trp core sequence [1], it is hypothesized that incorporation of reverse turn mimetic into the superpotent chimeric AGRP-Melanocortin template [2] will result in the potent and selective ligands. To test the hypothesis "Focused" peptide-peptidomimetic library was synthesized, characterized and pharmacologically evaluated at the MC1R and MC3-5R. The reverse turn mimetic incorporated into AGRP-melanocortin peptide template resulted in analogues possessing nanomolar activities at melanocortin receptors.

This work was supported by NIH RO1DK064250.

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P 304 NEWLY DEVELOPED HIGH STRENGTH AND CHEMICALLY STABLE SILICA GEL BASED PREPARATIVE REVERSED PHASE PACKING MATERIALS

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YMC Co., Ltd., Ishikawa, Japan

A new product based on high strength silica gel and a superior bonding technology has been developed to provide improved recovery, selectivity, and longer life time for the preparative HPLC peptide separations. The novel preparative silica particle was successfully prepared by a new generation process, which allows for higher gel density than typical silica gel and a particle size distribution that is mono-disperse. For effective reversed phase peptide separations, the pore size and pore volume of these new particles are optimized depending on the molecular weight of the peptides. To enhance chemical stability and selectivity under the typical peptide purification conditions, the combination of chemical bonding method and functional group density was optimized for maximum performance.

The poster shows how repeated packing and unpacking of a large dynamic axial compression column with this new material generated no fines and no hence no increase in back pressure occurred compared to other commercially available packing materials. Cost effective peptide purification with high loadability, productivity, and recovery was achieved with significant small and large peptides.

P 305 SYNTHETIC ANALOGUES OF α-CONOTOXINS IN RESEARCH OF BINDING SITES OF NICOTINIC RECEPTORS

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α-Conotoxins, short neurotoxic peptides from the poisonous marine mollusks of the *Conus* genera are at present the excellent molecular probes for discrimination of various subtypes of nicotinic acetylcholine receptors (nAChRs). The purpose of this work was synthesis of α-conotoxins' analogues using the different procedures of disulfide bond formation and evaluation of their efficiency. Syntheses of α-conotoxin Vc1.1 and [Y0]MII-analogue were carried out with orthogonal Cys protection. The first and the third Cys residues had stable Cys(*S*-tBu) protection, whereas the second and the fourth Cys residues had acid-labile Cys(*S*-Trityl) protection. The syntheses of α-conotoxin PnIA variants with additional single or multiple amino acid substitutions we performed with simultaneous disulfide bonds formation. α-Conotoxin ImII[W10Y] has been synthesized by both the methods. To remove the tBu-groups we used a solution of trifluoracetate thallium (III) in trifluoracetic acid. For destruction of the complex formed in the course of this reaction, EDTA solution was used. Biological activity of the synthesized peptides was evaluated in competition with radioactive α-bungarotoxin for binding to the nAChRs and acetylcholine-binding proteins.

This work was supported by a grant of the Russian Foundation for Basic Research No-09–04-01476

P 306 A SECRETION BASED PROCESS FOR THE LARGE-SCALE RECOMBINANT PRODUCTION OF PEPTIDE HORMONES

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Manufacturing of therapeutic peptides has been accomplished historically using chemical synthesis or recombinant protein production using protein fusion partners. Unigene's *E. coli* based peptide expression technology has overcome some of the limitations of both the synthetic and the recombinant fusion protein based systems by exploiting the *E. coli* general secretory pathway for direct expression of recombinant peptides that can be harvested from the conditioned culture medium. Intensive expression vector and fermentation development efforts have resulted in enhanced expression and increased diffusion of secreted peptides, and have yielded a process that leads to the accumulation of high levels of intact and biologically active peptides in the culture medium. For peptides that require C-terminal amidation for biological activity, an *in vitro* amidation process using recombinant peptidylglycine α-amidating monooxygenase has been developed. Examples that demonstrate the advantages of an *E. coli* secretion process for peptide manufacturing, including increased peptide yields, simplified purification strategies, high purity and ease of large-scale manufacturing, will be presented.

P 307 CHEMICAL SYNTHESIS OF FULL-LENGTH PARATHYROID HORMONE-RELATED PROTEIN-(1-141)

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Parathyroid hormone-related protein (PTHrP) is a 141-residue long protein that plays critical roles in bone and tissue development. The intact protein can be obtained by recombinant methods, but shorter-length synthetic fragments, such as PTHrP(1-36), are used in most studies. Knowledge on the structural and functional properties of intact PTHrP is thus limited. No attempt at chemical synthesis of full-length PTHrP has been reported. Herein we present the chemical synthesis of full-length PTHrP-(1-141). We used stepwise, Fmoc-based solid-phase chemistry and an automated peptide synthesizer. Residues 61-141 were coupled sequentially using HBTU/HOBt/DIEA activation chemistry, and the remaining amino acids were coupled via DIC/HOBt activation. Selective capping was performed at positions G^{60}, R^{37}, S^{14} and A^{1}, and the N-terminal Fmoc group was left intact. After cleavage and deprotection, the Fmoc-PTHrP(1-141) was purified by reverse-phase HPLC. Removal of the Fmoc group and re-purification by HPLC yielded PTHrP(1-141), as verified by mass spectrometry, and co-elution with recombinant (r)PTHrP(1-141) on analytical HPLC. Tryptic peptide mass analysis of the chemically synthesized and purified product revealed a profile identical to that obtained for rPTHrP(1-141). Studies performed on the PTH/PTHrP receptor showed the synthetic protein to be functionally equivalent to the recombinant protein. This work shows that peptides longer in length than previously appreciated can be approached via the automated synthesis route.

P 308 A PHOTOLABILE BACKBONE AMIDE LINKER FOR THE SOLID- PHASE SYNTHESIS OF CYCLIC PEPTIDES

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Purdue University, West Lafayette, IN

A new backbone amide linker has been developed for the synthesis of cyclic and C-terminally modified peptides that permits photochemical detachment of the synthesized peptide from the solid support, thus avoiding the problems associated with acid deprotection conditions. An initial survey of known photolabile motifs for their ability to produce a linker-bound model dipeptide in high yield and their ability to undergo efficient photochemical detachment of the model dipeptide found that the 6-nitroveratryl (Nve) motif afforded the most efficient release of dipeptide. The problematic acylation of Nve-bound amino esters was solved through the development of the 2-hydroxy-4-carboxy-6-nitrobenzyl (Hcnb) linker, which utilizes an O-to-N transacylation to afford efficient acylation of even sterically hindered linker-bound amino esters. The Hcnb linker was found to afford high yields of amino acid loading, acylation and photolytic cleavage of model dipeptides. Attachment of the Hcnb linker to the aminoethyl TG resin permitted the solid phase synthesis of several representative cyclic peptides in high overall yield and purity.

P 309 PROTEIN SYNTHESIS USING THIOACID CAPTURE LIGATION

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Thioacid capture ligation involves the reaction between a peptide thioacid and an N-ter ys(Npys)-peptide for specific peptide bond formation.1 Although highly efficient in nature, this ligation chemistry has not been used in the synthesis of any proteins. A major constraint has been the difficulty in obtaining the thioacid peptide building blocks. To overcome this limitation, we have recently developed several chemical, enzymatic and biosynthetic methods for the synthesis of thioacid peptides and proteins. We show here that easy accessibility to these thioacid building blocks has made thioacid capture ligation practically very useful for protein synthesis. Several examples will be provided.

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P 310 APPLICATION OF CDFSS TOOLS TO THE OXIDATION OF A PEPTIDE CONTAINING CYSTEINE RESIDUES

M. Steele, T. Osiek, A. Casciola, T. Gordon
Covidien

For many businesses, success and growth are measured by how efficient they are at launching new products. The strategy and tools utilized during research and development directly impacts the success of a product's launch. CDFSS (Chemical Design for Six Sigma) provides the methodology needed to allow customer requirements to be built into every aspect of the development process using quantifiable metrics. Understanding our customer expectations of our products and correctly implementing these tools will ensure success. In an effort to meet the demands of a current customer, we have begun implementing Six Sigma tools for the oxidation of a peptide containing cysteine residues. Tools including process mapping, cause & effect matrices and FMEA as they relate to the oxidation will be outlined in this poster.

P 311 NATIVE LIGATION WITH POST-TRANSLATIONAL MODIFICATIONS USING C-TERMINAL, *N*ACYLUREA PEPTIDES

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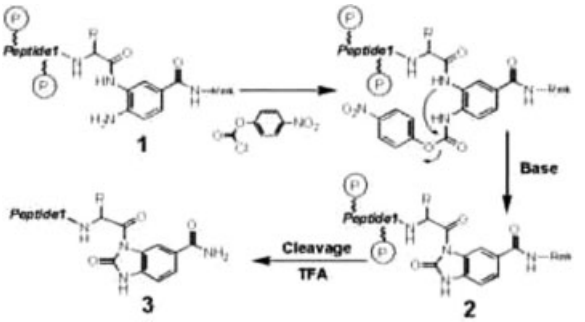
C-terminal activated peptides such as thioesters and esters are important intermediates that give access to a variety of complex synthetic targets. They have been widely used in the synthesis of proteins by chemical ligation methods (e.g. native chemical ligation)[1] and have a promising future in the synthesis of homogeneous glycoproteins. Despite the development of numerous strategies for generating these peptides using Fmoc chemistry,[2] simple and reliable access to their preparation remains a challenge. We have recently reported the use of C-terminal *N*-acylureas as versatile precursors to thioesters.[3] The key steps of the synthesis is the chemoselective acylation with p-nitrophenylchloroformate of C-terminal free amine of diaminobenzoic group 1, followed by a intramolecular attack of the anilide affording a final *N*-acyl-benzimidazolinone 2.



We have elaborated the approach through the synthesis of a range of glyco-, phospho- and lipo-peptides, suggesting a broad utility in the synthesis of post-translationally modified proteins.

1. P. E. Dawson, T. W. Muir, I. Clark-Lewis, S. B. H. Kent *Science* 1994, *266*, 776– 779.
2. J. A. Camarero, A. R. Mitchell *Prot. Pept. Lett.* 2005, *12*, 723–728 and references therein.
3. J. B. Blanco-Canosa, P. E. Dawson *Angew. Chem. Int. Ed.* 2008, *47*, 6851–6855.

P 312 INSULIN CHEMICAL SYNTHESIS USING A TWO-STEP ORTHOGONAL FORMATION OF THE THREE DISULFIDES

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A novel approach to the synthesis of insulin analogs from individual A and B chain functionalized peptide derivatives was achieved through a two-step formation of the three disulfide bonds. The first step is the formation of a single disulfide bond (A20-B19) or (A7-B7). The formation of this single bond is highly efficient and renders the formation of the remaining two disulfide bonds an intramolecular event, independent of peptide concentration. The discovery of reaction conditions where the two bonds can be formed in high efficiency was achieved under acidic conditions. The synthesis of insulin by this method can be achieved from relatively impure A and B chains which minimizes intermediate chromatographic purification and enhances the total yield of product. Most importantly, successfully application of the methodology is not dependent upon native higher order structure, as analog of low and high bioactivity were synthesized in nearly equal yield. This synthetic approach eliminates the commonly recognized challenges in preparation of various insulin analogs by the conventional one-step disulfide exchange reaction that employs S-sulfocysteine A and B chain derivatives at alkaline pH.

P 313 THE SYNTHESIS OF HYDROCARBON-STAPLED PEPTIDE (*I* AND *I*+7)

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The use of non natural amino acids containing olefinic side chains to generate an all-hydrocarbon "staple" by ruthenium-catalyzed olefin metathesis has been reported by Verdine's group [Schafmeister (*J. Am. Chem. Soc.* 2000, *122*, 5891)]. These hydrocarbon-stapled peptides are helical, protease-resistant, and cell-permeable that bind with increased affinity to some target proteins. Hydrocarbon stapling of native peptides may provide a useful strategy for experimental and therapeutic modulation of protein-protein interactions in many signaling pathways [Walensky (*Science,* 2004, *305,* 1466)].
Here we report a synthesis procedure for hydrocarbon-stapled peptides (*i* and *i*+7). Asymmetric synthesis of (R)-Fmoc-a-(2'-octenyl)alanine was successfully prepared via a D-Ala-Ni (II)-BPB-complex method in three steps with 30% total yield. Several peptides containing (R)-a-(2'-octenyl)alanine and (S)-a-(2'-pentenyl)alanine on position *i* and *i*+7 were successfully synthesized by Fmoc solid phase synthesis method. After olefin metathesis and cleavage, these peptides were purified by HPLC to obtain the desired hydrocarbon-stapled peptide (*i* and *i*+7).

P 314 DEVELOPMENT OF A NOVEL SOLID SUPPORT FOR THE ECONOMICAL SYNTHESIS OF LEUPROLIDE DRUG

M. Davis, K. Srivastava, M. Davis
Covidien

The secretion of luteinizing hormone-releasing hormone/follicle stimulating hormone (LH-RH/FSH) is under the stimulatory control of the gonadotropin releasing hormone (GnRH), a decapeptide amide. Leuprolide, a nonapeptide with a c-terminal alkylamide moiety, is an analog of LH-RH/FSH and has been approved and marketed as a drug for prostate cancer. Leuprolide has been prepared by both solution phase and solid phase methods; but, synthesis is not very straight forward. During the synthesis, the c-terminal amino acid of leuprolide has either a benzyl ester group or is attached to a solid support via a benzyl ester linkage. This requires alkylaminolysis at the end of the synthesis to yield the protected leuprolide followed by its acidolysis resulting in leuprolide. Now-a-days, the most popular way to synthesize peptides quickly is via solid phase approach, where the success is heavily determined by the solid support and its performance. Currently, there is no general rule to decide on the most convenient and effective solid support for a particular peptide synthesis. However, it is important to consider the type of the chemistry to be carried out during the synthesis, resin-reagent compatibility, swelling-to-solvent ratio, and the length and sequence of the desired peptide. As a result, we decided to examine the different solid supports employing different synthesis strategies. Subsequently, we conceived an idea to develop a new solid support and executed it for an economical and reproducible synthesis of Leuprolide. Details of the investigation will be presented.

P 315 EVALUATION OF ALTERNATIVES TO HOBT DURING SOLID PHASE PEPTIDE SYNTHESIS

M. Davis, K. Srivastava
Covidien

In peptide synthesis, coupling of two amino acids - one with a free acid function, and the other with a free amine group - is generally carried out with carbodiimide (DCC/DIC). Sometimes other coupling reagents such as EDC (EADC) or CDI have also been used. These coupling reagents are known to suffer from high levels of racemization and other side reactions. As a consequence, coupling reactions of molecules containing various functional groups have generally been performed with HOBT as a racemization suppressant. Recently, benzotriazole derivatives like HOBT, HOAT, HBTU, TBTU, etc... are considered potentially explosive and their transport/delivery, storage and handling needs special attention. More so, HOBT has been placed under an explosive category and as a result, its availability on a large quantity is not possible because of transport limitations. A few years back, replacement of HOBT by 2-MBT (2-mercaptobenzothiazole) was proposed by Reaxa company. Luxemborg Biotechnologies Ltd. is now advertising oxymapure as the most effective racemization suppressing coupling additive available. We, as a contract manufacturer of peptide API are considering substituting 2-MBT or oxy-

mapure for HOBT in our synthesis processes. As a result, we decided to study their applicability by synthesizing several peptides of different types such as peptide-acids, peptideamides and peptide-alkylamides. The details of the exploration will be presented.

P 316 SPIRAL COUNTERCURRENT CHROMATOGRAPHY: A NEW TECHNIQUE FOR PURIFICATION

M. Knight
CC Biotech LLC

Countercurrent chromatography (CCC), a liquid-liquid partitioning method carried out in flow-through tubing coils mounted in planetary centrifuges, has served well in laboratoryscale preparative purification of small molecules, including peptides. Recent breakthroughs in the design of the CCC rotors have enabled the use of aqueous-organic solvent systems containing the heavy alcohols (polar solvent systems) and the aqueous two phase solvent systems (TPAS) which extend the technique to the large molecules such as proteins, other polymers and many more types of peptides. We made new spiral rotors and applied them in the separation of peptides and proteins with the different solvent systems to assess their performance. The flow tubing is spaced farther apart with a higher pitch. This increases the centrifugal force to hold more stationary phase which increases the sample capacity per total volume (stationary phase ~70% versus 5-15% in packed columns) and with the appropriate solvent system, the resolution of components. Highly charged peptides to the very hydrophobic low-water soluble peptides are handled by the wide range of solvent systems able to be used in this method. Amino acid derivatives and protected fragments can be purified, as well. Non-denaturing solvent systems are possible for proteins, minimizing the need for extensive re-folding procedures.

P 317 EFFICIENT PEPTIDE LIGATION USING AZIDO- AND PYRUVOYL- PROTECTED PEPTIDES VIA THE Ag+-FREE THIOESTER METHOD

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Thioester method is one of the ligation method useful for (glyco) protein synthesis [1]. This method has the advantage that there is no restriction on the ligation site, whereas the native chmical ligation [2] can be applicable only for the Xaa-Cys site. Instead, to achieve the chemoselective ligation, side chain amino and thiol groups have to be protected during segment coupling in the thioester method. To realize this, the Boc groups were usually reintroduced to the side chain amino groups, which were made free during the deprotection step after SPPS. In this presentation, we examined the direct preparation of side chain *N*-protected peptide segments by introducing azido- or pyruvoyl-protected lysine during SPPS. As an example, pigment dispersing hormone (PDH-1) (NSELINSLLGIPKVMTDA-NH2) was synthesized. The peptide was divided between G10–I11. N-terminal peptide thioester was prepared by the *N*-alkylcysteine (NAC)-assisted thioesterification [3]. C-Terminal peptide was prepared by the conventional Fmoc method using Fmoc-Lys(N3 or CH3COCO) for the introduction of K13. After two segments were condensed by the Ag+-free thioester method [3], the side chain protecting groups were removed by An/AcOH (N3-) or *o*-phenylenediamine (CH3COCO-) to give desired PDH-1 in good yield, demonstrating the usefulness of the protecting groups for the thioester method. Synthesis of glycopeptides using pyruvoyl group will be also presented.

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[3]. H. Hojo, Y. Murasawa, H. Katayama, T. Ohira, Y. Nakahara, Y. Nakahara, *Org. Biomol. Chem.*, 6, 1808 (2008).

P 318 PEPTIDE SYNTHESIS IN WATER USING BOC-AMINO ACID NANOPARTICLES (NO. 2)

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Kobe Gakuin University

In the development of green sustainable chemistry, one of the most important technologies is to reduce the use of organic solvents and, instead, utilize less toxic solvents. During the last few years, we have focused on developing an organic solvent-free peptide synthesis method using aqueous solvents. One of the most important, and sometimes difficult, aspects of peptide synthesis is to choose the best amino acid derivatives for the synthetic reaction to proceed. Currently, Boc- and Fmoc-amino acids are routinely used as building blocks for peptide synthesis. However they are sparingly soluble in water. Recently, we succeeded in developing an organic solvent-free solid-phase synthesis method using water-dispersible Fmoc-amino acid nanoparticles. This novel technology uses suspended nanoparticle reactants for the coupling reaction. Here, we investigated the feasibility of this method for in-water solution-phase synthesis of peptides using water-dispersible Boc-amino acid nanoparticles. And, we focused on the coupling method in aqueous media using Boc-amino acid nanoparticles. For efficient reaction in water using nanoparticles, the coupling reagents must be soluble and stable in water and also require high reactivity in water. We examined the in-water efficiency of water-soluble coupling reagents, WSCD, DMT-MM and combination of several types of additive.

P 319 $^{1}H,^{13}C$-HSQC HRMAS-NMR AS A TOOL FOR INVESTIGATING THE QUALITY OF FMOC-AA-WANG RESINS FOR SPPS

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Synthesis of Fmoc-AA-Wang resins for SPPS is usually completed by endcapping the residual free hydroxyl groups of Wang. This final step is assumed to proceed completely and irreversibly and therefore is not investigated in routine quality control of final resin products. One dimensional (1D) 13C Magic Angle Spinning (MAS) NMR with conventional MAS probes has already been used successfully to determine quantitatively the loading of resins using internal references [1]. The significantly better resolution of High Resolution (HR) MAS probes enabled quantitative results from 1D 1H-MASNMR spectra as well [2, 3]. Owing to overlap of resonances, the 1D NMR methods cannot always be successfully applied, particularly in cases when weak resonances are present.
An analytical method is presented here to determine quantitatively the share of Fmoc-AA loaded, endcapped and free Wang resin using 1H,13C-Heteronuclear Single Quantum Coherence (HSQC) HRMASNMR. Evaluation of the corresponding cross signal enables to quantify low concentrations of free Wang in Fmoc-AA-Wang resins down to 1% by mass. The analytical method presented is generally considered to be a powerful tool for the investigation of the quality of resins used in SPPS.

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P 320 NOVEL PLATFORM FOR BIOSYNTHESIS OF LARGE OR COMPLEX PEPTIDES

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CC Biotech LLC

A novel bioprocess platform for rapid, scaleable, economical synthesis of large or complex peptides as C-terminal fusions with an ubiquitin-like (UBL) protein expressed in bacteria followed by proteolytic release has been developed. UBL-peptide fusion yields are typically 4-12 grams per liter and peptide yields depend upon the size of the peptide; in general, the larger the peptide, the greater the yield. UBL fusions are purified by immobilized metal affinity chromatography (IMAC) utilizing a histidine tag in the UBL component. Separation of peptide from UBL is achieved by an UBL-specific protease that mediates efficient cleavage in 2 hours at enzyme/substrate ratios of 1/104-106, representing a significant improvement over existing fusion protein-protease platforms The peptide is then purified away from the reaction mixture by countercurrent chromatography. A total of six demonstration peptides have been synthesized as fusion proteins and we have demonstrated the entire process for a 9-amino acid anti-inflammatory peptide.
Preliminary studies and cost projections suggest that synthesis of peptides ranging from about 20 to 70 amino acids using the UBL platform is more cost-effective than chemical synthesis. The requirements for costly reagents and large quantities of organic solvents are eliminated.

P 321 ANGIOGENESIS INHIBITION USING VEGF RECEPTOR BLOCKAGE APPROACH

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Angiogenesis is the process of new blood vessel formation from existent vascular network and it is tightly regulated by a balance between pro- and anti-angiogenic factors. Vascular endothelin growth factor (VEGF) is a glycoprotein consisted of an anti-parallel homodimer structure containing inter and intra disulfide bounds, characteristics that include it to the PDFG cystine-knot family. In this study we evaluated VEGF activated pathway and a approach to target angiogenesis, the blockage of VEGF and VEGFR-2 receptor interaction using peptides designed to mimic the VEGF binding site. We utilized an angiogenesis model cell line, HUVEC that respond to VEGF activation in several *in vitro* assays: proliferation, matrigel tube formation and VEGFR-2 phosphorylation and we observed the ability of VEGF mimic peptides to block the interaction between VEGF and VEGFR-2. We also evaluated the ability of the retro-inverso peptide to demonstrate anti-angiogenic effects. We tested two different animal models, where tumor growth is dependent (RIP1-TAG2) or accelerated ($VEGF^{+/-}$/Neu $2\text{-}5^{+/-}$) upon VEGF expression.

P 322 DESIGN OF STRUCTURED gp41 N-HEPTAD REPEAT PEPTIDES AS HIV-1 VACCINE CANDIDATES

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Efforts to develop an HIV-1 vaccine have focused on conserved structural elements which are the target of broadly neutralizing monoclonal antibodies (mAb). In recent years we have validated the N-heptad repeat (NHR) region of gp41 as a potential vaccine target, based on the identification of mAb D5 which binds to a highly conserved hydrophobic pocket on the NHR coiled-coil (Miller *et al*., Proc. Natl. Acad. Sci. U.S.A. 102 (2005) 14759).

To identify a candidate vaccine, able to elicit a D5-like antibody response, we designed a series of peptides in which the minimal D5 epitope-containing gp41 sequence (N17) or an extended NHR region (N36) are displayed as highly structured coiled-coil trimers. The series was evolved towards increased structural stabilization and focusing of the immune response on desired regions of the molecule. Comparative immunogenicity studies were performed in Guinea pigs and rabbits, showing that (i) purified IgG from vaccinated animals reproducibly neutralizes both a D5 hypersensitive mutant and wild type HXB2 virus, (ii) potency correlates both with overall antibody titer and with the concentration of specific D5-like antibodies. Moreover, we obtained definitive evidence that unfractionated sera raised against the most potent vaccine candidates can neutralize the virus, and performed a preliminary analysis of the breadth of neutralization on primary isolates. To our knowledge, these findings are the first demonstration of the ability to elicit a broadly neutralizing response against a highly conserved gp41 NHR epitope.

P 323 POSITRON EMISSION TOMOGRAPHY IMAGING OF VEGF RECEPTOR EXPRESSION IN POST-STROKE ANGIOGENESIS

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Vascular endothelial growth factor (VEGF) and VEGF receptors (VEGFRs) play important roles during neurovascular repair after stroke. In this study, we non-invasively imaged VEGFR expression after stroke in a rat model with positron emission tomography (PET).

Female Sprague-Dawley rats after distal middle cerebral artery occlusion (dMCAo) surgery were subjected to weekly MRI, 18F-FDG PET, and 64Cu-DOTA-VEGF121 PET scans. Histologic analysis and ex vivo autoradiography on stroke brain tissue slices was carried out to validate the in vivo findings. T2-weighed MRI correlated with the "cold spot" on 18F-FDG PET for dMCAo rats. 64Cu-DOTA-VEGF121 uptake in the stroke border zone peaked at about 10 days after surgery, indicating neovascularization as confirmed by histology (VEGFR-2, BrdU, and lectin staining). VEGFR specificity of 64Cu- DOTA-VEGF121 uptake was confirmed by significantly lower uptake of 64Cu-DOTA-VEGFmutant in vivo and intense 125I-VEGF165 uptake ex vivo in the stroke border zone. No appreciable uptake of 64Cu- DOTA-VEGF121 was observed in the brain of sham-operated rats.

For the first time, we successfully evaluated the VEGFR expression kinetics non-invasively in a rat stroke model. Multimodality imaging was used to assess the temporal profile of the lesion size, metabolic activity, and angiogenesis in the same animals after stroke. Imaging of VEGFR expression could become a significant clinical tool to plan and monitor therapies aimed at improving post-stroke angiogenesis.

P 324 A COMBINATORIAL APPROACH TO IDENTIFY NOVEL PEPTIDE LIGANDS TARGETED AGAINST THE CANCER SPECIFIC RECEPTOR MUTATION EGFRvIII

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Early diagnosis and staging is of critical importance for correct treatment of cancer. Therefore, the identification of novel cancer specific targets and receptor ligands for Positron Emission Tomography (PET) imaging is of great interest.

We report here the design and synthesis of a positional scanning synthetic combinatorial library (PS-SCL) for the identification of novel peptide ligands for the cancer specific epidermal growth factor tyrosine kinase receptor mutation (EGFRvIII). The library consisted of six individual positional sub-libraries in the format, H-O1-6XXXXX-NH2, O being one of the 19 proteinogenic amino acids (cysteine omitted) and X an equimolar mixture of these. Using a biotin-streptavidin assay, the binding of each sub-library to NR6M, NR6W-A and NR6 cells was tested. These cells express EGFRvIII, EGFR and neither of the receptors, respectively. We identified one peptide, H-FALGEA-NH2, which showed more selective binding to the mutated receptor than the EGFRvIII specific peptide PEPHC1.

C. Lund Denholt et al. (2009) Biopolymers (Peptide Science) 91, 201–206.

P 325 TIDE QUENCHER™-BASED FRET PROTEASE SUBSTRATES AND THEIR APPLICATIONS IN DRUG DISCOVERY AND DISEASE DIAGNOSIS

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Proteases play essential roles in protein activation, cell regulation and signaling, as well as in the generation of amino acids for protein synthesis or utilization in other metabolic pathways. Although EDANS/DABCYL and MCA/DNP are widely used to develop a variety of FRET protease substrates, their short absorption wavelengths and low extinction coefficients have limited their use in the development of sensitive protease assays. We have developed a class of non-fluorescent Tide Quencher™ dyes to eliminate these limitations.

Tide Quencher™ (TQ) dyes are excellent dark quenchers that are individually optimized to pair with all of the popular fluorescent dyes such as fluoresceins and rhodamines. Our TQ series of non-fluorescent dyes cover the full visible spectrum with unusually high quenching efficiency. Tide Quencher™ 2 (TQ2) has absorption maximum perfectly matching the emission of FAM while Tide Quencher™ 3 (TQ3) is proven to be the best quencher for TAMRA. We have used TQ dyes to develop new FRET peptide substrates for high throughput analysis of protease activities and screening of protease inhibitors. Our TQ2-based HIV protease substrates have demonstrated significantly enhanced

assay window. Excellent performance has also been observed for our new MMP protease substrates that incorporate TQ2 or TQ3 as acceptor. We have also used TQ2 and TQ3 to develop novel protease substrates for analysis of secretases, HDAC, HCV, HBV and caspases.

P 326 BRIGHT TIDE FLUOR™-BASED FLUORESCENT PEPTIDES AND THEIR APPLICATIONS IN DRUG DISCOVERY AND DISEASE DIAGNOSIS

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Fluorescent peptides are widely used as important tools in drug discovery and disease diagnosis. Although EDANS, FAM and TAMRA are widely used to label peptides, their short absorption wavelengths, low extinction coefficients and pH-sensitive fluorescence have limited their use in the development of sensitive fluorescent peptide probes. We have developed a class of strongly fluorescent Tide Fluor™ dyes to eliminate these limitations.

Strongly fluorescent Tide Fluor™ (TF) dyes are extremely bright and photostable dyes. They are individually optimized for labeling peptides and nucleotides. Our TF series of fluorescent labeling dyes cover the full visible spectrum with unusually high labeling efficiency. Tide Fluor™ 2 (TF2) has spectral properties essentially identical to FAM and FITC. It is 3 times brighter and 4 times more photostable than FAM under the physiological conditions or in cells. TF dyes have fluorescence that is insensitive to pH fluctuation from pH 4 to pH 10 while the fluorescence intensity of FAM and FITC is strongly dependent on pH in the same range. We have used TF dyes to develop new fluorescent peptides for high throughput analysis of protease and protein kinase activities and screening of protease and protein kinase inhibitors. Excellent spectral properties and labeling efficiencies have also been observed for TF3, 4 and 5.

P 327 ARTIFICIAL PEPTIDE SUBSTRATES FOR INTRACELLULAR PHOSPHORYLATION ANALYSIS

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Our lab uses chemical biology and proteomics to develop biosensors for intracellular phosphorylation signaling. We use synthetic peptide and protein chemistry to design and synthesize probes for multiplexed analysis of disease-related signaling proteins e.g. Abl, other Src-family kinases, receptor tyrosine kinases, etc. We are exploring a wide variety of multidisciplinary analytical methods including mass spectrometry, fluorescence microscopy and flow cytometry to develop novel techniques for monitoring these signaling proteins and their activities. Our technology development goals will lead us to new methods for asking increasingly complex questions about multiple signaling activities, their disruption in disease, and response of signaling profiles to treatment.

P 328 DEVELOPMENT OF IMAGINABLE *CLOSTRIDIUM DIFFICILE* TOXINS FOR CELLULAR IMAGING AND TRAFFICKING STUDIES

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C. *difficile* is a gram-positive, strictly anaerobic, spore-forming bacillus which can cause pseudomembranous colitis requiring colectomy and resulting in death in hospitalized patients. Currently the treatment of C. *difficile* colitis is inadequate and relapse rates are close to 50%. Pathogenic strains of C. *difficile* produce toxins. The toxins can cause severe inflammatory changes of colonic mucosa, leading to formation of an inflammatory pseudomembrane. However, the mechanism of the pathogenesis of the toxins to cause the disease is not clear. Development of imaginable toxins will allow us to better understand the interactions of the toxins with human cells.

The toxin peptide was synthesized and conjugated with different kind of near-infrared (N I R) dye. The near infrared (NIR) dye labeled toxins, unlabeled toxin and commercial toxin protein were compared against known positive and negative control samples. The toxin interaction with human cells and cellular trafficking imaging studies were concluded using confocal microscope.

The study results demonstrated that NIR dye-labeled peptide toxins maintained the same properties as the unlabeled peptide toxin and commercial toxin protein. Cellular confocal imaging revealed that the labeled toxin trafficked into human cells. We found that the labeled toxin can be used to visualize the toxin interaction with the host for *in vitro* and *in vivo* studies. Therefore, imaginable toxins can be applied as tools to study the toxin mechanisms, as well as possess the potential to develop inhibitors, blocking agents and therapeutic agents.

P 329 A NOVEL BIOCHIP SYSTEM FOCUSING ON PROTEIN DETECTION BY THE USE OF LABELED GLYCOPEPTIDES ARRAYED ON A NOVEL CHIP-MATERIAL

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We have proposed a novel biochip-concept for a protein detection system involving labeled structured peptides as capture molecules and the 'protein-fingerprint' method, while the protein-protein interaction can be mimicked by a protein-peptide interaction. In the present paper fluorescent labeled glycopeptides have been synthesized by the improved Fmoc-SPPS to construct libraries. The novel chip-material based on an amorphous carbon with a special surface chemistry has been also developed to be an ideal biochip material. The peptide solution (350 pL) was immobilized on to this novel material and toxic proteins were applied to show different responses to different glycopeptides in a dose dependent manner and finger prints could be obtained. In fact, solution assay requires larger amounts of capture peptides and analytes. One spot of the present arrayed peptides were 9 femto mole and toxicant protein as an analyte was ca 20 ng. The detection was also performed in the presence of 2% milk to simulate practical conditions. Thus glycopeptide arrays show promising applications as a toxin detection tool. It is emphasized that the present biodetection is practical, since the dried array recognized analytes, in fact lectin or antibody arrays often extinguish recognition abilities after dryness.

P 330 A NOVEL VIRTUAL ANALYTE NT-proXNP AS A PROGNOSTIC BIOMARKER IN ACUTE HEART FAILURE

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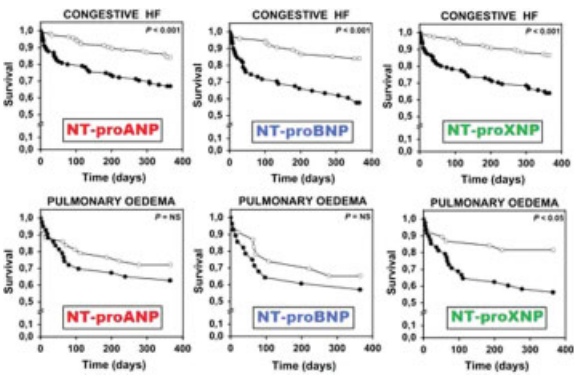
Activation of the neurohumoral system and subsequent elevation of natriuretic peptide (ANP, BNP) levels in circulation is a well recognized phenomenon in heart failure (HF). However, due to divergent expression, production and secretion of the ANP- and BNPderived peptides the circulating levels in different pathophysiological conditions may differ markedly. In the present study, a novel radioimmunoassay (referred to as NT-proXNP), which utilizes a recombinant peptide construct with amino acid sequences from both NT-proANP and NT-proBNP [NT-proBNP1-37 . NT-proANP29-98], was used to assess whether the differential activation of the neurohumoral system in two patient groups with different clinical manifestations (pulmonary oedema, n=86; congestive HF, n=230) was translated into diverse 12-month survival patterns as well. The survival analysis demonstrated that although all the natriuretic peptides were equal in predicting 12-month survival in the entire patient cohort as well as in the subgroup of



patients with congestive HF ($P<0.001$ for all), only NT-proXNP retained its prognostic power when estimating the outcome among patients with pulmonary oedema ($P=0.018$). These results suggest that although the traditionally applied natriuretic peptide assays identify .less severe. AHF patients with poor prognosis, the novel NT-proXNP assay seems to have characteristics advantageous for discriminating the survival in patients with AHF regardless of the clinical manifestation of the disease. Thus, the novel virtual analyte NT-proXNP appears as a new valuable biomarker of disturbed cardiac function.

P 331 NEW STRATEGY FOR PROTEIN IDENTIFICATION - IMPROVING SIGNAL INTENSITY AND SENSITIVITY OF MALDI MASS SPECTROMETRY BY SPECIFIC PEPTIDE DERIVATIZATION

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General approaches used in protein identification and characterization involve consecutive purification steps. Then the desired protein extract is submitted to mass spectrometry analysis (LC-MS/MS) after enzymatic digestion. Technical difficulties are involved in determining the PMF of a protein particularly in relative low abundance. A typical protein will give rise to at least twenty to thirty peptides after trypsin digestion. Not all of these peptides will appear in MALDI analysis. One factor that is believed to cause incomplete detection is competition for protonation during the ionisation process inducing ion discrimination.
We have recently developed a new technology allowing specific labeling of lysine residues in proteins and easy MALDI-MS detection and identification of labeled peptides following protein hydrolysis[1]. N-hydroxysuccinimide ester of α-cyano-4-hydroxycinnamic acid (CHCA) was used as a labeling reagent to increase MALDI signal of Lysine-containing peptides in Cytochrome C proteolytic mixture.
This original approach enables to discriminate labeled peptides of interest among other abundant peptides. Herein, we report the optimization process to investigate the limits of this tool and its application to specific membrane protein detection.

[1] D. Lascoux, D. Paramelle, G. Subra, M. Heymann, C. Geourjon, J. Martinez and E. Forest. *Angew. Chem. Int. Ed.*, 2007, 119(29); 5690–5693.

P 332 LHRH-II ANALOG DESIGN: STRUCTURE-FUNCTION STUDIES TOWARD THE DEVELOPMENT OF A LHRH-II BASED RADIOTHERAPEUTIC AGENT

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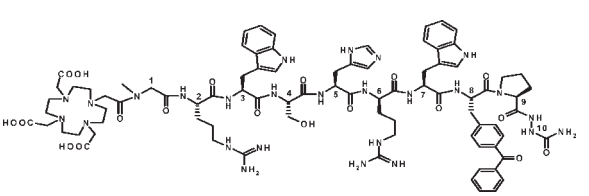
In the past few years accumulating evidence that normal and cancer tissues locally produce LHRH and express LHRH-I and LHRH-II binding sites suggests that LHRH agonists and antagonists have action at these peripheral targets. We therefore modified LHRH-II analogs for the development of radiotherapeutics and developed SPPS methods which allowed the preparation of analogs bearing the metal chelating agent DO3A10CM at the N-terminus of the peptide sequence (e.g. DO3A10CM-Sar^1-Arg^2-Trp^3-Ser^4-His^5-Darg^6-Trp^7-Bpa4^8-Pro^9-azaGly^{10}-NH_2), the substitution of various lipophilic and hydrophilic amino acids in the sequence, and, placement of various alkylamines or oxyalkylamines at the C-terminus. These analogs were tested for specific *in vitro* binding to human ovarian cancer EFO27 cells and their relative affinities were determined. The replacement of azaGly^{10} with oxyalkylamines, the placement of D-arginine at position 6 and the substitution at positions 1 and 2 with basic lipophilic amino acids, especially arginine, were emphasized in the development study of a LHRH-II analog with high potency *in vitro*. Key findings were that the introduction of the chelating agent at the N-terminus was optimal and that a benzoylphenylalanine derivative at position 8 increased the binding potency of the peptide. Based on the EC_{50} data, the structure-function relationship of these LHRH-II analogs was performed and results will be described.



P 333 HETEROBIVALENT LIGANDS CROSSLINK MULTIPLE RECEPTORS FOR TARGETING OF PANCREATIC BETA-CELL TO MONITOR CHANGES IN BETA-CELL MASS

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Dysfunction and loss of pancreatic β-cells is the primary cause of Diabetes, and is observed during the immunological destruction of β-cells in non-insulin dependent (Type 1) Diabetes. The ability to monitor changes in β-cell mass (BCM) and/or function is critical to analyzing the developing pathology, as well as the efficacy of treatments to preserve β-cells. Specific ligands targeting single-receptor have been limited by poor discrimination between β-cells and other cells within the imaging field such as pancreatic cells, and hepatocytes. Recently, we have demonstrated multiple surface receptors can be cross-linked using a synthetic heterobivalent ligand against hMC4R and d-opioid receptors. Multivalent interactions were characterized by enhanced affinities (avidities) and enhanced specificities when binding was compared to cells with only one of the targeted receptors. We applied multivalent ligand design to a several β-cell receptors; the Glucagon-like peptide 1 receptor (GLP1R), the Sulfonylurea receptor 1 (SUR1), and 5-Hydroxytryptamine 1F receptor (5-HT_{1F}R). The heterobivalent GLP-1/SUR1 ligand, labeled with Cy5, binds at low concentrations (>5 nM) to βTC3 cells, but not to a cell line expressing only one receptor. Single cells imaging demonstrates that with 10 minutes of binding, the ligand is capped, and taken from the cell surface. Our findings indicate that development of heterovalent ligands may provide an approach for specific targeting, and therefore analysis of β-cells in vivo.
Supported by grant from the Juvenile Diabetes Research Fundation.

P 334 INVESTIGATION OF UPTAKE EFFICIENCY AND CELL CYCLE- INDEPENDENT LOCALIZATION OF NOVEL NUCLEOLAR TARGETING PEPTIDES (NrTPs)

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Nucleolar targeting peptides (NrTPs) are recently developed sequences, 12-13 amino acids long and with two repetitive KKG triads, generated by structural minimization of a snake toxin (Rádis-Baptista et al., J Med Chem 2008, 50:7041-7044). Functionally, as probed by live cell confocal microscopy, NrTPs such as NrTP1 (YKQCHKKGGKKGSG) or its retro analogue are capable of penetrating immortalized somatic and tumoral cell lines, and compartmentalizing in the nucleolus of cells from human pancreatic adenocarcinoma (BxPC-3), human ductal mammary gland carcinoma (BT-474), human colorectal adenocarcinoma (Caco2) and human cervix epithelial carcinoma (HeLa). Both NrTP1 and its retro version are also able to penetrate and accumulate in the nucleolus of mouse embryonic fibroblasts (NIH 3T3) and in mouse neuroblastoma (N2a) cells. Since (1) nucleolar assembly and disassembly depends on the dynamic traffic of cell cycle-dependent proteins, and (2) the uptake efficiency and cell preference of penetrating peptides might also be potentially affected by the cell cycle, we have investigated this point by multi-parametric high resolution flow cytometry analysis, in combination with fluorescence confocal microscopy, and found that the nucleolar localization of NrTPs is independent of the cell cycle phase.

P 335 TOTAL CHEMICAL SYNTHESIS OF BIMODAL-LABELED RANTES AS A PET/TPLSM PROBE FOR *IN VIVO* MOLECULAR IMAGING OF ATHEROSCLEROTIC PLAQUE INSTABILITY

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Vulnerable plaque rupture is the main cause of life-threatening cardiovascular events such as myocardial infarction and stroke. Comparison of chemokine receptor 5 (CCR5) mRNA and protein expression levels from ruptured and stable plaque biopsies showed specific upregulation of CCR5 in ruptured atherosclerotic plaques. CCR5 is a macrophage biomarker known to play a pivotal role in the atherosclerotic process.
To provide a clinical tool for vulnerable plaque detection, the natural ligand for CCR5, RANTES, was chemically synthesized using SPPS and native chemical ligation. After oxidative folding, site-specific C-terminal labeling of RANTES was performed either with rhodamine by Michael addition or with bimodal DTPA-rhodamine conjugate by aniline-catalyzed imine chemistry.
Two photon laser scanning microscopy (TPLSM) showed RANTES rhodamine internalization in macrophages present in murine atherosclerotic lesions. *In vivo* PET experiments using bimodal RANTES-rhodamine-DTPA-68Ga were suggestive for non-invasive plaque detection. Subsequent *ex vivo* microPET imaging of the murine aortic arch showed contrast enhancement at the sites of atherosclerotic lesions.
Targeting CCR5 with RANTES-based contrast agents is a promising tool for non-invasive biomolecular imaging of plaque formation.

P 336 SELECTIVE CAPTURE AND DETECTION OF MISFOLDED PRION PROTEINS USING PRION-DERIVED PEPTIDES

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Novartis Vaccines and Diagnostics

Misfolded proteins are associated with a large number of diseases, including Creutzfeldt-Jakob (CJD, or prion) diseases, Alzheimer's, Parkinson's, and Diabetes Type II. Despite the broad health impact of these diseases, diagnosis of misfolded protein diseases can be challenging. For example, the development of blood test for prion disease is complicated by the fact that the pathogenic species is an endogenous protein, thereby rendering approaches like traditional ELISAs and PCR of little diagnostic value. Here we show that we can employ prion-derived peptides to selectively bind and capture the misfolded form of the prion protein, in the presence of thousands-fold excess of the properly folded form. This selective capture allows us to detect amol levels of misfolded prion, providing a basis for a highly sensitive diagnostic test for prion disease.

P 337 DISCOVERY AND STRUCTURAL OPTIMIZATION OF HIGH AFFINITY CO-AGONISTS AT THE GLUCAGON AND GLP-1 RECEPTORS

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Glucagon and Glucagon-like peptide (GLP-1) are two structurally homologous hormones that physiologically derive from a common biosynthetic precursor but differ appreciably in their endocrine function. Glucagon is a potent hormonal stimulus for liver glucose production, while GLP-1 acts principally in the gastrointestinal tract and at the pancreas to facilitate mealtime glucose management. The structural basis for the biological selectivity demonstrated by these two hormones is not known and might serve as a basis for improved management of diabetes and obesity.
We have embarked on the identification of co-agonists using glucagon as a structural point of origin and have identified potent co-agonists. Starting from the GLP-1 sequence has proven much more challenging and seemingly not possible without the introduction of multiple amino acid substitutions. Apparently, the GLP-1 receptor is considerably more pleiotropic than the homologous glucagon receptor in selection of high affinity ligand structures. We have identified significant increase in potency at the GLP-1 receptor on glucagon resulting from specific mutations. These analogs are associated with increased alpha helicity. Ligands of low nanomolar affinity, full agonism and nearly equal potency at the human glucagon and GLP-1 receptors have been identified. The structural optimization has been investigated through the application of backbone stabilization through covalent stapling at various positions using linkers of differing length. The results of this work will be reported.

P 338 IDENTIFICATION OF A HIGH POTENCY GLUCAGON AGONIST WITH ENHANCED BIOPHYSICAL STABILITY AND AQUEOUS SOLUBILITY

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Insulin and glucagon are the two counter-acting hormones responsible for regulating blood glucose. Glucagon is produced within pancreatic alpha cells and stimulates the liver to synthesize and release glucose. It is relatively simple linear peptide composed of 29 amino acids. Its primary sequence is shared by all vertebrates studied to date. Despite its critical role in moderating blood glucose levels, glucagon agonism has received limited medicinal attention relative to antagonists. Consequently, we consider glucagon to be the "forgotten hormone" and a source of significant medical importance.
For more than half a century it has been used as a critical care medicine in the treatment of life-threatening insulin-induced hypoglycemia. The biophysical properties of natural sequence glucagon are not conducive to formulation in a patient-friendly form. The hormone is poorly soluble at physiologic pH and prone to physical aggregation with formation of insoluble fibrils. Consequently, glucagon is commercially supplied as a lyophilized powder to be solubilized in dilute aqueous HCl immediately prior to administration. To a patient that is semi-conscious or unconscious this represents an obstacle to proper administration and could constitute a fatal flaw. We have explored the structural modification of native glucagon with the intent of enhancing the physical properties with minimal change to pharmacology. A series of amino acid changes and additions to native glucagon were studied relative to bioactivity and physical stability. A set of glucagon analogs was identified that appear to fulfill all physical, biological and synthetic criteria for further development.

P 339 CHEMICAL SYNTHESIS OF INSULIN ANALOGS

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Insulin constitutes a hormone of central importance in physiology and a vital element in glucose management. Its use in diabetes care has been of seminal significance for nearly a century. The advent of rDNA biosynthesis provided human insulin in virtually unlimited quantity. More importantly, it provided a mechanism by which improved biosynthetic insulin analogs could be synthesized, evaluated and registered as new medicines. The advent of chemical biotechnology (biosynthesis with unnatural amino acids) provides a new venue for optimizing insulin pharmacology through the use of synthetic chemistries that otherwise would be prohibitively expensive for commercialization. Multiple prior observations establish a foundation for application of unnatural amino acids as a route to insulin pharmacology that may not be obtainable with natural amino acids alone rDNA biosynthesis with non-native amino acids provides a means to lower cost, high integrity commercial synthesis but it is slow relative to chemical approaches in exploring diverse structural space. To facilitate the identification of insulin analogs with enhanced pharmacologic performance we have explored the synthesis of single chain insulin analogs using thiol-mediated peptide ligation. Selective enzymatic digestion provides the traditional two-chain heterodimeric insulin peptides for purposes of comparative analysis. Individual insulin-related peptide fragments have been prepared biosynthetically and used in conjunction with synthetic fragments that contain amino acids that could not be easily prepared by biosynthesis. The collective toolbox of synthetic methodologies provides a diverse and powerful means to synthesis of insulin analogs that were previously difficult to assemble by classical two-chain disulfide exchange in alkaline aqueous buffers.