YI-P058 Total Synthesis of Teixobactin

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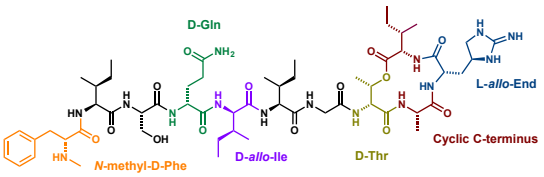

Figure 1. Structure of teixobactin showing key disconnections (red and blue) and the uncommon amino acid L-*allo*-enduracididine (green).

The emergence of drug resistant strains of pathogenic organisms proves to be a developing global health crisis and has already compromised the effectiveness of many clinical antibiotics. One such example of a pathogenic organism in which drug resistance is rapidly developing is *Mycobacterium tuberculosis* (*Mtb*), the etiological agent of tuberculosis (TB). Of the anti-TB drugs currently in clinical trials, many are simple variations on current drug architectures. As such, novel drugs with unique scaffolds and modes of action are greatly needed to combat the development of resistance. One possible source for such drugs lies in the privileged chemical space of natural products. In 2015, Ling *et al.*[1] reported the isolation of the peptidic natural product teixobactin from the previously uncultivable *Eleftheria terrae* using iChip technology. Structurally, teixobactin is an undecadepsipeptide (Figure 1) with four D-amino acids, a cyclised C-terminus and also features the uncommon L-*allo*-enduracididine cassette. Initial studies found teixobactin to be potent against a wide range of gram-positive bacteria including *staphylococcus aureus* (MRSA, MIC = 0.25 μg mL^{-1}), and *Mtb* (H37Rv, MIC = 0.125 μg mL^{-1}). The exciting biological properties and interesting structural features of teixobactin make it an appealing tuberculosis drug lead.

The work herein describes the first published total synthesis of the natural product teixobactin by an on-resin Fmoc solid-phase peptide synthesis (SPPS) strategy, amenable to rapid analogue generation for future structure-activity studies.[2] Initial studies focussed on producing the amino acid building blocks that would be required for the synthesis of the natural product, namely suitably protected N-methyl-D-phenylalanine and L-*allo*-enduracididine. With both building blocks in hand, attention then turned to the solid-phase assembly of the peptidic core of teixobactin which included a key on-resin esterification. Following global deprotection, macrolactamization and purification, the natural product teixobactin was afforded over 24 linear steps.

A resazurin based assay was used to determine activity against *Mtb* in addition to a high-throughput screen against a range of Gram-positive and Gram-negative bacteria. Synthetic teixobactin exhibited potent activity against Gram-positive bacteria including the drug-resistant *Staphylococcus aureus* MRSA strain (MIC = 1.1 μM) and against *Mtb* H37Rv (MIC = 1.5 μM), with no activity against Gram-negative pathogenic bacteria. These biological results suggest that teixobactin is a promising candidate for future lead optimisation as a potential drug for the treatment of TB and Gram-positive bacterial infections.

1. Ling, L. L. et al. Nature 2015, 517, 455.
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YI-P260 In Vitro and In-Silico Analysis of the Effects of a Natural Antifungal Peptide (Bacitracin) on Some Human Pathogenic Fungi

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Bacitracin is an antimicrobial peptide produced by the bacteria Bacillus subtilis and Bacillus licheniformis. There is voluminous literature confirming its activity against bacteria, but little is known about it when it comes to fungi. Henceforth, in order to fill in this gap, this study is dedicated to understand the effect of this natural antimicrobial peptide on Cryptococcus neoformans, Cryptococcus gattii, Candida albicans, Exophiala dermatitidis and Alternaria alternata.

Initially, the activity of bacitracin was tested through in-silico methods. A number of bioinformatics tools such as homology modelling, structure validation, docking, active site prediction and structural stability prediction on the basis of total energy have been used successfully to achieve this goal. In addition to the above stated studies, disc diffusion testing and minimum inhibitory concentration assay was also performed following the CLSI guidelines. Minimum inhibitory concentration of the natural antifungal peptides against the different fungi was carried out at a range of 5.5 μg/μl to 44 μg/μl and useful results have been obtained.

This study can prove to be a spearhead in the treatment of infections caused by fungi. A little modification in its structure, like backbone extension, cyclization, heterocyclic generation, N alkylation and Cα substitution can lead to the synthesis of efficient peptidomimetics, helping us to fight the fungal diseases in a better way.

P261 Selective Inhibition of Liver Cancer Cell Proliferation and Migration with Snail Venom Peptide Tv1

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Hepatocellular carcinoma is the third leading cause of cancer deaths worldwide, with over 500,000 people affected. The most common treatment for patients with liver cancer, hepatocellular carcinoma (HCC), is chemotherapy with doxorubicin, 5-fluorouracil or cisplatin, or targeted therapy with sorafenib. Peptidic compounds with high specificity for tumor cells provide a route for killing cancer cells while protecting normal cells and have several advantages, such as low molecular weight, and selectivity for a specific target, organelles or cells with minimal toxicity compared to chemotherapy or radioactive treatment.

This work illustrates the cytotoxic and anticancer properties of Tv1, a small disulfide rich peptide from a terebrid snail *Terebra variegate.* Tv1 appears to inhibit the cell proliferation via apoptosis using Cox-2 pathway in mouse liver cancer BNL 1ME A.7R.1 (chemically transformed from normal liver cells) cells. In-vivo treatment in allograft mouse tumor models also showed significant reduction in tumor sizes over a three week period of treatment. Immunofluorescent co-localization studies indicate Tv1's mechanism of action is inhibition of TRPC6 channels, which are over expressed in cancer cells compared to normal liver cells. Tv1's inhibition of TRPC6 results in downregulation of Cox-2 expression and further PGE2 release, two proteins involved in cell proliferation. Our results suggest the anticancer property of Tv1 can be exploited to contribute to selective HCC therapy.

P262 TransCon PTH - A Sustained-Release PTH Prodrug for the Treatment of Hypoparathyroidism

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The only approved hormone replacement therapy for hypopara-thyroidism (HP) is once-daily sc injection of PTH(1-84) adjunct to calcium and vitamin D. This treatment incompletely controls urinary calcium due to the short 3-hour half-life of the peptide.[1,2] In contrast, continuous infusion of PTH(1-34) to HP patients has been demonstrated to provide simultaneous control of serum and urinary calcium, as well as bone turnover.[3]
In the present work, we show the design of TransCon PTH, a sustained-release prodrug of PTH(1-34). To ensure an infusion-like profile, PTH is transiently bound to a PEG carrier that prolongs the peptide's serum half-life. Through auto-catalytic cleavage only controlled by temperature and pH, unmodified PTH is released independently of enzymatic activity. Several TransCon PTH variants were synthesized with modifications in the molecular structure of the TransCon linker, thereby providing different PTH half-lives. The optimized lead candidate was studied in single-dose pharmacokinetic (PK) studies in which TransCon PTH was administered to rats and monkeys. The individual PK profiles demonstrated mean half-lives of 28 h and 34 h, respectively. Based on this data, chronic daily TransCon PTH dosing at steady state was modelled, suggesting that physiological PTH replacement can be achieved, providing a continuous infusion-like profile. This makes TransCon PTH a promising candidate for treatment of HP.

1. Natpara Product Label
2. J. Clin. Endocrinol. Metab. 101(6): 2273-2283, 2015
3. J. Clin. Endocrinol. Metab. 97: 391–399, 2012

P263 Re-Evaluating the Stability of COMU in Different Solvents

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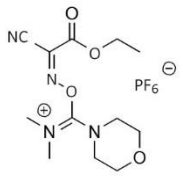
COMU is uronium-type coupling reagent based on OxymaPure. It showed several advantages over classical benzotriazole-based coupling reagents such as higher solubility, efficient coupling performance, water-soluble byproduct, and monitoring the reaction by changing of color. Although COMU is well known to perform excellent in solution but its hydrolytic stability in DMF limits its use in automatic peptide synthesizer. Herein, we evaluated the hydrolytic stability of COMU in γ-valerolactone (GVL), acetonitrile (ACN) and *N*-formylmorpholine (NFM) and compared its stability against DMF. The stability of COMU after 24 h was found to be 88% and 89% in GVL and ACN respectively, when compared in DMF (14%). Further, the demanding Aib-ACP decapeptitde was successfully synthesized using COMU as coupling reagent in GVL and ACN as solvent, respectively.



COMU

P264 Bioinspired PH-responsive Cell-Penetrating Foldamers for Intracellular Delivery of Nucleic Acids

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Natural and synthetic cell penetrating peptides (CPPs) have recently emerged as promising nucleic acid (NA) delivery system mainly due to their limited and well-defined size, their easy tailoring by sequence manipulation and their low cytotoxicity. LAH4 is a His-rich synthetic amphipathic cationic peptide that efficiently deliver plasmid DNA (pDNA) within the cell. It has been demonstrated that due to its pH-responsiveness, the presence of several His residues in CPP sequences can overcome NA endosomal entrapment (proton-sponge effect). From a structural standpoint, this peptide has a strong propensity to fold into an α-helical conformation with all His residues located on one face of the helix.

Mimicking this peptide architecture with synthetic folded oligomers (i.e. Foldamers) constitutes a valuable approach towards the design of original NA transport agents with improved transfection efficiency and enzymatic stability. However, despite significant achievements in Foldamer research, no investigation on their potential use to deliver nucleic acids within the cells has been reported so far.

Herein we will report the design of original pH-responsive bioreducible foldamer architectures based on a small amphipathic oligourea sequence exhibiting His-type units. The capacity of these cell penetrating foldamers (CPFs) to assemble with pDNA to form Foldaplexes (by analogy to polyplexes) and to mediate its delivery will be discussed in details as well as their apparent non-cytotoxicity.

P265 Design and Structure Calculation of a Composite Zinc Finger Tertiary Fold containing a Non-Peptide Foldamer Helical Domain

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De novo protein design has been implemented with the aim to provide new functions to artificial proteins beyond the ones accomplished by Nature. Towards this goal, over the last two decades intense research efforts have been devoted to identify artificial folded architectures (i.e. foldamers) exhibiting well-defined secondary structural preferences and thereby capable of reproducing the structural complexity of natural folding such as helices and β-sheets.

A high number of foldamer backbones are now available, and their high predictability and tunability constitute a great potential for their future applications. However, the design de novo of complex tertiary/quaternary structures using a foldamer-based approach has proved exceptionally challenging. Alternatively, one can consider interfacing synthetic foldamers with natural proteins by replacing a folded segment of a target protein by a complementary foldamer structure.

In this work we have designed a composite protein tertiary fold by swapping the natural α-helical (metal-binding) domain of a zinc finger motif by a non-peptide helical foldamer segment (e.g. peptidomimetic aliphatic N,N'-linked oligoureas). As a first target we selected the third domain of the transcription factor Zif268. This protein which has been extensively studied adopts a well-defined and well characterize tertiary fold and display unique DNA–binding properties.

We have investigated synthetic approaches to access this chimeric zinc finger domain, and have studied in details its metal binding properties, the effect of this 'α- to urea- helix swap' on the overall folding, and herein we will report the first structural elucidation of this α,urea-composite protein domain in solution.

P266 The Convergent Synthesis of a Triple Bridged Insulin Derivative

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The human insulin A- and B-chains were connected together by an additional covalent bond between the side-chain carboxyl function of Glu(A21) and the amino side-chain function of Lys(B29). The suitably protected A- and B-chains were prepared on 2-chlorotrityl resin. In the case of the A-chain the synthesis was started with the esterification of the side-chain carboxyl function of Glu(A21) on the resin. The preparation of the B-chain was started with resin-bound Lys(Mmt). Both protected peptides were cleaved from the resin by treatment with 1.5% TFA in DCM. In the case of the B-chain, the Mmt function was removed concurrently with the cleavage of the B-chain from the resin. Acm and Trt groups were used for the protection of the six Cys residues in various combinations. The protected A-chain was transformed to the corresponding pentafluorophenyl ester and then condensed on the unprotected Lys(B29) side-chain amino function. The obtained single chain insulin was deprotected and oxidized. Best results concerning the disulfide bond pattern was obtained when Cys(A7) and Cys(B7) were protected with Acm and the other Cys residues with Trt group. In this case insulin with the native disulfide bond pattern was obtained as the main product, as it was shown by Glu-C enzymatic degradation and peptide mapping. The third disulfide and the two chains connecting bond was then formed by oxidative removal of the Acm groups by iodine treatment. The opportunities opened to design the third interchain linkage in order to optimize and fine tune the peptide IP, the conformation, the lypophilicity and the enhanced chemical and biological stability of the three bridged chemically synthesized insulin derivatives will be discussed.

P267 Biomimetic Synthesis of Cyclo-Oligomers Using Butelase 1

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Bioactive peptide cyclic dimers such as theta-defensins are highly versatile antimicrobials with multiple functions. Their biosynthetic pathway involves homodimerization and backbone cyclization. Here we report a convenient biomimetic enzymatic approach to prepare cyclodimers and cyclotrimers in a one-pot reaction using an Asn/Asp-specific peptide ligase butelase 1[1]. This plant-derived ligase displays excellent catalytic efficiency and broad substrate tolerance. It recognizes a C-terminal recognition motif Asn-Xaa-Xaa on a peptide substrate and release the Xaa-Xaa tail by an incoming peptide through the formation of a new peptide bond. The incoming amino acid (S1' site in the reaction pocket) tolerates almost any amino acids including D-amino acids[2]. We demonstrated this broad specificity by preparing an all-D-version of [Asn]-theta-defensin using butelase. By introducing short precursor peptide with 7-9 residues, we obtained cyclodimers (10, 12 or 14 residues) and cyclotrimers (15, 18 or 21 residues). Our data also showed that precursors with more than 9 residues favors cyclomonomers. We have established the substrate-length rule in the butelase-mediated oligocyclization. Collectively, our results provide a promising approach to develop stable oligomeric macrocycles for drug design and delivery.

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2. Nguyen GKT, Hemu X, Quek J-P, Tam JP (2016) Butelase-Mediated Macrocyclization of d-Amino-Acid-Containing Peptides. Angew Chem Int Ed 55 (41):12802-12806. doi:10.1002/anie.201607188

P268 Ring Expansion of Cyclic Peptide Thioamides via Imide Intermediates: An Amino Acid Insertion Strategy

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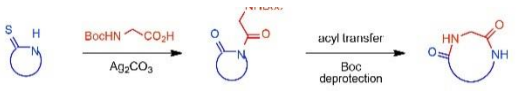
Cyclic peptides are typically constructed by head-to-tail cyclization, coupling the terminal amine and carboxylic acid groups. However, small cyclic peptides are not accessed efficiently via head-to- tail macrocyclization due to conformational constraints inherent in medium sized rings.

Herein we report the development of a ring expansion process that inserts an amino acid residue into a thioamide bond of a thiolactam or cyclic peptide thioamide, via an imide intermediate. The imides generated are *N*-(⍰-aminoacyl) lactams; once formed the exocyclic N nucleophile undergoes nucleophilic attack on the endocyclic carbonyl group, generating a new amide bond with concomitant cleavage of the original 'lactam' bond, thereby generating the ring expanded product. The cyclic peptide imide is produced through use of a site-selective silver(I)-promoted coupling of thioamides with protected ⍰-amino acids recently developed in our laboratory (Scheme 1).[1-3]



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C. A. Hutton, J. Shang and U. Wille, *Chem. Eur. J.* 2016, *22*, 3163–3169.

P269 Assessment of Cell Permeability of Cyclic Peptoids and Linear Peptoids

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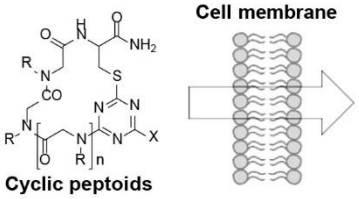
Peptoids, oligomers of *N*-substituted glycines, have several desirable features as peptidomimetics such as ease of synthesis and proteolytic stability. Importantly, peptoids are known to be more cell-permeable than native peptides. Due to the lack of backbone chirality and hydrogen bonding sources, however, peptoids generally have relatively flexible structures, making it difficult to develop peptoid- based protein ligands with high affinity. One possible strategy to restrict conformational flexibility of peptoids is macrocyclization. Cyclic peptoids are anticipated to have conformational rigidity and pre-organized structure, enabling them to bind more tightly to target proteins without major entropy penalty. Because cyclic peptoids do not possess amide protons in their backbones, cyclic peptoids are seemingly cell permeable like linear peptoids. However, no systematic evaluation for cell permeability of cyclic peptoids has been described yet. Herein, we present the assessment of cell permeability of cyclic peptoids relative to their linear counterparts. We have synthesized triazine-bridged cyclic peptoids of different side chains and sizes and the corresponding linear peptoids as fluorescently-labeled analogs. Using fluorescence-activated cell sorting and confocal microscopy, we have demonstrated that cyclic peptoids are far more cell permeable than linear peptoids. Given their excellent cell permeability, along with conformational rigidity and proteolytic stability, we believe that triazine bridged cyclic peptoids may serve as an excellent source of protein binding molecules, particularly targeting intracellular proteins.



P270 One-bead, One-compound Macrocycle Libraries: High Throughput Screening and Sequencing

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Current drug discovery efforts favor small molecules that can be described by Lipinski's "rule of 5", limiting their size and hydrogen donor/acceptor counts. This restricts drug targets to proteins with defined small molecule binding sites. However, important protein-protein interfaces (PPi) remain "undruggable" by small molecules. Although biologics may target PPi's, these molecules are not orally bioavailable and can only target extracellular receptors. The intermediate size of natural product-inspired macrocycles lends the ability to target PPi's while maintaining passive cellular permeability. "One Bead, One Compound" (OBOC) libraries containing $1x10^6$ of diverse compounds with only one compound per "bead" of resin are quickly generated using solid phase peptide synthesis (SPPS). While high-throughput production and screening of OBOC libraries are becoming increasingly robust, identifying hits cyclic peptides by tandem mass spectrometry is known to be inconclusive due to random ring opening during fragmentation. Sequencing has been improved via linearization using cyanogen bromide, but side reactions confound sequencing results. Using a novel incorporation of a backbone ester linkage allows for facile linearization and sequencing for hit identification.

P271 Improvement of the Activity of Intracellular Functional Peptides Using Peptide Array-Based Screening System

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Intracellular functional peptides that play a significant role inside cells have been receiving a lot of attention as regulators of cellular activity. Previously, we proposed a novel screening system for intracellular functional peptides[1)]; it combined a photo-cleavable peptide array system with cell-penetrating peptides (CPPs). Various peptides can be delivered into cells and then intracellular function of each peptide can be assayed by means of our system. In the present study, our purpose was to demonstrate that our screening system is useful for identifying peptides with higher activity. The cell death-inducing peptide (LNLISKLF)[2)] served as an original peptide for screening of peptides with higher activity. As a result of modification of the peptide sequence, we obtained 4 peptides with higher activity, in which we substituted serine (S) at the fifth position with phenylalanine (F), valine (V), tryptophan (W), or tyrosine (Y). During analysis of the mechanism of action, the modified peptides induced an increase in intracellular calcium concentration, which was caused by the treatment with the original peptide. Thus, the present work suggests that our screening system is useful for identification of intracellular functional peptides with higher activity.

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2) Kim JY. *et al.*, Journal of Peptide Science, 19, 8, 2013

P272 DNA-encoded Synthetic Peptides for Ligand Discovery and Enzyme Activity Sensing

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The use of DNA-encoded chemical libraries have emerged as new approach to ligand discovery. A major advantage of this approach is the ability to assess many molecules collectively in an in vitro selection. We apply in vitro selections on DNA-linked peptides for both discovery of ligands and also in a new approach for enzyme activity detection, which we call selection-based sensing. We present the synthesis of both combinatorial and scanning positional DNA-encoded libraries of peptides and peptidomimetics. We present novel peptidomimetic ligands against the trimethyllysine binding domain of the chromobox 8 (CBX 8) protein. In addition, we demonstrate the use of DNA-linked peptide substrates to enable enzymatic assays by DNA sequence analysis (qPCR or parallel DNA sequencing). Development of assay approaches are presented for various activities including protein kinase and protease. Progress is presented towards the use of collections of DNA-encoded peptide substrates for kinase proteomic activity profiling as an alternative to the use of peptide microarrays.

P273 Conjugation of Aurein 2.2 to HPG yields an Antimicrobial with Better Properties

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With the advent of bacterial resistance, it has become crucial to find substitutes to conventional antibiotics. Antimicrobial peptides (AMPs) are considered to be viable alternatives, because they are

broad spectrum and since bacteria develop little or no resistance towards AMPs. Interestingly, only few AMPS are used as therapeutics, due to problems such as toxicity, short circulation half-life, and rapid kidney clearance. Recently, hyperbranched polyglycerol (HPG) has gained attention due to its excellent biocompatibility, multifunctionality, compactness and long blood circulation time, which can be tuned by changing its molecular weight. HPGs have been used as scaffolds for the development of drug delivery vehicles, protein conjugates, and for cell surface modifications. Recent progress on methods to maintain activity and decrease toxicity of these peptides by conjugating to hyperbranched polyglycerol (HPGylation) systems will be presented. The HPGylation of aurein 2.2 decreased the antimicrobial activity (~3-7-fold) and the alpha helical content (~3 fold). On the other hand, the HPGylation of the aurein 2.2 decreased the interaction with blood components, especially to red blood cells and cell cultures (fibroblasts). In order to understand the mechanism of action (MOA) of these bioconjugates, we isotopically labelled aurein 2.2 (using a recently developed expression). This system will permit the determination of bioconjugate structure and interaction with live bacterial cells using NMR to further optimize bioconjugate design. This will also provide an understanding of the MOA of these novel conjugates relative to unconjugated peptides.

P274 Assembly of Tyrosine Rich Peptides and Various Applications

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Peptide self-assembly has received much attention because it can lead to various nanostructures with unique properties. Among amino acid residues, tyrosine has many important roles as a proton-coupled electron transporter in photosystem II, redox catalysts in several enzymes, and a high energy source when cross-linked. Here, we systematically introduced repeating tyrosine units into peptides to study the role of the peptide sequence on self-assembly. We have found that specific sequences of tyrosine-rich peptides (TRPs) could be organized into densely packed 2D film at air/water interface, acting as a redox-active scaffold for polypyrrole film formation [1]. Recently, we have further expanded our study on the TRP's assembly by exerting external stimuli such as surface pressure, metal ions, solvent, and light. For large-scale fabrication and easy transfer of peptide film, Langmuir Blodgett and Schaeffer methods were also utilized to form a monolayer of peptide molecules, accompanied by the conformational change of the peptide molecules. In addition, the peptide conformation was also changed by addition of palladium (Pd) ion, producing the peptide-Pd NPs hybrid nanostructure that showed an efficient catalytic property in the Sonogashira reaction in water. The TRP could also be cross-liked by UV radiation, forming nano-sheets and hollow nano-capsule depending on solvent condition, which could be further applied in various fields.

Reference
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Keywords: Peptide material, Self-assembly, Bio-catalyst, Hybrid material

P275 Fast and Cost Effective Solid Phase Synthesis of High Quality Crude Peptides

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We have studied the factors that affect the quality of crude peptides prepared by solid phase methods with the goal of producing crude peptides with increased yield and purity utilizing overall cost effective procedures. We have found that delay gradient heating during coupling is most effective in minimizing racemization and accelerating coupling rate. Crude peptides prepared with delay gradient heating had higher purity and yields compared to peptides prepared at room temperature, with rapid conventional heating, microwave heating or gradient heating. To reduce solvent waste, we derived a formula to calculate the amount of wash needed to efficiently remove piperidine following Fmoc deprotection. In addition, we developed an algorithm to predict difficult couplings in a peptide sequence. Utilizing this algorithm, we were able to successfully predict when double coupling would be required, thus improving synthesis efficiency and reducing unnecessary and wasteful double coupling.

P276 Apolipoprotein E Mimetic: From Theory to Therapy

Palgunachari N. Mayakonda, Geeta Datta, Roger C. White, David W. Garber, Dennis I. Goldberg, G.M. Anantharamaiah

Background: Dyslipidemia and inflammation are major determinants of atherosclerosis. Apo E (299 amino acid residues) associated with VLDL acts as a ligand for several receptors to clear apo B-containing atherogenic lipoproteins from the plasma. The use of apo E for the treatment of hypercholesterolemia and atherosclerosis is impractical since it is difficult to obtain therapeutic quantities of the protein. We have synthesized a peptide hE-18A that has a class A helical domain (which imparts apoA1 properties) at its C-terminus attached to amino acid residues 141-150 of apo E. Apo E 141-150 domain at N terminus confers several apo E mimetic properties to the peptide including the ability to associate with atherogenic lipoproteins to target them to hepatocytes for clearance. Therefore, it is hypothesized that this novel peptide, containing only 28 amino acid residues that has both apoA1 and apo E mimetic properties, will have anti-inflammatory and anti-dyslipidemic effects.
Aim: To evaluate the anti-inflammatory and anti-dyslipidemic effects of hE-18A peptide.
Results: In apoE null mice administration of 100 □g/mouse of hE18A dramatically reduced plasma cholesterol. In high fat fed rabbit the cholesterol levels in the peptide treated groups were lower at different time intervals as compared to the controls (1082± 119 m/dl in 7.5 mg/kg peptide group vs. 2146± 210 mg/dl in control p<.0.005 day 13). Sub-fractionation of total cholesterol revealed a predominant lowering of VLDL like particles in the peptide treated groups compared to controls. The endothelium dependent relaxation was significantly impaired in saline treated hypercholesterolemic rabbits. Peptide administration significantly improved vessel relaxation in hypercholesterolemic rabbits suggesting increased nitric oxide bioavailability. There was dose dependent decrease in total cholesterol compared to controls after peptide administration in homozygous WHHL rabbits. The effect of cholesterol lowering was seen as early as 30 minutes after peptide administration. The maximum lowering of plasma cholesterol levels was observed at 7 hours. Recently in cholesterol fed monkeys, a single administration of the peptide maintained the plasma cholesterol lower than the baseline for one week.

Conclusions: The apo E mimetic peptide dramatically lowers the atherogenic lipoproteins in mouse models, two dyslipidemic rabbit models and in monkey model of dyslipidemia. There is improved endothelial function that may be attributable to decreased cholesterol levels. Clearance of plasma cholesterol in WHHL rabbit model indicates that the peptide is enhancing the clearance of atherogenic lipoproteins through a mechanism other than LDL

receptor pathway. Combining the observations made in the two dyslipidemic rabbit models, we propose that the peptide is enhancing either the levels of apo E (the ligand for remnant lipoproteins), LDL receptor like proteins or enhancing lipoprotein lipase levels which enhances the lipoprotein metabolism via a more abundant heparin sulphate proteoglycan pathway. The peptide also has dramatic anti-inflammatory effect. This peptide has shown promise in Phase I clinical trials.

P277 Improving Biopharmaceutical Properties of Human Glucagon with Selective Histidine Substitutions

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Glucagon is a 29 amino acid pancreatic hormone. While physiologically important for maintaining glucose homeostasis, it is inadequate as a drug candidate due to poor aqueous solubility and high tendency for fibrillation. However, with increasing insulin usage by the diabetic population, there is a growing need for a safer and more user-friendly means of delivering this life-saving drug, either via a ready to use emergency device or a bi-hormonal pump. We have previously reported glucagon analogs with selective substitutions at positions 6, 10 and 13 with 3- and 4-pyridyl alanine (3 and 4-Pal). These analogs have shown a dramatic increase in solubility at physiological conditions (pH 7.4) with full retention of biological activity. Incorporation of Aib at position 16 additionally prevented aggregation of these glucagon analogs. Expanding on the concept of aromatic hydrophilicity we investigated a series of glucagon analogs selectively substituted with histidine. Similarly to the Pal series, analogs with His substitutions at position 10 and 13 exhibited high *in vitro* bioactivity. In contrast to the Pal analogs, histidine substitution was not tolerated at position6. Glucagon analogs doubly substituted at positions 10 and 13 in combination with previously studied Aib16, exhibited full *in vivo* potency with a more rapid onset of action and clearance when compared to native glucagon.

P278 Simultaneous Label-Free Analysis of 1485 Antibody-Antigen Interactions

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Protein-protein interactions (PPIs) are crucial for most cellular and biochemical processes and represent emerging drug targets. Label-free biosensor systems allow for a real-time monitoring and the precise characterisation of PPIs including the exact determination of kinetic parameters. However, until now, this analysis method was rarely combined with other high throughput technologies such as high density microarrays. Biametrics' SCORE technology (single colour reflectometry) implements the interference of monochromatic light for high throughput measurements at high spatial resolution based on affordable glass-type transducers in a conventional microscope slide format. It hence provides the advantages of label-free techniques meeting the requirements of multiplex analysis of large chemical libraries. Here, we characterise the binding of an anti-FLAG antibody to 1485 FLAG epitope variants which were produced through alanine scanning mutagenesis. By means of photolithographic immobilisation, high-density peptide microarrays were first generated. Without further processing, the arrays were then analysed using Biametrics' b-screen device. All binding events were detected simultaneously and exact kinetic parameters were determined. This study establishes SCORE as a robust and cost-effective technique to characterise PPIs within few minutes.

P279 Chemical Protein Synthesis of Bovine Pancreatic Trypsin Inhibitor: The Utility of Isonitrile-Activated C-terminal Thiocarboxylic Acids for Peptide Coupling

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We are interested in developing efficient ligation methods for peptide synthesis. The Danishefsky laboratory has demonstrated the chemoselective activation of C-terminal thiocarboxylic acids using hindered isonitriles to form native amide bonds. When combined in series with native chemical ligation (NCL), this isonitrile-activated coupling method enabled the synthesis of a large 174-residue containing human protein, granulocyte colony-stimulating factor (G-CSF). During a several month internship with the Danishefsky laboratory, I developed linear and convergent routes for the chemical synthesis of bovine pancreatic trypsin inhibitor (BPTI) to demonstrate an expanded utility of this method. BPTI, also known as aprotinin, is a 58-residue serine protease inhibitor and an ideal benchmark target for our method. This challenging protein target contains three disulphide linkages comprised from seven cysteine (Cys) residues, four charged lysine (Lys) residues and a troublesome hydrophobic region. Herein we demonstrate optimized isonitrile-activated coupling procedures for the synthesis of BPTI using minimally protected peptides.

P280 Towards Cost-Efficient, Scalable Green SPPS

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In recent years there has been a considerable focus on use of green chemistries in the manufacturing of chemicals and pharmaceuticals. Due to the enormous amounts of solvents used in these industries a specific point of emphasis has become the utilization of greener solvents and various green solvent guidelines have been put forth. In the field of synthetic peptides great advances have been made in the past decades while the impact of peptide chemistry on the environment has remained largely unaddressed. In fact, the vast majority of amide bond formations are still carried out in DMF and CH2Cl2, two solvents that are questionable in sustainable chemical processes. In the realm of synthetic peptides SPPS constitutes a prevalent methodology and several reports on greening of SPPS have appeared. Nevertheless, the reported green solid-phase peptide syntheses hinge on use of solvents that are compatible with expensive PEG resins and require use of large excesses of AA raw materials in couplings, or entirely new sets of protected amino acid derivatives. Herein we report on an efficient Fmoc-based SPPS of a classical, difficult 10-mer peptide which i) does not require use of hazardous solvents such as DMF ii) is carried out on an inexpensive PS resin iii) does not use the controlled substance piperidine in Fmoc deprotections iv) employs low AA equivs in couplings throughout. Details of a scaled up green SPPS will also be disclosed.

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P281 Cell Wall Piracy by a Synthetic Analog Reveals Metabolic Adaptation in Vancomycin Resistant Enterococci

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Drug-resistant bacterial infections threaten to overburden the healthcare system and disrupt modern medicine. A large class of potent antibiotics, including vancomycin, operate by interfering with bacterial cell wall biosynthesis. Vancomycin-resistant Enterococci (VRE) evade the blockage of cell wall biosynthesis by altering cell wall precursors, rendering them drug insensitive. Herein, we reveal, for the first time, the phenotypic plasticity and cell wall remodeling of VRE in response to vancomycin in live bacterial cells. Synthetic cell wall analogs were designed and constructed to monitor cell wall structural alterations. Our results demonstrate that the biosynthetic pathway for vancomycin-resistant precursors can be hijacked by synthetic analogs. Finally, we describe a rapid proof-of-principle diagnostic tool based on our synthetic cell wall reporter strategy for the visual classification of VRE. Together, the direct monitoring of VRE cell wall remodeling by our probes establishes the contribution of individual metabolic processes to the evolution of drug resistant phenotypes.

P282 De novo Design of a Hyperstable, Non-natural Protein-ligand Complex with Sub-Å Accuracy

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If we truly understand proteins, we should be able to design functional proteins purposefully from scratch. While the de novo design of proteins has seen many successes, no small molecule ligand- or organic cofactor-binding protein has been designed entirely from first principles to achieve i) a unique structure and ii) *a predetermined binding-site geometry with sub-Å accuracy*. Such achievements are prerequisites for the design of proteins that control and enable complex reaction trajectories, where the relative placements of cofactors, substrates, and protein side chains must be established within the length scale of a chemical bond. We designed a novel protein, PS1, which binds a highly electron-deficient, non-natural porphyrin at temperatures up to 100 °C. The high-resolution structure of holo-PS1 is in sub-Å agreement with the design. The structure of apo-PS1 retains the remote core packing of the holo, predisposing a flexible binding region for the desired ligand-binding geometry. Our results illustrate the unification of core packing and binding site definition as a central principle of ligand-binding protein design. Further application of this principle should facilitate the design of proteins that bind small molecules, peptides, and larger proteins in precisely predetermined configurations.

P283 Fast and Cost Effective Solid Phase Synthesis of High Quality Crude Peptides

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We have studied the factors that affect the quality of crude peptides prepared by solid phase methods with the goal of producing crude peptides with increased yield and purity utilizing overall cost effective procedures. We have found that delay gradient heating during coupling is most effective in minimizing racemization and accelerating coupling rate. Crude peptides prepared with delay gradient heating had higher purity and yields compared to peptides prepared at room temperature, with rapid conventional heating, microwave heating or gradient heating. To reduce solvent waste, we derived a formula to calculate the amount of wash needed to efficiently remove piperidine following Fmoc deprotection. In addition, we developed an algorithm to predict difficult couplings in a peptide sequence. Utilizing this algorithm, we were able to successfully predict when double coupling would be required, thus improving synthesis efficiency and reducing unnecessary and wasteful double coupling.

P284 Group-Assisted Purification (GAP) Technology for Peptide Synthesis

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The peptide therapeutics market has been growing rapidly for years, both financially and as an increasing sector of the pharmaceutical industry.[1] This market is projected to continue growing; therefore, new synthetic methodologies to reduce the cost of peptide synthesis and enable larger scale syntheses are highly desired.[2] We have developed a solution-phase, Fmoc / tBu peptide synthesis method utilizing Group-Assisted Purification (GAP) chemistry, by design of a small-molecule (MW = 292 Da) protecting group. This protecting group, equipped with a phosphine oxide moiety, facilitates selective precipitation of the peptide from solution after each coupling step, with an average isolated yield per coupling of 97%. All fully protected peptides are white, crystalline solids that are easy to handle and can be stored for over 1 year without noticeable degradation. Reactions are run in homogeneous solution without the use of bulky polymers, leading to highly efficient couplings and high crude peptide purity. GAP peptide synthesis is amenable to a wide variety of coupling strategies, protecting groups, and deprotection reagents. Our process is also semi-automated, and we are working towards full automation. Examples of peptides synthesized using the GAP process are presented.

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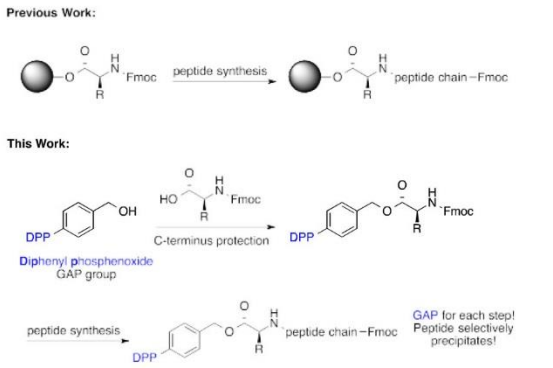

P285 Porphyrin-peptoid Conjugates as Novel Biomimetic Light-Harvesting Complexes

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Porphyrins, a class of naturally occurring pigments, have been actively investigated due to their wide applications ranging from photosensitizers to new optoelectronic materials. There has been much effort to construct a defined arrangement of porphyrins, particularly, in a face-to-face arrangement of porphyrin π planes. In this presentation, we wish to present an efficient strategy for (1)

face-to-face arrangement of porphyrins, and (2) energy donor-acceptor dyad synthesis using Zn-porphyrin and free base porphyrin on peptoid (or oligo-N-substituted glycine) helices. Distance and orientation of porphyrins are precisely defined employing characteristic structural features of peptoid helices, which results in distinct optical properties and energy transfer efficiency of the porphyrin-peptoid conjugate.
A set of porphyrin-peptoid conjugates displaying porphyrins in various spatial arrangements was synthesized and characterized by UV-Vis, circular dichroism (CD), and emission spectroscopy, which provided strong evidences for intermolecular and intramolecular interactions between porphyrins.1 Exciton-coupled CD (ECCD) spectral signature of porphyrins indicated handedness and structural integrity of peptoid helices. In addition, donor-acceptor molecular dyads were constructed using zinc porphyrin and free base porphyrin, and energy transfer efficiency was evaluated. Depending on the relative spatial arrangement and distance of donor and acceptor, precisely controlled energy transfer efficiency was demonstrated by stationary fluorescence emission and time-resolved transient absorption spectroscopy. The porphyrin-peptoid conjugates will be useful for understanding the optoelectronic behavior of porphyrins and potentially for the development of photosensitizers, light-harvesting systems, and sensors.
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P286 Pre-purify Peptides with Flash Chromatography to Increase Purification Throughput

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Peptides are often used as active site models in drug discovery and as Active Pharmaceutical Ingredients (APIs). The increased use of peptides necessitates improved purification techniques. Purification of synthesized peptides is a bottleneck. Impurities, and the injection solvent required to dissolve the sample and impurities, limit the amount of the desired peptide that can be loaded in a single purification run. Pre-purification of peptides by flash chromatography improves throughput by reducing impurities, that have significantly different polarities when compared to the target compound, loaded on the preparative column. Removal of these impurities also allows a more appropriate injection solvent that results in sharper peaks permitting an even higher sample loading.

P287 Mapping the Passive Permeability Landscape of Cyclic Hexapeptides

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Passively permeable (and orally bioavailable) cyclic hexapeptides such as 1NMe3 showed that this class of molecules has exciting potential as drug molecules, but is 1NMe3's passive permeability exceptional for cyclic hexapeptides? To answer this question, an OBOC library of 1800 cyclic hexapeptides and hexapeptomers was synthesized and a data analysis pipeline for highly multiplex PAMPA of one-bead one-compound (OBOC) libraries was developed. After PAMPA permeability scores were assigned to each chromatographic peak at each mass of interest, tandem mass spectroscopy data was used to sequence all peptides. Sequencing was accelerated by limiting the search space to only those sequences which are part of the OBOC library design. Virtual fragmentation spectra of potential sequences were compared to observed spectra and a similarity score was generated. The best scoring sequence at each neighborhood of MS^2 spectra was then matched to the PAMPA data by retention time. The high passive permeability of the library indicates that 1NMe3 is far from alone, but the extent to which these compounds tolerate addition of biologically relevant functional groups remains to be seen.

P288 Little Science - Big Difference in Peptide Purification Processes

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DAISO Fine Chem USA, Inc.

Peptide-based APIs are getting more complex and the bar for their downstream purification is constantly raised. Screening for the optimal silica-based stationary phase in Reversed Phase High Pressure Liquid Chromatography (RP HPLC) is often time-consuming and a financial burden in peptide manufacturing. Usually the last step, polishing for purification >95%, has strong impact on process economy, especially in the scale-up of RP HPLC processes.

This presentation aims to:
•Describe the important physicochemical parameters of silica particles and bonded media;
•Highlight pore and particle size, and carbon density to point out key roles they play in the purification process;
•Outline in-house pharma communication to develop efficient RP HPLC processes for lead compounds to manufacturing.