Y1. Abstract number: 403

'Clicking' peptides: Disulfide bond minetics by the CuAAC reaction.

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The CuAAC reaction introduced in 2001 by Tornøe et al.1 is a quantitative and completely chemo-selective reaction. In contrast to the chemo-selective formation of disulfide bonds used by Nature to stabilize the protein fold, the triazole formation is irreversible. The triazole has been found to mimic disulfides quite well as deemed by the bioactivity measured on compounds containing this mimetic. Furthermore, due to the quantitative nature of the reaction it is feasible to perform formation of several triazoles as mimetics of knots in peptides containing several disulfide bonds. The reactions can be performed on solid support to yield the mimetic peptides directly upon cleavage from the support. We performed the reaction on tachyplesin 1 analogs and conotoxin analogs with incorporation of propargyl glycine and either L-5-azido-2-Fmoc-amino-pentanoic acid (Orn) or L-4-azido-2-Fmoc-amino-butyric acid (Dab) as the reaction partners in place of the endogenous Cys. The biological activity of a tachyplesin analog was investigated and the antibacterial activity corresponded excellently with that of the natural compound. Calculation of the conformation of the analog and comparison with the known NMR structure showed that the triazole analog adopt conformations that are very close to that of the natural compound. We find that 1,4 substituted 1,2,3-triazoles are excellent disulfide bond mimetics and the CuAAC reaction can be a valuable alternative when metabolic stability of a disulfide bond is an issue.

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Y2. Abstract number: 129

Cellular expression of the human Angiotensin II type 1 receptor containing the non-canonical photolabelling amino acid Bpa

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The incorporation of non-coded amino acids into functional proteins in mammalian cells has recently been achieved by several laboratories, mostly through engineered tRNA-aminoacyl-tRNA synthetase pairs for the specific incorporation of unnatural amino acids through recognition of an AMBER codons. We applied this approach to a G Protein Coupled Receptors (GPCR) in order to ultimately identify the binding partners (e.g. ligands, signal transduction- and regulatory proteins) of these receptors through photocross-linking. The human angiotensin II type 1 receptor (hAT_1), a peptidergic GPCR, has been extensively studied using synthetic AngII analogues containing the photoreactive residue p-Benzoyl-L-Phenylalanine (Bpa); this has allowed to map the ligand binding domain of hAT_1 and to deduce its structure. As a proof-of-concept for later protein-receptor interaction studies we applied this approach for an inverse labelling study. The Amber codon TAG was inserted in a site specific manner (Lui et al., 2007) into the hAT_1 receptor gene at known ligand-interaction residues. Following co-transfection of this gene, together with a CUA bst tRNA, its engineered cognate Bpa specific aminoacyl-tRNA synthetase, and in presence of free Bpa, the mammalian cell line COS-7 was able to expressed two such hAT_1 mutants (i.e. F293Bpa hAT_1 and C296Bpa hAT_1). Both receptor mutants displayed native hAT_1 like binding affinity towards Ang II and [Sar^1, Ile^8] Ang II albeit with reduced expression rates. In absence of either one of the transfection elements or of free Bpa, no receptor expression was observed. hAT_1 and both mutant receptors were photolabelled with ^{125}I-[Sar^1, Bpa^8] Ang II and all gave identical CNBr digestion patterns, proving the integrity of the engineered protein. Synthesis on non-oxidized ^{125}I-[Sar^1, Met^8] Ang II as a Bpa selective bait ligand was not successful and an alternative identification strategy had to be found. The constitutively active N111G hAT_1 template tolerates N-terminal ligand modifications. For this purpose, [Iminobiotinyl-Aca^0, Gly^1, Met^8] AngII was synthesized (KD16 nM on N111G hAT_1; >500 nM on hAT_1) and N111G hAT_1 was mutated with the same TAG codons to produce the N111G/F293Bpa and N111G/C296Bpa hAT_1; they will be used for photo cross-link experiments with the iminobiotin ligand and HRP-Streptavidin detection of the cross-linked complex.

Y3. Abstract number: 415

Metal-Ion Directed Self-Assembly of Insulin Derivative

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Insulin, a small protein with three disulfide bridges, is a widely used biopharmaceutical drug for the management of glucose levels in diabetic patients. Human insulin forms a hexamer around a Zn(II) ion, as insulin has an inherent ability to form hexamers by combining three dimers, where each dimer has an antiparallel orientation. Several variants of insulin have been developed that are either fast- or long-acting, mainly by controlling the diffusion rates from subcutaneous depots via manipulations of insulin oligomer states. Both fast- and long-acting insulin doses are needed in diabetes treatment in order to approach the release of insulin in a healthy person, thus allowing optimal blood sugar control around the clock. New methods to control insulin self-assembly and dissociation are thus highly relevant.

Here we describe the introduction of a second metal ion binding site in insulin for controlled nano-scale self-assembly and its characterization by UV-Vis, CD and SEC. We utilized an insulin variant with two strategic amino acid substitutions relative to human insulin (B9Asp+B27Glu), which consequently has a low tendency to form a hexamer. In order to induce selectivity over the native His binding site we chose a metal ion chelator different from those known in native biomolecules. We selectively attached a non-natural bipyridine, a well characterized and redox stable compound. Adding iron(II) at physiological pH, cause the derivative to self-assemble into a three-blade propeller shaped supermolecule. The understanding and design of functional larger dynamic clusters that allow release of individual molecules in a controlled manner, could lead towards new long acting drugs.

Y4. Abstract number: 325

The novel fully-synthetic receptor for phosphoproteins

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Protein phosphorylation is a critical modulator of the most important of the cellular processes. In signal transduction

cascades, reversible phosphorylation of serine, tyrosoine and threonine backbones influences and governs the selective intra-protein interactions. Many biologically relevant species (like: enzymes) are activated/deactivated through the conformational changes induced by the phosphorylation events. Despite the fact that these processes are widespread in the biological systems the methods allowing for sensitive detection of phosphorylated proteins still remain imperfect and based mostly on post-modification techniques. Herein, we present an alternative approach to detect phosphorylated proteins. The technique is based on peptide-small molecule hybrid which can be readily prepared by means of organic synthesis. The artificial receptor is not only capable to extract phosphorylated proteins from complex biological mixtures but is also able to discriminate between smaller phosphorylated species and folded proteins. The presented technique is completely bio-compatible, not-toxic and stable in physiological conditions. Flexible structure of the binder opens the possibilities for further modification according to the desired need. This property enables us to utilize the receptor both for diagnostic and monitoring purposes. Here we describe the design, synthesis and the performance of the binder.

Y5. Abstract number: 445

Synthesis and Structural Characterization of All Four Diastereoisomers of the Crossed Alkene-Bridged Nisin DE-Ring Mimic.

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The lantibiotics represent a class of antimicrobial peptides, in which the unusual amino acids dehydroalanine and dehydrobutyrine and the intramolecular thioether bridges (lanthionines) are important structural features for bioactivity. Nisin is the most-prominent representative of the lantibiotics, and it inhibits the bacterial cell-wall biosynthesis by binding to lipid II via its N-terminus. The lipid II - nisin complex is responsible for pore-formation since the C-terminal part of nisin is inserted into the bacterial cell membrane which ultimately results in cell leakage and collapse of vital ion gradients. In order to increase the metabolic stability of nisin, the oxidation-sensitive thioether bridges can be replaced by metabolically stable dicarba moieties, as successfully demonstrated by the synthesis of nisin AB(C) analogs containing alkane/alkene bridges.[1] The most interesting fragment of nisin is the C-terminal intertwined DE-ring which has a i->i+3, i+2->i+5 connectivity pattern. To obtain more insight into the importance of this cross-bridged structure on nisin's bioactivity, we synthesized a series of all four diastereomers of the crossed alkene-bridged DE-ring mimic. This synthesis is based on the cyclization of a linear allylglycine-containing hexapeptide into the correctly knotted 1->4, 3->6 bicyclic hexapeptide using ring-closing metathesis, and all four diastereoisomers were obtained by HPLC and structurally characterized by NMR spectroscopy. An orthogonal protection scheme was used, to enable the independent N- or C-terminal modification of the bicyclic hexapeptides with azide/alkyne functionalities. Via Cu(I)-catalyzed cycloaddition chemistries, alkyne-functionalized natural ABC-fragments of nisin, which were obtained by tryptic digestion of full length nisin followed by HPLC purification, have been conjugated to synthetic DE-ring mimics to obtain novel nisin derivatives and their affinity toward lipid II and pore-forming capacity have been studied. Herein, we report on the details of the synthesis and characterization of the geometric isomers of the synthetic DE-ring mimics, and their use as synthons in Cu(I)-catalyzed click chemistry to obtain newly designed nisin hybrids as potential novel peptide antibiotics.

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Y6. Abstract number: 361

The Engineering of an Orally Active Conotoxin for the Treatment of Neuropathic Pain

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Neuropathic pain is caused by nerve damage and is among the most severe forms of chronic pain, affecting more than 60 million people. Current medications have severe limitations and there is an urgent need for new treatments. Peptides derived from cone-snail venoms have recently attracted attention as leads for the treatment of pain. These conotoxins have exquisite selectivity and potency for receptors involved in pain pathways. However, like most peptides, native conotoxins suffer from disadvantages of short half-lives, susceptibility to proteolysis, and poor oral bioavailability, limiting their value as therapeutics. Here we show that by re-engineering a conotoxin with a cyclic peptide backbone we overcame these limitations and produced an orally active analgesic with exciting potential for the treatment of neuropathic pain in humans. The cyclic conotoxin is more than two orders of magnitude more potent than the leading clinically used human therapeutic, gabapentin, in a rat model of neuropathic pain, and has enhanced receptor specificity and stability over its linear counterpart. Our approach of engineering a cyclic backbone onto bioactive peptides is applicable to the stabilization of a broad range of peptide-based therapeutics and has the potential to unlock the hitherto unrealised potential of peptides as next generation drugs.

Y7. Abstract number: 46

Enhancement of Receptor Selectivity of Cilengitide by Multiple N-Methylation

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In recent years multiple *N*-methylation of peptides has proven to be a powerful technique for medicinal chemists to increase the potential of peptides as drugs. *N*-Methylation not only improves the biological activity and selectivity profiles of peptides [1] but also may overcome their pharmacokinetic limitations i.e. increasing their metabolic stability and bioavailability [2].

In the present study we focused on Cilengitide [3], an integrin binding peptide cyclo[RGDf(N^{Me})V], which is now in clinical phase III for treating brain tumors and phase II for non-small cell lung cancers. To study the effect of multiple *N*-methylation on this pharmacologically relevant peptide, all ten dimethylated analogues of Cilengitide were synthesized and their binding affinity for different integrin receptors subtypes (αvβ3, αvβ5, α5β1 and αIIbβ3) was evaluated. The analogues in which Val was non-methylated showed a dramatic decrease in integrin affinity, regardless of the position of the *N*-methylated residues. However, when Val was presented as *N*-methylated, the resulting analogues showed low nanomolar affinities for the αvβ3 integrin receptor and improved selectivities for other receptor subtypes (800-fold over αvβ5; 50 to 100-fold over α5β1) compared to the parent peptide Cilengitide, thus improving its selectivity profile.

Furthermore, the effect of *N*-methylation on the oral bioavailability of these peptides is under investigation.

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[2] M. A. Dechantsreiter, et al., *J. Med. Chem.* **1999**, *42*, 3033-3040.
[3] J. Chatterjee, et al., *Accounts. Chem. Res.* **2008**, *41*, 1331-1342.

Y8. Abstract number: 144

Enzymatic synthesis of C-terminally activated peptide esters and their use in industrial chemo-enzymatic peptide synthesis

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To reduce the high costs associated with state-of-the-art chemical peptide synthesis, DSM embarked on a research programme on industrial chemo-enzymatic peptide synthesis. In order to avoid the inevitable enzymatic hydrolysis within the fragments, we focused on the use of near-anhydrous conditions applying enzymes that are stable in organic solvents.

We developed a high yielding enzymatic peptide synthesis strategy using an N-terminally protected amino acid (AA) C-terminal alkyl ester and a C-terminal AA tert-butylester as nucleophile. Aqueous removal of the tert-butyl group (enzymatic or by mineral acid) is often accompanied by side reactions such as hydrolysis. We discovered that the C-terminal tert-butyl ester protecting group can be enzymatically interconverted in one single step to give an alkyl ester which can be re-used for a new coupling on the C-terminus. Hence, deprotection and activation are performed simultaneously. We now disclose the elaboration of this enzymatic tert-butyl ester interconversion strategy towards the industrial synthesis of various pharmacologically active peptides up to the pentamer level.

However, when using alkyl esters for C-terminal activation of AAs and peptides, the incorporation of some AAs, e.g. proline, unnatural- or D-AAs remains a challenge. Several strategies are known from the literature to solve these issues, of which activation of the AA by ”substrate mimetics”such as guanidinophenyl2 esters or very activated esters such as carbamoylmethyl (Cam)3 or trifluoroethyl (Tfe) are the most successful ones. Nevertheless, chemical synthesis of these esters is laborious, cost-inefficient and accompanied by racemisation.

We now additionally disclose for the first time the enzymatic synthesis of AA and peptide C-terminal Cam and Tfe esters and their applicability in enzymatic peptide synthesis, with challenging nucleophiles such as proline and D-AAs being smoothly incorporated. Finally, a novel and industrially attractive ”two enzyme-one-pot strategy”will be disclosed, wherein two peptide fragments are coupled in the presence of an activating alcohol, an activating enzyme and a coupling enzyme giving the desired peptides in excellent yields.

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2. Bordusa, F. et. all. Angew. Chem. Int. Ed. 1997, 36, 2473-2475.
3 Miyazawa, T. et. all. Chem. Soc., Perkin Trans. 1, 2002, 390-395.

Y9. Abstract number: 167

Promising Tools for Breast Cancer Therapy: Carbaborane-Containing NPY Analogs

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The human Y_1-receptor subtype was found to be overexpressed in more than 90% of breast cancer patients and in 100% of breast cancer derived metastases [1]. Peptides that selectively bind to receptors over-expressed in the membrane of cancer cells are a promising tool for tumor diagnosis and therapy. Neuropeptide Y (NPY) as well as pancreatic polypeptide (PP) and peptide YY (PYY) selectively bind at Y-receptors (Y_1, Y_2, Y_4 and Y_5) which belong to the rhodopsin-like G-protein coupled receptors (GPCRs). Binding of ligands to Y-receptors leads to ligand induced internalization of the receptor. Using NPY analogs with high affinity to only one receptor subtype allows to selectively target only one Y-receptor subtype [2].

Boron Neutron Capture Therapy (BNCT) is a binary therapy using nontoxic ^{10}B, which is able to absorb nontoxic thermal neutrons to result in an excited state ^{11}B. ^{11}B decomposes to form highly toxic ^{4}He particles (alpha particles) and ^{7}Li with a short radiation range of 9 or 5 µm inside the cell. Using these effects, BNCT can be applied in tumor therapy [3].

In this work the combination of both therapeutic approaches is described. Carbaborane-modified amino acids were introduced into receptor-selective NPY analogs by Fmoc/*t*-butyl solid phase peptide synthesis. The resulting peptides were tested for their affinity towards Y_1-receptors, their ability to induce signal transduction and receptor internalization.

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Y10. Abstract number: 3

Bicyclic peptides with tailored binding specificities

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We are generating bicyclic peptide ligands with high affinities and specificities for disease targets using an approach that I had recently developed with Sir Greg Winter at the Laboratory of Molecular Biology (LMB) in Cambridge, UK (1). Briefly, linear peptides on phage are chemically modified to obtain phage-encoded combinatorial libraries of bicyclic peptides and binders are isolated in affinity selections. With this approach, inhibitors with nanomolar affinites to the human disease targets plasma kallikrein and cathepsin G could be generated. The bicyclic peptides combine key qualities of antibody therapeutics (high affinity and specificity) and advantages of small molecule drugs.

Currently, we are generating bicyclic peptide inhibitors of proteases that play important roles in tumour growth and metastasis formation. First potent and selective inhibitors of such a protease, the urokinase-type plasminogen activator (uPA), could be isolated and suppressed tumour cell migration in an in vitro assay.

(1) Heinis, C., Rutherford, T.; Freund, S.; Winter, G., Phage-encoded combinatorial chemical libraries based on bicyclic peptides. Nat Chem Biol (2009).

Y11. Abstract number: 311

Peptide architecture: A two-helix variant of PYY3-36 for improved membrane binding and receptor affinity

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The prevalence of obesity is increasing worldwide, which is reflected in increased occurrence of obesity-linked diseases, such as type II diabetes, and there is a need for anti-obesity drugs. The peptide hormone PYY3-36 has appetite suppressing potential by activating Y2 receptors. Thus novel analogues of PYY3-36 with high Y2 receptor selectivity and potency pose potential as drugs for the treatment of obesity. We have previously hypothesized that PYY3-36 binds to the membrane prior to interaction with its receptors. Based on this hypothesis, and that the N terminus of PYY has been shown to be a key segment for Y1 receptor selectivity, we have designed a number of novel PYY3 36 analogues for increased membrane binding affinity.

Here we suggest substituting the whole N-terminal segment of PYY3-36 with a non-native amphipathic α-helix to create a two-helix analog of PYY3-36. A number of different amphipathic heptad repeat sequences were incorporated. The design resulted in a helix-loop-helix structure, where the N-terminal helix originates from a heptad repeat sequence and the loop as well as the C-terminal helix originates from the native PYY sequence. Affinity as well as functionality studies of the two-helix analogues revealed that heptad repeat sequences with a net positive change were preferred. One of the most promising analogues was further investigated by solution NMR techniques in the presence of phospholipid micelles. In solution the peptide exits in an oligomeric state preventing its detailed structural analysis. In contrast in presence of dodecylphosphocholine (DPC) micelles that mimic biological membranes, the peptide is characterized by two well-defined α-helices. One of those is constituted by the C-terminal α-helical segment that is conserved in peptides of the NPY family both in solution and in the membrane-associated state. The second helix comprises the amphiphilic N-terminal segment and forms a ninety-degree angle with the C-terminal helix. The improved biological activity of the novel analogues provides further support for the hypothesis that some peptide hormones bind to the membrane prior to activating the membrane-bound receptor.

Y12. Abstract number: 550

Design and Total Chemical Synthesis of an Achiral Protein

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All natural proteins comprise polypeptide chain(s) made up of L- amino acids and are inherently chiral. One way to make an achiral protein would be to artificially symmetrize the molecule by creating an inversion center within the protein molecule itself. This could be achieved by covalently joining in cyclic fashion the polypeptide chains of an L-protein and its mirror image D-protein {i.e. composed of all D- amino acids with opposite handedness}. The resulting meso-protein molecule will be achiral and thus devoid of net optical activity. Such a hypothetical achiral protein molecule is unknown in nature and can only be realized using chemistry. To demonstrate the feasibility of preparing such an intricate molecule we have designed a cyclic protein molecule based on the natural product plectasin where half of the molecule will be made up of L-amino acids and the other half will be composed of D-amino acids. The L- and D- polypeptide are covalently joined, either with additional spacers or linked by direct amide bond in head-to-tail fashion, such that the resulting protein molecule will posses a center of symmetry. Total chemical synthesis of the achiral ‘meso-plectasin’ protein molecule was highly challenging and necessitated the use of fully convergent synthesis from three peptide segments using kinetically controlled chemical ligation. Folding behavior of the 80 residue synthetic polypeptide will be reported, along with physicochemical properties and structure of this unique protein molecule.