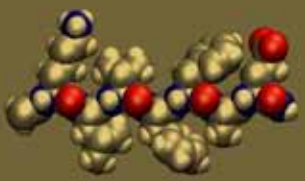
October 2-5, 2012

The Sixth Peptide Engineering Meeting

Emory University Conference Center–Atlanta, Georgia



PEM6

Learning from Nature to Engineer Peptides

Co-Chairs

Lila M. Gierasch, UMass Amherst
Joel Schneider, National Cancer Institute
David G. Lynn, Emory University

International Program Committee

J. Schneider (USA)
D. Lynn (USA)
L. Gierasch (USA)
C. Toniolo (Italy)
E. Giralt (Spain)
J. Clayden (England)
K. Mihara (Japan)
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Welcome to PEM6
Learning from Nature to Engineer Peptides

The co-chairs of the sixth Peptide Engineering Meeting, PEM6, would like to welcome you to Atlanta, Georgia for this exciting international workshop addressing structure-based approaches to peptide design, peptide-protein interactions, peptide-membrane interactions, peptide bioactivity, and peptide-based materials. The theme of this year's meeting is *Learning from Nature to Engineer Peptides*.

The PEM series was established in 1997 by Professors Susumu Yoshikawa, Kyoto University, and Claudio Toniolo, University of Padova. These special meetings are held every three years and are designed to present cutting-edge advances in peptide science from leading international speakers, and have young scientists and students as major participants. The PEM series serves as a superb vehicle to foster international collaboration and exchange of ideas, and exposure of trainees to new opportunities outside their own countries. From a tour of venues over the past 15 years, the meeting comes now to the United States to grace the beautiful city of Atlanta, Georgia.

We would like to thank our program committee: C. Toniolo (Italy), E. Giralt (Spain), J. Clayden (England), K. Mihara (Japan), M. Nomizu (Japan), S. Futaki (Japan) for their help in assembling a fantastic program that covers themes in peptide science ranging from chemical biology to protein folding, from materials science to enzymology, and more.

The venue for PEM6 is a wonderful conference center and hotel complex at Emory University. The Conference Center architecture was inspired by Frank Lloyd Wright, the famous American architect whose structures were designed in harmony with Nature following his personal philosophy of "organic architecture". The setting provocatively links nature and design and provides perfect inspiration for the goals of PEM6.

We look forward to seeing old friends and making new ones and most importantly, to discussing the exciting peptide science that has evolved since the last meeting in Barcelona.

Lila M. Gierasch, University of Massachusetts Amherst
David G. Lynn, Emory University
Joel Schneider, National Cancer Institute

The History of the Peptide Engineering Meeting (PEM) Series

In 1994, during his sabbatical leave at the Institute for Protein Research, Osaka University, Japan, Claudio Toniolo of the University of Padua, Italy, was contacted by Susumu Yoshikawa (Osaka National Research Institute, ONRI) who shared his desire to start a series of international meetings, termed PEMs (Peptide Engineering Meetings), to be held every three years with a rotation system among Asian (or Australasian), European, and American countries. These meetings were to be innovative in terms of their small number of participants, their choice of speakers from a range of countries who present exciting research in peptide engineering, their highly interdisciplinary topics, the organization of the program to encourage interaction, and a focus on young scientists as the principal target attendees. Thus was born the PEM series.

Claudio Toniolo and Susumu Yoshikawa (now Professor at Kyoto University) co-chaired PEM 1, which was held at ONRI, Ikeda (Osaka) in 1997.

The PEM series has continued to be held and to adhere to the attributes first articulated by Susumu and Toniolo. The sites, dates, and chairs of PEMs 1-5 are listed below.

To enhance the legacy and long term significance of the highly successful, interactive, international PEM series, manuscripts submitted by invited speakers at each PEM have been published in dedicated issues of *Biopolymers Peptide Science*, coordinated by its Editors-in-Chief, first the late Murray Goodman, then Lila Gierasch, and most recently, Joel Schneider

PEM1
Venue: Ikeda (Osaka), Japan, 1997 – Chairs: S. Yoshikawa, C. Toniolo

PEM2
Venue: Capri (Naples), Italy, 2000 – Chairs: E. Benedetti, C. Toniolo

PEM3
Venue: Boston (MA), USA, 2003 – Chair: C. M. Deber

PEM4
Venue: Yokohama, Japan, 2006 – Chair: H. Mihara

PEM5
Venue: Barcelona, Spain, 2009 – Chairs: E. Giralt, C. Toniolo

Schedule of Events

Registration on Tuesday and and the Welcome Reception are in the Great Hearth Room. Registration on Wednesday is adjacent to the Emory Amphitheatre on the Garden Level where all lectures will be held. Breaks are in the Emory Break Area, lunches are in the Dining Room, and posters are in Salon III/IV/V with exhibits and poster refreshments in the Garden Overlook and adjacent area. The banquet will be held in the Silver Bell Pavilion. [See floorplan on page 12.]

Tuesday, October 2, 2012

4:00-7:30 pm	Registration
7:00-7:15	Welcome from Co-chairs David Lynn, Emory University Lila Gierasch, University of Massachusetts Amherst Joel Schneider, National Cancer Institute
5:30-7:30	Welcome Reception

Wednesday, October 3, 2012

7:30 am -4:30 pm	Registration
7:15-8:15 am	Continental Breakfast and Poster Set-up
8:15-8:20	Opening Remarks, Lila Gierasch
8:20-9:05	Opening Keynote Lecture (sponsored by Aileron, Inc.) Laura Kiessling, USA – **Controlling cell fate decisions with peptides**

SESSION I (Chair: Claudio Toniolo, Italy)

9:05-9:40 am	Sheena Radford, UK (sponsored by Lilly, Inc.) – **Molecular Conversion in Amyloid Formation**
9:40-10:15	Joan Emma Shea, USA (sponsored by CSBio) – **Simulations of peptide aggregation**
10:15-10:30	Ulla Gerling, Germany – **The influence of fluorinated amino acids on amyloid forming model peptides**
10:35-11:05	Coffee Break/Poster Session

SESSION II (Chair: Shiro Futaki, Japan)

11:05-11:40 am	Annalisa Pastore, UK (sponsored by CSBio) – **Functional interactions as a survival strategy against abnormal aggregation: The example of ataxin-3**

11:40-12:15	Sihyun Ham, Korea – **Understanding protein aggregation in water**
12:15-12:35	Bradley Nillson, USA – **Rippled β-sheet fibrils from co-assembly of enantiomeric amphipathic peptides**
12:35 pm	Lunch and Posters

SESSION III (Chair: Ernest Giralt, Spain)

2:10-2:45 pm	Gilles Guichard, France – **Structural characterization of foldamers comprising urea linkages**
2:45-3:20	Annelise Barron, USA (sponsored by Waters, Inc.) – **World's most dangerous chemotherapeutics: Peptoid mimics of host defense peptides**
3:20-3:40	Jonathan Clayden, UK – **Conformational switching for transmembrane communication**
3:40-4:00	Hisakazu Mihara, Japan – **Self-assembling peptide materials for cell scaffolds**
4:00-4:30	Coffee Break/Poster Session

SESSION IV (Chair: Hisakazu Mihara, Japan)

4:30-5:05 pm	Ron Wetzel, USA – **Studying polyglutamine amyloid formation and structure using β-turn mimics**
5:05-5:40	Takaki Koide, Japan – **Engineering and applications of collagen-like triple helical peptides**
5:40-6:00	Anil Mehta, USA – **Battle for supremacy in peptide amphiphile macromolecular assembly**
6:00-8:00	Poster Session with Refreshments

Thursday, October 4, 2012

7:30-8:30 am Continental Breakfast

8:30-8:35 Opening Remarks, David Lynn

SESSION V (Chair: Jonathan Clayden, UK)

8:35-9:10 am Robert Hancock, Canada (sponsored by CSBio) –
Designing antimicrobial, anti-biofilm and immunomodulatory peptides for combating infections

9:10-9:45 William DeGrado, USA (sponsored by Biotage) –
From peptides to proteins: Analysis of the hierarchic organization of proteins and de novo design of protein-like architectures

9:45-10:05 Jianmin Gao, USA –
Targeting membrane lipids with designed peptides

10:05-10:25 Alex Smirnov, USA –
Interactions of peptides with lipid nanotubular bilayers of defined curvatures

10:25-10:55 Coffee Break/Poster Session

SESSION VI (Chair: Ikuo Fujii, Japan)

10:55-11:30 am Margaret Sunde, Australia –
Using amyloid to your advantage

11:30-12:05 Aphrodite Kapurniotu, Germany –
Peptide-based inhibition of protein aggregation in amyloid disease: from self- to cross-recognition

12:05-12:25 pm Hirokazu Tamamura, Japan –
HIV gp41 trimer mimics for vaccines and fusion inhibitors

12:25-12:45 Alessandro Moretto, Italy –
Peptide self-assembly and microstructure formation

12:45 Lunch and Posters

SESSION VII (Chair: Rudi Fasan, USA)

2:30-3:05 pm	Tomi Sawyer, USA – **Stapled peptide drugs: Mechanism of cell penetration**
3:05-3:40	Junko Ohkanda, Japan – **Natural product-based chemical probes for detecting 14-3-3-phospholigand interactions**
3:40-4:00	Ikuo Fujii, Japan – **Post-antibodies: Generation of molecular-targeting peptides by directed evolution in phage-displayed libraries**
4:00-4:30	Coffee Break/Poster Session

SESSION VIII (Chair: Hirokazu Tamamura, Japan)

4:30-5:05 pm	Bing Xu, USA (sponsored by American Peptide Society) – **Nanofibrils of small peptide derivatives formed by molecular self-assembly**
5:05-5:40	Chie Kojima, Japan (sponsored by CSBio) – **Preparation of artificial proteins based on dendrimers**
5:40-6:00	Ana Salome Veiga, Portugal – **Arginine containing self-assembling peptides form potent antibacterial hydrogels**
7:00	Banquet with multimedia performance **First Life: Imagining the Chemical Origins of Life**

Friday, October 5, 2012

7:30-8:30 am	Continental Breakfast
8:30-8:35	Opening Remarks, Joel Schneider

SESSION IX (Chair: Motoyoshi Nomizu, Japan)

8:35-9:10 am	Seiji Sakamoto, Japan – **Design of a sensing system for caspase activities using a combination of split-fluorescent proteins and split-intein**
9:10-9:45	Meritxell Teixido, Spain – **Present and future of peptides as blood-brain barrier shuttles**
9:45-10:05	Giovanna Ghirlanda, USA – **De novo design of functional protein assemblies: towards hydrogen production**
10:05-10:35	Coffee Break/Poster Session

SESSION X (Chair: Yoon-Sik Lee, Korea)

10:35-11:10 am	Vincent Conticello, USA – **Peptide nanotubes from lock-washer stacking of coiled-coil assemblies**
11:10-11:45	Hanna Rapaport, Israel – **Self-assembled anionic β-sheet peptides in monolayers and hydrogels**
11:45-12:20 pm	Hee-Seung Lee, Korea – **3D organic molecular architectures: Folding into shape**
12:20-1:05	Isabella L. Karle Keynote Lecture Honoring the occasion of her 90th birthday (with support from Bruker AXS, Inc.) P. Balaram, India (sponsored by CSBio) **Engineering peptide structures: Architecture, design and construction**
1:05 pm	Closing Remarks: Lila Gierasch, David Lynn, Joel Schneider

First Life:
Imagining the Chemical Origins of Life

– string quartet, narration, and live audio and video

- music/video – Steve Everett (Emory University)
- performers – Vega String Quartet
- narration/text - Martha Grover (Ga Tech) and David Lynn (Emory University)

The creation of First Life draws upon stochastic modeling of chemical data provided by researchers in Martha Grover's Research Group at the School of Chemical and Biomolecular Engineering at Georgia Institute of Technology. Each section of the work is constructed from contingent outcomes drawn from Grover's research on early Earth formations of organic compounds, as well as earlier experiments by Miller-Urey. Data representation types in this composition include discrete, continuous, stochastic, and interactive forms. This project attempts to create data-driven auditory models, or sonifications, of many of the elemental and environmental conditions present in early Earth thus providing a new way to imagine the salient biochemical morphologies at play in the origins of evolution. First Life is supported by the NSF/NASA Center for Chemical Evolution.

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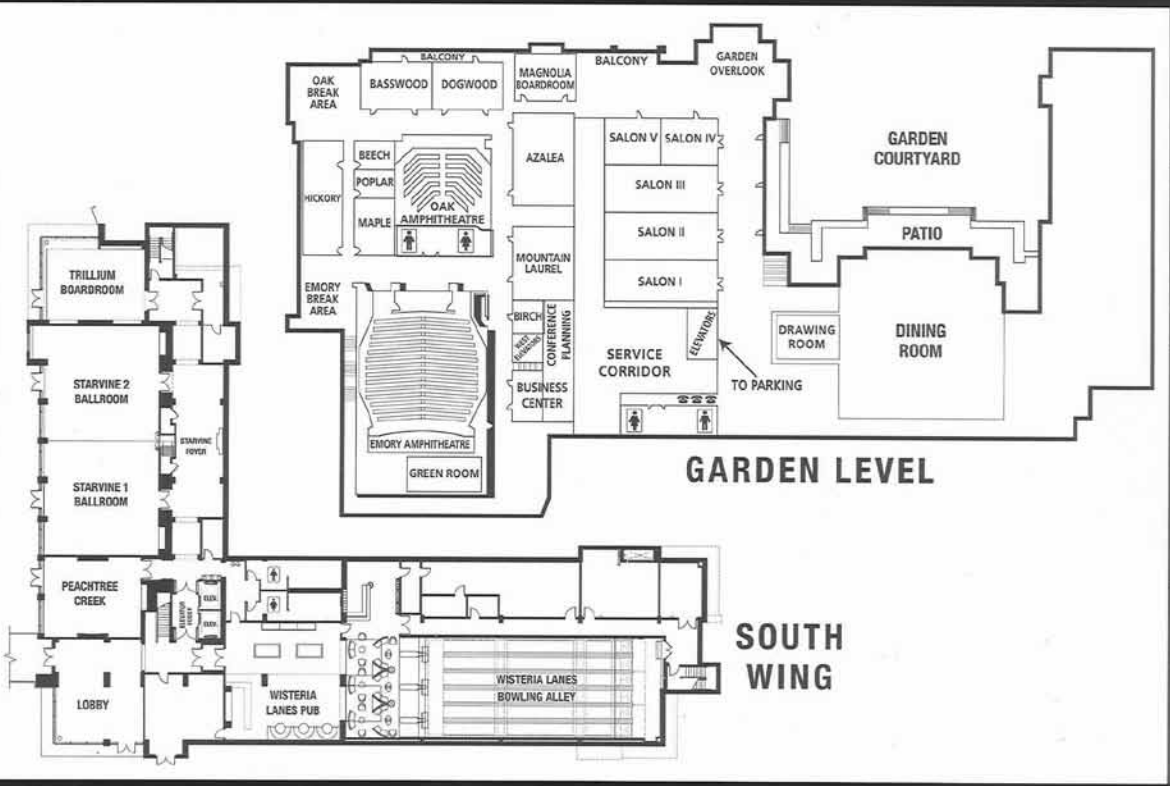
MEETING SPACE FACILITIES
FLOOR SPECIFICATIONS



Speaker Abstracts

Controlling cell fate decisions with peptides

Laura L. Kiessling
Departments of Chemistry and Biochemistry, University of Wisconsin, Madison, WI U.S.A.

Human pluripotent stem cells (human embryonic stem cells and induced pluripotent stem cells) are extraordinary. They can self-renew indefinitely, and they can differentiate into all cell types. Coaxing them to either propagate or differentiate remains a challenge. Because most culture conditions are chemically undefined, it has been difficult to determine the requisite signaling pathways that must be activated or suppressed. Our goal is to identify chemically defined conditions to control human pluripotent stem cell self-renewal and differentiation. In physiological settings, the microenvironment of the niche transmits information through three types of components: soluble molecules, neighboring cells, and the collection of secreted proteins and glycans that is termed the extracellular matrix. In seeking conditions for human pluripotent stem cell culture and differentiation, researchers have focused identifying soluble components that influence cell decisions. We postulated that insoluble components also deliver signals that direct cell decisions. To test this hypothesis, we devised arrays of chemically defined surfaces composed of self-assembled monolayers that display peptides. From these arrays, we identified peptide-substituted surfaces with surprising assets: They not only permit human pluripotent stem cells to differentiate to specific lineages but even *instruct* them to do so. Our findings highlight the dramatic effects of insoluble cues on stem cell pluripotency and differentiation.

Molecular conversion in amyloid formation

Sheena E. Radford
School of Molecular and Cell Biology, Astbury Centre for Structural Molecular Biology, University of Leeds, Leeds LS2 9JT UK (Amherst, MA USA)

Most proteins fold efficiently to their native structures in vivo, assisted by molecular chaperones. It is now widely known, however, that proteins do misfold and that misfolding events can result in conformational disease. Work in our laboratory aims to elucidate the mechanisms by which proteins fold or misfold and aggregate, so as to provide information key to the development of therapeutics against misfolding disease. In the lecture I will discuss the nature of protein folding and misfolding landscapes and discuss recent data from our laboratory that have focused on developing an armoury of biochemical and biophysical methods to elucidate the mechanism of amyloid formation at a structural molecular level. In particular, focusing on the protein, beta-2-microglobulin, I will discuss our new insights into the structural molecular mechanism of amyloid assembly and describe recent data from our laboratory which suggest that amyloid fibrils may be more than just the inert end products of protein fibrillation.

Simulations of peptide aggregation

Joan-Emma Shea
Department of Chemistry and Biochemistry and Department of Physics, University of California Santa Barbara (Santa Barbara, CA USA)

A number of diseases, known as amyloid diseases, are associated with the pathological self-assembly of peptides. I will discuss fully atomic simulations of the early stages of aggregation of the Alzheimer Amyloid-beta peptide implicated in Alzheimer's Disease. I will also introduce a novel off-lattice coarse-grained peptide model that can be used to simulate the entire aggregation process from monomers to fibrils. The effects of beta-sheet propensity and of surfaces on the morphology of the aggregates will be discussed.

The influence of fluorinated amino acids on amyloid forming model peptides

Ulla I. M. Gerling, Mario Salwiczek, and Beate Koksch
Department of Biology, Chemistry and Pharmacy, Institute of Chemistry and Biochemistry, Freie Universität Berlin (Berlin, Germany)

Due to the unique stereoelectronic properties of fluorine, fluorinated amino acids can have dramatic effects on protein stability as well as on protein-protein interactions.[1] However, systematic approaches towards assessing their properties have mainly focused on α-helical systems, although fluoro-amino acids were shown to exhibit lower intrinsic helix propensities compared to their hydrocarbon analogues.[2] Furthermore, fluorination of specific β-sheet positions within globular proteins has already been shown to have a stabilizing effect suggesting that fluorinated amino acids may generally be well suitable for non-helical structures such as β-sheets. *De novo* designed amyloid forming peptides offer interesting applications as self-assembling biomaterials and in this context, fluorine modifications may add a new dimension to the design principles. Our aim was to substitute natural amino acids within a *de novo* designed coiled-coil based amyloid forming model peptide by amino acids that contain different amounts of fluorine in their side chain to allow for a systematic evaluation of fluorine's impact on amyloid formation. The structure of the model peptide is based on an engineered coiled-coil folding motif that was designed to provide an α-helical starting structure and can fold into β-sheets and amyloids at certain conditions.[4] Substitution with fluorinated amino acids was accomplished for the two neighbouring valine residues 13 and 14. The resulting peptides with a different fluorine content show unexpected folding behaviours that cannot be easily rationalized based on stereoelectronic reasoning.

References

1. M. Salwiczek, E.K. Nyakatura, U.I.M. Gerling, S. Ye, B. Koksch, Chem. Soc. Rev. 2012, 41, 2135.
2. H. P. Chiu, R.P. Cheng, et al. J. Am. Chem. Soc. 2006, 128, 15556.
4. U.I.M. Gerling, B. Koksch, Biomacromolecules, 2011, 12, 2988.

Functional Interactions as a survival strategy against abnormal aggregation: The example of ataxin-3

Annalisa Pastore
MRC National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK

Protein aggregation is associated to an increasing numbers of diseases which have a large impact in our society. It is thus in order to wonder how and to which extent protein native states are protected against aggregation. Valuable insights for understanding protective mechanisms can be gained through the study of the relatively few proteins that aggregate under native conditions. Ataxin-3, the protein responsible for Spinocerebellar ataxia type 3, a polyglutamine expansion disease, represents one of such examples. Polyglutamine expansion is central for determining solubility and aggregation rates of ataxin-3, but these properties are profoundly modulated by its N-terminal Josephin domain which is per se prone to aggregate and form fibres. In my talk, I shall present data from an extensive study aiming at characterizing the aggregation pathway of both ataxin-3 and the isolated Josephin domain and identifying the regions that promote Josephin fibrillogenesis. Using different biophysical techniques, aggregation propensity predictions and rational design of aminoacid substitutions, I shall show that Josephin has an intrinsic tendency to fibrillize under native conditions and that fibrillization is promoted by two solvent-exposed patches, which are also involved in recognition of natural substrates, such as ubiquitin. Indeed, designed mutations at these patches or substrate binding significantly reduce Josephin aggregation kinetics. Our data allow us to rationalize the mechanisms that protect Josephin and non-expanded ataxin-3 from aberrant aggregation and provide evidence that protein non-pathologic function can play an active role in preventing protein aggregation.

Understanding protein aggregation in water

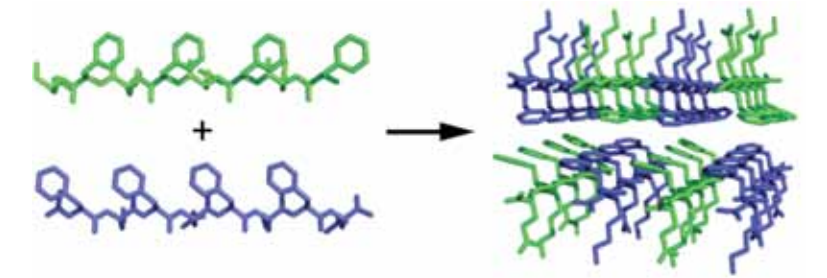
Sihyun Ham
Department of Chemistry, Sookmyung Women's University, Seoul, Korea

Globular proteins may convert their native conformations into non-native forms due to the intrinsic or external perturbations in microenvironmental conditions. When a protein is in its non-native state, it can further unfold or self-assembly to form amyloid aggregates, which are presumably toxic and consequently can cause various diseases. In this regard, protein aggregation is one of the most actively investigated issues in relation to the development of the therapeutics and medical applications for the cure of protein aggregation diseases. We recently developed novel theory and computational methods to effectively execute thermodynamic quantities on the dynamical process of protein, especially protein misfolding and aggregation processes. By taking advantage of this novel method, we have successfully provided the molecular basis on the experimentally observed folding, misfolding, and aggregation phenomena on various proteins. Here, I report our recent efforts on the structural and thermodynamic investigations on the folding, misfolding and aggregation processes of various proteins using the unguided, fully atomistic, explicit-water molecular dynamics (MD) simulations as well as the integral-equation theory of liquids. The MD simulated atomic-level structures of various aggregation-prone proteins are provided and verified by the experimentally available structural data. Gibbs free energy and its constituents including protein internal energy, protein configurational entropy, and solvation free energy are reported to elucidate thermodynamic driving forces for the misfolding and aggregation processes of proteins in water. I will address the role of water as well as the role of key residues in initiating the misfolding and aggregation processes of various proteins associated with diseases.

Rippled β-Sheet Fibrils From Coassembly of Enantiomeric Amphipathic Peptides

Bradley L. Nilsson, Ria J. Swanekamp, and John T. M. DiMaio
Department of Chemistry, University of Rochester (Rochester, NY USA)

Amphipathic peptides with alternating hydrophobic and hydrophilic amino acids readily self-assemble into β-sheet fibrils that have been exploited in a number of biotechnology applications. We recently discovered that mixtures of enantiomeric amphipathic peptides efficiently coassemble into hybrid nanofibrils composed of alternating enantiomeric sequences consistent with Pauling's prediction of "rippled β-sheet" structures (*Proc. Natl. Acad. Sci. U.S.A.* 1953, 39, 253–256). Specifically, equimolar mixtures of L- and D-(FKFE)2 peptides selectively coassemble into rippled β-sheet fibrils rather than self-sort into enantiomeric all-L and all-D fibrils (*J. Am. Chem. Soc.* 2012, 134, 5556–5559). Isotope-edited infrared and fluorescence FRET spectroscopy support the coassembled rippled β-sheet packing orientation. Isothermal titration calorimetry indicates that formation of coassembled rippled β-sheet fibrils is thermodynamically preferred compared to formation of self-assembled all-L and all-D fibrils. Rippled β-sheet fibrils are a fundamentally new class of engineered peptide biomaterial that increases the complexity that can accessed in the design of noncovalent peptide architectures.



Structural characterization of foldamers comprising urea linkages

Gilles Guichard
Université de Bordeaux – CNRS, UMR 5248, institut Euroipéne de Chimie et biologie, (Bordeaux, France)

Structures and functions of biopolymers have inspired the design, synthesis and characterization of a multitude of synthetic oligomeric backbones with well defined and predictable folding patterns, *i.e. foldamers*.[1,2] Recent advances in *foldamer* chemistry have opened avenues towards the use of *foldamers* to interfere with biological functions. The diversity of *foldamer* structures originates from the chemical diversity of constituent units that have been developed to impose conformational restriction and promote folding. Many *foldamer* backbones are built from amino acid type units expressing diversity at the side chain level (e.g. peptoids, aliphatic β- and γ-peptides, aromatic δ-peptides). In the past few years, we have been studying folding propensities of oligomers without amide linkages. Aliphatic urea oligomers are a class of peptidomimetic *foldamers* that form a well defined and stable helical fold akin to the α-helix.[3] We have now delineated the extent to which the canonical helix geometry is affected by the introduction of alternative substitution patterns and "minimal" backbone modifications. This of practical utility if one aims to utilize the oligourea scaffold to place side chains in the 3D-space and to elaborate functional helices. We will also mention our efforts towards the elaboration of more sophisticated folded molecular architectures (*i.e.* long helices, supersecondary structures).

References
1. Gellman, S. H. (1998) Foldamers: A Manifesto, Acc. Chem. Res. 31, 173-180.
2. Guichard, G., and Huc, I. (2011) Synthetic foldamers, Chem. Commun. (Camb) 47, 5933-5941.
3. Fischer, L., and Guichard, G. (2010) Folding and self-assembly of aromatic and aliphatic urea oligomers: Towards connecting structure and function, Org. Biomol. Chem. 8, 3101-3117.

World's most dangerous chemo-therapeutics: peptoid mimics of host defense peptides

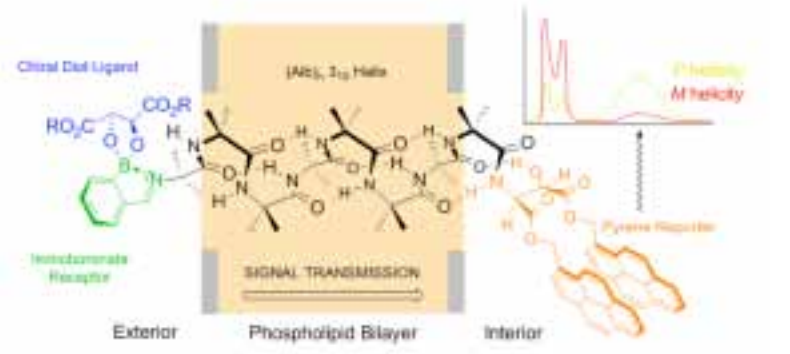
Annelise E. Barron
Department of Bioengineering, Stanford University (Palo Alto, CA, USA)

We have developed peptoid mimics of host defense peptides (**HDP**s). We designed 5-17mer peptoids as physical structural mimics of the cationic, helical HDPs (a.k.a. antimicrobial peptides), such as the magainins and cathelicidins. Highly active peptides can incapacitate pathogens at low-μM concentrations, while highly selective compounds have relatively low toxicity to host cells and/or whole animals at effective anti-pathogenic concentrations. We studied peptoid/peptide mechanisms of action and determinants of selectivity, working with almost 100 peptoid sequence variants and always comparing directly with real HDPs. For natural HDPs and peptoids, activity and selectivity are governed by relative extents of net positive charge, hydrophobicity, amphipathicity, and we believe, self-association into meta-stable assemblies that dissemble on encountering electronegative cell membranes. Our biophysical mechanistic studies utilized peptoids with orthogonal activity/selectivity profiles, which displayed either high (or low) activity, and either high (or low) selectivity, creating a grid of mechanistic data with comparisons to selective and non-selective HDPs. Vesicle leakage, membrane depolarization, and TEM data have yielded surprising information about the inter-relationships between mechanisms of exerting damaging effects on bacteria, fungi, and mammalian host cells. The most active antibiotic peptoids were cytotoxic; this is also true of important classes of HDPs. We posit that bacteria, fungi, and mammalian cells have all evolved mechanisms of resisting and recovering from the damage HDPs can cause. I will describe the striking mechanistic similarities between antibacterial and cytotoxic activities of our peptoid HDP mimics and natural HDPs. I hypothesize a central role for HDPs in the etiology of human degenerative disease, including Alzheimer's and other amyloid diseases (atherosclerosis, diabetes, and other maladies) as a consequence of the HDPs' many interrelated, highly potent mechanisms of action—both beneficial and damaging.

Conformational switching for transmembrane communication

Robert Brown, Simon Webb, and Jonathan Clayden
School of Chemistry, University of Manchester, Oxford Road, Manchester M13 9PL, UK

G-Protein coupled receptors (GPCRs) are one of nature's principal ways of addressing the challenge of communicating information through a cell membrane. In essence, such receptors are switchable structures in which a conformational change, initiated by binding of a ligand, propagates over nulti-nanometre distances and leads to a remote chemical effect. We are developing functional analogues of GPCRs likewise capable of communicating information through impermeable barriers, but based on much simpler structures: namely, the 310 helix of Aib (2-aminoisobutyric acid) homopeptides. An N-terminal binding site allows ligands to induced a bias towards either M or P helicity, detectable by a C-terminal reporter. Recent results show that a boronic acid receptor which binds chiral diols may induce a screw-sense preference in the otherwise achiral peptide, with the screw-sense in turn modifying the conformation, and hence excimer/monomer emission, of a C-terminal chiral fluorophore [figure 1]. Based on the homology of the peptides with known membrane active structures (eg the cephaibols) we are exploring translation of this principle from solution to the membranes of synthetic vesicles, leading to artificial transmembrane signalling.



Self-assembling peptide materials for cell scaffolds

Masaki Tsuchiya, Kazuto Fukunaga, Hiroshi Tsutsumi, and Hisakazu Mihara
Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology

Self-assembly of biomolecules has attracted increasing interests for fabrication of nano- and micro-scaled biomaterials. In particular, some of designed peptides that self-assemble into nanofibers can form a hydrogel and be utilized as biomaterial scaffolds for cell engineering with biocompatibility and biodegradability. Designed peptides have advantages in modulating molecular assembly and introducing functionality[1].

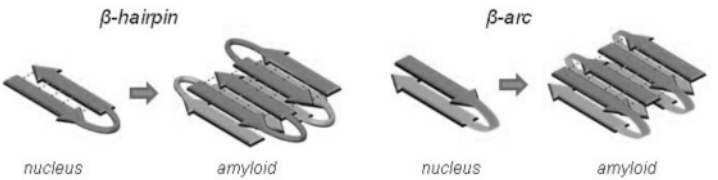
We have found that *de novo* designed short peptides, Y9 (Ac-YKYEYKYEY-NH_2), self-assembled into highly-networked fibrillar structures with β-sheet conformations in aqueous solution. In addition, we have constructed self-assembling peptide nanofibers with responsive ability to calcium ion (Ca^{2+}) to control assembled structures. A various number of Glu residues were conjugated to the N termini of Y9 as a Ca^{2+} responsive site (EnY9). Additionally, the cell-adhesive peptide (RGDS) was introduced to the C termini of E1Y9 to functionalize as cell-adhesive scaffolds (E1Y9-RGDS). In the presence of $CaCl_2$, EnY9 and E1Y9-RGDS peptides assembled into wider tape-like structures than Y9, and formed stable hydrogels. On the hydrogels composed of E1Y9, E1Y9-RGDS and Ca^{2+}, 3T3-L1 cells were cultured more than 7 days without any toxicity. The self-assembling peptide materials based on the Y9 peptide are useful for cell scaffolds.

Reference
1. (a) T. Sawada, T. Takahashi, H. Mihara. J. Am. Chem. Soc., 2009, 131, 14434–14441. (b) T. Sawada, M. Tsuchiya, T. Takahashi, H. Tsutsumi, H. Mihara, Polymer J. 2012, 44, 651-657.

Studying polyglutamine amyloid formation and structure using β-turn mimics

Ronald Wetzel
Department of Structural Biology, University of Pittsburgh School of Medicine (Pittsburgh, PA USA)

In contrast to many other amyloids, the fibrils formed by disease-associated polyglutamine (polyQ) sequences appear to be composed of antiparallel β-sheet. While the simplest structures of anti-parallel fibrils consist of strands connected by β-turns within β-sheets, it is also possible that the β-strands are connected by β-arcs spanning two sheets, as in the case of the parallel β-sheet in Aβ amyloid. Similarly, the critical nucleus for polyQ amyloid formation is a high energy monomer that has been modeled as a folded molecule with one or more β-hairpins; but alternative folds involving β-arcs are at least theoretically feasible, as shown below. To investigate these questions, we have been studying short



polyQ sequences containing various well-characterized β-hairpin encouraging motifs, such as charge complementary termini, D-Pro-Gly, trpzip motifs, and disulfide bonding. We previously showed that while K_2QNK_2 peptides with N = 26 or longer aggregate via a monomeric nucleus, peptides with N = 23 or shorter require a low efficiency tetrameric nucleus. Here we show that if any of the β-turn mimics are installed into polyQ of about N = 23, aggregation is greatly enhanced and nucleus size (n*) drops to ~1. Furthermore, the amyloid fibrils produced are also more stable, exhibiting ΔΔG values compared with $K_2Q_{23}K_2$ fibrillization that are consistent with the known β-hairpin enhancing power of the installed motifs. The amyloid fibrils produced exhibit EM morphology, FTIR and ssNMR spectral features, and cellular activities identical to fibrils from unbroken polyQ sequences. These data support the β-turn model for nuclei and fibrils. The β-turn motifs also allow us to predict the fold of the nucleus and of polyQ within the fibril, allowing us to engineer molecules for particular tasks. In one example, N-methylation of a specific backbone amide group essentially completely eliminates aggregation to generate a peptide with strong amyloid inhibitory activity.

Engineering and applications of collagen-like triple helical peptides

Takaki Koide
Department of Chemistry & Biochemistry, School of Advanced Science and Engineering, Waseda University (Tokyo, Japan)

The collagen triple helix consisted of tandem repeats of Xaa-Yaa-Gly tripeptide units is a unique tertiary structure that characterizes the collagen family proteins (Figure). To date, various collagen-like peptides that mimic the native triple helical structure have been synthesized and characterized. The accumulated knowledge has enabled us to prepare triple helical peptides with various chain arrangements and amino acid sequences, and to control the thermal stability of triple helices.

Here, I will talk about two topics related to the design, synthesis and applications of chemically prepared collagen-like peptides.

1) Development of supramolecular architectures based on collagen-like peptides
Challenges have been made to develop totally synthetic collagen surrogates which are useful in regenerative medicine. I will report recent progress on designing collagen-like supramolecules via self-assembly of peptides.

2) Biomedical applications of soluble triple helical peptides
Taking advantages of the unique features of the collagen triple helix, we have been seeking possible biomedical applications of synthetic collagen-like peptides. Here, some applications of the peptides *in vitro* and *in vivo* will be presented.

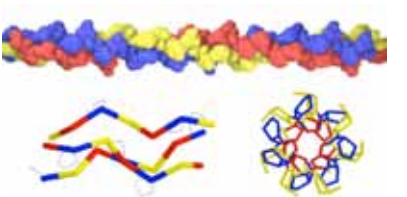
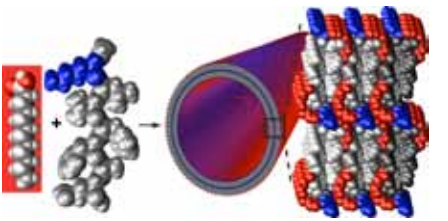


Figure: The collagen triple helix

Battle for supremacy in peptide amphiphile macromolecular assembly

Anil K Mehta, Rong Ni, W. Seth Childers and David G. Lynn
Department of Chemistry, Emory University (Atlanta, GA USA)

Peptide amphiphiles are promising candidates for the construction of highly ordered, functional, self-healing assemblies for use in nanotechnology and biomedicine. Covalent hybrids of traditional biological macromolecular families (e.g. proteins with lipids) have created novel self-assembling materials, but it has been the models of short peptides as structural and functional bilayer membranes that has created the greatest materials interest. For all peptide amphiphiles presented to date, the hydrophobic lipid tail has directed assembly. Here we demonstrate an entirely new model in which the peptide core directs self-assembly. X-ray diffraction and solid-state NMR $^{13}C\{^{15}N\}$ REDOR heteronuclear distance measurements between the ^{13}C enriched alkyl chain and ^{15}N enriched peptide backbone, assign specific distance restraints on the positions of the peptides and alkyl chains. In contrast to previous peptide amphiphile models where the alkyl chain is proposed to form worm-like micelles, our atomic-level distance measurements demonstrate that the alkyl chains in fact are buried between two β-sheets. This discovery suggests that a vast array of higher-order structures and morphologies may be accessible to these chimeric self-assembling materials and that we are now armed with the techniques to determine these structures and assign the rules directing peptide amphiphile assembly.



Designing antimicrobial, anti-biofilm and immunomodulatory peptides for combating infections

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Antibiotics are the underpinning of all modern medicine, but are being undermined by an explosion of (multidrug) resistance, and a dearth of new antibiotics. We are developing novel therapeutics for antibiotic resistant bacteria. Cationic host defence (antimicrobial) peptides are produced by virtually all organisms, ranging from plants and insects to humans, as a major part of their innate defences against infection. Cationic antimicrobial peptides with direct antimicrobial activity are being designed based on peptide array and computationally driven QSAR (machine learning) approaches. We have also demonstrated that cationic peptides have potent activity against biofilms, and when covalently associated with surfaces, and intriguingly each of these activities has different structural determinants compared to direct antimicrobial activities.

Host defence peptides also profoundly modulate innate immunity. Microarrays, sophisticated bioinformatics, and pathway and transcription factor studies have demonstrated that these peptides stimulate innate immunity/inflammation in a unique fashion, boosting protective immunity while suppressing harmful inflammation/sepsis. Structure activity relationship studies reveal that unlike the other activities discussed above immune modulation demonstrates a consensus sequence for the best available peptides. Using this principle of selective boosting of innate immunity we have developed novel small peptides with no direct antibacterial activity, that can protecting against diverse infections in animal models, including antibiotic resistant infections and cerebral malaria, as well as inflammatory diseases, providing a new concept in anti-infective and anti-inflammatory therapy.

From peptides to proteins: Analysis of the hierarchic organization of proteins and de novo design of protein-like architectures

William F. DeGrado
Dept. of Pharmaceutical Chemistry, Investigator, Cardiovascular Research Institute, University of California, San Francisco

The folding of water-soluble and membrane proteins reflects the hierarchic assembly of peptide-like segments into a structural and functional unit. This talk will focus on the principles governing this assembly process, with particular reference to the analysis and design of: 1) natural and designed membrane proteins including the M2 proton channel from influenza A virus[1-3] and a designed proton/Zn(II) antiporter; 2) metalloproteins[4]; 3) nanostructured materials[5].

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Targeting membrane lipids with designed peptides

Jianmin Gao, Hong Zheng, Fang Wang, and Christopher Pace
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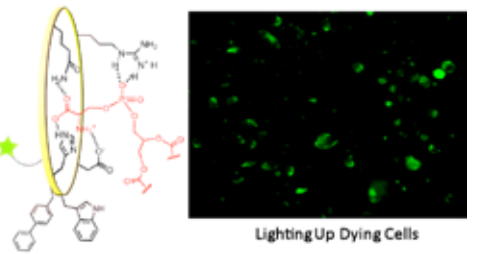
Cell membranes comprise a large variety of lipids, the composition and dynamic distribution of which have significant ramifications to cell physiology and disease. Molecules that target specific lipids may serve as powerful tools for probing membrane biology; they may also enable new technologies for molecular imaging and drug delivery. The overarching goal of our research program is to develop small molecules that recognize membrane lipids with protein-like specificities. By rationally targeting phosphatidylglycerol (PG), a phospholipid abundant in bacterial membranes yet absent on mammalian cell surfaces, we have successfully converted the nonselective channel-forming toxin gramicidin A into bacterium-specific channels. On a related subdirection, we have developed cyclic peptides that preferentially bind to phosphatidylserine (PS) over other commonly seen membrane lipids. These cyclic peptides were designed to mimic the structure and function of the PS-binding protein lactadherin. With fluorophore labeling, they specifically stain cells at early stages of apoptosis as the parent protein does. The design and optimization of both classes of lipid-targeting peptides will be discussed. Particular emphasis will be put on the new knowledge gleaned on protein-lipid interactions.



Lighting Up Dying Cells

Interactions of peptides with lipid nanotubular bilayers of defined curvatures

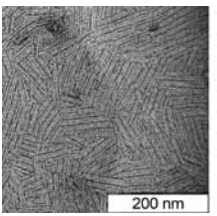
Peter Kett, Antonin Marek, Maxim A. Voinov, and Alex I. Smirnov
Department of Chemistry, North Carolina State University (Raleigh, NC USA)

It is now established that many proteins preferentially bind to curved lipid bilayer membranes. The ability of proteins to specifically detect membrane curvature has a number of important implications in cellular processes including endocytosis, exocytosis, and vesicle trafficking as well as pathogenic processes associated with viral infections or protein aggregation disorders. Experimentally, binding of proteins to convex membranes with positive curvature is readily measured using liposomes as model membrane structures. The curvature of liposomes can be controlled by sizing multilamellar vesicles by extruding them through nanoporous filters and the exterior of the liposomes is easily accessible to proteins added from solution. On the contrary, currently only limited data are available on binding of proteins to lipid bilayers possessing negative curvature. Here we describe the use of lipid nanotube arrays as a novel type of substrate-supported lipid bilayers with tunable curvature radii ranging from ca. 40 to 250 nm to study peptide binding and membrane-induced aggregation phenomena. The nanotubular lipid bilayers are formed by self-assembly inside cylindrical nanopores of anodic aluminum oxide (AAO). These systems impose essentially no restrictions on the lipid composition and have the inner surface of negative curvature accessible for solute molecules and peptide binding. The AAO nanopores are macroscopically aligned to a high degree and so are the lipids and peptides self-assembled within the nanopores. This alignment simplifies solid-state (ss) NMR studies. AAO membrane is essentially transparent to light allowing for optical studies of binding events. Here we provide examples of the use of lipid nanotube arrays for studying peptide-lipid bilayer interactions ranging from such small antibacterial peptides as melittin and alamecitin to synaptotagmin, among others. Further opportunities for using these structures for layered lipid-peptide nanotubes will also be discussed.

Using amyloid to your advantage

Margaret Sunde, Qin Ren, Vanessa Morris, Ingrid Macindoe, and Ann Kwan
School of Medical Sciences and School of Molecular Bioscience, University of Sydney (Sydney, NSW Australia)

The hydrophobins are a family of small proteins found in all filamentous fungi. They self-assemble specifically at hydrophilic:hydrophobic interfaces to form highly ordered amphipathic monolayers. Some hydrophobins form monolayers that are composed of fibrils that share many of the structural characteristics of amyloid. For the fungus this amyloid layer is functional and plays important roles in the life-cycle.



Monolayer of hydrophobin rodlets produced *in vitro* from recombinant protein

We are able to compare the structure and assembly process for different hydrophobins and to identify key features of this directed aggregation process. We have probed the process of self-assembly by hydrophobins using mutagenesis, in order to identify the conformational changes that occur upon fibril formation and the residues that form the ordered core of the fibrils. We have studied the role of the interface in triggering this protein aggregation. Surface tension is critical for the the conformational changes and intermolecular assembly which occur upon amyloid formation by hydrophobins. The amphipathic nature of the hydrophobin monolayers makes them attractive coatings for nanomaterials such as graphene that are otherwise incompatible with biological solutions. We have started a program of engineering hydrophobins to display functional groups and aim to exploit the self-assembly properties and the amphipathic nature of hydrophobin proteins for synthesis of functional nanosurfaces.

Peptide-based inhibition of protein aggregation in amyloid disease: from self- to cross-recognition

Aphrodite Kapurniotu
Division of Peptide Biochemistry, Technische Universität München, Freising, Germany

A chemical strategy to devise inhibitors of protein self-assembly in amyloid disease features the use of short peptide segments derived from self-recognition regions in their native or chemically modified forms. Few years ago, we have extended the above concept and have shown that it is possible to redesign the 37 residue, highly insoluble and amyloidogenic islet amyloid polypeptide (IAPP) (type 2 diabetes (T2D)) into a soluble nonamyloidogenic IAPP mimic (IAPP-GI) and high affinity inhibitor of IAPP aggregation and cell toxicity via a minimal chemical modification of a self-recognition region (Yan et al., PNAS (2006)). Unexpectedly, IAPP-GI has been also found to be a nanomolar affinity inhibitor of cytotoxic self-assembly of the Alzheimer's (AD) key amyloid polypeptide Aβ (Yan et al., Angew. Chem. (2007); Andreetto, Yan, et al., Angew. Chem. (2010)).

Further, nonfibrillar and nontoxic Aβ and IAPP species have been shown to form high affinity nonfibrillar and nontoxic hetero-oligomers which results in suppression of cytotoxic self-assembly of both polypeptides (Yan et al., Angew. Chem. (2007)). Together with recent results on other cross-amyloid interactions such as the insulin-IAPP, the Aβ-PrP, the Aβ-tau interaction, or the Aβ-α-synuclein interaction, our findings suggest that, in addition to interactions involved in self-recognition, cross-amyloid interactions may play a crucial role in pathogenic protein self-association processes.

Here I will present results of our studies on the molecular mechanism of interaction of Aβ with IAPP and IAPP-GI and on novel designed IAPP mimics as high affinity inhibitors of cytotoxic self-assembly of both IAPP and Aβ. Such peptides could become leads for therapeutics in AD, T2D, or both diseases.

HIV gp41 trimer mimics for vaccines and fusion inhibitors

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Department of Medicinal Chemistry, Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University (Tokyo, Japan)

Several useful anti-AIDS drugs have been discovered, however, the antibody therapy is still a promising and desirable treatment. To develop effective HIV vaccines and fusion inhibitors, artificial molecules have been synthesized based on HIV-1 envelope proteins such as gp120 and gp41. Gp41 plays a pivotal role in the membrane fusion process of HIV-1 infection, and is divided by the N-terminal helix region (N36) and the C-terminal helix region (C34). In the membrane fusion process, a six-helical bundle structure of gp41 is formed, which consists of a trimeric coiled-coil of N36 surrounded by three strands of C34 in an antiparallel fashion. Thus, it suggests that antibodies which recognize the N36 or C34 trimer might block HIV-1-entry. To construct an N36 trimer mimic, three strands of the N36 peptides were assembled on a C3-symmetric template with three equivalent linkers by thiazolidine ligation for chemoselective coupling of a Cys-containing unprotected N36 peptide with an aldehyde scaffold-containing three arms. Sera produced by immunization of the synthetic N36 trimer antigen showed structural preference in binding to the N36 trimer and higher potent neutralizing activity, compared to sera produced by the N36 monomer immunization. In design of the C34-derived peptides, glycine thioester was fused to the C-terminus of C34 sequence. To form a triple helix corresponding precisely to the gp41 pre-fusion form, the novel C3-symmetric template was designed. This approach used native chemical ligation for chemoselective coupling of unprotected C34REG-thioester with a three-armed cysteine scaffold to produce triC34e. The potency of this C34 trimer mimic as an antigen and as a fusion inhibitor were tested. As an antigen, the trimer induced structural specific antibody as N36 peptides. Moreover, the C34 trimer showed a 100-fold increase of inhibition activity compared to the monomer. Our present results would be useful for HIV vaccine and fusin inhibitor design based on the natural structure of proteins correlated to HIV fusion mechanisms.

Peptide self-assembly and microstructure formation

Alessandro Moretto
Department of Chemistry, University of Padova, 35131 Padova, Italy

We recently developed a new family of nano- and microstructures, based on water-compatible peptides, able to incorporate a variety of molecules and release them under appropriate stimuli. Applications include: (i) new drug-delivery platforms, (ii) imaging and (iii) artificial photosynthetic systems.

We showed that short (2-10 residue) peptides containing non-coded α-amino acids spontaneously self-assemble in water. They form vesicle-like aggregates (peptosomes) that may range from 50 nm to over 1 μm in diameter, depending on processing conditions and amino acid composition. Moreover, we were able to load these peptosomes with a variety of molecules, including drugs. Because of the presence of non-coded amino acids, the peptosomes should be able to evade the body's immune system and "cryptically" deliver the enclosed substances to the appropriate cellular targets.

Also, we studied an interesting photoswitch moiety: a C^{α}-tetrasubstituted α-amino acid, namely bis[*p*-(phenylazo)benzyl]glycine (*p*azoDbg), which may reversibly change from the *cis*- to the *trans*-isomer. The two different spatial arrangements are endowed with significantly distinct physical and chemical properties, including dipole moments. Based on these findings, we synthesized the dipeptide Fmoc-L-Phe-*p*azoDbg-OH and the derivative H-(*p*azoDbg)-O-$(CH_2)_2$-NH-Boc to build up light-driven, in water self-assemblies. Our preliminary data in water revealed: (i) formation of microspheres, (ii) transitions to different microstructures upon photo-illumination or dilution in water, (iii) reversibility, (iv) encapsulation of metallic nanoparticles or drugs, (iv) release of encapsulated molecules.

Finally, we applied the 'mechanochemistry' refers to solid reactions. We recently set up a method by which, surprisingly enough, grinding together water-soluble microstructure-forming peptides with water insoluble compounds (e.g. organic chromophores), we were able to solubilize the insoluble part of the mixture in water. In this connection we also were able to drive, under external stimuli, the above mentioned solutions into well ordered microstructures (fibres, tapes, ribbons) in water.

Stapled peptide drugs: Understanding cell penetration

Tomi Sawyer
Aileron Therapeutics (Cambridge, MA USA)

Stapled peptides are a promising breakthrough therapeutic modality for the treatment of many diseases by modulating intracellular or extracellular targets. Specifically, α-helical protein-protein interactions provide broad target space for many diseases in which traditional small-molecule or biologic strategies have significant limitations. Aileron is developing promising stapled α-helical peptides for numerous intracellular targets and such studies have addressed the mechanism of cell penetration by this novel class of drugs. The use of fluorescently-labeling, cell-based imaging and known inhibitors of active transport pathways, biophysical properties and emerging knowledge of cell penetration for stapled α-helical peptides will be described.

Natural product-based chemical probes for detecting 14-3-3-phospholigand interactions

Junko Ohkanda
ISIR, Osaka University (Osaka, Japan)

In this presentation we will describe design and functional evaluation of a natural product-based chemical probe for fluorescent labeling of 14-3-3 protein surface. Diterpenoid ISIR-042, semi-synthetically derived from a natural product fusicoccin (FC), demonstrates exquisite antitumor activities by inducing cell differentiation. A critical step involved in the biological mechanism was thought to be binding of FC to a hydrophobic pocket of 14-3-3 proteins, and stabilizing the ternary complex of FC, 14-3-3, and a phosphorylated ligand protein. However, there was no experimental evidence to support that 14-3-3 proteins are the primary targets of FCs. We anticipated that identifying and proving 14-3-3 proteins as the molecular targets of FC in cells would provide an insight into the molecular mechanism of unusual biological activities of FC derivatives.
To this end, we performed the structure-based design of the fluorescent chemical probes for 14-3-3 by linking ISIR-042 and a fluorescent tag through a recent developed phenylsulfonate linkage. Incubation of 14-3-3 proteins with BODIPY-attached FC agent and a 14-3-3 consensus phosphopeptide resulted in yielding the 1:1 conjugate product. The labeling reaction did not proceed in the absence of the phosphopeptide, indicating that the ternary complex formation is a critical determinant for the labeling reaction. The reaction was also found to be highly selective for 14-3-3 zeta over sigma isoform, due to the site specific reaction at His164 in zeta. Endogenous 14-3-3 in human living cells were detected under hyperphosphorylated condition, demonstrating that 14-3-3 proteins are the primary target of FC[1]. Details of the effect of phosphopeptide sequence upon the labeling reaction will also be discussed.

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Post-antibodies: Generation of molecular-targeting peptides by directed evolution in phage-displayed libraries

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The aim of our study is to investigate molecular design relying on evolutionary processes, called as "directed evolution," to generate a novel class of biofunctional molecules. In our previous work, we have succeeded to develop a directed evolutionary process for improving catalytic efficiency and binding affinity of catalytic antibodies. Therefore, now, we apply our technology and know-how to directed evolution of antibody-like targeting peptides, which is named "microAntibodies."

At present, antibodies are indisputably the most successful reagents in molecular targeting therapy. However, use of antibodies has been limited due to the biophysical properties and the cost to manufacture. To enable new applications where antibodies show some limitations, we have developed an alternative-binding molecule with non-immunoglobulin domain. The molecule is a helix-loop-helix peptide, which is stable against natural enzymes in vivo and is too small to be non-immunogenic. Since the molecule folds by virtue of the interactions between the amino acid residues positioned inside the helix-loop-helix, the solvent-exposed, outside residues would be randomized to give a library of helix-loop-helix peptides. We constructed a phage-displayed library of the structured peptides and screened the library for G-CSF receptor. Finally, the screened peptides was cyclized by introduction of a disulfide-bond linkage into the N- and C-termini. The cyclic peptide showed strong binding affinity (Kd of 4 nM) to the receptor and a long half life (15 days) in mouse sera, proving an enzyme-resistant property. Futhermore, imminization of the peptide to mice elicited non-immunigenecity. The peptide is named "microAntibodes" due to haveing the same properties as those of antibodies. This semi-rational strategy, which combines phage-displayed libraries with de novo designed peptides, provides a new way to generate structured functional peptides for useful tools in the field of chemical biology as well as altanatives to antibody medicines.

Nanofibrils of small peptide derivatives formed by molecular self-assembly

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Department Chemistry, Brandeis University (Waltham, MA USA), 415 South St., Waltham, MA 02454, USA

This talk focuses on the design and applications of various types of supramolecular hydrogelators for the development of soft nanomaterials for a range of applications. Like certain proteins that form fibrillary nanostructures (e.g., actin filament, microtubules, or paired helical filaments), small peptide derivatives can self-assemble in water to afford supramolecular nanofibrils and result in hydrogels. While it is known that the intracellular filaments of the proteins intricately associate with normal cellular functions (e.g., cell movements) or illnesses (e.g., Alzheimer disease), the generation and destiny of the supramolecular nanofibers inside cells remain unexplored. We report a range of small molecules that undergo enzyme catalyzed transformation to form molecular nanofibrils/hydrogels and discuss the use of enzyme to trigger self-assembly for generating molecular nanofibrils and hydrogels after the designed substrates diffuse passively into cells. In addition, we show the potential applications of enzymatic hydrogelation in biomedicine. Besides illustrating the new opportunities presented by small peptides for molecular soft nanomaterials, we will also discuss the challenges that have yet to be met.

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Preparation of artificial proteins based on dendrimers

Chie Kojima
Nanoscience and Nanotechnology Research Center, Osaka Prefecture University (Osaka, Japan)

Dendrimers are synthetic polymers with unique structures, which is a candidate for a peptide scaffold. Multiple antigen peptides were previously produced by using dendrimer and antigen peptides, which inspired me to design artificial proteins using dendrimers and the model peptides. Three topics will be shown in the presentation.

1. Collagen-Mimic Dendrimers[1-5]: We have designed collagen-mimic dendrimers by using collagen model peptides, glycine-proline-(hydroxy)proline (GPP(O)) repeats. The collagen-mimic dendrimers exhibited the collagen-like higher order structure and formed temperature-dependent hydrogels, indicating that collagen-mimic dendrimers are a potent artificial collagen materials.

2. Elastin-Mimic Dendrimers: We have designed elastin-mimic dendrimers by using elastin model peptides, valine-proline-glycine-valine-glycine (VPGVG) repeats. The elastin-mimic dendrimers exhibited the temperature-dependent higher order structure, similar to elastin polypeptides. The dendrimers showed the phase transition, dependent in pH and salt concentration. The peptide length and the dendrimer generation influenced the phase transition behavior.

3. Dendritic Peptidomimetics[6]: We have prepared dendrimers conjugating to amino acids, which is a potent peptidomimetic.

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Arginine containing self-assembling peptides form potent antibacterial hydrogels

Ana Salomé Veiga[a,b], Chomdao Sinthuvanich[a,c], Diana Gaspar[b], Henri Franquelim[b], Miguel Castanho[b], and Joel Schneider[a]
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Bacterial infections are a common problem associated with dermal wounds. These infections can prolong or impair wound healing. Hydrogel materials that display inherent activity against bacteria can be used to directly treat accessible wounds to prevent or kill existing infection. Hydrogels composed of self-assembling β-hairpin peptides, having a high content of arginine, were found to be extremely effective at killing both gram-positive and gram–negative bacteria, including multi-drug resistant *P. aeruginosa*. No added antibacterial agents are necessary to realize activity. Using self-assembling peptides for material construction allows facile structure-activity relationships to be determined since changes in peptide sequence at the monomer level are directly transposed to the bulk material's antibacterial properties. SAR studies show that arginine content largely influences the hydrogel's antibacterial activity, and influences their bulk rheological properties. These studies culminated in an optimized gel, composed of the peptide PEP6R (VKVRVRVRV^{D}PPTRVRVRVKV). PEP6R gels prepared at 1.5 wt % or higher concentration, demonstrate high potency against bacteria, but are cytocompatible towards human erythrocytes as well as mammalian mesenchymal stem cells. Mechanistic studies suggest a mode of action that involves membrane disruption when cells come in contact with the gel's surface. Rheological studies indicate that the gel is moderately stiff and displays shear-thin recovery behavior, allowing its delivery via simple syringe.

Design of a sensing system for caspase activities using a combination of split-fluorescent proteins and split-intein

Seiji Sakamoto, Mika Terauchi, Anna Hugo, Yasuyuki Araki, and Takehiko Wada
Institute of Multidisciplinary Research for Advanced Materials, Tohoku University (Sendai, Japan)

Apoptosis is an essential process in the normal development and homeostasis of muticellular organisms, and it is known that defects in apoptosis mechanisms cause various diseases. In apoptosis pathways, caspases play crucial roles to mediate the initiation and propagation of signals. Therefore, the development of a highly sensitive and specific detection system for caspase activities will provide new insights into intricate apoptosis mechanisms. In this study, we have attempted to construct a new class of fluorescent indicator by utilizing split-GFP and split-intein (Fig. 1). In the strategy to create split-GFP that reassembles in response to caspase activities, N- and C-terminal ends of one of GFP fragments are ligated by the substrate sequence for a target caspase. We envisioned that the cyclization the split-GFP fragment should inhibit protein reassembly, while the digestion of the linker by the caspase would allow the self-association and functional recovery of the split-GFP. The *Synechocystis* sp DnaB split-intein was successfully employed for the preparation of a cyclic C-terminal fragment (cM4-DEVD). In the absence of caspase, reconstitution of split-GFP was scarcely observed. By contrast, addition of a target caspase resulted in cleavage of the cyclic peptide, leading to acceleration of protein reassembly and concomitant fluorescence recovery. This result demonstrates that the successful construction of the new fluorescent indicator for caspase activities by the combination of split-GFP and split-intein.

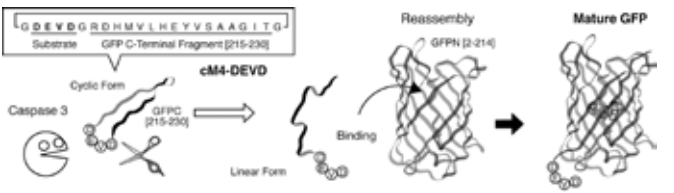

Fig. 1 Illustration of a sensing system for caspase activities using Split-GFP.

Present and future of peptides as blood-brain barrier shuttles

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Over recent years we have worked extensively on the use of peptides as BBB-shuttles to carry drugs that cannot cross the blood-brain barrier (BBB) and therefore cannot reach the brain unaided. The capacity of a drug to cross the BBB is crucial, as several major diseases require brain treatment. These include neurodegenerative disorders such as Parkinson's and Alzheimer's, but also central nervous system (CNS) diseases, such as schizophrenia, epilepsy and bipolar disorder. Cerebral cancer, HIV, and some aspects of obesity can also be included as pharmaceutical targets inside the brain (Malakoutikhah et al., Angew. Chem 2011).

Initially, we focused our efforts on passive diffusion as a transport mechanism (Teixidó et al., *JACS* 2007; Malakoutikhah et al., *J. Med. Chem.* 2008, *J. Med. Chem.* 2010). In these studies, we achieved molecules with 2-4 amino acids that act as passive BBB-shuttles and are efficient at carrying drugs such as L-Dopa, Baicalin, GABA, Nipecotic and Aminolevulinic acids. In some cases, the shuttle plays a dual role and once inside the brain acts as an enzyme inhibitor (Teixidó et al. 2012). In spite of their potential use for small molecules, passive diffusion shuttles have limitations for transporting macromolecules (proteins, mAbs, nanoparticles). This prompted us more recently to focus on the use of peptides recognized by receptors as actively transported BBB-shuttles (Prades et al. 2011).

In this communication, we will review our latest results of peptides as passive BBB-shuttles and present our unpublished results on peptide-shuttles that use active transport to cross the blood-brain barrier.

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De novo design of functional protein assemblies: towards hydrogen production

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The long-term goal of this project is to develop a reducing catalyst capable of generating molecular hydrogen from aqueous protons. We are using a bioinspired approach, which recapitulates all components of the natural hydrogenases in an integrated system: a catalytic center, the activity of which is modulated by the protein matrix in which it is embedded; a molecular wire, which forms the conduit to transfer electrons to the active site; and a designed protein-electrode interface, which connects the catalyst to the rest of the device. Towards this goal we designed a family of coiled-coil helical proteins, which we functionalize with organometallic active sites. Here, we present designed proteins that contain two iron sulfur clusters in the core of a closed dimeric three helix bundle. Exploiting the propertieds of coiled coils, we demonstrate that we can alter the distance between the clusters by copying and translating the site along the coil axis. The two peptides, DSD-[Fe_4S_4] A and B, were prepared by SPPS; both readily incorporate two iron sulfur clusters by self assembly. We confirmed the presence of the clusters in intact proteins by UV-vis spectroscopy, CD, ESI, and analytical ultracentrifugation. Incorporation of -[Fe_4S_4] results in increase in helical content and stabilization of the DSD proteins in both A and B versions, as assessed by CD monitored thermal denaturation. EPR analysis of reduced samples confirms the presence of [Fe_4S_4] clusters. Further. ELDOR experiments demonstrated electronic coupling between the two clusters; the data are consistent with the expected inter-cluster distances of 36 Å and 14 Å respectively for the two proteins, A and B. We further demonstrated catalytic production of hydrogen by direct incorporation into the helical framework of an intact (μ-SRS)[$Fe(CO)_3$]$_2$ cluster anchored via an artificial amino acid; the cluster mimics the active site of [FeFe] hydrogenases, and carries out photoinduced hydrogen production in water. Current work is focused on directly interfacing the [4Fe4S] electron relay with the catalytic site.

Peptide nanotubes from lock-washer stacking of coiled-coil assemblies

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The crystal structure of a *de novo*-designed peptide, GCN4-pAA (PDB ID: 2HY6), was recently reported in which the formation of a discrete seven-helix bundle was observed in which the coiled-coil defined a channel with an internal diameter of 7 Å.[1] This structure provides a prototype for the design of tubular channels from simple helical peptides. A key feature of the peptide sequence was an expansion of the hydrophobic interface to encompass the *a*-, *d*-, *e*-, and *g*-residues of the heptad repeats. Moreover, the crystallographic analysis of the GCN4-pAA structure indicated a single residue shift in registry between adjacent helices, which resulted in an overall shift of seven residues (i.e., one coiled-coil heptad repeat) upon closure of the bundle structure. In contrast, most coiled-coil structures have no corresponding shift in helix registry and result in blunt-ended helical bundles. The structure of the seven-helix bundle of GCN4-pAA resembles a screw (or lock washer) with an axial translation corresponding to seven residues within the helical assembly. We prepared a synthetic peptide **7HSAP1**, in which the sequence of GCN4-pAA was modified to promote end-to-end association between the surfaces perpendicular to the super-helix axis of the lock washer structure, such that the resulting helical bundle would self-associate into a high aspect-ratio fibril with a continuous channel throughout the assembly that would correspond in lateral dimensions to that of the seven-helix bundle observed in the crystal structure of the original peptide. Physical evidence derived from CD and LD spectropolarimetry, TEM/cryo-TEM, X-ray fiber diffraction, STEM mass per unit length measurements, and solid-state REDOR NMR of labeled peptides supports the structural model that we have proposed. A number of structural variants of the original **7HSAP** peptide have been prepared in which core/non-core positions have been mutated. Thus far, conservative mutations introduced into the peptide do not interfere the formation of the nanotube, but can alter the thermodynamic stability of the assembly vis-à-vis the parent system, the extent of end-to-end and lateral association, and the affinity for shape-selective binding of small-molecule substrates within the central channel. We present a description of the supramolecular materials derived from self-assembly of these peptides in terms of the structural model of GCN4-pAA. The potential for creation of novel nanoporous materials based on this assembly and related coiled-coil-based supramolecular materials will be described in terms of *de novo* peptide design.

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Self-assembled anionic β-sheet peptides in monolayers and hydrogels

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In recent years there has been growing interest in generating biomolecular architectures composed of designed peptides. We have focused on a group of amphiphilic and anionic β-sheet peptides that can be assembled in the form of monolayers at interfaces and as fibrillar bilayer structures capable of generating hydrogels in bulk. Structural characterization revealed that β-strands under pressure, may undergo elastic bending deformations.[1] Recently, we utilized this characteristic to study the effect of peptide monolayer rigidity and amino acid type on calcium-phosphate mineralization.[2]

These peptides may be triggered by alkaline solutions to immediately form hydrogels, stabilized by entangled matrix of assembled fibrils. The more hydrophobic peptides were found to form stable hydrogels at near neutral pH values, pointing to a positive shift in the pKa value of the anionic amino acids hence, to stabilizing intermolecular forces based on hydrogen bonds and hydrophobic interactions.[3] These hydrogels were studied in context of bone regeneration and drug delivery to bone tissue.[4,5] Enriched with calcium ions and loaded with the mineral tricalcium-phosphate the hydrogels were found to induce osteoblast differentiation in-vitro as well as to enhance bone healing in-vivo in small animal models.

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3D organic molecular architectures: Folding into shape

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Molecular self-assembly is the spontaneous assembly of molecules into structured aggregates by which nature builds complex functional systems. While numerous examples have focused on 2D self-assembly to understand the underlying mechanism and mimic this process to create artificial nano- and microstructures, a limited progress has been made toward 3D self-assembly at the molecular level. This lack of progress is partially due to the difficultly of designing and using nondirectional noncovalent interactions, such as van der Waals and hydrophobic interactions, in synthetic, nonbiological molecular systems. Thus, we sought to establish a set of self-assembling components that could be linked to observable 3D shapes by which the governing parameters of self-assembly could be disentangled and tractable.

Recently, we discovered for the first time that helical peptidic foldamers with well-defined hydrophobic surfaces self-assembled to form unprecedented 3D molecular architectures ("foldectures") in a controlled manner in aqueous solution. We anticipate that our strategy can be a starting point for the rational design of 3D organic molecular architectures with various functions.

Engineering peptide structures: Architecture, design and construction

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The construction of well ordered secondary structures, helices and hairpins, in short synthetic peptides, is readily achieved by the incorporation of backbone modified amino acids that serve as folding nuclei. Aib and DPro have been widely used to bias local conformational choices, with the former supporting helix formation, while the latter facilitates hairpin structures. The success of design principles is evaluated by definitive conformational characterization in crystals by X-ray diffraction. A correspondence between solid and solution state structures is made possible by comparing NMR structures and crystal state conformations.[1]

The incorporation of β and γ amino acid residues (backbone homologated residues) enhances the repertoire of hydrogen bonded structures that may be constructed in hybrid sequences containing α , β and γ residues.[2] Several examples of recently determined structures of (αβ)n, (αγ)n and (αγα)n sequences are described.[3] Comparisons are made between sequences containing the conformationally constrained γ-residue, gabapentin (Gpn),[4] and the unconstrained γ-Val residue derived by homologation of L-Val.[5] Hybrid sequences permit expansion of the structure space for designed peptides. The multiplicity of crystal structures presented, emphasizes the importance of definitive structural characterization in refining the principles of peptide design and engineering.

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Poster Abstracts

Insights into core-packing of an artificial hetero coiled coil assembly

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The intrinsic protease susceptibility as well as the low number of accessible functionalities in α-peptides have motivated the design of peptidic foldamers with unnatural residues to trigger the adoption of natural helical assemblies. Peptidic foldamers consisting homologous amino acids are shown to form, successfully, a variety of helical conformations at secondary, tertiary, and quaternary structure levels. Such efforts have also included the design of αβγ chimeras comprising an alternating sequence of β- and γ-amino acids as well as study of their corresponding hetero-assembly with natural α-helical partners as our specific approach in this context. A general challenge associated with design of such artificial quaternary structures is prediction of the geometry of the foldameric binding groove that interacts with α-peptides. The fact that amino acid side chains exhibit different characteristics depending on the sequence (i.e., structural) context further complicates the recognition of well-suited side chain compositions required for specific interaction between bioactive foldamers and natural targets.

Applying a phage display library, we broadly surveyed for suitable interacting peptides that provide ideal core packing when assembled with the αβγ chimeric sequence. This screen led to consensus sequences bearing a cystein in combination with an aromatic residue at the helical interfaces. Selected peptides are able to form tetrametric assemblies with αβγ-chimera and possess thermal stabilities that are similar to that of its parental system. Molecular dynamic simulations further confirm the unique core-packing of αβγ-chimera against its natural partner due to appearance of cystein residue at the hydrophobic core. An interhelical H-bond between the thiol side chain and a non-H-bonded backbone carbonyl of the β/γ-segment provides a selectivity for helical assembly as well as higher thermal stability.

Heterogeneous fluorescent amyloid nanotube structures exhibiting modulated long-range order

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Understanding the subtleties of the nucleation-dependent organization of cross-β peptides is extremely important in designing functional amyloid derived bionanomaterials. The past decade has seen multiple directions in studies of Amyloid-β (Aβ), ranging from the top-down investigations of neurodegenerative diseases, to bottom-up amyloid-derived self-assembling functional nanomaterials. We use a rhodamine 110-labeled Aβ(17-22) peptide, Rh1722, to investigate the nucleation and assembled structure of Aβ(16-22) peptide nanotubes. We have previously shown that small fractions of Rh1722 co-assemble with Aβ(16-22) to give unaltered nanotube structures. Using fluorescence lifetime imaging microscopy (FLIM) and the high sensitivity of Rh1722 to its local environment, we can spatially monitor lifetime differences along growing peptide nanotubes. We find that co-assembly ratios and air-solution interfaces can accelerate and influence nucleation pathways, leading to heterogeneous nanotube structures exhibiting modulated long-range order. We also characterize the transition from homogeneous to heterogeneous structures and discuss these findings in terms of functional nanotube design.

Engineering of peptide nucleation and functionalization of peptide nanotubes

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Controlling and manipulating the nucleation and self assembly pathways of peptide nanotubes as well as the functionalization of those nanotubes for optical and electronic emission is accomplished through both covalent and non-covalent attachment of different peptide modifiers. Here we detail the use of two-photon microscopy for the imaging, monitoring, and activation of a series of different peptide nano and microstructures ranging from dipeptides to heptapeptides. The core residue of the Aβ fibril, Aβ(16-22), assembles into bilayer tubes at pH 2. This peptide can be futher modified through the covalent addition of a Rhodamine fluorescent peptide. Once assembled the tubes can then be imaged optically due to their high local fluorescent properties. Aβ(16-22) derivatives E22L and E22V can also be functionalized through the non-covalent attachment of negatively charged photosensitizers and phosphorescence quenchers which allows for transfer of e- along the surface of the tube substrate. Diphenylalanine, which assembles into para-crystalline hexagonal tubes at high concentrations can be made to form completely different assemblies in the presence of Aβ(16-22), also exhibits auto-fluoresence due to the high concentration of Phe-Phe molecules in the volume of the structures.

Roles of cell-surface glycosaminoglycans in islet amyloid polypeptide-induced cytotoxicity

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Glycosaminoglycans (GAGs) are long linear sulfated polysaccharides that are prevalent in the extracellular matrix and at the cell surface where they are usually covalently linked to the protein core of proteoglycans. GAGs are found in association with virtually all extracellular amyloid deposits in humans[1]. Biophysical investigations have shown that GAGs, particularly heparan sulfate (HS) and its highly sulfated derivative heparin, promote and/or accelerate the aggregation of several pro-amyloidogenic peptides and proteins through, most likely, a scaffold-based mechanism. Particularly, GAGs are well-known to enhance fibrillogenesis of the islet amyloid polypeptide (IAPP)[2], an unusual amyloid-prone peptidic hormone that is co-secreted with insulin by the pancreatic islet β-cells. IAPP misfolding and aggregation into cytotoxic cross β-sheet aggregates, observed in the pancreas, accelerate the pathogenesis of type II diabetes mellitus by exacerbating β-cell degeneration and compromising insulin secretion[3]. Whereas the mechanism of GAGs-accelerated amyloidogenesis has been well characterized biophysically[4,5], the roles of GAGs located at the outer cell membrane on IAPP-induced cytotoxicity has been poorly explored. Herein, we initially evaluated how cell-surface GAGs modulate peptide adsorption to the plasma membrane and IAPP-amyloidogenesis. Using rat insulinoma β-cells (INS-1) and Chinese hamster ovary (CHO) cells that were genetically modified and/or enzymatically treated to modulate their cell-surface GAGs composition, we then investigated the effects of the sugar core of proteoglycans in IAPP-mediated toxicity.

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Peptide nanotube self-assembly

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Short peptides, KLVFFAL and KLVFFAV, assemble into beta-sheet rich nanotubes. These peptides are derived from the nucleating core of the Alzheimer's disease Aβ peptide, with KLVFFAE or Aβ(16-22) serving as the nucleation core. The KLVFFAL peptide assembles into tubes that have a homogeneous diameter but a structure distinct from KLVFFAV. In both tubes, beta sheets helically spiral upward, but the orientation of the beta sheets and wall structures are unique to each type of tube. This remarkable difference, mediated by the difference of a single methylene, is expected to differ at least in part by a difference in the rate of assembly of Aβ(16-22)E22L and Aβ(16-22)E22V nanotubes. We have developed approaches that allow us to determine the self-assembly rates using circular dichroism (CD) and fluorescence microscopy. Beta-sheet structures show a unique fingerprint in their CD spectrum, allowing the kinetics of peptide aggregation to be measured directly. However, fluorescence microscopy provides both the geometry of the assembled structure as well as individual nanotube rates in real time, linking the length of nanotubes and the polymer concentration together. Variations in environmental conditions, such as pH, temperature, and solvent, further allow us to define and control the formation of these nanostructures. Control of reaction rates, assembly structure, and fluorescence activity are helping us to build the necessary mathematic models for these assembling processes. We hope ultimately to understand the factors that control the diversity of and accessible energetic landscapes that dictate cross-β assembly.

Effect of conjugation site and hydrophobic modification on Tat-mediated drug delivery to multi-drug resistant cancer cells

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Overexpression of efflux transporters, such as P-glycoprotein (P-gp), can significantly reduce the cytotoxicity of chemotherapeutics by preventing their intracellular accumulation at sufficient concentrations to exert an effect – a phenomenon of multi-drug resistance (MDR). Conjugation to cell penetrating peptides (CPPs) is a popular method for bypassing these efflux processes, with the HIV-derived peptide, $GRK_2R_2QR_3P_2Q$ (Tat), the most commonly used. The ability of Tat to carry small molecules, peptides, proteins, oligonucleotides and nanoparticles across the cell membrane is well documented. Attachment of a cargo can, however, impact the ability of the Tat peptide to translocate, though no systematic study has been carried out to fully probe this.

Here, we will demonstrate how the conjugation site of the anticancer drug, doxorubicin, to the Tat peptide can affect its cellular translocation ability and how an increase in hydrophobicity can aid intracellular delivery in both doxorubicin-sensitive and resistant cancer cell lines. Specifically, we found that *C*-terminal conjugation results in greater uptake when compared with *N*-terminal, though both are not as effective as free doxorubicin in sensitive cells. In resistant cells, however, both conjugates exhibit greater uptake than free doxorubicin and prevent efflux of the drug. The addition of a lipophilic tail further enhances the ability of the Tat peptide to carry the drug cargo across the cell membrane, with little difference between sensitive and resistant cells. Cytotoxicity and drug release studies will also be presented.

Engineering intelligent peptide materials with reversible linkages

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Peptide materials can not only be designed by programming their amino acid sequences to generate various self-assembled nanostructures but also further engineered by replacing the amide bond with kinetically more accessible linkages, which yield dynamic chemical networks of peptide products. The dynamic network of these modified peptides have the ability to respond to the environment and the potential to learn and adapt from external changes, defining them as intelligent (switchable and adaptable) materials.

Here we demonstrate that by coupling modified amino acids phenylalanine aldehyde with serine, cysteine, and asparagine to form Ser-, Cys-acetal (five membered ring) and Asn-acetal (six membered ring) linkages, the engineered peptides will be generated with these reversible acetal linkages. To gain insight into the behavior of the dynamic linkages, we have systematically studied and quantified the kinetics and thermodynamics of the Ser-; Cys- and Asn-acetal linkages under a range of temperatures and in various solvents (benzene, acetonitrile and DMSO) by ^{1}H-NMR spectroscopy. Among these reactions, Asn-acetal condensation have the highest yields and greatest diastereoselectivity.

The insights gained from these models continue to shed light on the reversibility/stability of thess peptide materials. It is the coupling of the control of assembled morphology with the ability of these assemblies to direct chemical reactivity that will set the stage for the development of intelligent materials.

Creation of dynamic chemical networks for re-invention of proto-ribosomes

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In extant biology, the ribosome represents a synergistic collaboration of nucleic acid and amino acid polymers for molecular information translation, serving as the Darwinian Threshold for cellular life. To re-invent the emergence of the ribosome requires a dynamic Chemical Threshold for the creation of a diverse polymer network that allows for dynamic interaction, cooperation and co-evolution, which later can give rise to the multicomponent supramolecular assembly structure of the ribosome. Here we demonstrate a dynamic chemical network (DCN) constructed from simple reduced states of existing amino acids. By incorporating different reversible coupling elements (e.g. N,O-; N,S-; and N,N-acetal linkages) and recognition elements (amino acid side chain, nucleobases) , the DCN can be further diversified. We now demonstrate that sequence and conformational information can be non-randomly selected from randomly encoded information in DCN though supramolecular assemblies, which indicates the concept of chemical evolution. This very first step towards the construction of a prebiotic chemical network can now be further extended for testing symbiosis between modified nucleic/ amino acids oligomers to generate a proto-ribosome.

Role of both Ca^{2+} and CaM in the regulation of Connexin26

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Gap junction, allowing the intercellular transmission of molecules through its specialized cell membrane channels, plays a major role in intercellular calcium signaling between adjacent cells. In this report, the gating role of calcium and calmodulin (CaM) in the Connexin26 (Cx26) has been studied. According to the previous study identifying the binding domain of Cx32 with CaM, N-Termini of Cx26 predicted by CaM Target Database were synthesized in order to demonstrate the interaction between CaM and Cx26. Further, we have predicted several calcium binding pockets with recent resolved crystal structure of Connexin26 (Cx26) using our developed MUG algorithm. Biophysical results indicate that in the presence of Ca^{2+}, synthesized Cx26 peptide fragment encompassing predicted CaM binding regions are able to bind with high affinity to CaM using NMR and Fluorescence spectroscopy. Highly conserved amino acids in gap junction family around predicted calcium binding pockets in Cx26 by sequence alignment support the hypothesis that calcium may regulate the gating of hemi-channel of connexin via direct binding. The metal binding ability of Cx26 has been studied by entire protein purified from both Insect cell and bacteria. Our results elucidate the molecular mechanism of regulation of gap junction by both Ca^{2+} and Ca^{2+} dependent CaM actions and provide insight into molecular basis of human diseases.

Acknowledgements
This work is supported in part by NIH grants EY-05684 to CFL and JJY and GM-081749 to JJY.

Oligo(4-aminopiperidine-4-carboxylic acid): An unusual basic oligopeptide with an acid–induced helical conformation

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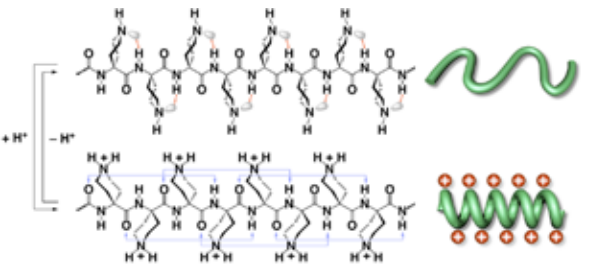
Natural polypeptides containing basic amino acids such as lysine and arginine play crucial roles in biological events.[1] Conformational studies on their synthetic analogues have indicated that protonation of the basic side chains of these amino acid residues results in a helix-to-coil transition as a result of disruption of the H-bond network along the peptide backbone by an electrostatic repulsion.[2] In sharp contrast with helical oligopeptides carrying basic side chains, homotropic oligopeptides (octamer; Api8) from non-natural achiral amino acid '4-aminopiperidine-4-carboxylic acid (Api)' adopt a helical conformation only in acidic media.[3] In a pH-titration with an Api8 oligomer having a leucine derivative at its N-terminus, a helix-to-coil transition takes place in a pH range of 7–10. Detailed NMR studies indicate that the lone pairs at the piperidine nitrogen atoms in Api8 regioselectively interact with proximal amide protons and disrupt the H-bond network along the peptide backbone, responsible for the helical conformation. The helical structure is induced not only by protonation of the piperidine groups but also acylation of their nitrogen atoms. We claim that oligo(4-aminopiperidine-4-carboxylic acid) (Apin) is the first basic oligopeptide adopting a stable helical conformation only in acidic media.



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Molecular docking and in silico structure-based design of D-Phe-Pro-D-Arg-derived direct thrombin inhibitors

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New peptidic non-covalent direct thrombin inhibitors (DTIs) were designed by generating peptide lead compounds derived from the substrate sequence Phe(P3)-Pro(P2)-Arg(P1). The free energy of interaction between each ligand and thrombin was calculated with the built-in molecular mechanics force field (MMFF) provided by the docking software *SCULPT* (Accelrys). During the original screening, the hexapeptides [D-Phe(P3)-Pro(P2)-D-Arg(P1)-P1′-P2′-P3′-CONH2] and pentapeptides [D-Phe(P3)-Pro(P2)-D-Arg(P1)-P1′-P2′-CONH2] were used as scaffolds for developing the optimized final tetrapeptide lead sequence, D-Phe(P3)-Pro(P2)-D-Arg(P1)-P1′-CONH2. Once the lead tetrapeptide scaffold was found to have higher affinity for thrombin than the hexa and pentapeptides, based on structure-activity relationship (SAR) studies on thrombin inhibition conducted in vitro, new peptide candidate inhibitors were further designed as derivatives of the tetrapeptide motif D-Phe(P3)-Pro(P2)-D-Arg(P1)-P1′-CONH2. New peptide sequences were developed by varying the P1′ positions both with L and D natural or non-natural amino acids, covering a wide range of chemical structures. Trials for optimization of P3 position were further performed with un-natural amino-acids, such as D-3,3-di-Phenylalanine, trans and dihydrocinnamic acids, (L)/(D)-Tic [1,2,3,4-tetrahydro-isoquinoline-3-carboxylic acid], (L)/(D)-Thi [Thienylalanine] and D-Naphthylalanine (D-Nal). The protein template used in all molecular docking experiments was the structure of human α-thrombin in complex with the covalent inhibitor PPACK (PDB entry 1ABJ). The thrombin:peptide complex was minimized using the *SCULPT* built-in molecular mechanics force field (MMFF94). After each round of minimization, the free energy of interaction (scoring function) was assessed using both Van der Waals and electrostatic force fields. Preliminary kinetics for in vitro inhibition of alpha thrombin cleavage of the chromogenic substrate S2238 established a new structure-activity relationship (SAR) for the tetrapeptides with new unnatural aminoacids at (P3) position.

Development of peptidomics assays for mapping the epitopes derived from collagen I and II processing by metalloproteases and cathepsins

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In recent years several lines of research indicate the relevance of extracellular matrix remodeling in tissue homeostasis. Several enzymes, including matrix metalloproteinases (MMPs), have been described as modulators of tissue microenvironment through degradation of the extracellular matrix (in particular collagen metabolism), processing of cytokines and growth factors and processing of cellular surface receptors. The MMPs are the primary enzymes involved in collagenolysis. Peptide fragments generated by collagen processing could be presented to the immune system for generation of either tolerance or immunity. In order to reveal new potential MHC class II restricted epitopes processed by MMPs that are distinct and additional to peptides processed by endosomal proteases, we developed peptidomics assays in which the antigen processing was coupled with nano-LC ESI MS/MS sequencing of the peptides epitopes. New peptides derived from collagen-I and -II processing by the recombinant cathepsins S, B, D and L and by the late endosomal fraction isolated from dendritic cells (DCs) were sequenced and compared with the epitopes derived from the activity of pure recombinant MMPs (such as MMPs 1, 2, 3, 7, 9, 13, 14)) and from the enzymatic activity of the plasma membrane fraction isolated from the human primary DCs, leading to the generation of new peptides epitopes (with molecular weights of 800-6,000 Da). The new discovered epitopes suggest new mechanisms for the extracellular antigen processing distinct from those present in the late endosomal compartment.

collagen II epitopes

Engineering the translation machinery to accept unnatural amino acids

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The cellular translation machinery is the optimal platform for the development and manufacture of therapeutic peptides but is currently hindered by its specificity for the set of natural amino acids. The set of 20 natural amino acids only capture a small amount of the chemical variety seen in current drug entities and unnatural amino acids have already been demonstrated to confer useful properties onto drugs such as increased stability and more efficient cellular delivery. A key step limiting the translation of unnatural amino acids is the delivery of tRNAs carrying them to the ribosome. This step is carried out by the protein EF-Tu, which has evolved over billions of years to specifically deliver only the set of 20 natural amino acid-tRNA species. I use computational evolutionary sequence analyses, along with structural knowledge, to design EF-Tu variants. These variants are assayed for their ability to deliver unnatural amino acids to the ribosome using an in vitro bacterial protein translation system. EF-Tu variants with both increased and decreased amino acid tolerances have thus far been identified. Mutations from promiscuous variants will guide further efforts to design an efficient and highly promiscuous EF-Tu protein for translation of unnatural amino acids. This work may thus expand the set of unnatural amino acids that can be readily used for translation of novel peptides.

Recombinant site specific labeling of proteins with IR probes

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Structural specificity in the study of ultrafast folding proteins can be achieved using temperature-jump infrared spectroscopy to monitor signal changes over time of infrared active functional groups at target sites in a protein sequence. In our group we have devised a method using recombinant genetics to introduce backbone infrared active labels at targeted sites. By introducing methionine point mutations at selected residues, a methionine auxotrophic *E. coli* cell line can be used to incorporate modified methionines. This method has been used successfully to introduce an infrared active backbone label. The carbonyl of the backbone of methionine was labeled with $^{13}C=^{18}O$ to introduce this backbone specific label. The use of point mutations and auxotrophic cells allows for a less complex and less expensive method of introducing a site specific label as compared to comparative methodologies.

Novel assay for screening of low affinity glycan-lectin interactions

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The growing awareness that the glycan component of cellular glycoconjugates stores biological information, embodied by the term 'sugar code', has attracted increasing attention to analyzing glycan-lectin interactions. Among lectins, the family of galactoside-binding lectins (galectins) has been recognized as "decoders" of biological information. Through the highly selective choice of binding partners galectins can modulate a variety of cellular functions in relation to both health and disease.

The development of high-throughput assays for identification of potent and selective inhibitors of galectins has been hampered by the weak binding affinities between galectins and their carbohydrate ligands. To circumvent this obstacle, we have developed a fast and simple assay based on AlphaLISA technology (Amplified luminescent proximity homogeneous assay, PerkinElmer) to screen for inhibitors of different members of galectin family. Due to the avidity of the AlphaLISA beads, this assay is ideally suited for characterization of low affinity protein-glycan interactions as well as for measuring binding affinities in complex systems. We designed and optimized the AlphaLISA-based method for assessment of galectin-3 binding affinities for β-galactose-containing glycans in a 384-well plate format by using the nickel-chelate Alpha screen kit. Histidine-tagged galectin-3 was bound to nickel-chelate donor beads, whereas biotinylated asialofetuin, a galectin-3 nanomolar binding partner, was bound to streptavidin coated acceptor beads. The natural ligands of galectin-3, *N*-acetyl-lactosamine and lactose, inhibited galectin-3/asialofetuin interaction with IC_{50} values that were in agreement with the K_d values previously determined by others and us. Furthemore, the assay was used to assess the binding affinities of galectin-3 towards its natural ligands, MUC1-derived glycosylated peptides that carry tumor associated Thomsen-Friedenreich (TF) antigen.

Use of organometallics to organize and control peptide conformation

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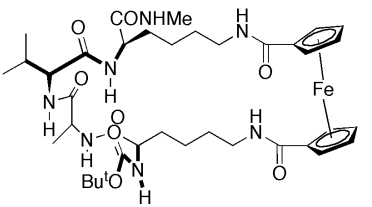
Although peptide conformation has typically been controlled by the introduction of covalent constraints, in recent years the use of organometallic moieties has received some attention. The use of organometallic moieties to constrain peptide conformation may make it possible to access molecular geometries not possible with covalent linkages, to provide peptides with new chromophores, and to introduce novel chemical reactivities to peptide amino acid side chains. In our lab we have been exploring the use of ferrocene and tungsten-alkyne coordination as peptide constraining elements. This presentation will describe how we have been able to produce peptides that have helical, turn and sheet conformations enforced by the organometallic moiety. Two representative species, **1** and **2**, are shown below. Details regarding the synthesis and conformational analyses of peptides constrained with ferrocene and tungsten-alkyne coordination will be presented.



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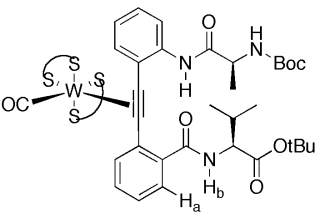

2

Peptide nanotubes as selective condensation catalysts

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Short peptides capable of stacking in rich β-sheet folds can access diverse microphases with patterened surfaces having remarkable long-range order. In recent years, we have detailed the surface of these nanosurfaces using X-ray diffraction, solid state NMR, CD and FT-IR spectroscopy and other electron microscopic techniques. In the present work, the surface of these assemblies have been exploited for the template-directed condensation via simple imine bonds. The reaction is achieved in aqueous milieu, an environment which is not conducive for dehydration reactions except on the template. Strikingly, the peptide assemblies templated the selective formation of imine dimers over. These results are significant as they reveal unique binding sites of the soluble microphases. Furthermore, these results motivated the use of activated nucleoside monophosphates with the prediction that the nucleobase can fit into the hydrophobic grove while the negatively charged phosphates can align the surface of the assemblies. Phosphoraimidazolides of adenosine monophosphates also condensed to form dimers in high yields when incubated with peptide assemblies. These results highlight the remarkable potential of these supramolecular surfaces as nanoreactors, which can yield unique and exclusive imine naphthalene dimers and also phosphodiester nucleotide dimers.

Heterogeneity in fast folding β-proteins

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We have taken a hierarchal approach to studying how β-rich proteins fold, starting from the smallest β-sheet structural motif, the β-hairpin, and building up to the WW domain, which incorporates 2 β-hairpins. We have studied CLN025, a synthetic β-hairpin that folds on the nanosecond timescale, and derivatives of FBP28 WW domain, which fold on the microsecond timescale. In an effort to speed up WW domain folding, we have systematically replaced each of the loops in the FBP28 WW domain by the faster folding β-hairpin, CLN025. A combination of Fourier transform infrared spectroscopy (FTIR) and laser induced temperature-jumps (T-jump) coupled with infrared and fluorescence spectroscopy are used to probe changes in the peptide backbone. The relaxation dynamics of the β-sheet and β-turn were measured independently by probing the corresponding bands assigned in the amide I region. We find that even for a simple β-hairpin structure folding is heterogeneous and cannot be described by a simple two state model. In the more complex WW Domain systems, we have observed as many as four relaxation lifetimes covering the nanosecond to millisecond timescale.

Theoretical characterization of low-energy particle deposition on PMMA surfaces

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The chemical processes that lead to polymer surface modification via low energy particle deposition were analyzed using molecular dynamics simulations (MD) and first principle calculations. The MD simulations performed used the reactive empirical bond order (REBO) potential to identify key products as a result of the interaction between the deposited particles and the surface of the polymer chains. Variety of particles (H^+, C_2H^+, C_2F^+, $CH_2^{+/\cdot}$ etc.) were deposited on the poly(methyl methacrylate) (PMMA) surface and, a large number of likely products were identified from the MD simulations. The reaction mechanisms to obtain the identified products were subsequently examined using Density Functional Theory (DFT) methods. The structures of the reaction species and energy barriers were determined using the B3LYP hybrid functional and subsequent CCSD (Coupled Cluster Singles and Doubles) calculations were performed on each optimized structure to obtain more accurate energetics. The calculations provide key information about the mechanism of surface reactions studied and will be further incorporated into studies to improve and extend the fidelity and capabilities of empirical potentials such as the REBO and COMP potentials. This work is supported by the National Science Foundation grant number CHE 0809376.

Effect on helical backbone conformation by insertion of extra CH_2 groups in the main chain of the model heptapeptides

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Peptides containing β-amino acids can fold into well defined secondary structures like α-amino acids in peptides and proteins[1,2]. Having an extra methylene group in the backbone can provide conformational flexibility to peptides. The peptides containing β-amino acids are also proteolytically more stable[3,4]. The present study focuses on the conformational properties of hepta peptides containing β, γ and ε-amino acids. We synthesized five hepta peptides with the sequence Boc-Ala-Leu-Aib-XXX-Ala-Leu-Aib-OMe (where XXX=Gly, Ala, β-Ala, γ-amino butyric acid(GABA) and ε-amino caproic acid). The peptides were designed based on the results obtained previously for the heptapeptide sequences Boc-Ala-Leu-Aib-XXX-Ala-Leu-Aib-OMe (where XXX =Met, SeMet and S-BzlCys) that gave mixed 310-/α- helical conformations in the solid state with helical transitions at the guest residue[5] to see whether β, γ and ε amino acid can be accommodated in 310-/α- helical conformation.We were able to crystallize three peptides(where XXX=Ala(**A**), β-Ala(**B**) and GABA(**C**)). All the structures were solved in $P2_12_12_1$ space group with R factors of 7.7, 4.43 and 5.29 respectively.The peptide A gave mixed 310-/α- helical conformation and the helix transition occurs at residue Aib(3) carbonyl as previously observed5 whereas peptide **B** and **C** gave folded structures with the centrally positioned β-Ala and GABA residue adopting gauche conformation (about C-C bonds of poly(methylene) units)which is necessary for folding). In peptide B and C there is one backbone hydrogen bond (1 ← 4) between Aib(3) carbonyl(O3) and Leu(6) NH group(N6) in both cases forming C11 and C12 ring respectively. There are other two successive solvated 1 ← 4 hydrogen bonds in both the peptides where OH group of methanol and water molecule forms bridge between C=O and NH group in the peptide backbone and these bridge forms between C=O of Ala(1) and NH of β-Ala(4) or GABA(4) and second one C=O of Leu(2) and NH of Ala(5) in both peptide **B** and **C**. Solution NMR study and CD analysis will be correlated with the crystal structure observations obtained for the peptides.

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Macrocyclic organo-peptidic hybrids from synthetic and genetically encoded precursors

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Macrocyclic peptides and peptide-containing molecules constitute attractive molecular scaffolds for the development of bioactive compounds to modulate biomolecular interactions. In this report, we describe a novel methodology for generating macrocycles with a hybrid organo-peptide backbone via the embedding of non-proteogenic, synthetic moieties into genetically encoded peptidic frameworks. This strategy relies on a tandem bioorthogonal ligation reaction between bifunctional synthetic precursors and recombinant protein precursors incorporating unnatural amino acids and intein moieties. The method offers the opportunity to readily diversify the composition, ring size, and architecture (cyclic, lariat, protein-tethered) of the resulting macrocycles by varying the nature of the synthetic and genetically encoded precursors. Our studies demonstrate the versatility and robustness of this approach toward generating diverse libraries of hybrid organo-peptide macrocycles.

Inhibition of human liver pyruvate kinase by phosphorylation and cysteine oxidation share a regulatory mechanism, removal of an activating interaction between the N-terminus and the main body of the protein

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We previously provided data supporting that phosphorylation of Ser12 of human liver pyruvate kinase reduces the protein's affinity for its substrate, phosphoenolpyruvate (PEP), by disrupting an activating interaction between the N-terminus and the main body of the protein. The primary supportive data were a truncation series that demonstrated removal of the N-terminus results in the same response as phosphorylation. In addition, the isolated non-phosphorylated N-terminal peptide can be added to the phosphorylated protein, resulting in a trans-acting "allosteric" activation. Therefore, the N-terminal peptide may serve as a drug lead for the goal of activating liver pyruvate kinase to counteract hyperglycemia.

Unfortunately, currently available crystal structures do not reveal where/how the N-terminus interacts with the main body of the protein. This is primarily because the N-terminus is either not present or disordered in those structures. A 1.8Å crystallographic structure of human liver pyruvate kinase (L-PYK) provides evidence for a sulfenic acid derivatization of a single cysteine residue. The oxidized residue was further probed to demonstrate energetic coupling with PEP binding. Mutant cycles provide evidence that the mechanism for regulation by oxidation is similar/equivalent to that caused by phosphorylation, disruption of an activating interaction between the N-terminus with the main body of the protein. Therefore, oxidation of the cysteine residue in crystallized protein may explain why the non-phosphorylated N-terminus is not ordered in currently existing crystal structures of human liver pyruvate kinase.

Nucleic acid driven peptide assembly

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This work describes the first steps toward engineering artificial enzymes from multiple peptides strategically arranged in a 3D DNA scaffold. The ultimate goal is to develop a peptide based artificial Oxygen Evolving Complex (OEC) that mimics the active site of Photosystem II (PSII). PSII evolves all the oxygen in the Earth's atmosphere and enables conversion of solar energy to bioenergy for the entire biosphere. However, the native system is unstable due to the accompanying chlorophyll-based photochemistry. Our goal is to assemble the peptides coordinating the metal cluster that catalyzes the water oxidation reaction inside a stable DNA nanocage. Due to the large and complex coordination environment surrounding the active site, three or more separate peptides with five or more DNA conjugation sites will be required, which is not feasible with conventional bioconjugation techniques. Peptide Nucleic Acid (PNA)[1] is a DNA analog synthesized like a peptide with an increased binding affinity to DNA, which allows many peptides to be incorporated into small DNA nanostructures. Here we report modifications to a previously described DNA tetrahedron design[2] to incorporate two short PNA-peptide conjugates into the DNA cage. Microwave assisted solid phase synthesis was used to make two fluorescently labeled PNA-peptides, which were subsequently hybridized by incubation with the preassembled DNA cage. The distance between the two PNAs bound to the cage was mapped by Resonance Energy Transfer. This method will form the basis for the peptide-based assembly of an artificial OEC inside a stable DNA Nanocage and serve as a model system for mimicking protein active sites.

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Post-antibodies: Generation of molecular-targeting peptides by directed evolution in phage-displayed libraries

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The aim of our study is to investigate molecular design relying on evolutionary processes, called as "directed evolution", to generate a novel class of biofunctional molecules. In our previous work, we have succeeded to develop a directed evolutionary process for improving catalytic efficiency and binding affinity of catalytic antibodies. Therefore, now, we apply our technology and know-how to directed evolution of antibody-like targeting peptides, which is named "microAntibodies"

At present, antibodies are indisputably the most successful reagents in molecular targeting therapy. However, use of antibodies has been limited due to the biophysical properties and the cost to manufacture. To enable new applications where antibodies show some limitations, we have developed an alternative-binding molecule with non-immunoglobulin domain. The molecule is a helix-loop-helix peptide, which is stable against natural enzymes *in vivo* and is too small to be non-immunogenic. Since the molecule folds by virtue of the interactions between the amino acid residues positioned inside the helix-loop-helix, the solvent-exposed, outside residues would be randomized to give a library of helix-loop-helix peptides. We constructed a phage-displayed library of the structured peptides and screened the library for G-CSF receptor. Finally, the screened peptides was cyclized by introduction of a disulfide-bond linkage into the N- and C-termini. The cyclic peptide showed strong binding affinity (Kd of 4 nM) to the receptor and a long half life (15 days) in mouse sera, proving an enzyme-resistant property. Futhermore, imminization of the peptide to mice elicited non-immunigenecity. The peptide is named "microAntibodes" due to haveing the same properties as those of antibodies. This semi-rational strategy, which combines phage-displayed libraries with *de novo* designed peptides, provides a new way to generate structured functional peptides for useful tools in the field of chemical biology as well as altanatives to antibody medicines.

Creation of artificial epidermal growth factor receptor activated by coiled-coil peptides

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Epidermal growth factor receptor (EGFR) is the prototype of ErbB family receptor tyrosine kinases, which regulates various signaling pathways to induce cellular functions (e.g. proliferation, motility, survival). If the activation of this receptor could be controlled on demand, the techniques contribute to a lot of valuable aspects of cellular regulations, including therapeutic applications. However, because overexpression and overactivation of EGFR lead to cancer-like modality, the control is difficult using intact receptor and ligands. From the points of view, our research objects are to create artificial EGFR on cell membranes, which can be specifically activated with artificial ligands that do not exist in any animals.

Coiled-coil has been used in numerous applications such as affinity purification, miniaturized antibodies, and receptor imaging[1]. Using the advantage of the coiled-coil peculiarities, we designed artificial EGFR, of which the receptor dimerization can be controlled by coiled-coil interactions[2]. Growth factor binding to the extracellular region of EGFR promotes dimerization of the receptor and increases the tyrosine kinase activity of its intracellular domain. To create the artificial EGFR and ligands, coiled-coil peptides (E3 and K4)[3] were used. We designed the surface-exposed tag sequence E3 fused EGFR (E3-EGFR) lacking the region of domain I~III and a portion of domain IV, which participate in dimerization of EGFR after the natural ligand (e.g. EGF) binding to the receptor. To dimerize the E3-EGFR, we synthesized conjugates of two K4 peptides (K4-conjugates), which linker lengths (~10 angstrom) mimic the distance during dimerization of the EGFR.

To test receptor activation, E3-EGFR expressed CHO cells were treated with artificial ligands, and this was followed by the western blot assay for detection of the phosphorylation of tyrosine 1173 of the receptor. Treatment of the cells with the K4-conjugates increased the level of phosphorylation of E3-EGFR in ~5 min. On the other hand, only marginal activation of E3-EGFR was observed for monomeric K4 peptide. The enhanced lamellipodia formations and migrations of the E3-EGFR ex pressed cells were also observed by the treatment of the K4-conjugates as are observed for the wild-type EGFR stimulated with EGF. This model-receptor system should be applicable to the design of receptors and other membrane-associated proteins to attain the control of cellular functions.

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Controlled degradation of self-assembled β-hairpin hydrogels by proteolysis with matrix metalloproteinase-13

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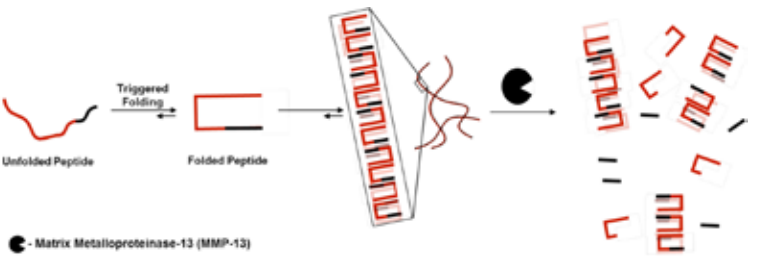
Hydrogels are a class of biomaterials that are useful for tissue regenerative therapies. Controlling biodegradation of the hydrogel can aid in its integration into newly formed tissue. In general, the rate of hydrogel degradation should approximate the rate of new tissue formation. Upon tissue injury, the surrounding cells secrete proteases that hydrolyze the amide-containing components of the extracellular matrix. If the hydrogel is peptide-based, proteases can provide a means to degrade the hydrogel scaffold with temporal resolution. Herein, five hydrogels formed from self-assembling β-hairpin peptides were designed to have varying susceptibilities towards matrix metalloproteinase-13. Hydrogel degradation was assessed by oscillatory rheology, HPLC and mass spectrometry. The rheological results demonstrated degradation of peptide hydrogels can be controlled by varying the peptide's amino acid sequence. Furthermore, degradable peptide hydrogels showed that SW1353 cells can migrate through the hydrogel at rates consistent with enzymatic degradation.



Expanding biology: Engineering tRNAs for delivery of unnatural amino acids to the ribosome

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The effective delivery of unnatural amino acids to ribosomes during the translation process could allow for the use of unnatural amino acids in drug design or any other number of applications involving synthetic molecules. "Unnatural" amino acids are amino acids that are not a part of the 20 natural amino acids typically used in cells. Amino acids are attached to transfer ribonucleic acids (tRNAs) and delivered by elongation factor proteins (EF-Tus) to the ribosome to become a part of a protein that will take physiological action within the body. EF-Tu is very specific for the binding of both the amino acid and the tRNA portions of the aminoacyl-tRNA (amino acid bound to a tRNA). There is a concept called thermodynamic compensation that says if there is a "weak" binding of the amino acid to EF-Tu, then there should be a "strong" binding of tRNA to EF-Tu, or vice versa (LaRaiviere, F., et al. 2001). Noncognate amino acid-tRNA pairs and unnatural amino acids are often not bound to EF-Tu with the correct strength. My project involves using mutant valine tRNAs that bind with increased or decreased affinity for EF-Tu. These mutant tRNAs are paired with unnatural amino acids for delivery by EF-Tus to the ribosome. More efficient overall binding with the entire mutant tRNA-unnatural amino acid pair would allow for a more effective delivery of the unnatural amino acid to the ribosome. This efficiency is measured with an in-vitro translation assay using S35 methionine labeling. Overcoming the specificity problem would be a major step in synthetic delivery of the amino acid to a ribosome.

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Exploring binding specificities of HIV-1 gp120 and CXCR4 through peptide-peptide interactions

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The design and generation of peptides capable of mimicking the binding and/or functional sites of proteins, represents a promising strategy for the exploration and modulation of protein function through controlled interference with the underlying protein-ligand interactions[1].

We have recently designed a soluble synthetic peptide that functionally mimics the HIV-1 coreceptor CXCR4, which belongs to the family of seven-transmembrane GPCRs[2]. This CXCR4 mimetic peptide, termed CX4-M1, presents the three extracellular loops (ECLs) of the receptor[3]. In binding assays involving recombinant proteins, as well as in cellular infection assays, CX4-M1 was found to selectively recognize gp120 from HIV-1 strains that use CXCR4 for cellular entry (X4 tropic HIV-1).

We could now show that CX4-M1 selectively recognizes not only gp120 from X4 tropic HIV-1, but also synthetic peptides presenting the V3 loops of these gp120 proteins. The V3 loop is thought to be an essential part of the coreceptor binding site of gp120 that contacts the second ECL of the coreceptor[4]. We were able to experimentally confirm this notion in binding assays using substitution analogs of CX4-M1 and the V3 loop peptides, respectively. These results clearly demonstrate the feasibility of mimicking protein-protein interactions through peptide-peptide interactions, as well as the utility of these mimetic peptides to explore protein-protein interactions at the molecular level.

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Modular protein templates for assembly and patterning of nanostructured materials

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Fundamental advances in biotechnology, medicine, environment, electronics and energy require methods for control of structure on nanometer scales. Assemblies that rely on highly specific biomolecular interactions are an attractive approach for the tailoring of nanostructure and bottom-up design of materials with sophisticated properties. Here we discuss design of modular repeat-protein arrays that serve as templates for nanostructured materials.

In first example we use the intrinsic self-assembling properties of the designed, rod shaped, superhelical **c**onsensus sequence **t**etratrico**p**eptide **r**epeat protein, **CTPR** comprising of 18 tandem repeats to generate self-supporting, uniformly birefringent films. CD and FTIR measurements show that the individual CTPR molecules retain their secondary structure in the film. Furthermore, CTPRs maintain their ligand binding functionality within the material. We will discuss structural and physical properties of this nanostructured material, and its ability to impose order on the otherwise disordered molecules such as fluorescent dyes.

In the second example we create biohybrid materials with tunable morphology by using click chemistry to cross-link CTPR arrays and synthetic polymers. We have designed CTPR array containing 20 repeats in which select positions have been replaced with amino-acids carrying alkyne functionality such that reactive moieties are precisely spaced along the superhelical array. We will discuss effects of different geometry of cross-linking functionalities along the CTPR array and the length and the functionality of the cross-linking polymer on physical properties of biohybrid materials.

This work opens the door to generation of innovative biomaterials with tailored structural and functional properties.

Local structure, global patterning and impact of Cu^{2+} binding in fibrillar amyloid-β protein

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The amyloid-β (Aβ) protein forms fibrils and higher-order, plaque aggegrates in Alzheimer's disease (AD) brain. Cu^{2+} ion is found at high concentrations in plaques, but its role in AD etiology is unclear. We use pulsed-electron paramagnetic resonance spectroscopy to characterize local coordination structure and global patterning of Cu^{2+} in fibrils of full-length Aβ(1-40). The results reveal a bis-cis-histidine (His) equatorial Cu^{2+} coordination geometry, and participation of all three N-terminal His residues in Cu^{2+} binding. A model is proposed, in which Cu^{2+}–His6/His13 and Cu^{2+}–His6/His14 sites alternate along the fibril axis on opposite sides of the β-sheet fibril structure. This structure precludes facile multi-electron and bridged-metal site reactivity, indicating that the fibrillar form of Aβ suppresses Cu redox cycling and reactive oxygen species (ROS) production. The insulator configuration suggests use of Cu^{2+}-Aβ fibrils as an amyloid architecture for switchable electron charge/spin conduction and redox reactivity.

Inhibiting EGFR dimerization: A peptide based approach

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Overexpression of epidermal growth factor receptor (EGFR) leads to activation of downstream signaling pathways that promote oncogenic activity. Extensive efforts have been put forth to target EGFR, leading to the development of various inhibitors including small molecule tyrosine kinase inhibitors and monoclonal antibodies. However, current therapies have numerous limitations including acquired resistance. Thus, there is a significant need for the development of an alternative approach to EGFR inhibition. The goal of this project is to develop an alternative targeting strategy in which novel, peptide-based inhibitors of EGFR disrupt key protein-protein interactions that mediate EGFR dimerization, which is a prerequisite for EGFR activation. A series of peptides were synthesized that mimic key structural features of the receptor dimer interface. Various chemistries were tested so as to synthetically constrain the peptides into a β-loop conformation. The proteolytic stability of the peptides was evaluated by trypsin degradation analysis and indicated that a chemically-stabilizing triazole linker provided increased stability. The peptides were evaluated for their ability to inhibit EGFR dimerization and autophosphorylation in cell-based assays. Western blot analysis indicated that a selection of the peptides reduced the relative expression levels of the EGFR dimer as well as phosphorylated EGFR. The results of this research indicate that these synthetically constrained peptides may show promise as an alternative targeting strategy for EGFR inhibition.

Peptidic cysteine protease inhibitors with an arylketone warhead for SARS

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A novel coronavirus was the causative agent of severe acute respiratory syndrome (SARS). In 2003, its epidemic resulted in over 8,000 infections with the mortality rate of about 10%. On the efforts towards the development of anti-SARS agents in case the disease re-emerges, we have focused on a viral cysteine protease (SARS-CoV $3CL^{pro}$), which is critical for the replication. From its native substrate sequences, we designed tripeptide-type $3CL^{pro}$ inhibitors focused on two strategies; i) side chain modification at the P1 position (Gln) and ii) introduction of chemically reactive warhead; an electron-withdrawing arylketone. As a result, Z-Val-Leu-Ala(pyrrolidone-3-yl)-2-thiazole (**1**) with a pyrrolidone side chain at the P1 site and thiazolyl-2-ketone structure at the warhead part (P1') exhibited a strong inhibitory activity[1,2]. In the present study, we further investigated SAR from **1**. According to the molecular modeling study of **1** with the protease, to fill the predicted larger space in the S1' pocket, a benzothiazole unit was introduced in place of the thiazole moiety. The P4 position, which stuck out from the binding pocket of the protease in the modeling, was further modified to increase the hydrophilic property. These modifications resulted in the discovery of potent SARS-CoV $3CL^{pro}$ inhibitor with the Ki values of nanomolar level. It was suggested that the arylketone structure, which can form a reversible hemi-thioacetal bonding with the catalytic cysteine-SH group in the protease, is useful machinery for cysteine protease inhibition. Development of dipeptide-type $3CL^{pro}$ inhibitors will also be discussed.

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Thermal stabilization of myocilin, a novel-calcium binding protein, prevents amyloid aggregation

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Approximately 4% of adult-onset and 10-33% of juvenile-onset primary open angle glaucoma cases are caused by mutations in the gene encoding for myocilin. The vast majority of mutations are within the 30 kDa C-terminal olfactomedin (OLF) domain. Disease-causing myocilin variants aggregate within human trabecular meshwork (TM) cells. This accumulation taxes the cells and leads to TM cell death, leading to increased intraocular pressure, and a hastening of glaucoma-associated vision loss. Our lab has demonstrated that the myocilin OLF domain harbors a novel Ca^{2+} binding site and has a propensity to grow amyloid fibrils under mildly destabilizing conditions. The latter feature is accelerated for destabilized, disease-causing variants. We hypothesize that small molecules capable of stabilizing the native conformation of the OLF domain could prevent amyloid aggregation and subsequent damage to the TM cells. Here we investigate the structural and biophysical effects of adding stabilizing compounds, such as osmolytes and Ca^{2+}, to the myocilin-OLF domain and evaluate their ability to prevent myocilin-OLF amyloid aggregation.

Synthetic biology of self-propagating beta-sheet assemblies

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Protein mis-folding diseases encompass a wide range of medical disorders in which a native protein undergoes a conformational transition that results in the formation of insoluble protein aggregates with commensurate loss of native function and/ or gain of toxic function. However, native protein sequences can vary significantly in their proficiency as templates for the conformational conversion, which may be related to the transmissibility of the phenotypes associated with the aggregated protein. In order to address these questions, we have constructed a reporter system based on structural modification of the yeast prion [*PSI*$^{+}$]. This prion state acts as an epigenetic modulator of biochemical behavior in *S. cerevisiae* strains and arises from the self-assembly of the native yeast protein Sup35p into a structurally defined supramolecular aggregates. We have created chimaeric Sup35p fusion proteins in the knockout *S. cerevisiae* strain 74D-694 (*sup35::kanMX4*) in which self-assembling peptide sequences from alpha-synuclein have been substituted in place of the native prion nucleating domain ($NQ_{1\text{-}39}$) of the wild-type Sup35p sequence. *In vivo* assays have demonstrated that these chimaeric sequences can maintain a prion state that displays sequence selectivity in transmission. *In vitro* seeding assays indicate that the selectivity resides in the identity of the amyloidogenic sequence.

Mixed peptide scaffolds with environmentally-responsive domains

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Short peptides have the ability to self-assemble into a variety of environmentally-responsive macromolecular morphologies. Amyloidogenic peptide sequences, such as KLVFFAE, the nucleating core of Aβ(1-42), have been shown to assemble into β-sheet rich nanostructures in a nucleation-dependent process. We show that short fragments from ubiquitous β-sheet rich protein folds can also self-assemble into diverse nanoscale morphologies, though not all have β-sheet character. The peptide fragments from the core of the protein assemble into the most interesting morphologies--large, dynamic spherical particles. These exogenous peptide particles can be used to alter the nucleating unit cell of the KLVFFAE peptide assemblies, leading to the propagation of new macromolecular morphologies without changing the environmental conditions of the system. The degree of mixing can also be used to tune the dimensions of the nanostructures, allowing for nucleation-directed control of thermostable products. The exogenous peptide appears to partition into domains within the larger structure and can be ejected from the scaffold in a salt-dependent manner to yield the two parent morphologies in a reversible manner. This responsive and reversible molecular partitioning will allow for the development of nanostructures that can access new morphologies via an environmental triggering mechanism.

Reactivation of p53 by stapled peptides

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Many protein-protein interactions are involved in signaling pathways that are of therapeutic importance in various malignant states e.g. tumour suppression by p53, c-myc transcriptional activity and anti apoptotic BCL-2. Most of these interactions used to be regarded as undruggable. Several of these interactions involve an α-helix. The design of compounds that can mimic the interactions of these helices is quite popular. Peptidic inhibitors can be fine tuned for greater specificity than small molecules. However, peptides are susceptible to proteolytic degradation. These problems have been partly overcome by the technique of stapling these peptides which also offer the advantage of preorganizing them into helices, yielding higher affinities and also renders them cell permeable.
Bernal et al[1] have previously reported the successful stabilization of the α-helix in p53 peptides interacting with the suppressor of p53 MDM2. Our atomistic simulations of these peptides revealed that the highest affinity is achieved when the staples engage the surface of MDM2[2] (subsequently validated in a crystallographic study[3]). Simulations revealed that the location of the staple is a key determinant for optimizing the interactions with the target MDM2; these notably involve exploiting hydrophobic patches on the target surface that can be engaged by the hydrophobic staple. The designed peptides were investigated in a series of biophysical, molecular biology and cell biology experiments which have revealed some novel aspects which will be discussed.

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Remodeling cross-β surfaces with analog chimeras

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The nucleating core of the Amyloid β peptideassociated with Alzheimer's disease is able to organize into a bilayer architecture with dimensions similar to biological phospholipid membranes. Although the dynamics of the hydrogen-bonded peptides are certainly different from the flexible alkanes of the lipid membranes, the plasticity of the laminate packing prompted us to consider their potential to respond dynamically to environmental fluctuations. Here we will review two ways to remodel cross-β surfaces inspired by the similarity between these self-assembled peptide membrane architechtures and lipid membranes. 1) Chemical modification of both the lipid chain and the peptide sequence directs morphology, demonstrating a delicate balance between the roles of lipid and peptide functional groups in directing self-assembly. 2) Mixing of peptide membranes with positively- and negatively-charged surfaces led to similar architectures with surfaces having new charge properties via EFM analysis. Our results show that these peptide membrane scaffolds are dynamic, –similar to lipid membranes, and their plastic properties and ability to self-organize offer a new opportunity for engineering specific molecular recognition elements into the peptide assemblies.

Heterogeneous seeding effect in self-assembly

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Peptide self-assembly has been shown to be susceptible to many environmental factors, including pH, temperature and salt concentration. For example, a truncation of the nucleating core of Aβ peptide: Ac-KLVFFAE, assembles into fibers at neutural pH, while at acidic pH, the glutamic acid will be protonated, leading to the formation of tubes. When the glutamic acid is mutated to leucine, the mutant peptide forms tubes under either condition. Here, by seeding Ac-KLVFFAE with sonicated Ac-KLVFFAL tubes, we demonstrate that even under neutral pH, a transient state of tube formation from Ac-KLVFFAE can be observed. The addition of the tube seed changes the original assembly process through a kinetic mechanism: the end of the mutant tube provides an ideal surface for wild-type (WT) monomer to attach to, helping monomer to skip the nucleating phase. However, since such a conformation is not the most thermodynamically stable one, the WT monomers will rearrange themselves within the tube and generate a new conformation--bundled fibers. Our study discovered that seeds can be used to shift the self-assembly process and kinetic processes can divert the assembly process to a new pathway.

Design, synthesis and pharmacology of fluorescently labeled ligands for ORL1 and Mu receptor binding assays

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Opioid receptors are widely known pharmacological targets for a number of drug discovery therapeutic areas. Development of high through-put methods to evaluate the affinity of novel pharmacophores greatly enhances these lead identification and development efforts. Fluorescent derivatives of endogenous ORL1 and Mu ligands were developed for use in fluorescent polarization assays. The affinities of these ligands were measured in traditional radio-ligand filtration and GTPγS assay formats in comparison with their unlabeled or tritiated endogenous counterparts. FP ligands with equivalent affinity measurements to the endogeneous ORL1 and Mu ligands were then further developed for 384-well assay formats. In the development of the FP assay, a ligand depletion correction factor was determined for each receptor for ORL1 and Mu, respectively. The results of the corrected FP assay Ki end-points were then profiled against filtration and GTPγS formats using known standards and were found to be equivalent. The overall outcome of this assay development effort was the generation of a homogeneous 384-well assay capable of rapid and predictable Ki values to support drug discovery efforts for identification and lead development With our developed method, triples labeled (^{2}H, ^{13}C, ^{15}N) CaM has been expressed and purified with very high yield (40 mg/L medium) for obtaining high quality NMR spectrum of the huge CaM complex. Isotope labeled membrane fragments synthesis has been developed using a vector that contains an enterokinase cleavage site, which can be used to get the clean desired peptide without any additional tag. Instead of the expensive chemical synthesized peptides with only selective labeling, uniform label isotopes of CaM target peptide with ^{15}N and ^{13}C can be labeled in bacterial synthesize system. This study will provide more efficient and affordable way to study CaM binding mode with more detailed structure.

Protein and peptide engineering of calmodulin target proteins

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Ca^{2+} is involved in many cellular functions, such as muscle contraction, fertilization, cell differentiation and proliferation. Calmodulin (CaM) is essential for many cellular calcium signaling events by binding and regulating many different target proteins. Ca^{2+} binds to CaM cause a conformational change, the loop connect the two helices become more helical and exposed more hydrophobic residues to the surrounding surface, which make CaM more easy to bind with specific proteins for specific response. Because the diversity of regulation of CaM, it is important for us to understand the different CaM binding mode in structure level.

Many CaM target are membrane proteins; therefore, it is difficult to study the complex due to the challenge of expression and purification of the membrane proteins. CaM binding peptides and fragments become ideal model for studying the CaM interaction. In this study, we focus on design CaM binding peptides base on three CaM binding proteins, Ryanodine receptor 1 (RyR1) of SR membrane, Connexin 26 (Cx26) and Connexin 43 (Cx43) of the cytoplasm membrane. RyR1 controls the releasing of Ca^{2+} from the SR that is necessary for the skeletal muscle contraction. Lots of life threat diseases are due to the mutation of RyR1, such as malignant hyperthermia and central core disease. CaM also regulates the connexin family, which are channel for the communications of cells. Many diseases that cause by abnormal function of specific connexin are genetic deafness, skin disease, and cataracts.

Isotope labeled peptides and proteins are essential for the NMR structure study. With our developed method, triples labeled (^{2}H, ^{13}C, ^{15}N) CaM has been expressed and purified with very high yield (40 mg/L medium) for obtaining high quality NMR spectrum of the huge CaM complex. Isotope labeled membrane fragments synthesis has been developed using a vector that contains an enterokinase cleavage site, which can be used to get the clean desired peptide without any additional tag. Instead of the expensive chemical synthesized peptides with only selective labeling, uniform label isotopes of CaM target peptide with ^{15}N and ^{13}C can be labeled in bacterial synthesize system. This study will provide more efficient and affordable way to study CaM binding mode with more detailed structure.

Comparative analysis of the subunit interfaces of the sliding clamps PCNA and Rad9-Rad1-Hus1

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The Proliferating Cell Nuclear Antigen (PCNA) is well known cyclic protein for DNA clamp or sliding clamp in eukaryotic cells. PCNA was originally identified as an antigen that is expressed in the nuclei of cells during the DNA synthesis phase of the cell cycle. PCNA helps to hold the DNA polymerase and support the high processivity of them during DNA synthesis or repairing. PCNA achieves this processivity by encircling the DNA, thus creating a topological link to the genome and serving as a DNA clamp. Two structurally similar trimeric ring-shaped complexes, the PCNA and 9-1-1 complex are central players in different DNA transactions. The homotrimetic PCNA ring is crucial for DNA replication and replication-linked functions, whereas 9-1-1 complex plays a special role in checkpoint activation. PCNA contains three same subunits but 9-1-1 contains three different subunits. The opening and closing of the PCNA interfaces is the activated process. During the opening of PCNA interfaces, it requires the opening of the subunit-subunit interfaces which are stabilized by the network of hydrogen bonds, salt-bridges and hydrophobic interaction. In this current theoretical and computational study, we applied Steered Molecular Dynamics (SMD) on the PCNA interfaces to study the opening mechanism. We performed the series of PCNA pulling run to identify a directional breaking down mechanism and reasonable free energy profile for opening the interfaces.

Helix-constrained antagonists of the oncogenic AP-1 transcriptional regulator, cFos

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Protein-based therapeutics feature large interacting surfaces. Protein folding endows structural stability to localised surface epitopes, imparting high affinity and target specificity upon interactions with binding partners. However, short synthetic peptides with sequences corresponding to such protein epitopes are unstructured in water and promiscuously bind to proteins with low affinity and specificity. Here we combine structural stability and target specificity of proteins, with low cost and rapid synthesis of small molecules, towards meeting the significant challenge of antagonising transcriptional regulation. By iteratively truncating a Jun-based peptide from 37 to 22 residues, strategically incorporating helix-inducing constraints, and positioning unnatural amino acids, we have produced short water-stable, α–helical peptides. A three dimensional NMR-derived structure for the most helical peptide (24) in water confirmed a highly stable α–helix across the molecule, while serum studies demonstrated resistance to proteolytic degradation. These short structured peptides bind with high affinity and specificity to cFos, a key component of the oncogenic transcriptional regulator Activator Protein-1 (AP-1), and competitively antagonize the cJun–cFos interaction. While truncating the Jun-based peptide by 15 residues decreased the binding enthalpy for cJun by ≤ 9 kcal/mol, this was compensated by increased conformational entropy ($T\Delta S \leq 7.5$ kcal/mol). This study demonstrates rational design of short, water-stable, α-helical peptides using cyclic pentapeptide modules that confer high helicity, target affinity and retain the specificity of the parent peptide for cFos. These are important steps towards more druggable small molecule antagonists of the cJun-cFos interaction that mediates gene transcription in cancer and inflammatory diseases.

Development of peptide-bindng antibodies as generic crystal chaperones

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Hydrophobic membrane proteins perform a variety of important functions in the cell, but their structures are notoriously difficult to solve. Thus, new strategies to obtain crystals of membrane proteins for structure determination are critical. We aim to develop a toolbox of orthogonal single-chain antibody fragments engineered for hyper-crystallizability and specific recognition of short peptides. These peptide sequences can be introduced into flexible internal loops or at the termini of membrane proteins without interfering with protein function. The resulting scFv-membrane protein complex is expected to form a crystal lattice mediated by chaperone interactions, resulting in high-resolution crystal structures.

We have developed candidate scFv chaperone proteins binding hexa-histidine, EYMPME (EE) and FLAG tags. The scFv's were engineered via site-directed and random mutagenesis in conjunction with phage display to improve biophysical features influencing crystallization propensity, including peptide affinity and specificity, stability, solubility and expression level. Selected variants exhibit high solubility (up to 16.6 mg/ml) and nanomolar peptide binding affinities; complexes of one chaperone with the signal peptide peptidase integral membrane proteins harboring an internal EE tag has been isolated by gel filtration. Three of these scFv chaperones have been crystallized. The 3.1 Å resolution structure of this chaperone reveals a binding surface complementary to the EE peptide and a ~52 Å channel in the crystal lattice. These engineered scFvs represent a new class of chaperones that may eliminate the need for de novo identification of candidate chaperones from large antibody libraries.

Interactions between sulfated glycosaminoglycans and the peptide hormone secretin

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Secretin is a 27-residue peptide that exerts a large array of pharmacological effects and biological activities, mainly observed in the gastrointestinal tract, pancreas and other endocrine glands. To perform its functions, secretin interacts with the specific membrane-bound G protein-coupled receptor. Owing to its cationic and highly-flexible nature, secretin could bind electrostatically to various anions that decorate the outer leaflet of the cell membrane, such as O-linked glycosaminoglycans (GAGs). GAGs, which are linear sulfated polysaccharides abundant at the cell surface as part of proteoglycans, are already known to act as templates that can bridge growth factors and chemokines to their specific receptors. In this context, we investigated the kinetics and thermodynamics of secretin binding to sulfated glycosaminoglycans by a combination of affinity chromatography, isothermal titration calorimetry and surface plasmon resonance. We observed that secretin, that comprises a consensus heparin-binding motif, binds avidly by electrostatic interactions to heparin, as elution of this peptide from a heparin-Sepharose column required as much as 1M NaCl. Isothermal titration calorimetry revealed a high affinity of secretin for several sulfated glycosaminoglycans and this binding event is an exothermic reaction. Besides, using far-UV circular dichroism spectroscopy, we noticed that the interaction of secretin with sulfated GAGs induces a conformational transition of the peptide from a random coil to an α-helix. Herein, we propose a model for secretin-sulfated-GAGs complex and discuss the potential biological significance of this interaction. This study suggests that cell surface sugars could facilitate the initial adsorption of secretin to the cell membrane microenvironment.

Peptide-polysaccharide matrices as a synthetic basement membrane

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Biomaterials that provide a support or scaffold for tissue formation play key roles in virtually tissue engineering approaches. Extracellular matrix (ECM) components including laminin, collagen, and fibronectin, and their active peptides are potential candidates for affording the cell binding activities to materials. Laminins are a major component of the basement membrane, a thin ECM, and have diverse biological activities. Our goal is to identify active sequences from laminins and to use the biologically active peptides for biomaterials as a synthetic basement membrane. We already have identified various biologically active peptides from laminins using more than 3,000 synthetic peptides. These peptides recognized various cellular receptors and have the potential ability to serve as bio-adhesiveness for tissue engineering. We prepared peptide-polysaccharide matrices using three laminin active peptides, A99 (AGTFALRGDNPQG, bind to integrin $\alpha v\beta 3$), AG73 (RKRLQVQLSIRT, bind to syndecans), and EF1zz (ATLQLQEGRLHFXFDLGKGR, X = Nle, bind to integrin $\alpha 2\beta 1$), and three polysaccharides, chitosan, alginate, and agarose, and examined their biological activities. Most of the peptide-polysaccharide matrices showed cell attachment activity and neurite outgrowth activity. The morphological appearance of the attached cells was found to depend on the peptides and physical properties of the polysaccharides. Further, the mixed AG73/EF1zz-chitosan matrices mimicked the cell adhesion of a multifunctional protein, suggesting that the mixed peptide-polysaccharide matrices are useful as a multifunctional biomaterial for tissue engineering. These results suggest that the receptor type of ligands and physical properties of scaffolds are critical for cellular functions. The peptide-polysaccharide matrices have a potential to be used as a synthetic basement membrane for tissue engineering.

Accommodating fluorinated amino acids in helical peptide environments

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Structural modifications of peptides and proteins using non-natural amino acids provide the opportunity to improve their biophysical and pharmaceutical properties as well as to modulate their biological activity. The successful application of fluorine in the development of small molecule pharmaceuticals also motivates the interest in using fluorine as a heteroatom in amino acid side chains.[1] Since fluorine is absent in native amino acids, it is especially desirable to discover fluorophilic environments within native protein surroundings.

To this end, we applied the phage display technology in search for preferred interaction partners of four different fluorinated amino acids using a heterodimeric α-helical coiled coil model system. The predefined secondary and tertiary structure of the model allows to randomize the direct interaction partners of the respective amino acid and therefore to select specific binders for fluorinated amino acids out of the pool of the canonical amino acids.

We find that despite the polarity that is induced by partial fluorination of aliphatic amino acids these building bocks preferably interact with hydrophobic side chains within the hydrophobic core of the coiled coil.

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Self-propagating minimal peptide catalyst

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Although the proportion of functional folded structures that exist within protein sequence space may be small, most peptides appear to access the cross-β fold. These stacks of β-sheets of the cross-β protein fold, best known in protein misfolding diseases, form highly ordered soluble networks that can access polymorphically diverse micro-phases that are highly responsive to environmental fluxuations. Here we report that simple 7-residue peptide assemblies can bind small molecules as determined by fluorescence microscopy and function as specific catalysts for controlling carbon-carbon bonds by having detectable retroaldolase activity. These β-rich assemblies are enantioselective and subtle changes in peptide sequence and/or assembly conditions can significantly impact both final morphology and catalyst efficiency. Eliminating the binding pocket or adding a charge abolishes catalytic activity. These results suggest that simple self-propagating peptide assemblies can produce new enzymes, diversify any chemical inventory and provide new functional materials.

N-sulfanylethylanilide peptide for peptide engineering

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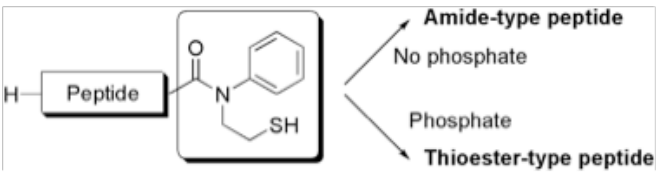
N-Sulfanylethylanilide (SEAlide) peptides were developed with the aim of achieving facile synthesis of peptide thioesters by 9-fluorenylmethyloxycarbonyl (Fmoc)-based solid-phase peptide synthesis (Fmoc SPPS). Initially, SEAlide peptides were found to be converted to the corresponding peptide thioesters under acidic conditions. However, the SEAlide moiety was proved to function as a thioester in the presence of phosphate salts and to participate in native chemical ligation (NCL) with N-terminal cysteinyl peptides, and this has served as a powerful protein synthesis methodology. The reactivity of a SEAlide peptide (anilide vs thioester) can be easily tuned with or without the use of phosphate salts. This interesting property of SEAlide peptides allows sequential three-fragment or unprecedented four-fragment ligation for efficient one-pot peptide/protein synthesis. Furthermore, dual-kinetically controlled ligation, which enables three peptide fragments simultaneously present in the reaction to be ligated in the correct order, was first achieved using a SEAlide peptide. Beyond our initial expectations, SEAlide peptides have served in protein chemistry fields as very useful crypto-peptide thioesters. Furthermore, we recently found that the SEAlide moiety shows a potential utility in a ligand-mediated protein labeling useful for chemical biology researches.



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Rationale based, de novo design of dehydrophenylalanine containing antibiotic peptides and systematic modification in sequence for enhanced potency

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As part of an effort to develop new, low molecular mass peptide antibiotics we used a rationale based minimalist approach to design short, non hemolytic, potent and broad spectrum antibiotic peptides with increased serum stability. These peptides incorporated a non natural amino acid α, β-dehydrophenylalanine (ΔPhe), and were designed to attain an amphipathic structure in helical conformations.

ΔPhe residues are present in many naturally occurring antibiotic peptides like albonoursin. Presence of ΔPhe in peptides has been shown to increase the helicity of the peptides and increase the relative stability of peptides towards proteolytic degradation. An eleven residue peptide VS1 was used as the lead compound and its properties were compared with three series of derivates obtained by N-terminal amino acid addition (12-14 residues), systematic Tryptophan (Trp) substitution and peptide dendrimerization. The Trp substitution approach underlined the optimized sequence of VS2 in terms of potency, faster membrane permeation and cost effectiveness. VS2 (two Trp substituted variant of VS1) was found to exhibit good antimicrobial activity (5μM -25 μM) against both, Gram negative bacteria *E.coli* and Gram positive bacteria *S. aureus*. VS2 showed negligible hemolysis, non cytolytic activity and has the ability to permeate and depolarize the bacterial membrane. Lysis of *E.coli* inner membrane by the peptide VS2 was also confirmed by Scanning and Transmission Electron Microscopy.

A combination of small size, presence of unnatural amino acid, high antimicrobial activity, insignificant hemolysis and relative proteolytic resistance provides fundamental information towards the de novo design of an antimicrobial peptide useful for the management of infectious disease.

Novel cyclic peptides effective against multidrug-resistant Gram-negative

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The rapid rise of multi-antibiotic resistant bacteria has become a global public health problem, making the discovery and development of new antibiotics an imperative. The majority of life-threatening infections worldwide are caused by the ESKAPE pathogens (***Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species***). This group of bacteria is resistant to the most commonly used antibiotics today.

Fusaricidin class of naturally occurring cyclic depsipeptides, microbial secondary metabolites that contain one or more ester bonds in addition to the amide bonds, have emerged as an important source of novel antimicrobial agents. However, these natural products exhibit potent activity exclusively against Gram-positive bacteria and have a low stability in human serum due to ester bond hydrolysis. Synthetic modifications of fusaricidin that includes ester-to-amide substitution and introduction of multiple charges led to more stable analogs active against Gram-negative bacteria. Our synthetic peptides exhibit potent *in vitro* activities against trimethoprim and streptomycin resistant *E. coli* K-12 strain S4362 (MIC 8 μg/mL), and they possess strong anti-endotoxin activities. Analogs with modified sequence bind lipid A with an affinity comparable to polymyxin B (K_d 0.5 μM determined by isothermal titration calorimetry; reported value for polymyxin B is 0.4 μM), and effectively inhibits TNFα cytokine release from LPS-stimulated human MDA-MB-231 model cells with IC_{50} of 1.3 μM; a value slightly higher than the value determined for polymyxin B (IC_{50} 0.5 μM). In addition, confocal fluorescence microscopy study shows that fluorescein-labeled analogs can efficiently enter *E. coli* K-12 strain S4362 (ATCC 29181) cells, suggesting that our synthetic peptides exert their antibacterial effect after translocating into the cytoplasm.

Antimicrobial peptides and macromolecular crowding

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Cationic Antimicrobial Peptides (CAMPs) represent an ancient defense mechanism against pathogenic bacteria and are essential elements of innate immunity in higher organisms. These peptides have been shown to exert broad-spectrum antimicrobial activities, via mechanisms that involve targeting the anionic lipid membrane of bacteria, or even enveloped viruses[1]. With large proteins and polysaccharides occupying significant portions of extracellular (8% in plasma) and intracellular space (20-30% in cytoplasm), macromolecular crowding is a significant factor in these environments[2]. While macromolecular crowding has been shown to impact protein structural and functional properties, CAMPs have not generally been the subject of such investigations. Accordingly, the studies reported here focus on a series of CAMPs that includes NA-CATH, from the elapid *Naja atra*, and truncated NA-CATH variants and the effect that macromolecular crowding agents have on their performance. It was found that molecular crowding agents can dramatically impact CAMP performance, both in a positive and a negative capacity.

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Design protein-based MRI contrast agents for the molecular imaging of prostate cancer

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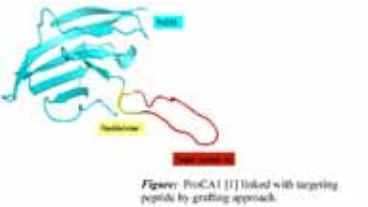

Figure: ProCA1 [1] linked with targeting peptide by grafting approach.

Many biomarkers such as gastrin releasing peptide receptor (GRPR) are overexpressed on the surface of diseased cells. Meanwhile, short peptides such as gastrin releasing peptide (GRP) have been often used to target these biomarkers to image the mobility. This approach is limited by the short biologic half-life, less binding affinity, less specificity and pharmacologic side effects of short peptides. Magnetic resonance imaging (MRI), which provides good contrast between different soft tissues is a useful tool in the disease diagnostics because of its non-invasive, three-dimensional and high depth penetration properties. ProCA1[1] is a protein-based MRI contrast agent designed by our lab which adds a Gd3+ binding site on a stable protein (domain 1 of rat CD2) surface. Compared to clinical used MRI contrast agent, ProCA1 shows much higher longitudinal and transverse relaxation rate. Moreover, it also performs better than Gd-DTPA on respects of contrast enhancement and blood retention time. Gastrin-releasing peptide (GRP) is one of a well-characterized group of mammalian bombesin-like peptides. We previously linked ProCA1 with GRP (named ProCA1.GRP(52)), a targeting sequence which specifically binds to GRPR highly expressed on tumor surface[2]. Tumor enhancement was achieved under MRI through intratumoral injection. In this poster, we show our progress in developing protein-based MRI contrast agents for the molecular imaging of disease biomarkers with improved targeting capability.

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Molecular imaging and monitoring HER2 biomarker by targeted protein based MRI contrast agents

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The application of magnetic resonance imaging (MRI) to non-invasively assess disease biomarkers has been hampered by the lack of desired contrast agents with high relaxivity, targeting capability, and optimized pharmacokinetics. We have developed a novel MR imaging probe targeting to HER2; a biomarker for various cancer types and a drug target for anti-cancer therapies. This multimodal HER2-targeted MR imaging probe integrates a *de novo* designed protein contrast agent with a high affinity HER2 affibody and a near IR fluorescent dye. Our probe can differentially monitor tumors with different expression levels of HER2 in both human cell lines and xenograft mice models. In addition to its 100-fold higher dose efficiency compared to clinically-approved non-targeting contrast agent DTPA, our developed agent also exhibits advantages in crossing the endothelial boundary, tissue distribution, and tumor tissue retention over reported contrast agents as demonstrated by even distribution of the imaging probe across the entire tumor mass. Furthermore, by treating the cancer cells or the xenograft mice with commercial drugs against HER2, our contrast agent can monitor the effectiveness. This contrast agent will provide a powerful tool for quantitative assessment of molecular markers, and improved resolution for diagnosis, prognosis and drug discovery.

Directed self-assembly of peptides using small molecules

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The cross-β fold of natural polypeptides achieve long-range order and their diversity is generated at the level of conformational richness seen in protein folding and amyloid aggregates. Cross-β peptide conformation and assembly are determined by a combination of physicochemical forces such as van der Waals, ionic, aromatic and hydrogen-bonding interactions. As a result, these assemblies and morphological diversities are acutely responsive to fluctuating environmental factors and to changes in amino acid sequence. Using Aβ(16-22) KLVFFAE as a model system, the central nucleation core of the Aβ peptide associated with Alzheimer's Disease (AD), it has been shown that a single residue mutation dramatically alters peptide conformation and morphology. Given the context dependent effects on Aβ(16-22) assembly which gives rise to different peptide aggregates we want to explore the possibility of incorporating small molecules to drive the formation of a specific peptide self assembling morphology. By incorporating a single mutation to Aβ(16-22)E22L changing the V18 to an aspartic acid (D) we can select for positively charged small molecules to bind in a site specific manner to any given peptide they encounter. If incorporation of a hydrophilic residue within the hydrophobic core of Aβ(16-22) does not disrupt assembly, then using such a mutation to incorporate small molecules within the peptide assembly becomes a possibility. Here I show that we can regulate morphological 'expression' of this self-assembling system by incorporating the V18D mutation, moving us a step closer to generating a responsive material that can not only adapt, but can also integrate "factors" present in its environment.

Development of a cobalt center-protein catalyst for biofuels production

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Our aim is to design and construct protein-based artificial photosynthetic systems capable of catalyzing the environmentally favorable carbon dioxide (CO_2) reduction and hydrogen (H_2) production reactions. We have selected the robust and adaptable $(\beta\alpha)_8$, TIM-barrel protein of the EutB subunit of B_{12} (adenosylcobalamin)-dependent ethanolamine ammonia-lyase from *Salmonella typhimurium* for use as a scaffold. The fully-reduced, Co(I) forms of the native cobalamin (Cbl) and cobinamide (Cbi) possess relatively low redox potentials, that are commensurate with the target reactions. Efficacy of Cbi as a catalyst was assesed by gas chromatography (GC) of the products of steady-state photo-initiated H_2 production in solutuion at pH 7.0 with Eosin Y and triethanolamine as sacrificial electron donor. Co(III)(dmgH)2pyCl (Cbx) was used as control. The rate of Cbi-catalyzed H_2 production was observed to be maximal at low catalyst concentrations, [50 μM; $4.4 \pm 1.8 \times 10^{-3}$ turnover number per hour (TON/h)] in pure water. Under the same illumination and sampling conditions, Cbx in 1:1 actonitrile:water gave optimized H^2 production of 6.0 ± 1.6 TON/h, and retained activity of 1.7 ± 0.2 TON/h in pure water. A system was developed for genetic manipulation, overexpression (in *Escherichia coli*), and purification of EutB. The isolated EutB protein forms the native hexamer. Assembly of the cobinamide-cofactor/protein subunit was assessed from the dissociation constant of methyl-Cbi for EutB of 2.1×10^{-10} M, which was measured by using endogenous tryptophan fluorescence quenching. A long lived (lifetime > 104 seconds) catalytically active Co(I) state was formed by photo-induced reduction of the protein-bound Cbi cofactor, indicating the assembly of the catalytically active construct. Protein light scattering measurements (600 nm) and ultra-violent/visible absorption spectroscopy were used to further confirm assembly and stability. The EutB protein with bound Co(I) was shown to be stable under steady-state irradiation (>7 minutes, 75 mW, 520 nm) in the presence of Eosin Y. The results demonstrate that the native EutB protein is a viable scaffold for cobinamide-mediated H_2 production, and other reduction reactions.

Cloning, expression, and purification of a centrin biological target: Krr1, kh domain

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The origin of many disease states has been linked to genetic mutations, defects in gene expression, nuclear excision repair and ribosome biogenesis. Centrin, a calcium binding protein, which has recently been found to regulate some of these processes along other target proteins within the nucleus. One target, Krr1, contains a K homology (KH) domain; which has been identified as a nucleic acid recognition motif, required for proper processing of pre-rRNA, for synthesis of 18S rRNA, and for the assembly of the 40S subunit. Our initial findings have identified a putative centrin binding site located within the KH domain of Krr1 using bioinformatics tools. In this study, the KH domain (192 bp) was amplified by PCR and then ligated to the expression vector pET100. Colony PCR was performed to identify the E. coli colonies that have been transformed effectively with the desired recombinant. The Krr1 KH domain fragment was then overexpressed. An isolation and purification protocol has been designed which includes preparative centrifugation, cross flow and tangential flow filtration and two ion exchange chromatography's. The evaluation of the purification of the KH domain fragment has been performed by SDS-PAGE and peptide samples have been sent for MS analysis and partial amino acid sequencing.

Design and synthesis of a peptide based functional mimic of [FeFe] hydrogenase

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Natural hydrogenases catalyze the reduction of protons to molecular hydrogen reversibly under mild conditions. The relevance of this reaction to energy production, and in particular to sustainable fuel production, has spurred considerable interest around these enzymes. The catalytic site of hydrogenases, termed the H-cluster, is composed by a [4Fe4S] cluster and a [FeFe] site coordinated by a non-protein dithiolate bridging ligand as well as carbon monoxide (CO) and cyanide (CN) ligands. The site is anchored to the protein through a cysteine that bridges the proximal iron-sulfur cluster and one iron. Such an unusual cluster is assembled in vivo through complex biosynthetic machinery. For these reasons, direct mimicking of natural hydrogenases has proven to be a daunting task.

Here, we describe an alternative approach that utilizes an artificial amino acid containing a 1,3 dithiol moiety as an anchor to covalently secure the diiron–cluster at any position in an artificial protein scaffold[1]. We verified the correct incorporation of the cluster into a model helical peptide UV-Vis, FTIR, ESI-MS and CD spectroscopy. Most importantly, we found that the peptide complex, 1-[$Fe_2(CO)_6$], catalyses photo-induced hydrogen production in the presence of a photosensitizer and a sacrificial reducing agent in water with remarkable efficiency. Our approach allows for the directed incorporation of hydrogenase mimics into any peptide scaffold and opens the way for the design of more elaborate peptide-based architectures. With such artificial proteins, it will be possible to explore the effect of second sphere interactions on the activity of the diiron center, and to include in the design properties such as compatibility with conductive materials and electrodes.

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The dynamics of Mastoparan X insertion into DPPC lipid vesicles

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Understanding the mechanism by which both naturally occurring and synthetic peptides fold and traverse membrane bilayers has been long studied but poorly understood. Such peptide-membrane interactions are hallmark to many fundamental physiological processes including those involving the structure and function of membrane-associated peptides and even a variety of host-pathogen interactions. Herein, we describe both the steady state conversion of solvated to buried helix using FTIR and the dynamics of insertion of alpha helical peptide mastoparan X (MpX) into d62-DPPC lipid vesicle bilayers using temperature-jump infrared spectroscopy. This work aims to couple dynamic changes of the membrane in conjunction with those of lipophilic MpX to further elucidate the mechanism of invasive peptide-membrane interactions.

Interactions of peptides with lipid nanotubular bilayers of defined curvatures

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It is now established that many proteins preferentially bind to curved lipid bilayer membranes. The ability of proteins to specifically detect membrane curvature has a number of important implications in cellular processes including endocytosis, exocytosis, and vesicle trafficking as well as pathogenic processes associated with viral infections or protein aggregation disorders. Experimentally, binding of proteins to convex membranes with positive curvature is readily measured using liposomes as model membrane structures. The curvature of liposomes could be controlled by sizing multilamellar vesicles by an extrusion through nanoporous filters and the exterior of the liposomes is easily accessible to proteins added from solution. On the contrary, currently only limited data are available on binding of proteins to lipid bilayers possessing negative curvature. Here we describe the use of lipid nanotube arrays as a novel type of substrate-supported lipid bilayers with tunable curvature radius ranging from ca. 40 to 250 nm to study peptide binding and membrane-induced aggregation phenomena. The nanotubular lipid bilayers are formed by self-assembly inside cylindrical nanopores of anodic aluminum oxide (AAO). These systems have essentially no restrictions on the lipid composition and have the inner surface of negative curvature accessible for solute molecules and peptide binding. The AAO nanopores are macroscopically aligned to a high degree and so are the lipids and peptides self-assembled within the nanopores. This alignment simplifies solid-state (ss) NMR studies. AAO membrane is essentially transparent to light allowing for optical studies of binding events. Here we provide examples of the use of lipid nanotube arrays for studying peptide-lipid bialyer interactions ranging from such small antibacterial peptides as melittin and alamecitin to synaptotagmin among the others. Further directions of using these structures for layered lipid-peptide nanotubes will also be discussed.

pH sensitive EPR labels to probe local dielectric gradients in protein-membrane interface

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Nitroxide spin-labeling in combination with EPR spectroscopy has found many applications in studying structure and dynamics of proteins and biological membranes. Recently, there has been a substantial interest in utilizing EPR to characterize local effects of polarity and hydrogen bonding in these systems. Here we report on employing an arsenal of advanced spin-labeling EPR methods to profile heterogeneous dielectric and hydrogen bonding environment along the α-helical chain of an alanine-rich WALP peptide that is anchored in a lipid bilayer in a transmembrane orientation. A series of WALP single cysteine mutants was labeled with a pH-sensitive nitroxide IMSTL (S-(1-oxyl-2,2,3,5,5-pentamethylimidazolidin-4-ylmethyl) ester) that is similar in molecular volume to phenylalanine. The protonation state of this nitroxide could be directly observed by EPR allowing us to follow proton gradient across the membrane in the vicinity of the WALP α-helix, and, thus, to reconstruct the gradient in the effective dielectric constant across the membrane on membrane-protein interface. Q-band DEER experiments with symmetric double-labeled WALPs were employed to derive positions of nitroxides upon protonation. This system provided another estimate of the local dielectric constant. Local polarity was also evaluated from characteristic changes in EPR spectra that were enhanced by the use of perdeuterated and 15N-substituted nitroxides and high field EPR at 130 GHz (D-band). Formation of hydrogen bonds between the nitroxides and membrane-penetrating water molecules was observed directly in HYSCORE X-band experiments. Such measurements allowed us to derive experimental profiles of heterogeneous dielectric and hydrogen bonding environment along a typical transmembrane α-helix.

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Using replica exchange molecular dynamics to predict the helicity of short stapled peptides

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Short, stapled peptides have drawn great interest recently due to their potential applications on inhibiting Protein/Protein interaction and interacting with Class B GPCRs. Being able to accurately predict the secondary structure of these peptides is critical for peptide design. Herewith we present our application of replica exchange molecular dynamics (REMD) method in predicting the helicity of stapled peptides. REMD uses high temperature simulation to cross energy barriers and room temperature simulation to collect structures mimicking experimental conditions. The results agree with the published CD and NMR data. Furthuer structural analysis and potential applications on peptide permeability are also included in this presentation.

Bacterial expression of a self-assembling peptide hydrogelator

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Peptide hydrogels have been developed via de novo design as scaffolds for tissue regeneration and vehicles for drug delivery. Hydrogelation can be accomplished by the triggered self-assembly of individual peptides in response to changes in pH, temperature and salt concentration. Simple modifications of the amino acid content of the peptide further allow the tuning of the material's biological and mechanical properties to provide diverse materials with many biotechnological uses. In this study, bacterial expression was investigated as an alternative method to chemical synthesis for the production of a self-assembling peptide that can form a rigid hydrogel under physiological conditions. Our lab has designed and characterized a 20 amino acid beta-hairpin forming amphiphilic peptide containing a D-residue in its turn region (MAX1). As a result, this peptide must be prepared chemically. Peptide engineering, using the sequence of MAX1 as a starting point, afforded a small family of peptides having different beta-turn sequences. These new sequences consist of natural amino acids and are thus amenable to bacterial expression. Each sequence was initially chemically synthesized to quickly assess the material properties of each of their respective gels. The peptide P26EC was found to be the best candidate among all. Gel formation characterization showed that P26EC afforded a more rigid hydrogel than MAX1 at the end of one hour under physiological conditions. DNA constructs facilitating the expression of P26EC were designed in such a way that the peptide could be expressed with different fusion partners and cleaved by chemical means to afford the free peptide. Optimization studies were performed to increase the yield of pure peptide, that ultimately allowing 50 mg of pure peptide to be harvested from one liter of culture, providing an alternate means to produce this material-forming peptide.

CD44 derived glycopeptide inhibitors of matrix metalloproteinases

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Glycosylation has been considered one of the most important post-translational modifications that proteins undergo. Aberrant changes in protein glycosylation patterns have been associated with malignant cell transformations. Consequently, the hydrolysis of glycoproteins (glycoproteolysis) may result in a multivariate diagnostic tool for cancer initiation and progression.

CD44 is broadly distributed cell surface glycoprotein implicated in many biological and pathological processes such as lymphocyte homing, T-cell activation, wound healing, and angiogenesis. CD44 is shed by proteases implicated in cancer initiation and progression (MMPs and ADAMs). The proteolytic cleavage of CD44 from the cell surface plays a critical role in the migration of tumor cells. At present, the physiological mechanism of CD44 shedding is poorly understood and protease(s) responsible for shedding of CD44 have not been clearly identified.

To analyze CD44 shedding we have designed, synthesized, and characterized 8 peptides spanning the stem region. The peptides were examined as potential substrates for MMP-2, -8, -9, -14, ADAM10 and ADAM17. Suprisingly, two of the peptides, named CD44-1 and CD44-1G, having the sequence:

CD44-1	E^{185}RSSTSGGYIFYTFFST^{200}
CD44-1G	E^{185}RSST*SGGYIFYTFFST^{200}

(where T*=Thr(α-D-GalNAc) residue) turned out to be MMP inhibitors and did not inhibit either of the ADAM enzymes. We presently describe the synthesis of the O-glycosylated Thr and Ser residue building blocks, the synthesis and characterization along with enzymatic activity evaluation and structure determination of CD44-derived peptides.

Structure of serine proteinase [Nlys]5SFTI-1 peptide-peptoid inhibitor in complex with trypsin

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Peptoids (poly *N*-substituted glycine residues) and peptide – peptoid hybrid polymers are interesting compounds mimicking structure and function of biologically active peptides. They represent a class of polymers that are not found in nature. Among desirable properties of peptoids as peptidomimetics, two of them are most notable and desirable: bioavailability and protease resistance. Peptoids can be easily incorporated into native peptide sequences and the synthetic methodology is fully compatible with existing Fmoc SPPS protocols.

We hereby present the structure of analog of the trypsin inhibitor SFTI-1 modified at the P1 position. Substitution of Lys5 in SFTI-1 by N-(4-aminobutyl)-glycine, which mimic Lys residue made this analog completely protease-resistant at P1-P1' reactive site. The obtained analog appeared to be potent inhibitor of bovine β-trypsin.

Up to date there is no report in the literature for explanation of proteolytic resistance of peptoids or peptide-peptoid hybrids. Herein we report the crystal structure of [Nlys]5SFTI-1 inhibitor in complex with bovine β-trypsin at 1.29Å resolution. Surprisingly, the reported structure is almost identical with the structure of native SFTI-/trypsin complex. What's interesting, the reported complex structure extends the view for the possible mechanism of action of serine proteinases. The P1-P1' cleavage site in our structure remains the same as in the native complex structure, revealing the key role of Ser^{218} residue in the mechanism of proteolysis of serine proteinases.

Evolutionary strategy for the self-assembling intelligent peptide materials

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Evolution generally achieves increasing complexity (randomly encoded information) and ingenious adaptations (non-random survival) to fit the environment and generate living matter as the most intelligent materials in Nature. The diversification and selection increase molecular complexity and organization, fundamental to Chemical Evolution. Reinventing and testing these processes as they may have informed the biological and biochemical evolution of inanimate substances to living matter also provides us a new evolutionary strategy to construct intelligent materials.

By changing the oxidation state of phenylalanine C-terminus from carboxylic acid to aldehyde, reactive short peptides (TF-CHO, TFTF-CHO and NF-CHO) have been designed, combining the kinetically accessible, reversible imine condensation linkage with intramolecular ring-closure to mimic amide linkages via N, O-, and N, N-acetals. Different Dynamic Chemical Networks (DCN) are formed via spontaneous oligomerization of the differentially reactive peptides, represented by diversities in sequence length and geometry (linear and ring-closed oligomers). We have demonstrated that not only are these libraries dynamic, but that encoded sequence information can be selected via environmental inputs (pH, temperature, exogenous templates, etc.). Ordered structures generated from the networks through the supramolecular self-assembly of the dynamic components by selection and propagation of peptide cross-β conformational information. This dynamic network elicits a fundamental chemical level of natural selection as represented by Darwinian evolution, and sheds light on novel strategies for constructing hierarchical composed intelligent materials.

Chirality as a tool to engineer peptide-based biomaterials

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In addition to its central importance to the origin and evolution of life, chirality opens new possibilities to control the assembly and bulk mechanical properties of peptide-based biomaterials. When designing de novo peptide-based biomaterial, the interplay between homochirality and heterochirality could have a wide range of structural implications which further translate into radically different bulk mechanical characteristics of biomaterials. In this work, we explored in detail different homochiral and heterochiral combinations of a pair of self-repulsive but mutually attractive oligopeptides that co-assemble into hydrogels. Using dynamic rheometry, it has been shown that homochiral peptide-based hydrogels have advantageous mechanical characteristics—almost 20 fold higher elastic moduli. On the other hand, two co-assembling peptides of opposite chirality demonstrate the kinetic advantages—much faster gelation and assembly into the fibrous network. At the level of the individual hydrogel fiber, the structural studies using small-angle neutron scattering (SANS) as well as the dynamic monitoring of the structural changes during gelation by means of small-angle X-ray scattering (SAXS) suggest that both homochiral and heterochiral hydrogels are assembled from fibers interconnected by lappet-like webs. However, as opposed to the heterochiral hydrogels, the homochiral system is formed by thicker and denser fibers, and this evidently translates into its improved bulk biomaterial properties. In summary, this allows one to look at the biohomochirality from an angle of its use in the engineering of biomaterials with tunable and predictable mechanical and structural characteristics. From a fundamental viewpoint, the structural data on the homochiral and heterochiral biomaterials in combination with their material properties could help to understand a break in mirror symmetry in bioorganic realm and could support the suggestion that homochiral life has evident selective advances in the natural selection process.

Creation of tubular α-helical assemblies through seven-helix coiled coils

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Native coiled-coil sequences can form large bundles composed of four or more helices in which the helix packing arrangements define continuous channels throughout the supermolecular structure. If the number of helices within the assembly and the inter-helical packing arrangements could be controlled, then one might be able to tailor the channel dimensions and chemistry to facilitate binding of specific classes of guest molecules. To better understand the self-assembly of α-helical peptides and to explore the potential applications of coiled-coil peptide assemblies to selectively encapsulate small molecules in the channel, a nanoscale peptide fibrous assembly was designed based on the seven-helix coiled-coil motif. Various characterization methods including Electron Microscopy, Analytical Ultracentrifugation, Solid-state NMR, Small Angel X-ray Scattering and Fluorescence Spectroscopy have been employed to investigate the structure of the nano-fibrous assembly and its ability to encapsulate guest molecules.

Molecular imaging of cancer biomarkers by protein-based MRI contrast agents

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MRI is a promising non-invasive imaging technique with high depth penetration and high spatial resolution. MRI contrast agents with high metal binding affinity, high relaxivity, low toxicity and targeting capabilities are highly desired for the preclinical and clinical imaging. ProCAs are newly designed MRI contrast agents with high relaxivity and tumor targeting property. The novel protein-based MRI contrast agents, ProCA3, with multiple Gd^{3+} binding sites are designed based on the native protein. By optimizing Gd^{3+} binding ligands, the per particle relaxivity of ProCA3 increased to above 100 $mM^{-1}s^{-1}$ (60 MHz 37 ˚C), which is more than 20-fold higher than that of Gd-DTPA. Strong Gd^{3+} stability and metal selectivity are achieved by manipulating Gd^{3+} binding ligands of ProCA3. ProCA3 present high stability, no cell toxicity and acute toxicity and proper blood circulation time for MR angiography. Mice MR angiography was obtained under 4.7 T scanner after injection of 0.02 mmol/kg ProCA3 for 50 minutes with 3D gradient echo sequence. We further constructed different classes of targeted MRI contrast agents for biomedical imaging. Mice models of prostate and breast tumors were established. We then developed targeted ProCA3 for various biomarkers, such as gastrin-releasing peptide receptor, HER2/neu and integrin $\alpha v\beta 3$. MR images of tumor mice were obtained before and after the injection of targeted ProCA3 under 4.7 T and 7 T MRI scanner. The biomarker positive tumor is enhanced under MRI after injection of ProCA3 for 1 day. Tumor enhancement in MRI was further confirmed by immunofluorescence and ICP-OES. Non-targeted ProCA3 was applied for dynamic contrast-enhanced MRI to probe tumor vasculature in mice.

Three-dimensional cell culture system using peptide-hyaluronate hydrogels

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Tissue engineering is an important approach for therapeutic applications. Recently, cell behavior in three-dimensional (3D) matrices in vitro has been shown to be different from that in two-dimensional (2D) matrices, suggesting that functional cell and tissue systems are reconstructed in a 3D matrix environment. In native tissues, cells are held within 3D extracellular matrices (ECMs), which have critical roles in maintaining tissues, and guiding development, regeneration, and homeostasis. Mimicking ECM as a matrix is a common goal in biomaterial studies for tissue engineering. We have identified a number of cell adhesive peptides from laminins, which are major components of basement membrane, a thin layer of ECM. Cell adhesive peptides derived from ECM proteins are potential candidates for incorporating cell-binding activities into materials for tissue engineering. Previously, we conjugated the laminin active peptides to polysaccharides, including chitosan and alginate, and demonstrated that the peptide-chitosan and -alginate matrices have various biological activities. Here, we focused hyaluronate (HA) as a scaffold material beause HA is a biodegradable, biocompatible, and non-immunogenic polysaccharide, which has been used for various medical applications. Laminin-derived cell adhesive peptides, A99 (AGTFALRGDNPQG, bind to integrin $\alpha v\beta 3$) and AG73 (RKRLQVQLSIRT, bind to syndecans), were conjugated to 3D HA matrices and biological activities were examined using human dermal fibroblasts (HDFs) and PC12 cells. As a result, HDF spreading and neurite outgrowth of PC12 cells were observed in the 3D peptide-HA matrices. These results suggest that the 3D HA matrices can interact with cells via the peptides and control cellular function. The 3D cell culture system using cell adhesive peptides and HA hydrogels will be a useful tool for tissue engineering and clinical applications.

Engineering protein ligands to measure cellular forces

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Mechanical forces play an essential role in many biological processes, such as transcription, the action of efflux pumps and mitosis. Most of these physical inputs are sensed and transduced through the receptors on the cell surface. Recently, our lab developed a FRET-based method to quantify the mechanical tension exerted by the EGF receptor during endocytosis. This method, however, is limited to small molecule ligands and proteins due to the narrow dynamic range of FRET (3-8 nm). Moreover, for accurate force quantification, a 1:1 stoichiometry between the FRET pair and the appended ligand is needed. To address these limitations and expand the scope of force probes for the study of diverse biological systems, a more modular design is needed. In this poster, we present the synthesis and application of a universal force sensor that reports forces across specific proteins in living cells. The sensor contains a donor and an acceptor fluorophore at each end of a peptide. When the peptide experiences mechanical tension it will extend, thus physically separating the donor from the acceptor and increasing the intensity of donor emission. The sensor thereby transduces mechanical forces into an optical signal that can be imaged using a standard fluorescence microscope. The force probe was synthesized with a solid phase peptide synthesizer, and on-bead dye labeling was performed to introduce one of the dyes. To conjugate recombinant proteins to the peptide terminus, we employed the native chemical ligation strategy to couple protein α-thioesters to the peptide force sensor N-terminus. The desired protein was conjugated to the peptide force sensor through a stable peptide bond, thereby increasing the stability and biological activity. The modularity and adaptability of this sensor will allow it to serve as a universal platform for the study of mechanical forces between virtually any receptor and their cognate protein ligand that can be expressed *in vitro*.

Synthetic coiled coil glycopeptides as drug carriers

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Peptides represent the ideal scaffold for the presentation of ligands due to their functionality and folding properties. In particular, the α-helical coiled coil folding motif can be used as a central organizing element and is the basis for the *de novo* design of peptides. The unique feature of the coiled coil is that, by changing very few positions on the oligomer-forming sequence, it is possible to obtain an aggregating system for the presentation of ligands in an even more multivalent fashion[1].

In this project, the focus is to optimize the presentation of sugar moieties for the interaction with biological macromolecules. This goal can be achieved by using PEG linkers to connect scaffold-peptide and ligand. We are currently working on the synthesis of glycopeptides for the development of a potential drug carrier through the improvement of the interaction with the asialo-glycoprotein (ASGP) receptor on the surface of liver cells. We established a new technique for the complete solid phase synthesis of the complex peptide-PEG linker-sugar and we are ready to proceed with the biological assays. Furthermore, we want to use the coiled coil peptide as a tool to investigate the unknown structure of the ASGP receptor by using various lengths for the linker and different ligand distribution on the scaffold.

Further ahead, we will create a heteromeric design concept to cover the largest possible variability of ligand intervals without expensive synthesis, obtaining a library of multivalent peptide nanofibers in which the glycosyl ligands will be found, by statistical distribution, within different intervals of each other.

Reference

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The bended tail: Novel mechanistic insights of histone modification and function

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The core histone tails of eukaryotic nucleosomes are subject to a variety of covalent modifications, including acetylation, methylation, and phosphorylation. These highly dynamic posttranslational modifications (PTMs) drastically modulate the structure of local chromatins and constitute a fundamental epigenetic mechanism for the regulation of essentially all DNA-mediated transactions such as DNA transcription, replication, repair and recombination. Notably, a growing body of evidence suggests extensive cross-talk exists between different epigenetic pathways and multiple histone modifications may exert their effects on nucleosomes in a coordinated manner, resulting in the proposition of so-called histone codes. Histone-modifying enzymes have been found to associate with many essential cellular phenotypes such as proliferation, growth, apoptosis and differentiation. Despite intensive studies in this field, the molecular understanding of how histone modification, especially on the amino-terminal tail regions, is poorly defined. Recently, we set out to investigate the substrate specificity regulation of several key histone acetyltransferases and methyltransferases by combining a number of biochemical and biophysical methods. Our data surprisingly revealed that the histone tails have strong tendency to form folded structures, instead of being disordered random coils as previously visioned. This work offers a novel understanding of the mechanism and function of histone modifications in epigenetic pathways.

Identification of the calmodulin binding domain of connexin 45

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Calmodulin, as an intercellular calcium receptor, has been found to function in Ca^{2+}-dependent regulation of gap junctions. Our research has identified several CaM-binding sites in the intracellular loop of Cx43, Cx44 and Cx50 in alpha gap junction family. In the present study, we found a CaM-binding motif in the intracellular loop of Cx45 which is a gamma family member playing crucial roles in the nervous system and cardiovascular system. The synthesized peptide containing this CaM-binding motif displayed the strong interaction with Ca^{2+} loaded CaM with 1:1 stoichiometry by applying different biophysical approaches, including circular dichroism, fluorescence spectroscopy, surface plasmon resonance and nuclear magnetic resonance. Fluorescence coupled with NMR studies indicated that conformational changes of both the peptide and CaM are induced by the formation of CaM: peptide complex. But the alternation of structure can be only observed when Ca^{2+} is supplied. The fluorescence study shows that Cx45 peptide directly bound with Ca^{2+}-CaM with a dissociation constant of 5nM. Using N-domain and C-domain of CaM, we further demonstrates that only the N-domain of CaM can interact with Cx45 peptide in 5mM Ca^{2+}. No interaction between C-domain of CaM and Cx45 peptide can be detected in 5mM EGTA or Ca^{2+} buffer by monitoring dansyl-labled CaM, which is unlike the alpha subgroup gap junction members. Further cell assay and electrophysiological experiment will be applied to confirm the interaction between CaM and the putative CaM-binding motif of Cx45 and provide molecular basis for nervous and cardiovascular diseases related with Cx45.

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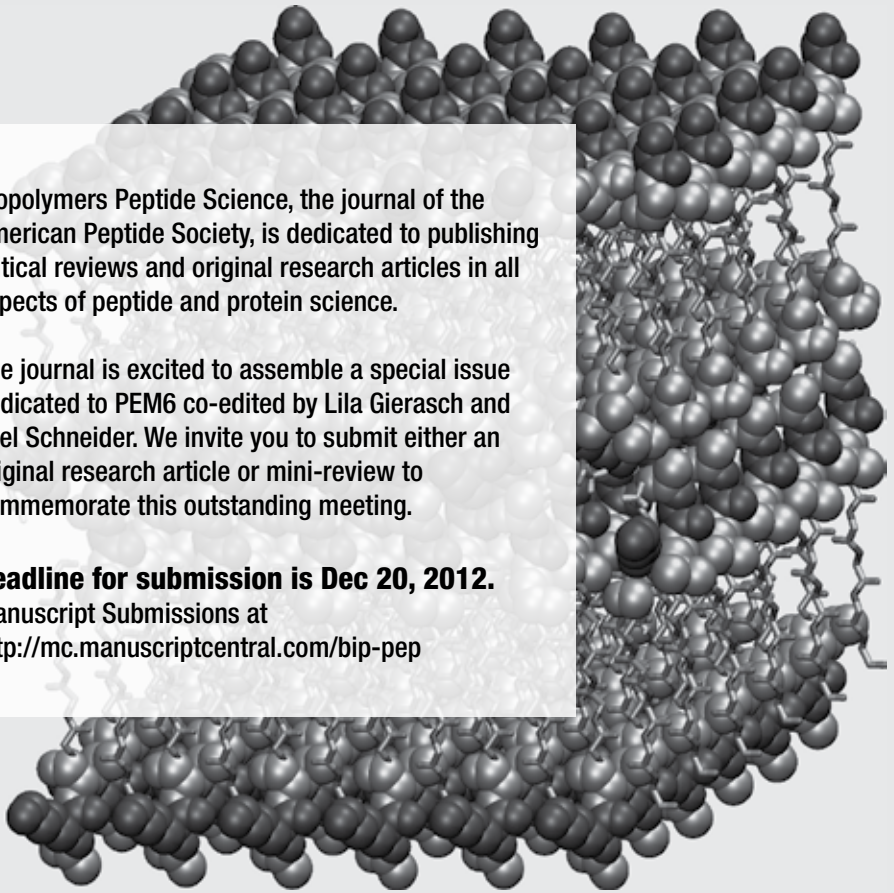

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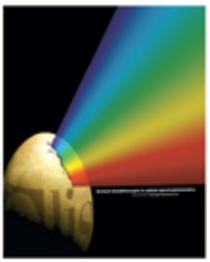

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