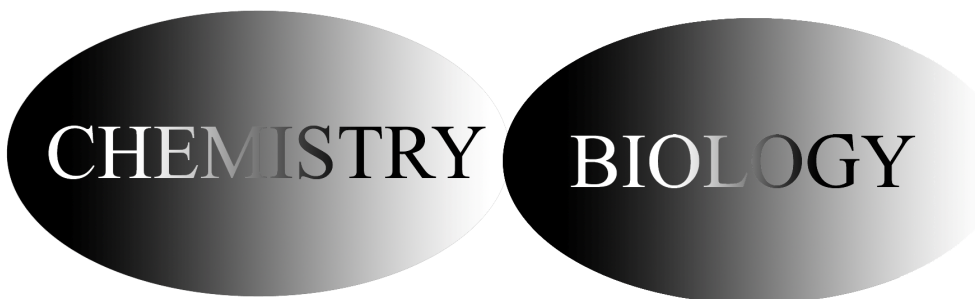


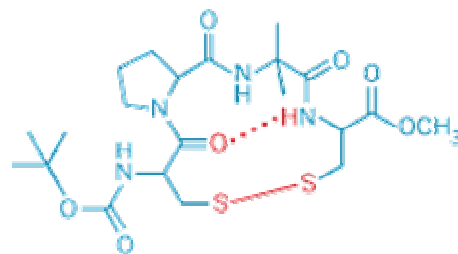
Chmielewski Group Literature Abstracts



Highlight of the Month

Peptides Caught In The Act

Peter Hamm of the University of Zurich and his colleagues have figured out to watch molecular movies of an unfolding peptide on a picosecond timescale (*Nature* 2006, 444, 469). The researchers investigated a string of four amino acids cinched into a cyclic structure by a S-S bond and an O...H hydrogen bond (red). This structure models β -turns in full-scale proteins. The researchers then snapped the disulfide bridge with UV light, thereby allowing the tetrapeptide to unfold. As it did, the hydrogen bond stretched, and the vibrational interactions among the amino acid keto groups evolved. Hamm's group tracked the changes with a rapid-fire series of 2-D IR spectra. The technique could help discern molecular dramas that have been too small and too fast to see before.



November 2006

Contributing Editors:

Song-Gil Lee (*PNAS*)

Stefan Hershberger (*Science*)

Marcos Pires (*Nature and Nature subdivisions/Angewandte Chemie*)

Brandon Gaddis/Iris Geisler (*JACS*)

Jee Yeon Lee (*JBC*)

Dawn Ernenwein (*ACS Chem Biol/Chem & Bio/Chem Biol & Drug Design*)

Dave Przybyla (*Org Lett*)

Nature

Watching hydrogen-bond dynamics in a bold beta-turn by transient two-dimensional infrared spectroscopy

Nature 444, 469-472 (23 November 2006)

Christoph Kolano¹, Jan Helbing¹, Mariusz Kozinski¹, Wolfram Sander² and Peter Hamm¹

X-ray crystallography and nuclear magnetic resonance measurements provide us with atomically resolved structures of an ever-growing number of biomolecules. These static structural snapshots are important to our understanding of biomolecular function, but real biomolecules are dynamic entities that often exploit conformational changes and transient molecular interactions to perform their tasks. Nuclear magnetic resonance methods can follow such structural changes, but only on millisecond timescales under non-equilibrium conditions. Time-resolved X-ray crystallography has recently been used to monitor the photodissociation of CO from myoglobin on a subnanosecond timescale¹, yet remains challenging to apply more widely. In contrast, two-dimensional infrared spectroscopy, which maps vibrational coupling between molecular groups and hence their relative positions and orientations^{2, 3, 4, 5, 6, 7, 8, 9, 10, 11}, is now routinely used to study equilibrium processes on picosecond timescales. Here we show that the extension of this method into the non-equilibrium regime^{12, 13} allows us to observe in real time in a short peptide the weakening of an intramolecular hydrogen bond and concomitant opening of a beta-turn. We find that the rate of this process is two orders of magnitude faster than the 'folding speed limit' established for contact formation between protein side chains.

Nature Biotechnology

FRET analysis of protein conformational change through position-specific incorporation of fluorescent amino acids

Nature Methods - 3, 923 - 929 (2006)

Daisuke Kajihara^{1, 2}, Ryoji Abe², Issei Iijima¹, Chie Komiyama², Masahiko Sisido² & Takahiro Hohsaka

We designed and synthesized new, fluorescent, non-natural amino acids that emit fluorescence of wavelengths longer than 500 nm and are accepted by an Escherichia coli cell-free translation system. We synthesized p-aminophenylalanine derivatives linked with BODIPY fluorophores at the p-amino group and introduced them into streptavidin using the four-base codon CGGG in a cell-free translation system. Practically, the incorporation efficiency was high enough for BODIPYFL, BODIPY558 and BODIPY576. Next, we incorporated BODIPYFL-aminophenylalanine and BODIPY558-aminophenylalanine into different positions of calmodulin as a donor and acceptor pair for fluorescence resonance energy transfer (FRET) using two four-base codons.

Fluorescence spectra and polarization measurements revealed that substantial FRET changes upon the binding of calmodulin-binding peptide occurred for the double-labeled calmodulins containing BODIPY558 at the N terminus and BODIPYFL at the Gly40, Phe99 and Leu112 positions. These results demonstrate the usefulness of FRET based on the position-specific double incorporation of fluorescent amino acids for analyzing conformational changes of proteins.

ACS Chemical Biology

A Comparative Study of Bioorthogonal Reactions with Azides

ACS Chem. Biol. **1** (10), 644–648

Jeremy M. Baskin, Jennifer A. Prescher, Anderson Lo, and Carolyn R. Bertozzi

Detection of metabolites and post-translational modifications can be achieved using the azide as a bioorthogonal chemical reporter. Once introduced into target biomolecules, either metabolically or through chemical modification, the azide can be tagged with probes using one of three highly selective reactions: the Staudinger ligation, the Cu(I)-catalyzed azide-alkyne cycloaddition, or the strain-promoted [3 + 2] cycloaddition. Here, we compared these chemistries in the context of various biological applications, including labeling of biomolecules in complex lysates and on live cell surfaces. The Cu(I)-catalyzed reaction was found to be most efficient for detecting azides in protein samples but was not compatible with live cells due to the toxicity of the reagents. Both the Staudinger ligation and the strain-promoted [3 + 2] cycloaddition using optimized cyclooctynes were effective for tagging azides on live cells. The best reagent for this application was dependent upon the specific structure of the azide. These results provide a guide for biologists in choosing a suitable ligation chemistry.

Chemistry and Biology

A Fluorescent Broad-Spectrum Proteasome Inhibitor for Labeling Proteasomes In Vitro and In Vivo

Chemistry & Biology **13**, 1217–1226, November 2006

Groothuis⁴, Michiel A. Leeuwenburgh¹, Huib Ovaa³, Jacques J. Neefjes⁴, Dmitri V. Filippov¹, Gijs A. van der Marel¹, Nico P. Dantuma² and Herman S. Overkleeft¹,

The proteasome is an essential evolutionary conserved protease involved in many regulatory systems. Here, we describe the synthesis and characterization of the activity-based, fluorescent, and cell-permeable inhibitor Bodipy TMR-Ahx3L3VS (MV151), which specifically targets all active subunits of the proteasome and immunoproteasome in living cells, allowing for rapid and sensitive in-gel detection. The inhibition profile of a panel of commonly used proteasome inhibitors could be readily determined by MV151 labeling. Administration of MV151 to mice allowed for in vivo labeling of proteasomes, which correlated with inhibition of proteasomal degradation in the affected tissues. This probe can be used for many applications ranging from clinical profiling of proteasome

activity, to biochemical analysis of subunit specificity of inhibitors, and to cell biological analysis of the proteasome function and dynamics in living cells.

Stattic: A Small-Molecule Inhibitor of STAT3 Activation and Dimerization

Chemistry & Biology **13**, 1235–1242, November 2006

Jochen Schust¹, Bianca Sperl¹, Angela Hollis¹, Thomas U. Mayer² and Thorsten Berg¹,

Signal transducers and activators of transcription (STATs) are a family of latent cytoplasmic transcription factors that transmit signals from the cell membrane to the nucleus. One family member, STAT3, is constitutively activated by aberrant upstream tyrosine kinase activities in a broad spectrum of cancer cell lines and human tumors. Screening of chemical libraries led to the identification of Stattic, a nonpeptidic small molecule shown to selectively inhibit the function of the STAT3 SH2 domain regardless of the STAT3 activation state in vitro. Stattic selectively inhibits activation, dimerization, and nuclear translocation of STAT3 and increases the apoptotic rate of STAT3-dependent breast cancer cell lines. We propose Stattic as a tool for the inhibition of STAT3 in cell lines or animal tumor models displaying constitutive STAT3 activation.

Chemical Biology and Drug Design

Synthesis of Novel Peptide Inhibitors of Thrombin-induced Platelet Activation

Chem Biol Drug Des **2006; 68: 235–238**

Fernanda M. Burke¹, Mark Warnock², Alvin H. Schmaier³ and Henry I. Mosberg

Inhibitors of the activation of platelet aggregation have promise as important therapeutic agents for the management of acute coronary syndrome (ACS). Platelet activation by thrombin, a serine protease, occurs by binding to and cleavage of the extracellular N-terminal domains of protease-activated receptors 1 and 4 (PAR1 and PAR4). The proteolysis of the PARs exposes new tethered ligands that then signal through transmembrane domains to initiate platelet activation as a downstream effect. A pentapeptide cleavage product of bradykinin with the sequence Arg-Pro-Pro-Gly-Phe serves as a thrombin inhibitor by blocking α - and γ -thrombin-induced platelet aggregation. Analogs of RPPGF have been prepared that result in improved inhibition of thrombin activation of platelets. Specific amino acid residues required for activity against platelet aggregation have been identified, and a lead compound, rOicPaPhe(p-Me)-NH₂ (FM19), has been developed. FM19, which completely inhibits threshold γ -thrombin-induced platelet aggregation at a concentration of $16 \pm 4 \mu\text{M}$, represents an important lead compound in the development of inhibitors of thrombin-mediated platelet aggregation for treatment of ACS.

The Indirect Generation of Long-distance Structural Changes in Antibodies upon their Binding to Antigen

Chem Biol Drug Des 2006; 68: 276–283

Barbara Piekarska^{1,*}, Anna Drozd¹, Leszek Konieczny¹, Marcin Król², Wiktor Jurkowski², Irena Roterman², Paweł Spólnik¹, Barbara Stopa¹ and Janina Rybarska

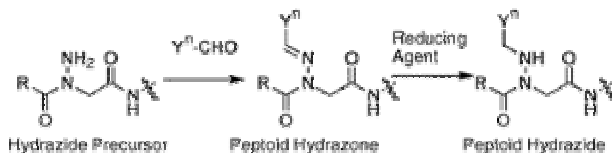
An allosteric mechanism for the generation of long-distance structural alterations in Fab fragments of antibodies in immune complexes has been postulated and tested in theoretical and experimental analysis. The flexing and/or torsion-derived forces exerted on the elbow region in Fab arms of bivalent antibodies upon binding to antigen were assumed to drive the disruption of hydrogen bonds which stabilize N- and C-terminal chain fragments in V-domains. This allows an extra movement in the elbow followed by a relaxation in the Fab arm and may generate long-distance effects if, in particular, the structural changes are generated asymmetrically involving one chain of the Fab arm only. This mechanism was studied by simulation of molecular dynamics. The local instability in the area involving the site of packing of the N-terminal chain fragment allows penetration and binding of the supramolecular dye Congo red that hence becomes an indicator of the initiated relaxation process and is also the prospective ligand in studies of designing drugs. The susceptibility to dye binding was observed in complexation of bivalent antibodies only, supplying the evidence that constraints associating the interaction with randomly distributed antigenic determinants drive the local structural changes in the V-domain followed by long-distance effects.

Organic Letters

Hydrazone- and Hydrazone-Containing N-Substituted Glycines as Peptoid Surrogates for Expedited Library Synthesis: Application to the Preparation of Tsg101-Directed HIV-1 Budding Antagonists

Org. Lett., 8 (22), 5165 -5168, 2006.

Fa Liu, Andrew G. Stephen, Catherine S. Adamson, Karine Gousset, M. Javad Aman, Eric O. Freed, Robert J. Fisher, and Terrence R. Burke, Jr



Replacing the Pro6 in the p6Gag-derived 9-mer "P-E-P-T-A-P-P-E-E" with N-substituted glycine (NSG) residues is problematic. However, incorporation of hydrazone amides ("peptoid hydrazones") can be readily achieved in library fashion. Furthermore, reduction of these hydrazones to N-substituted "peptoid hydrazides" affords a facile route to library diversification. This approach is demonstrated by application to Tsg101-binding compounds designed as potential HIV budding antagonists.

Switchable Dual Binding Mode Molecular Shuttle

Org. Lett., 8 (23), 5377 -5379, 2006.

David A. Leigh* and Andrew R. Thomson

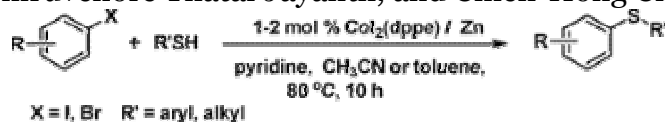


Protonation controls the location of a dual binding mode macrocycle in a [2]rotaxane. In the neutral form, amide-amide hydrogen bonds hold the macrocycle over a dipeptide residue; when the thread is protonated, polyether-ammonium cation interactions dominate and the macrocycle changes position.

Cobalt-Catalyzed Aryl-Sulfur Bond Formation

Org. Lett., **8** (24), 5613-5616, 2006.

Ying-Chieh Wong, Thiruvellore Thatai Jayanth, and Chien-Hong Cheng



A new cobalt-catalyzed coupling of aryl halides with thiophenols and alkanethiols is reported. A variety of aryl sulfides can be prepared in excellent yields under mild reaction conditions using 1-2 mol % of $\text{CoI}_2(\text{dppe})$ and Zn. This new cobalt-catalyzed coupling represents an interesting addition to previously known methods to synthesize thioethers.

Science

FG-Rich Repeats of Nuclear Pore Proteins Form a Three-Dimensional Meshwork with Hydrogel-Like Properties

Science Vol 314, Issue 5800, 815-817, 3 November 2006

Steffen Frey,^{1,2} Ralf P. Richter,^{3,4} Dirk Görlich

Nuclear pore complexes permit rapid passage of cargoes bound to nuclear transport receptors, but otherwise suppress nucleocytoplasmic fluxes of inert macromolecules ≥ 30 kilodaltons. To explain this selectivity, a sieve structure of the permeability barrier has been proposed that is created through reversible cross-linking between Phe and Gly (FG)-rich nucleoporin repeats. According to this model, nuclear transport receptors overcome the size limit of the sieve and catalyze their own nuclear pore-passage by a competitive disruption of adjacent inter-repeat contacts, which transiently opens adjoining meshes. Here, we found that phenylalanine-mediated inter-repeat interactions indeed cross-link FG-repeat domains into elastic and reversible hydrogels. Furthermore, we obtained evidence that such hydrogel formation is required for viability in yeast.

A Bacterial Protein Enhances the Release and Efficacy of Liposomal Cancer Drugs

Science Vol 314, Issue 5803, 1308-1311, 24 November 2006

Ian Cheong, Xin Huang, Chetan Bettgowda, Luis A. Diaz, Jr., Kenneth W. Kinzler, Shibin Zhou,* Bert Vogelstein

Clostridium novyi-NT is an anaerobic bacterium that can infect hypoxic regions within experimental tumors. Because *C. novyi*-NT lyses red blood cells, we hypothesized that its membrane-disrupting properties could be exploited to enhance the release of liposome-encapsulated drugs within tumors. Here, we show that treatment of mice bearing large, established tumors with *C. novyi*-NT plus a single dose of liposomal doxorubicin often led to eradication of the tumors. The bacterial factor responsible for the enhanced drug release was identified as a previously unrecognized protein termed liposomase. This protein could potentially be incorporated into diverse experimental approaches for the specific delivery of chemotherapeutic agents to tumors.

PNAS

Crystal structure of HIV-1 protease in situ product complex and observation of a low-barrier hydrogen bond between catalytic aspartates

PNAS | December 5, 2006 | vol. 103 | no. 49 | 18464-18469

HIV-1 protease is an effective target for designing drugs against AIDS, and structural information about the true transition state and the correct mechanism can provide important inputs. We present here the three-dimensional structure of a bi-product complex between HIV-1 protease and the two cleavage product peptides AETF and YVDGAA. The structure, refined against synchrotron data to 1.65 Å resolution, shows the occurrence of the cleavage reaction in the crystal, with the product peptides still held in the enzyme active site. The separation between the scissile carbon and nitrogen atoms is 2.67 Å, which is shorter than a normal van der Waal separation, but it is much longer than a peptide bond length. The substrate is thus in a stage just past the G'Z intermediate described in Northrop's mechanism [Northrop DB (2001) Acc Chem Res 34:790–797]. Because the products are generated in situ, the structure, by extrapolation, can give insight into the mechanism of the cleavage reaction. Both oxygens of the generated carboxyl group form hydrogen bonds with atoms at the catalytic center: one to the OD2 atom of a catalytic aspartate and the other to the scissile nitrogen atom. The latter hydrogen bond may have mediated protonation of scissile nitrogen, triggering peptide bond cleavage. The inner oxygen atoms of the catalytic aspartates in the complex are 2.30 Å apart, indicating a low-barrier hydrogen bond between them at this stage of the reaction, an observation not included in Northrop's proposal. This structure forms a template for designing mechanism-based inhibitors.

Anthrax pathogen evades the mammalian immune system through stealth siderophore production

PNAS | December 5, 2006 | vol. 103 | no. 49 | 18499-18503

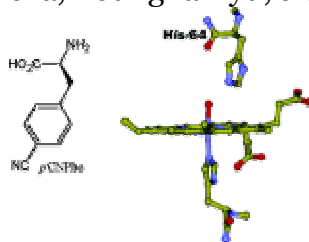
Systemic anthrax, caused by inhalation or ingestion of *Bacillus anthracis* spores, is characterized by rapid microbial growth stages that require iron. Tightly bound and highly regulated in a mammalian host, iron is scarce during an infection. To scavenge iron from its environment, *B. anthracis* synthesizes by independent pathways two small molecules, the siderophores bacillibactin (BB) and petrobactin (PB). Despite the great efficiency of BB at chelating iron, PB may be the only siderophore necessary to ensure full virulence of the pathogen. In the present work, we show that BB is specifically bound by siderocalin, a recently discovered innate immune protein that is part of an antibacterial iron-depletion defense. In contrast, neither PB nor its ferric complex is bound by siderocalin. Although BB incorporates the common 2,3-dihydroxybenzoyl iron-chelating subunit, PB is novel in that it incorporates the very unusual 3,4-dihydroxybenzoyl chelating subunit. This structural variation results in a large change in the shape of both the iron complex and the free siderophore that precludes siderocalin binding, a stealthy evasion of the immune system. Our results indicate that the blockade of bacterial siderophore-mediated iron acquisition by siderocalin is not restricted to enteric pathogenic organisms and may be a general defense mechanism against several different bacterial species. Significantly, to evade this innate immune response, *B. anthracis* produces PB, which plays a key role in virulence of the organism. This analysis argues for antianthrax strategies targeting siderophore synthesis and uptake.

Journal of the American Chemical Society

A Genetically Encoded Infrared Probe

J. Am. Chem. Soc., 2006, 128 (43), 13984 -13985

Kathryn C. Schultz, Lubica Supekova, Youngha Ryu, Jianming Xie, Roshan Perera

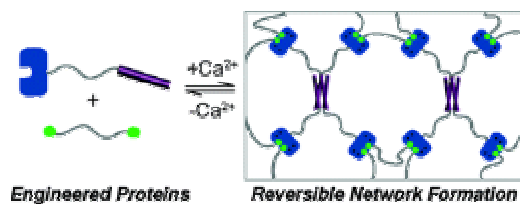


An orthogonal tRNA/aminoacyl-tRNA synthetase pair has been evolved that makes it possible to selectively and efficiently incorporate para-cyanophenylalanine (pCNPhe) into proteins in *E. coli* at sites specified by the amber nonsense codon, TAG. Substitution of pCNPhe for histidine-64 in myoglobin (Mb) affords a sensitive vibrational probe of ligand binding. This methodology provides a useful infrared reporter of protein structure, biomolecular interactions, and conformational changes.

A Genetic Toolbox for Creating Reversible Ca²⁺-Sensitive Materials

J. Am. Chem. Soc., 2006, 128 (43), 13994 -13995

Shana Topp,[¶] V. Prasad,[‡] Gianguido C. Cianci,[‡] Eric R. Weeks,[‡] and Justin P. Gallivan

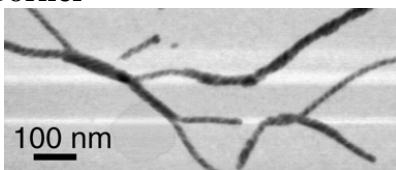


A major goal of polymer science is to develop "smart" materials that sense specific chemical signals in complex environments and respond with predictable changes in their mechanical properties. Here, we describe a genetic toolbox of natural and engineered protein modules that can be rationally combined in manifold ways to create reversible self-assembling materials that vary in their composition, architecture, and mechanical properties. Using this toolbox, we produced several materials that reversibly self-assemble in the presence of Ca^{2+} and characterized these materials using particle-tracking microrheology. The properties of these materials could be predicted from the dilute solution behavior of their component modules, suggesting that this toolbox may be generally useful for creating new stimuli-sensitive materials.

Peptide-Directed Microstructure Formation of Polymers in Organic Media

J. Am. Chem. Soc., 2006, 128 (43), 14142 -14149

Jens Hentschel and Hans G. Börner



Synthesis and peptide-guided self-assembly of an organo-soluble peptide-polymer conjugate, comprising a sequence-defined polypeptide and a poly(*n*-butyl acrylate), are described. The amino acid sequence of the peptide encodes a high tendency to adopt an antiparallel β -sheet motif, and thus programs the formation of tapelike microstructures. Easy synthesis and controllable self-assembly is ensured by the incorporation of structure breaking switch defects into the peptide segment. This suppresses temporarily the aggregation tendency of the conjugate as shown by circular dichroism, infrared spectroscopy (FT-IR), and atomic force microscopy (AFM). A pH-controlled rearrangement in the switch segments restores the native peptide backbone, triggering the self-assembly process and leading to the formation of densely twisted tapelike microstructures as could be observed by AFM and transmission electron microscopy. The resulting helical superstructures, when deposited on a substrate, are 2.9 nm high, 10 nm wide, and up to 2.3 μm long. The helical pitch is about 37 nm, and the pitch angle is 48° . The helical superstructures undergo defined entanglement to form superhelices, leading to the formation of soft, continuous organo-gels. A twisted two-dimensional core-shell tape is proposed as a structure model, in which the peptide segments form an antiparallel β -sheet with a polymer shell.

A Minimalist Approach toward Protein Recognition by Epitope Transfer from Functionally Evolved β -Sheet Surfaces

J. Am. Chem. Soc., 2006, 128 (44), 14356 -14363

Srivats Rajagopal, Scott C. Meyer, Aaron Goldman, Min Zhou, and Indraneel Ghosh

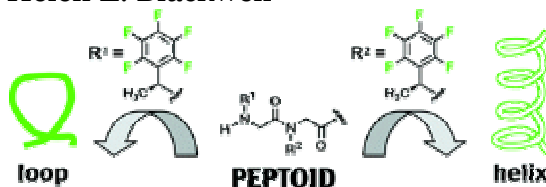


New approaches for identifying small molecules that specifically target protein surfaces as opposed to active site clefts are of much current interest. Toward this goal, we describe a three-step methodology: in step one, we target a protein of interest by directed evolution of a small β -sheet scaffold; in step two, we identify residues on the scaffold that are implicated in binding; and in step three, we transfer the chemical information from the β -sheet to a small molecule mimic. As a case study, we targeted the proteolytic enzyme thrombin, involved in blood coagulation, utilizing a library of β -sheet epitopes displayed on phage that were previously selected for conservation of structure. We found that the thrombin-binding, β -sheet displaying mini-proteins retained their structure and stability while inhibiting thrombin at low micromolar inhibition constants. A conserved dityrosine recognition motif separated by 9.2 Å was found to be common among the mini-protein inhibitors and was further verified by alanine scanning. A molecule containing two tyrosine residues separated by a linker that matched the spacing on the β -sheet scaffold inhibited thrombin, whereas a similar dityrosine molecule separated by a shorter 6 Å linker could not. Moreover, kinetic analysis revealed that both the mini-protein as well as its minimalist mimic with only two functional residues exhibited noncompetitive inhibition of thrombin. Thus, this reductionist approach affords a simple methodology for transferring information from structured protein scaffolds to yield small molecule leads for targeting protein surfaces with novel mechanisms of action.

Tuning Peptoid Secondary Structure with Pentafluoroaromatic Functionality: A New Design Paradigm for the Construction of Discretely Folded Peptoid Structures

J. Am. Chem. Soc., 2006, 128 (44), 14378 -14387

Benjamin C. Gorske and Helen E. Blackwell



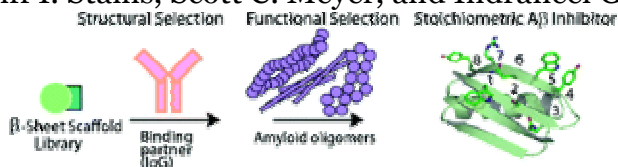
Peptoids, or oligomers of N-substituted glycine, are an important class of non-native polymers whose close structural similarity to natural α -peptides and ease of synthesis

offer significant advantages for the study of biomolecular interactions and the development of biomimetics. Peptoids that are N-substituted with α -chiral aromatic side chains have been shown to adopt either helical or "threaded loop" conformations, depending upon solvent and oligomer length. Elucidation of the factors that impact peptoid conformation is essential for the development of general rules for the design of peptoids with discrete and novel structures. Here, we report the first study of the effects of pentafluoroaromatic functionality on the conformational profiles of peptoids. This work was enabled by the synthesis of a new, α -chiral amine building block, (S)-1-(pentafluorophenyl)ethylamine (S-2), which was found to be highly compatible with peptoid synthesis (delivering (S)-N-(1-(pentafluorophenyl)ethyl)glycine oligomers). The incorporation of this fluorinated monomer unit allowed us to probe both the potential for π -stacking interactions along the faces of peptoid helices and the role of side chain electrostatics in peptoid folding. A series of homo- and heteropeptoids derived from S-2 and non-fluorinated, α -chiral aromatic amide side chains were synthesized and characterized by circular dichroism (CD) and nuclear magnetic resonance (NMR) spectroscopy. Enhancement of π -stacking by quadrupolar interactions did not appear to play a significant role in stabilizing the conformations of heteropeptoids with alternating fluorinated and non-fluorinated side chains. However, incorporation of (S)-N-(1-(pentafluorophenyl)ethyl)glycine monomers enforced helicity in peptoids that typically exhibit threaded loop conformations. Moreover, we found that the incorporation of a single (S)-N-(1-(pentafluorophenyl)ethyl)glycine monomer could be used to selectively promote looped or helical structure in this important peptoid class by tuning the electronics of nearby heteroatoms. The strategic installation of this monomer unit represents a new approach for the manipulation of canonical peptoid structure and the construction of novel peptoid architectures.

Inhibition of β -Amyloid Fibrillization by Directed Evolution of a β -Sheet Presenting Miniature Protein

J. Am. Chem. Soc., 2006, 128 (45), 14456 -14457

Thaddeus J. Smith, Cliff I. Stains, Scott C. Meyer, and Indraneel Ghosh

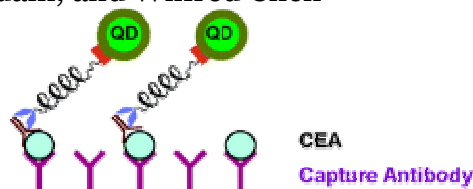


We describe the directed evolution of a miniature β -sheet protein for targeting β -amyloid oligomers implicated in Alzheimer's disease. Circular dichroism spectroscopy, thermal denaturation experiments, and immunoglobulin binding assays established that our β -amyloid-targeted miniature protein, TJ10, presents a well-folded thermostable β -sheet. TJ10 was found to prevent β -amyloid fibrillization at stoichiometric concentrations and was also an effective inhibitor at substoichiometric concentrations. Thus our results provide a new and potent β -sheet chemical template for effectively targeting β -amyloid while also demonstrating a general strategy for targeting proteins implicated in other amyloidogenic diseases.

Simple Conjugation and Purification of Quantum Dot-Antibody Complexes Using a Thermally Responsive Elastin-Protein L Scaffold As Immunofluorescent Agents

J. Am. Chem. Soc., 128 (46), 14756 -14757, 2006.

U Loi Lao, Ashok Mulchandani, and Wilfred Chen

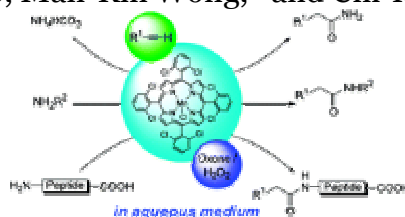


Fluorescent quantum dots (QDs), because of their tunable spectral properties, are ideal for simultaneous multiplexed detection in an antibody array format. Despite these advantages, their widespread usage is limited by the costly and tedious conjugation and separation protocol. Herein, we report a simple platform for the direct conjugation and separation of highly luminescent CdSe-ZnS QD-antibody complexes using a genetically engineered polyhistidine tagged elastin-protein L fusion (His-ELP-PL). The principle of immunoassay-ready conjugates was to take advantage of the direct conjugation of QDs via metal coordination with the His tag, the unique temperature-responsive property of ELP, and the high affinity of the antibody-binding protein L domain toward IgGs. Simple separation of the QD- His-ELP-PL-IgG complex was achieved by thermally triggered precipitation without any interference on the QD functionality. The utility of the biofunctionalized QD probes was demonstrated in an antibody array for the detection of carcinoembryonic antigen.

Oxidative Amide Synthesis and N-Terminal α -Amino Group Ligation of Peptides in Aqueous Medium

J. Am. Chem. Soc., 128 (46), 14796 -14797, 2006.

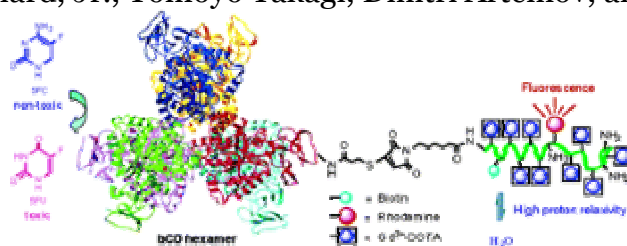
Wing-Kei Chan, Chi-Ming Ho, Man-Kin Wong,* and Chi-Ming Che



A new method for oxidative synthesis of amides from alkynes and amines in high yields (up to 96%) using [Mn(2,6-Cl₂TPP)Cl] 1 as a catalyst and Oxone/H₂O₂ as an oxidant in aqueous medium has been developed. This method could be used for N-terminal α -amino group ligation of unprotected peptides with aryl, aliphatic, and internal alkynes under mild conditions.

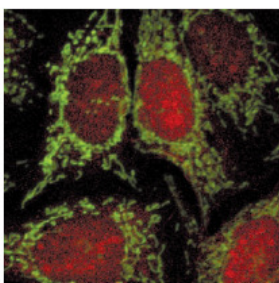
Multimodal Image-Guided Enzyme/Prodrug Cancer Therapy

J. Am. Chem. Soc., 128 (47), 15072 -15073, 2006.



The conjugate of bacterial cytosine deaminase (bCD) and poly-L-lysine (PLL) that was functionalized with biotin, rhodamine, and Gd³⁺-DOTA was synthesized and characterized. It demonstrated high relaxivity, improved enzymatic specificity to prodrug 5-fluorocytosine, low cytotoxicity, efficient cell uptake, and high enzymatic stability in fresh mouse serum and human breast cancer cell culture.

Angewandte Chemie



Playing tag: Noncanonical amino acid tagging enables the selective fluorescent visualization of newly synthesized proteins in mammalian cells (see the picture). Susceptibility to tagging is determined by the spatial and temporal character of the protein synthesis, thus providing a complement to methods which identify relevant members of the proteome.

Fluorescent Probes

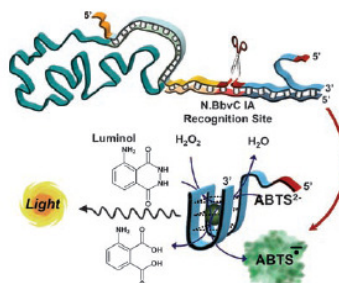
K. E. Beatty, J. C. Liu, F. Xie, D. C. Dieterich, E. M. Schuman, Q. Wang, D. A. Tirrell* ————— 7364 – 7367

Fluorescence Visualization of Newly Synthesized Proteins in Mammalian Cells

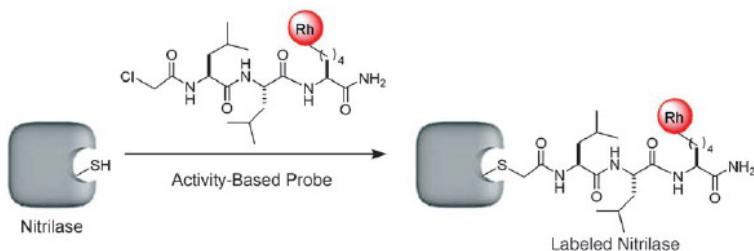
DNA Structures

Y. Weizmann, M. K. Beissenhirtz, Z. Cheglakov, R. Nowarski, M. Kotler, I. Willner* ————— 7384 – 7388

A Virus Spotlighted by an Autonomous DNA Machine



Highly sensitive: Analysis of viral DNA (M13 phage) is possible by using a DNA-based machine that is fueled by dNTPs and synthesizes DNAzyme reporter units. The DNAzymes mimic peroxidase and lead to a colorimetric or chemiluminescent readout of the analyzed DNA.



Up and at it: A set of activity-based proteomics probes containing a dipeptide α -chloroacetamide scaffold that targets the nitrilase family of enzymes is described. One member of the nitrilase

class, ureidopropionase- β , was found to react selectively with a single probe that mimics the enzyme's endogenous substrate *N*-carbamoyl β -alanine.

Activity-Based Proteomics

K. T. Barglow, B. F. Cravatt* . 7408 – 7411

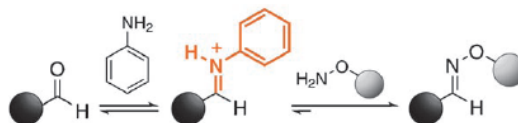
Substrate Mimicry in an Activity-Based Probe That Targets the Nitrilase Family of Enzymes

Oxime Ligation

A. Dirksen, T. M. Hackeng,
P. E. Dawson* 7581–7584

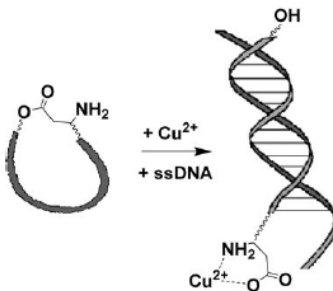
Nucleophilic Catalysis of Oxime Ligation

Aniline acts as a nucleophilic catalyst of oxime ligation in aqueous solution through formation of the orange-colored intermediate. The method is demonstrated by acceleration of the oxime ligation of



peptides at pH 4.5 and pH 7, which extends the scope of this reaction to conditions relevant for the bioconjugation of macromolecules (see scheme).

Copper-sensitive cycle: A cyclic peptide nucleic acid (PNA) that does not bind DNA and RNA can be prepared by placing an optimized linker between the termini. Cu^{II} ions, but not other tested metal ions, catalyze the transformation of the cyclic PNA into the linear PNA, which is a strong binder of nucleic acids (see picture). This effect is used for the fluorimetric detection of Cu^{II} . ssDNA = single-stranded DNA



Peptide Nucleic Acids

J. Kovács, T. Rödler,
A. Mokhir* 7815–7817

Chemodosimeter for Cu^{II} Detection Based
on Cyclic Peptide Nucleic Acids

Journal of Biological Chemistry

Self-association of Collagen Triple Helic Peptides into Higher Order Structures

J. Biol. Chem., Vol. 281, Issue 44, 33283-33290

Karunakar Kar¹, Priyal Amin¹, Michael A. Bryan², Anton V. Persikov³, Angela Mohs⁴, Yuh-Hwa Wang, and Barbara Brodsky

Interest in self-association of peptides and proteins is motivated by an interest in the mechanism of physiologically higher order assembly of proteins such as collagen as well as the mechanism of pathological aggregation such as β -amyloid formation. The triple helical form of (Pro-Hyp-Gly)₁₀, a peptide that has proved a useful model for molecular features of collagen, was found to self-associate, and its association properties are reported here. Turbidity experiments indicate that the triple helical peptide self-assembles at neutral pH via a nucleation-growth mechanism, with a critical concentration near 1 mM. The associated form is more stable than individual molecules by about 25 °C, and the association is reversible. The rate of self-association increases with temperature, supporting an entropically favored process. After self-association, (Pro-Hyp-Gly)₁₀ forms branched filamentous structures, in contrast with the highly ordered axially periodic structure of collagen fibrils. Yet a number of characteristics of triple helix assembly for the peptide resemble those of collagen fibril formation. These include promotion of fibril formation by neutral pH and increasing temperature; inhibition by sugars; and a requirement for hydroxyproline. It is suggested that these similar features for peptide and collagen self-association are based on common lateral underlying interactions between triple helical molecules mediated by hydrogen-bonded hydration networks involving hydroxyproline.

Real-time and Single Fibril Observation of the Formation of Amyloid β Spherulitic Structures

J. Biol. Chem., Vol. 281, Issue 44, 33677-33683

Tadato Ban¹, Kenichi Morigaki², Hisashi Yagi³, Takashi Kawasaki⁴, Atsuko Kobayashi⁵, Shunsuke Yuba⁶, Hironobu Naiki⁷, and Yuji Got

In Alzheimer disease, amyloid β , a 39-43-residue peptide produced by cleavage from a large amyloid precursor protein, undergoes conformational change to form amyloid fibrils and deposits as senile amyloid plaques in the extracellular cerebral cortices of the brain. However, the mechanism of how the intrinsically linear amyloid fibrils form spherical senile plaques is unknown. With total internal reflection fluorescence microscopy combined with the use of thioflavin T, an amyloid-specific fluorescence dye, we succeeded in observing the formation of the senile plaque-like spherulitic structures with diameters of around 15 μm on the chemically modified quartz surface. Real-time observation at a single fibrillar level revealed that, in the absence of tight contact with the surface, the cooperative and radial growth of amyloid fibrils from the core leads to a huge spherulitic structure. The results suggest the underlying physicochemical mechanism of senile plaque formation, essential for obtaining insight into prevention of Alzheimer disease.

Characterization and Classification of ATP-binding Cassette Transporter ABCA3 Mutants in Fatal Surfactant Deficiency

J. Biol. Chem., Vol. 281, Issue 45, 34503-34514

Yoshihiro Matsumura¹, Nobuhiro Ban², Kazumitsu Ueda³, and Nobuya Inagaki

The ATP-binding cassette transporter ABCA3 is expressed predominantly at the limiting membrane of the lamellar bodies in lung alveolar type II cells. Recent study has shown that mutation of the ABCA3 gene causes fatal surfactant deficiency in newborns. In this study, we investigated in HEK293 cells the intracellular localization and N-glycosylation of the ABCA3 mutants so far identified in fatal surfactant deficiency patients. Green fluorescent protein-tagged L101P, L982P, L1553P, Q1591P, and Ins1518fs/ter1519 mutant proteins remained localized in the endoplasmic reticulum, and processing of oligosaccharide was impaired, whereas wild-type and N568D, G1221S, and L1580P mutant ABCA3 proteins trafficked to the LAMP3-positive intracellular vesicle, accompanied by processing of oligosaccharide from high mannose type to complex type. Vanadate-induced nucleotide trapping and ATP-binding analyses showed that ATP hydrolysis activity was dramatically decreased in the N568D, G1221S, and L1580P mutants, accompanied by a moderate decrease in ATP binding in N568D and L1580P mutants but not in the G1221S mutant, compared with the wild-type ABCA3 protein. In addition, mutational analyses of the Gly-1221 residue in the 11th transmembrane segment and the Leu-1580 residue in the cytoplasmic tail, and homology modeling of nucleotide binding domain 2 demonstrate the significance of these residues for ATP hydrolysis and suggest a mechanism for impaired ATP hydrolysis in G1221S and L1580P mutants. Thus, surfactant deficiency because of ABCA3 gene mutation may be classified

into two categories as follows: abnormal intracellular localization (type I) and normal intracellular localization with decreased ATP binding and/or ATP hydrolysis of the ABCA3 protein (type II). These distinct pathophysiologies may reflect both the severity and effective therapy for surfactant deficiency.