

Chmielewski Group Literature Abstracts

CHEMISTRY

BIOLOGY

CHEMICAL BIOLOGY

Modified Serine Controls Protein Phosphorylation

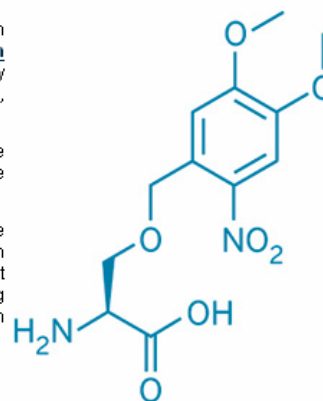
Light releases serine protecting group, permitting researchers to regulate cell-signaling mechanism

Celia Henry Arnaud

Protein phosphorylation, which is a major mechanism of cell signaling, can occur only on certain amino acids, including serine. [Peter G. Schultz](#) and coworkers at [Scripps Research Institute](#) report that they can control protein phosphorylation on serine residues by genetically incorporating a photoprotected serine analog into proteins (*Nat. Chem. Biol.*, DOI: 10.1038/nchembio.2007.44).

Phosphorylation can't occur at the protected serine residues. But shining blue light on the protein removes the 4,5-dimethoxy-2-nitrobenzyl protecting group, thereby exposing serine and making it available for phosphorylation.

The researchers used the serine analog to regulate phosphorylation of specific serine residues in Pho4, a yeast transcription factor that regulates genes and allows the organism to grow at different inorganic phosphate concentrations. By replacing one serine residue at a time with the photoprotected analog, the team was able to observe the effects of blocking phosphorylation at specific sites and measure dynamic responses to the phosphorylation event.



PHOTOCONTROL Light cleaves bulky nitrobenzyl group from serine. Once exposed, the serine residue can be phosphorylated.

September 2007

Contributing Editors:

Stefan Hershberger (*Science*)

Marcos Pires (*Nature and Nature subdivisions*)

Brandon Gaddis/Iris Geisler (*JACS*)

Jee Yeon Lee (*PNAS*)

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

Dave Przybyla (*Angewandte Chemie*)

Hilda Namanja (*Chem & Bio*)

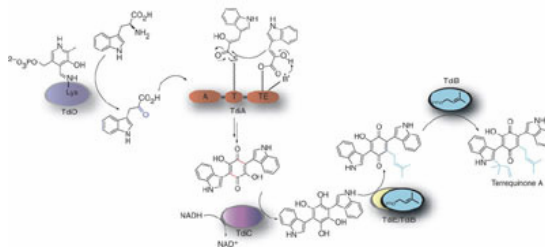
Nicole O'Neil (*Org Lett*)

Nature Chemical Biology

Terrequinone A biosynthesis through L-tryptophan oxidation, dimerization and bisprenylation

Nature Chemical Biology 3, 584-592 (2007)

Carl J Balibar, Annaleise R Howard-Jones & Christopher T Walsh



The antitumor fungal metabolite terrequinone A, identified in extracts of *Aspergillus* sp., is biosynthesized by the five-gene cluster *tdiA*–*tdiE*. In this work, we have overproduced all five proteins (TdiA–TdiE) in the bacterial host *Escherichia coli*, fully reconstituting the biosynthesis of terrequinone A. This pathway involves aminotransferase activity, head-to-tail dimerization and bisprenylation of the scaffold to yield the benzoquinone natural product. We have established that TdiD is a pyridoxal-5'-phosphate-dependent L-tryptophan aminotransferase that generates indolepyruvate for an unusual nonoxidative coupling by the tridomain nonribosomal peptide synthetase TdiA. TdiC, an NADH-dependent quinone reductase, generates the nucleophilic hydroquinone for two distinct rounds of prenylation by the single prenyltransferase TdiB. TdiE is required to shunt the benzoquinone away from an off-pathway monoprenylated species by an as yet unknown mechanism. Overall, we have biochemically characterized the complete biosynthetic pathway to terrequinone A, highlighting the nonoxidative dimerization pathway and the unique asymmetric prenylation involved in its maturation.

Science

Asymmetry in the Structure of the ABC Transporter-Binding Protein Complex BtuCD-BtuF

Science Vol 317, Issue 5843, 1387-1390, 7 September 2007

Rikki N. Hvorup,¹ Birke A. Goetz,¹ Martina Niederer,¹ Kaspar Hollenstein,¹ Eduardo Perozo,² Kaspar P. Locher

BtuCD is an adenosine triphosphate-binding cassette (ABC) transporter that translocates vitamin B12 from the periplasmic binding protein BtuF into the cytoplasm of *Escherichia coli*. The 2.6 angstrom crystal structure of a complex BtuCD-F reveals substantial conformational changes as compared with the previously reported structures of BtuCD and BtuF. The lobes of BtuF are spread apart, and B12 is displaced from the binding pocket. The transmembrane BtuC subunits reveal two distinct conformations,

and the translocation pathway is closed to both sides of the membrane. Electron paramagnetic resonance spectra of spin-labeled cysteine mutants reconstituted in proteoliposomes are consistent with the conformation of BtuCD-F that was observed in the crystal structure. A comparison with BtuCD and the homologous HI1470/71 protein suggests that the structure of BtuCD-F may reflect a posttranslocation intermediate.

Multicolor Super-Resolution Imaging with Photo-Switchable Fluorescent Probes

Science Vol 317, Issue 5845, 1749-1753, 21 September 2007

Mark Bates,¹ Bo Huang,^{2,3} Graham T. Dempsey,⁴ Xiaowei Zhuang

Recent advances in far-field optical nanoscopy have enabled fluorescence imaging with a spatial resolution of 20 to 50 nanometers. Multicolor super-resolution imaging, however, remains a challenging task. Here, we introduce a family of photo-switchable fluorescent probes and demonstrate multicolor stochastic optical reconstruction microscopy (STORM). Each probe consists of a photo-switchable "reporter" fluorophore that can be cycled between fluorescent and dark states, and an "activator" that facilitates photo-activation of the reporter. Combinatorial pairing of reporters and activators allows the creation of probes with many distinct colors. Iterative, color-specific activation of sparse subsets of these probes allows their localization with nanometer accuracy, enabling the construction of a super-resolution STORM image. Using this approach, we demonstrate multicolor imaging of DNA model samples and mammalian cells with 20- to 30-nanometer resolution. This technique will facilitate direct visualization of molecular interactions at the nanometer scale.

Structures of the CCR5 N Terminus and of a Tyrosine-Sulfated Antibody with HIV-1 gp120 and CD4

Science Vol 317, Issue 5846, 1930-1934, 28 September 2007

Chih-chin Huang,^{1*} Son N. Lam,^{2*} Priyamvada Acharya,¹ Min Tang,¹ Shi-Hua Xiang,³ Syed Shahzad-ul Hussan,² Robyn L. Stanfield,⁴ James Robinson,⁵ Joseph Sodroski,³ Ian A. Wilson,⁴ Richard Wyatt,¹ Carole A. Bewley,^{2†} Peter D. Kwong

The CCR5 co-receptor binds to the HIV-1 gp120 envelope glycoprotein and facilitates HIV-1 entry into cells. Its N terminus is tyrosine-sulfated, as are many antibodies that react with the co-receptor binding site on gp120. We applied nuclear magnetic resonance and crystallographic techniques to analyze the structure of the CCR5 N terminus and that of the tyrosine-sulfated antibody 412d in complex with gp120 and CD4. The conformations of tyrosine-sulfated regions of CCR5 (α -helix) and 412d (extended loop) are surprisingly different. Nonetheless, a critical sulfotyrosine on CCR5 and on 412d induces similar structural rearrangements in gp120. These results now provide a framework for understanding HIV-1 interactions with the CCR5 N terminus during viral entry and define a conserved site on gp120, whose recognition of sulfotyrosine engenders posttranslational mimicry by the immune system.

PNAS

Inhibitors of metabolism rescue cell death in Huntington's disease models

PNAS | September 4, 2007 | vol. 104 | no. 36 | 14525-14530

Hemant Varma*, Richard Cheng*, Cindy Voisine†, Anne C. Hart†, and Brent R. Stockwell

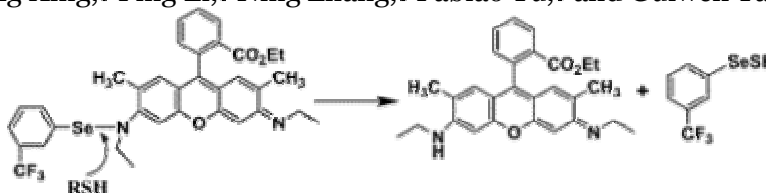
Huntington's disease (HD) is a fatal inherited neurodegenerative disorder. HD is caused by polyglutamine expansions in the huntingtin (htt) protein that result in neuronal loss and contribute to HD pathology. The mechanisms of neuronal loss in HD are elusive, and there is no therapy to alleviate HD. To find small molecules that slow neuronal loss in HD, we screened 1,040 biologically active molecules to identify suppressors of cell death in a neuronal cell culture model of HD. We found that inhibitors of mitochondrial function or glycolysis rescued cell death in this cell culture and in in vivo HD models. These inhibitors prevented cell death by activating prosurvival ERK and AKT signaling but without altering cellular ATP levels. ERK and AKT inhibition through the use of specific chemical inhibitors abrogated the rescue, whereas their activation through the use of growth factors rescued cell death, suggesting that this activation could explain the protective effect of metabolic inhibitors. Both ERK and AKT signaling are disrupted in HD, and activating these pathways is protective in several HD models. Our results reveal a mechanism for activating prosurvival signaling that could be exploited for treating HD and possibly other neurodegenerative disorders.

Journal of the American Chemical Society

A Rhodamine-Based Fluorescent Probe Containing a Se-N Bond for Detecting Thiols and Its Application in Living Cells

J. Am. Chem. Soc., 129 (38), 11666 -11667, 2007

Bo Tang,*† Yanlong Xing,† Ping Li,† Ning Zhang,† Fabiao Yu,† and Guiwen Yang

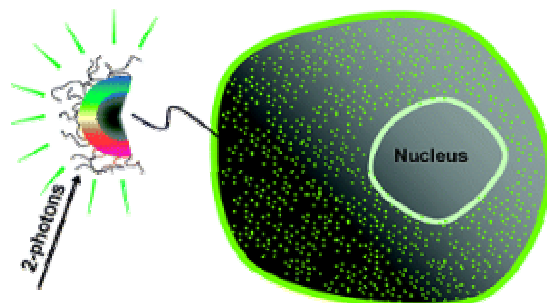


Here we report a new rhodamine-based fluorescent probe containing a selenium-nitrogen bond for detecting thiols based on the nucleophilic substitution of sulfhydryl. The probe was successfully applied to the imaging of thiols in both HL-7702 cells and HepG2 cells with high sensitivity and selectivity.

Carbon Dots for Multiphoton Bioimaging

J. Am. Chem. Soc., 129 (37), 11318 -11319, 2007.

Li Cao, Xin Wang, Mohammad J. Meziari, Fushen Lu, Haifang Wang, Pengju G. Luo, Yi Lin, Barbara A. Harruff, L. Monica Veca, Davoy Murray, Su-Yuan Xie, and Ya-Ping Sun

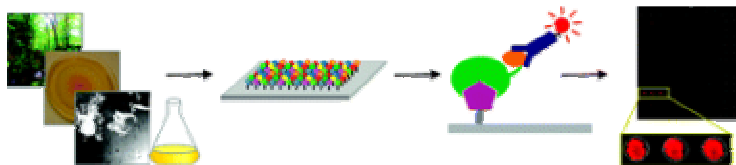


Carbon nanoparticles upon simple surface passivation exhibit bright photoluminescence. Reported here is a new finding that these carbon dots are also strongly two-photon luminescent with pulsed laser excitation in the near-infrared. The experimentally measured two-photon absorption cross-sections are comparable to those of the high-performance semiconductor quantum dots already available in the literature. The two-photon luminescence microscopy imaging of human breast cancer cells with internalized carbon dots is demonstrated.

Detecting Binding Interactions Using Microarrays of Natural Product Extracts

J. Am. Chem. Soc., **129** (37), 11346 -11347, 2007.

Katja Schmitz,[†] Stephen J. Haggarty,[‡] Olivia M. McPherson,[‡] Jon Clardy,[†] and Angela N. Koehler

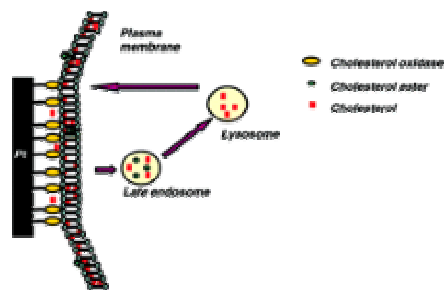


Small-molecule microarrays have been used to discover biologically active small molecules in collections of synthetic compounds. Here we utilize a versatile isocyanate chemistry to immobilize extracts from microorganisms in a microarray format. Specific bioactive small molecules are detected in crude extracts of *Streptomyces hygrosopicus*, and the amount of specific to nonspecific binding of a protein to an immobilized compound on a microarray can be estimated. These natural product-extract microarrays (NPEMs) provide new tools for characterizing the metabolic products of organisms and for discovering biologically active small molecules from nature.

Direct Electrochemical Evaluation of Plasma Membrane Cholesterol in Live Mammalian Cells

J. Am. Chem. Soc., **129** (37), 11352 -11353, 2007.

Dechen Jiang,[†] Anando Devadoss,^{†,‡} M. Simona Palencsar,[†] Danjun Fang,[†] Nicole M. White,[‡] Thomas J. Kelley,[‡] Jonathan D. Smith,[§] and James D. Burgess

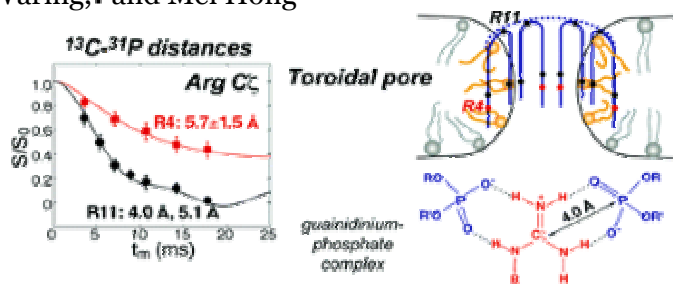


Cholesterol is a tightly regulated structural component of the cell plasma membrane. Dysfunctional intracellular cholesterol transport machinery causes disease states with altered plasma membrane cholesterol. This Communication reports microelectrode evaluation of plasma membrane cholesterol of single cells at physiological temperature. Electrochemical data indicate that transport of intracellular cholesterol to the plasma membrane is active in an atherosclerotic macrophage model

Phosphate-Mediated Arginine Insertion into Lipid Membranes and Pore Formation by a Cationic Membrane Peptide from Solid-State NMR

J. Am. Chem. Soc., **129** (37), 11438 -11446, 2007.

Ming Tang,[†] Alan J. Waring,[‡] and Mei Hong



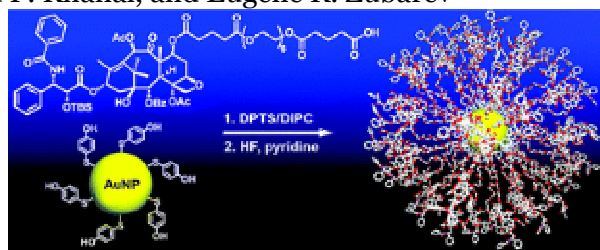
The insertion of charged amino acid residues into the hydrophobic part of lipid bilayers is energetically unfavorable yet found in many cationic membrane peptides and protein domains. To understand the mechanism of this translocation, we measured the ^{13}C - ^{31}P distances for an Arg-rich β -hairpin antimicrobial peptide, PG-1, in the lipid membrane using solid-state NMR. Four residues, including two Arg's, scattered through the peptide were chosen for the distance measurements. Surprisingly, all residues show short distances to the lipid ^{31}P : 4.0-6.5 Å in anionic POPE/POPG membranes and 6.5-8.0 Å in zwitterionic POPC membranes. The shortest distance of 4.0 Å, found for a guanidinium C^α at the β -turn, suggests N-H...O-P hydrogen bond formation. Torsion angle measurements of the two Arg's quantitatively confirm that the peptide adopts a β -hairpin conformation in the lipid bilayer, and gel-phase ^1H spin diffusion from water to the peptide indicates that PG-1 remains transmembrane in the gel phase of the membrane. For this transmembrane β -hairpin peptide to have short ^{13}C - ^{31}P distances for multiple residues in the molecule, some phosphate groups must be embedded in the hydrophobic part of the membrane, with the local ^{31}P plane parallel to the β -strand. This provides direct evidence for toroidal pores, where some lipid molecules change

their orientation to merge the two monolayers. We propose that the driving force for this toroidal pore formation is guanidinium-phosphate complexation, where the cationic Arg residues drag the anionic phosphate groups along as they insert into the hydrophobic part of the membrane. This phosphate-mediated translocation of guanidinium ions may underlie the activity of other Arg-rich antimicrobial peptides and may be common among cationic membrane proteins.

Paclitaxel-Functionalized Gold Nanoparticles

J. Am. Chem. Soc., **129** (37), 11653 -11661, 2007.

Jacob D. Gibson, Bishnu P. Khanal, and Eugene R. Zubarev*

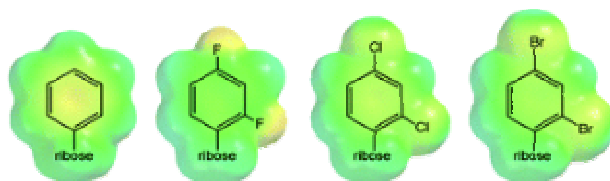


Here we describe the first example of 2 nm gold nanoparticles (Au NPs) covalently functionalized with a chemotherapeutic drug, paclitaxel. The synthetic strategy involves the attachment of a flexible hexaethylene glycol linker at the C-7 position of paclitaxel followed by coupling of the resulting linear analogue to phenol-terminated gold nanocrystals. The reaction proceeds under mild esterification conditions and yields the product with a high molecular weight, while exhibiting an extremely low polydispersity index (1.02, relative to linear polystyrene standards). TGA analysis of the hybrid nanoparticles reveals the content of the covalently attached organic shell as nearly 67% by weight, which corresponds to ~70 molecules of paclitaxel per 1 nanoparticle. The presence of a paclitaxel shell with a high grafting density renders the product soluble in organic solvents and allows for detailed ^1H NMR analysis and, therefore, definitive confirmation of its chemical structure. High-resolution TEM was employed for direct visualization of the inorganic core of hybrid nanoparticles, which were found to retain their average size, shape, and high crystallinity after multiple synthetic steps and purifications. The interparticle distance substantially increases after the attachment of paclitaxel as revealed by low-magnification TEM, suggesting the presence of a larger organic shell. The method described here demonstrates that organic molecules with exceedingly complex structures can be covalently attached to gold nanocrystals in a controlled manner and fully characterized by traditional analytical techniques. In addition, this approach gives a rare opportunity to prepare hybrid particles with a well-defined amount of drug and offers a new alternative for the design of nanosized drug-delivery systems.

RNA Probes of Steric Effects in Active Sites: High Flexibility of HIV-1 Reverse Transcriptase

J. Am. Chem. Soc., **2007**, **129** (35), 10626 -10627

Adam P. Silverman and Eric T. Kool

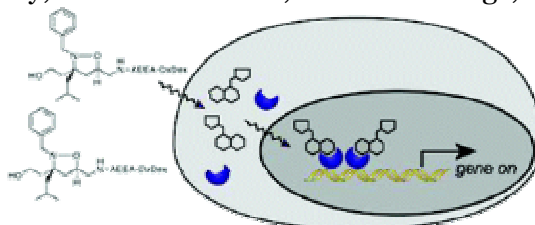


As part of viral replication, the HIV-1 reverse transcriptase (HIV-RT) makes a DNA copy of the RNA genome of the virus. It is a mutagenic polymerase, which leads to the rapid development of resistance in patients being treated with antiviral drugs. To study the mechanism by which this enzyme makes frequent errors, we have developed and used a set of variably sized nonpolar uridine analogues (rH, rF, rL, rB) in RNAs to probe its functional steric flexibility in nucleotide incorporation. Our kinetics studies show that the enzyme pairs all the analogues selectively with adenine, with a preference for dichloro analogues. However, unlike high-fidelity replicative DNA polymerases, this reverse transcriptase shows little sensitivity to size increases beyond the optimum, which gives evidence for unusually high steric flexibility. This suggests that at least part of the mutagenic behavior of the HIV-1 virus is caused by active site flexibility in its replicating enzyme.

Transcriptional Up-regulation in Cells Mediated by a Small Molecule

J. Am. Chem. Soc., 2007, 129 (35), 10654 -10655

Steven P. Rowe, Ryan J. Casey, Brian B. Brennan, Sara J. Buhrlage, and Anna K. Mapp

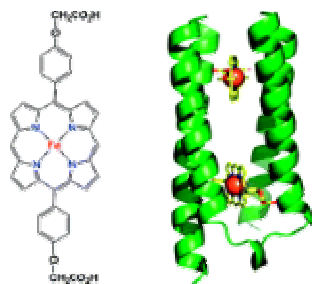


Activator artificial transcription factors, molecules that seek out specific genes and up-regulate their transcription, are desirable as mechanistic tools and as potential therapeutic agents. One challenge has been the identification of small molecule-based activator ATFs that function in cellular systems. Here we demonstrate that an amphipathic isoxazolidine activates transcription up to 80-fold in human cells when targeted to a specific promoter. Analogous to natural transcriptional activators, the function of the isoxazolidine appears to be independent of the DNA-targeting moiety, suggesting that the molecule will be effective in a variety of contexts. This is thus a critical step toward activator ATFs constructed from small molecule components.

De Novo Design of a Single-Chain Diphenylporphyrin Metalloprotein

J. Am. Chem. Soc., 2007, 129 (35), 10732 -10740

Gretchen M. Bender,[†] Andreas Lehmann,[‡] Hongling Zou,[‡] Hong Cheng,[‡] H. Christopher Fry,[‡] Don Engel,[†] Michael J. Therien,[‡] J. Kent Blasie,[‡] Heinrich Roder,[‡] Jeffrey G. Saven,[‡] and William F. DeGrado

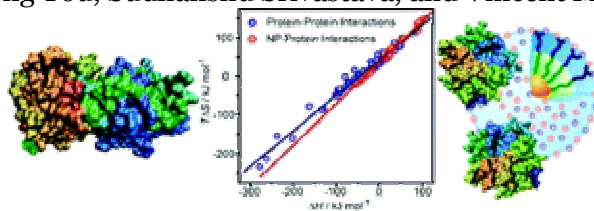


We describe the computational design of a single-chain four-helix bundle that noncovalently self-assembles with fully synthetic non-natural porphyrin cofactors. With this strategy, both the electronic structure of the cofactor as well as its protein environment may be varied to explore and modulate the functional and photophysical properties of the assembly. Solution characterization (NMR, UV-vis) of the protein showed that it bound with high specificity to the desired cofactors, suggesting that a uniquely structured protein and well-defined site had indeed been created. This provides a genetically expressed single-chain protein scaffold that will allow highly facile, flexible, and asymmetric variations to enable selective incorporation of different cofactors, surface-immobilization, and introduction of spectroscopic probes.

Biomimetic Interactions of Proteins with Functionalized Nanoparticles: A Thermodynamic Study

J. Am. Chem. Soc., 2007, 129 (35), 10747-10753

Mrinmoy De, Chang-Cheng You, Sudhanshu Srivastava, and Vincent M. Rotello*

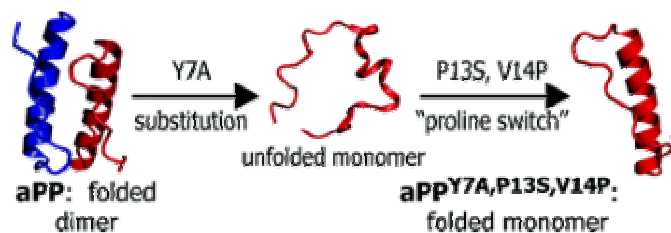


Gold nanoparticles (NPs) functionalized with L-amino acid-terminated monolayers provide an effective platform for the recognition of protein surfaces. Isothermal titration calorimetry (ITC) was used to quantify the binding thermodynamics of these functional NPs with α -chymotrypsin (ChT), histone, and cytochrome c (CytC). The enthalpy and entropy changes for the complex formation depend upon the nanoparticle structure and the surface characteristics of the proteins, e.g., distributions of charged and hydrophobic residues on the surface. Enthalpy-entropy compensation studies on these NP-protein systems indicate an excellent linear correlation between ΔH and $T\Delta S$ with a slope (α) of 1.07 and an intercept ($T\Delta S_0$) of 35.2 kJ mol⁻¹. This behavior is closer to those of native protein-protein systems ($\alpha = 0.92$ and $T\Delta S_0 = 41.1$ kJ mol⁻¹) than other protein-ligand and synthetic host-guest systems.

Engineering a Monomeric Miniature Protein

J. Am. Chem. Soc., 2007, 129 (36), 11024-11025

Abby M. Hodges and Alanna Schepartz

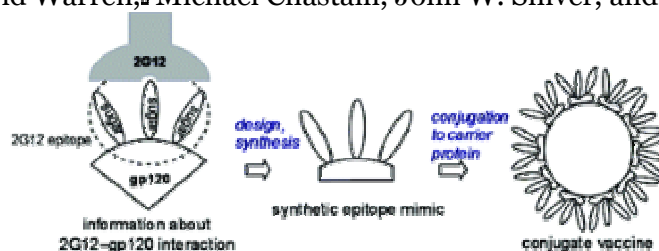


The stability, size, and structure of the avian pancreatic polypeptide (aPP) has made it an ideal starting point for the design of miniature proteins capable of binding DNA and protein surfaces with exceptionally high affinity and specificity, both in vitro and in mammalian cells and extracts. Despite these attributes, some miniature proteins based on the aPP self-associate at micromolar concentrations into dimers or higher order aggregates. Here we systematically isolate, quantify, and remove the two structural elements responsible for aPP dimerization and then install a new structural element—a "proline switch"—that single-handedly repacks aPP's signature fold. The result is a monomeric and well-folded miniature protein that should accelerate the in vitro and in vivo application of these molecules.

Fully Synthetic Carbohydrate HIV Antigens Designed on the Logic of the 2G12 Antibody

J. Am. Chem. Soc., 2007, 129 (36), 11042 -11044

Isaac J. Krauss, Joseph G. Joyce, Adam C. Finnefrock, Hong C. Song, Vadim Y. Dudkin,†
Xudong Geng,‡ J. David Warren,§ Michael Chastain, John W. Shiver, and Samuel J. Danishefsky



Di- and trivalent glycopeptide mimics of the HIV 2G12 epitope have been synthesized and evaluated for their comparative 2G12 binding characteristics. The epitope mimics consist of a cyclic peptide scaffold (unrelated to gp120 peptide sequences) attached via aspartate linkages to two or three copies of the high-mannose glycan, Man₉GlcNAc₂. The synthesis has been achieved via high-yielding double and triple Lansbury aspartylations of Man₉GlcNAc₂-NH₂ with peptides containing, respectively, two and three aspartate residues. Conjugation of such constructs with an immunogenic carrier protein, OMPC, has been accomplished through the peptide's cysteine sulfhydryl function, and Biacore assays have shown that binding affinity for 2G12 increases with increasing valency.

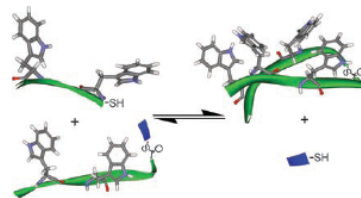
Angewandte Chemie

Protein Folding

E. B. Hadley, A. M. Witek, F. Freire,
A. J. Peoples,
S. H. Gellman* _____ 7056–7059

Thermodynamic Analysis of β -Sheet
Secondary Structure by Backbone
Thioester Exchange

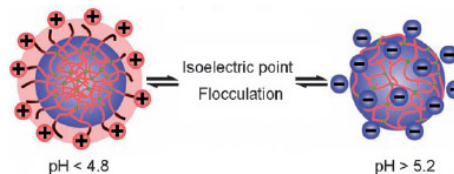
No need for references: The conformational stability of β hairpins has been examined by replacing a backbone amide group by a thioester (see picture). The increased flexibility causes a decrease in the β -hairpin stability without changing the structure of the folded conformation and allows the stability of the folded state to be measured under native conditions, without the need for reference compounds for the fully unfolded and folded states.



Stimuli-Responsive Polymers

X. Shen, L. Zhang, X. Jiang,* Y. Hu,
J. Guo _____ 7104–7107

Reversible Surface Switching of Nanogel
Triggered by External Stimuli



Inside out and back again: A novel nanogel composed of two biocompatible components, namely chitosan and ethylenediaminetetraacetic acid, is presented and shows novel surface switching of both composition and charge in response to

pH changes in the medium (see picture). The pH-dependent surface switch of this nanogel is fully reversible and the particle integrity is maintained in the entire pH range owing to the gel nature of the system.

ACS Chemical Biology

The New Biomimetic Chemistry: Artificial Transcription Factors

ACS Chem. Biol., 2 (9), 599–601

John T. Koh* and Jianfei Zheng

While many research programs have focused on the challenge of developing small molecules that can inhibit protein–protein interactions, some researchers have taken the problem one step further by attempting to develop small molecules that mimic the essential features of an entire protein. An area of particular interest has been in the field of artificial transcription factors (ATFs), where the essential function of some transcription factors is to recruit and promote the assembly of a larger transcription complex, leading to the expression of a gene of interest. The goal of synthesizing small-molecule ATFs holds promise as a means to independently control the expression of genes such as those that are misregulated in cancer and disease.

Design of Cyclic Peptides That Bind Protein Surfaces with Antibody-Like Affinity

ACS Chem. Biol., 2 (9), 625–634

Steven W. Millward[†], Stephen Fiocco[‡], Ryan J. Austin[‡], and Richard W. Roberts

There is a pressing need for new molecular tools to target protein surfaces with high affinity and specificity. Here, we describe cyclic messenger RNA display with a trillion-

member covalent peptide macrocycle library. Using this library, we have designed a number of high-affinity, redox-insensitive, cyclic peptides that target the signaling protein Gai1. In addition to cyclization, our library construction took advantage of an expanded genetic code, utilizing nonsense suppression to insert N-methylphenylalanine as a 21st amino acid. The designed macrocycles exhibit several intriguing features. First, the core motif seen in all of the selected variants is the same and shares an identical context with respect to the macrocyclic scaffold, consistent with the idea that selection simultaneously optimizes both the cyclization chemistry and the structural placement of the binding epitope. Second, detailed characterization of one molecule, cyclic Gai binding peptide (cycGiBP), demonstrates substantially enhanced proteolytic stability relative to that of the parent linear molecule. Third and perhaps most important, the cycGiBP peptide binds the target with very high affinity ($K_i \approx 2.1$ nM), similar to those of many of the best monoclonal antibodies and higher than that of the $\beta\gamma$ heterodimer, an endogenous Gai1 ligand. Overall the work provides a general route to design novel, low-molecular-weight, high-affinity ligands that target protein surfaces.

Chemistry and Biology

Semisynthetic Murine Prion Protein Equipped with a GPI Anchor Mimic Incorporates into Cellular Membranes

Volume 14, Issue 19, 21 September 2007, Pages 994-1006

Diana Olschewski, Ralf Seidel, Margit Miesbauer, Angelika S. Rambold, Dieter Oesterhelt, Konstanze F. Winklhofer, Jörg Tatzelt, Martin Engelhard and Christian F.W. Becker.

Conversion of cellular prion protein (PrP^C) into the pathological conformer (PrP^{Sc}) has been studied extensively by using recombinantly expressed PrP (rPrP). However, due to inherent difficulties of expressing and purifying posttranslationally modified rPrP variants, only a limited amount of data is available for membrane-associated PrP and its behavior in vitro and in vivo. Here, we present an alternative route to access lipidated mouse rPrP (rPrP^{Palm}) via two semisynthetic strategies. These rPrP variants studied by a variety of in vitro methods exhibited a high affinity for liposomes and a lower tendency for aggregation than rPrP. In vivo studies demonstrated that double-lipidated rPrP is efficiently taken up into the membranes of mouse neuronal and human epithelial kidney cells. These latter results enable experiments on the cellular level to elucidate the mechanism and site of PrP-PrP^{Sc} conversion.

The Biosynthesis of Teicoplanin-Type Glycopeptide Antibiotics: Assignment of P450 Mono-Oxygenases to Side Chain Cyclizations of Glycopeptide A47934

Volume 14, Issue 19, September 2007, Pages 1078-1089

Bianka Hadatsch, Diane Butz, Timo Schmiederer, Julia Steudle, Wolfgang Wohlleben, Roderich Süßmuth and Evi Stegmann.

Streptomyces toyocaensis produces A47934, a teicoplanin-like type-IV glycopeptide with antibiotic activity against methicillin-resistant *Staphylococcus aureus*. A47934 differs from the type-I vancomycin glycopeptides, which possess a tricyclic peptide backbone, by the presence of an additional ring closure between the aromatic amino acids 1 and 3. To elucidate the order of crosslinking reactions, P450 mono-oxygenase-inactivation mutants (Δ staF, Δ staG, Δ staH, and Δ staJ) of the A47934 producer were generated, and the accumulated intermediates were analyzed. Thus, the formation of each crosslink could unambiguously be assigned to a specific oxygenase. The structure of the released intermediates from the wild-type nonribosomal peptide synthetase assembly line facilitated the determination of the cyclization order. Unexpectedly, the additional ring closure in A47934, catalyzed by StaG, is the second oxygenase reaction.

Chemical Biology and Drug Design

Structure–activity Relationships of Amyloid Beta-aggregation Inhibitors Based on Curcumin: Influence of Linker Length and Flexibility

Chem Biol Drug Des 2007; 70: 206–215

Ashley A. Reinke and Jason E. Gestwicki

Self-assembly of amyloid beta into fibrillar plaques is characteristic of Alzheimer's disease and oligomers of this peptide are believed to be involved in neurodegeneration. Natural organic dyes, such as congo red and curcumin, bind tightly to amyloid beta and, at higher concentrations, block its self-assembly. The ability of these molecules to prevent amyloid accumulation has generated interest in understanding which of their structural features contribute to inhibitory potency. In general, amyloid beta ligands tend to be flat, planar molecules with substituted aromatic end groups; however, a comprehensive structure–activity study has not been reported. To better understand these ligands, we surveyed the effect of three prominent features on inhibition of amyloid aggregation: the presence of two aromatic end groups, the substitution pattern of these aromatics, and the length and flexibility of the linker region. We found that modification of any one of the modules has profound effects on activity. Further, we report that the optimal length of the linker lies within a surprisingly narrow regime (6–19 Å). These results offer insight into the key chemical features required for inhibiting amyloid beta aggregation. In turn, these findings help define the nature of the docking site for small molecules on the amyloid beta surface.

Suppression of Type 1 Diabetes in NOD Mice by Bifunctional Peptide Inhibitor: Modulation of the Immunological Synapse Formation

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The aim of this work was to design and utilize a bifunctional peptide inhibitor called glutamic acid decarboxylase–bifunctional peptide inhibitor to suppress the progression

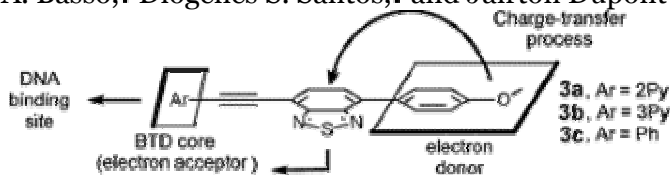
of type 1 diabetes in non-obese diabetic mice. The hypothesis is that glutamic acid decarboxylase–bifunctional peptide inhibitor binds simultaneously to major histocompatibility complex-II and intercellular adhesion molecule type 1 on antigen-presenting cell and inhibits the immunological synapse formation during T-cell–antigen-presenting cell interactions. Glutamic acid decarboxylase–bifunctional peptide inhibitor was composed of a major epitope of the type 1 diabetes-associated antigen, glutamic acid decarboxylase 65 kDa, covalently linked to a peptide derived from CD11a of lymphocyte function-associated antigen-1. The suppression of insulinitis and type 1 diabetes was evaluated using non-obese diabetic and non-obese diabetic severe combined immunodeficiency mice. Glutamic acid decarboxylase–bifunctional peptide inhibitor had the capacity to suppress invasive insulinitis in non-obese diabetic mice. CD4+ T-cells isolated from glutamic acid decarboxylase–bifunctional peptide inhibitor treated mice also suppressed insulinitis and hyperglycemia when transferred with diabetogenic non-obese diabetic spleen cells into non-obese diabetic severe combined immunodeficiency recipients. As predicted, the glutamic acid decarboxylase–bifunctional peptide inhibitor cross-linked a significant fraction of major histocompatibility complex class-II molecules to intercellular adhesion molecule type 1 molecules on the surface of live antigen-presenting cell. Intravenous injection of the glutamic acid decarboxylase–bifunctional peptide inhibitor elicited interleukin-4-producing T-cells in non-obese diabetic mice primed against the glutamic acid decarboxylase-epitope peptide. Together, the results indicate that glutamic acid decarboxylase–bifunctional peptide inhibitor induces interleukin-4-producing regulatory cells but does not expand the glutamic acid decarboxylase-specific Th2 population. Given that Th2 effector cells can cause pathology, the glutamic acid decarboxylase–bifunctional peptide inhibitor may represent a novel mechanism to induce interleukin-4 without Th2-associated pathology.

Organic Letters

New Sensitive Fluorophores for Selective DNA Detection

Org. Lett., **9** (20), 4001–4004, 2007.

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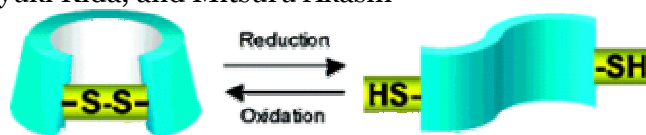


4,7-Disubstituted benzothiadiazoles containing 1-arylethynyl and 4-methoxyphenyl groups are selective photoluminescent "light up" probes to duplex DNA with unprecedented sensibility in both spectrophotometric and spectrofluorimetric measurements.

Synthesis of Stimuli-Responsive Cyclodextrin Derivatives and Their Inclusion Ability Control by Ring Opening and Closing Reactions

Org. Lett., 9 (20), 3909 -3912, 2007.

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Novel stimuli-responsive cyclodextrins (CDs), in which a disulfide unit was inserted into the rings of permethylated α - and β -CDs, were synthesized. Their inclusion ability was controlled by the opening and closing of the ring based on dithiol-disulfide interconversions.