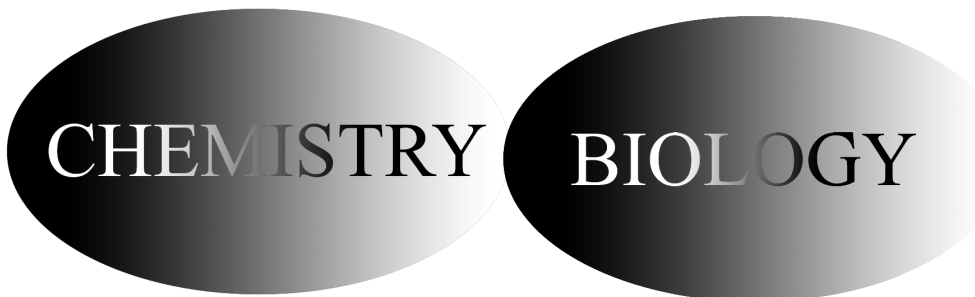


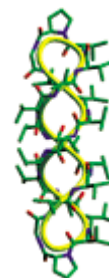
# Chmielewski Group Literature Abstracts



## Highlight of the Month

### First $\beta$ -hairpin $\beta$ -helix peptide

An unprecedented peptide structure—a cyclic  $\beta$ -hairpin  $\beta$ -helix (shown, with side chains)—has been designed, synthesized, and characterized by Thomas D. Clark and Christopher Brown of the Naval Research Laboratory, Washington, D.C., and Mallika Sastry and Gerhard Wagner of Harvard Medical School (*J. Am. Chem. Soc.*, DOI: 10.1021/ja062737f).  $\beta$ -Helices are formed by peptides composed of alternating d- and l-amino acids and are stabilized by  $\beta$ -sheet-type hydrogen bonding. The antibiotic gramicidin A is a naturally occurring  $\beta$ -helical peptide, and several attempts have been made to synthesize similar structures, but previous efforts resulted in single- and double-stranded helical mixtures. Now, Clark and coworkers have solved that problem by tying together two appropriate peptide strands into a cyclic structure with  $\beta$ -hairpin ends.



## August 2006

### **Contributing Editors:**

Song-Gil Lee (*PNAS*)

Yannick Fillon (*Angewandte Chemie*)

Stefan Hershberger (*Science*)

Marcos Pires (*Nature and Nature subdivisions*)

Brandon Gaddis/Iris Geisler (*JACS*)

Jee Yeon Lee (*JBC*)

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

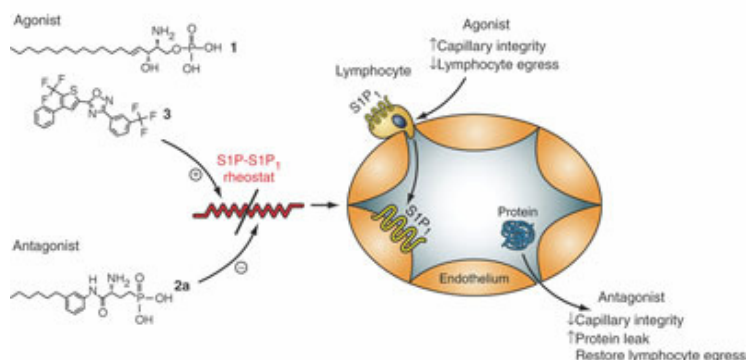
Dave Przybyla (*Org Lett*)

## Nature Chemical Biology

### Enhancement of capillary leakage and restoration of lymphocyte egress by a chiral S1P1 antagonist in vivo

*Nature Chemical Biology* **2**, 434-441 (2006)

M Germana Sanna<sup>1,6</sup>, Sheng-Kai Wang<sup>2,6</sup>, Pedro J Gonzalez-Cabrera<sup>1,3</sup>, Anthony Don<sup>1</sup>, David Marsolais<sup>1</sup>, Melanie P Matheu<sup>4</sup>, Sindy H Wei<sup>4</sup>, Ian Parker<sup>5</sup>, Euijung Jo<sup>1</sup>, Wei-Chieh Cheng<sup>2</sup>, Michael D Cahalan<sup>4</sup>, Chi-Huey Wong<sup>2</sup> and Hugh Rosen



Sphingosine 1-phosphate (S1P, 1) regulates vascular barrier and lymphoid development, as well as lymphocyte egress from lymphoid organs, by activating high-affinity S1P1 receptors. We used reversible chemical probes (i) to gain mechanistic insights into S1P systems organization not accessible through genetic manipulations and (ii) to investigate their potential for therapeutic modulation. Vascular (but not airway) administration of the preferred R enantiomer of an in vivo-active chiral S1P1 receptor antagonist induced loss of capillary integrity in mouse skin and lung. In contrast, the antagonist did not affect the number of constitutive blood lymphocytes. Instead, alteration of lymphocyte trafficking and phenotype required supraphysiological elevation of S1P1 tone and was reversed by the antagonist. In vivo two-photon imaging of lymph nodes confirmed requirements for obligate agonism, and the data were consistent with the presence of a stromal barrier mechanism for gating lymphocyte egress. Thus, chemical modulation reveals differences in S1P-S1P1 'set points' among tissues and highlights both mechanistic advantages (lymphocyte sequestration) and risks (pulmonary edema) of therapeutic intervention.

## Nature Structural & Molecular Biology

### Architecture of the SARS coronavirus prefusion spike

*Nature Structural & Molecular Biology* - **13**, 751 - 752 (2006)

Daniel R Beniac<sup>1</sup>, Anton Andonov, Elsie Grudskii & Tim F Booth

The emergence in 2003 of a new coronavirus (CoV) responsible for the atypical pneumonia termed severe acute respiratory syndrome (SARS) was a stark reminder that

hitherto unknown viruses have the potential to cross species barriers to become new human pathogens. Here we describe the SARS-CoV 'spike' structure determined by single-particle cryo-EM, along with the docked atomic structures of the receptor-binding domain and prefusion core.

## **ACS Chemical Biology**

### **Lighting Up the Nascent Cell Wall**

*ACS Chemical Biology*, **1 (7)**, 425–428

Wilfred A. van der Donk\*

Many antibiotics target the assembly of the cell wall of eubacteria, a netlike 3D structure composed of layers of peptidoglycan (PG). Very little is known about how the lipid precursor of PG, lipid II, is inserted into the existing cell wall in a growing and dividing cell. A new study provides a powerful tool for investigating this insertion process and opens the door to understanding the mechanism of eubacterial cell wall biogenesis.

### **Structural Insight into the Self-Sacrifice Mechanism of Eneidyne Resistance**

*ACS Chemical Biology*, **1 (7)**, 451–460

Shanteri Singh<sup>†,‡</sup>, Martin H. Hager<sup>\*</sup>, Changsheng Zhang<sup>‡</sup>, Byron R. Griffith<sup>‡</sup>, Min S. Lee<sup>†,||</sup>, Klaas Hallenga<sup>§</sup>, John L. Markley<sup>†,§</sup>, and Jon S. Thorson

The recent discovery of the first "self-sacrifice" mechanism for bacterial resistance to the enediyne antitumor antibiotics, where enediyne-induced proteolysis of the resistance protein CalC inactivates both the highly reactive metabolite and the resistance protein, revealed yet another ingenious bacterial mechanism for controlling reactive metabolites. As reported herein, the first 3D structures of CalC and CalC in complex with calicheamicin (CLM) divulge CalC to be a member of the steroidogenic acute regulatory protein (StAR)-related transfer (START) domain superfamily. In contrast to previous studies of proteins known to bind DNA-damaging natural products (*e.g.*, bleomycins, mitomycins, and nine-membered chromoprotein enediynes), this is the first demonstrated involvement of a START domain fold. Consistent with the CalC self-sacrifice mechanism, CLM in complex with CalC is positioned for direct hydrogen abstraction from Gly113 to initiate the oxidative proteolysis-based resistance mechanism. These structural studies also illuminate, for the first time, a small DNA-binding region within CalC that may serve to localize CalC to the enediyne target (DNA). Given the role of START domains in nuclear/cytosolic transport and translocation, this structural study also may implicate START domains as post-endocytotic intracellular chaperones for enediyne-based therapeutics such as MyloTarg.

## **Chemistry and Biology**

## **Rational Dissection of Binding Surfaces for Mimicking of Discontinuous Antigenic Sites**

*Chemistry and Biology*, 13, 815–823, August 2006

Judit Villén<sup>1, 5, 8</sup>, Ricard A. Rodríguez-Mias<sup>2, 6</sup>, José I. Núñez<sup>3, 4, 7</sup>, Ernest Giralt<sup>2, 6</sup>, Francisco Sobrino<sup>3, 4, 7</sup> and David Andreu

Peptide-based approaches to mimicking protein interactive regions have relied mainly on linear peptides; however, most binding sites are discontinuous and thus not easily reproducible by a linear sequence. Any attempt to replicate those sites by chemical means must not only integrate all residues involved in the recognition but also provide structural organization to native-like levels. Here we describe a surface mimic approach to the reconstruction of such complex molecular architectures, using as a model a discontinuous antigenic site of foot-and-mouth disease virus that is defined by residues belonging to three different capsid proteins. Our surface mimics are synthetic cyclic peptides, designed in silico, capable of binding antibodies directed to this site, and with demonstrated functional capabilities as vaccines in guinea pigs. Further, by saturation transfer difference NMR, we have determined several antibody binding residues on these peptides.

## **A Role for Sulfation-Desulfation in the Uptake of Bisphenol A into Breast Tumor Cells**

*Chemistry and Biology*, 13, 891–897, August 2006

Cheri L. Stowell<sup>1</sup>, Kevin K. Barvian<sup>1, 4</sup>, Peter C.M. Young<sup>2</sup>, Robert M. Bigsby<sup>2</sup>, Dawn E. Verdugo<sup>3</sup>, Carolyn R. Bertozzi<sup>3</sup> and Theodore S. Widlanski

Bisphenol A (BPA) is a widely used plasticizer whose estrogenic properties may impact hormone-responsive disorders and fetal development. In vivo, BPA appears to have greater activity than is suggested by its estrogen receptor (ER) binding affinity. This may be a result of BPA sulfation/desulfation providing a pathway for selective uptake into hormone-responsive cells. BPA is a substrate for estrogen sulfotransferase, and bisphenol A sulfate (BPAS) and disulfate are substrates for estrone sulfatase. Although the sulfated xenobiotics bind poorly to the ER, both stimulated the growth of receptor-positive breast tumor cells. Treatment of MCF-7 cells with BPAS leads to desulfation and uptake of BPA. No BPAS is found inside the cells. These findings suggest a mechanism for the selective uptake of BPA into cells expressing estrone sulfatase. Therefore, sulfation may increase the estrogenic potential of xenobiotics.

## **Chemical Biology and Drug Design**

### **Selective Peptide Chain Extension at the C-terminus of Aspartic and Glutamic Acids Utilizing N-protected ( $\alpha$ -aminoacyl)benzotriazoles**

*Chemical Biology and Drug Design*, 2006; 68: 42–47

Alan R. Katritzky, Ekaterina Todadze, Alexander A. Shestopalov, Janet Cusido, Parul Angrish

Aspartic and glutamic acids were selectively extended at each of the alternative C-terminals under mild conditions to afford diverse natural and unnatural N-protected dipeptides and tripeptides in yields of 73–96%. The reactions between N-protected ( $\alpha$ -aminoacyl)benzotriazoles and free amino acids or dipeptides proceeded with complete retention of chirality as supported by parallel experiments involving d-Ala, l-Ala, and dl-Ala in the preparation of dipeptides and tripeptides, monitored by NMR and HPLC analyses.

## **Design of Novispirin Antimicrobial Peptides by Quantitative Structure–Activity Relationship**

***Chemical Biology and Drug Design 2006; 68: 48–57***

Olivier Taboureau<sup>1</sup>, Ole Hvilsted Olsen<sup>2</sup>, Jesper Duus Nielsen<sup>1</sup>, Dora Raventos<sup>1</sup>, Per Holse Mygind<sup>1</sup> and Hans-Henrik Kristensen

Novispirin G10 is an  $\alpha$ -helical antimicrobial peptide designed in an effort to develop alternative treatments against multidrug-resistant micro-organisms. To further optimize the antimicrobial activity, 58 novispirin analogs were constructed and used to establish a quantitative structure–activity relationship model. A statistically significant model ( $r^2 = 0.73$ ,  $q^2 = 0.61$ ) was obtained using a set of 69 selected molecular descriptors. Among these, VolSurf and charged partial surface area descriptors played a dominant role. Analysis of the model indicated that hydrophobicity, amphipathicity and charge were the most important features influencing activity for this set of peptides. Furthermore, the ability of the quantitative structure–activity relationship model to predict bioactivity was evaluated by analyzing a set of 400 novispirin analogs designed by molecular modeling. Out of these 400, 16 new novispirins with a higher predicted antimicrobial activity were tested in the suicide expression system, and about three out of four appeared more potent than the parent novispirin G10. Combination of VolSurf and charged partial surface area descriptors seems relevant to depict the interaction between novispirin and its target(s), presumably the microbial cell membrane. The presented findings show that modeling and quantitative structure–activity relationship methods can be useful in the construction of and/or optimization of the bioactivity of antimicrobial peptides for further development as effective antibiotic therapeutics.

## **Prediction of Activity, Synthesis and Biological Testing of anti-HSV Active Peptides**

***Chemical Biology and Drug Design 2006; 68: 58–66***

Håvard Jenssen<sup>1</sup>, Tore J. Gutteberg<sup>1</sup>, Øystein Rekdal<sup>2</sup> and Tore Lejon<sup>3,\*</sup>

Herpes simplex virus infections can be treated with a number of drugs, but as for all pathogens, there is a constant need for new therapies. In the search for lead compounds some peptides have proven to possess an antiviral effect, but it is still unclear what

mechanisms are responsible for this effect. We wish to report on the use of principal properties of amino acids for developing quantitative structure–activity relationships (QSAR:s) as a tool for modelling peptide activity and predicting the activity of new peptides. In order to test the reliability of the method, new peptides have been designed by using multivariate methodology, synthesized and tested for a number of responses. Two of the new peptides synthesized were active at lower concentrations than experienced before regarding entry and herpes simplex virus activity, but they were not able to completely inhibit viral infection. This may reflect differences in mode of action of peptides depending on the amino acid content.

## **Discovery of Synthetic Penaeidin Activity against Antibiotic-resistant Fungi**

*Chemical Biology and Drug Design* **68: 120–127**

Brandon J. Cuthbertson<sup>1,\*</sup>, Erika E. Büllesbach<sup>2</sup> and Paul S. Gross

Penaeidins are antimicrobial peptides from shrimp that are constituted by divergent classes of peptide isoforms in an individual organism. Penaeidin sequence variation suggests functional diversity in the host and promises differential activities if applied to treat infections in humans. We have synthesized isoform 4 of penaeidin class 3 from the Atlantic shrimp, *Litopenaeus setiferus*, by native ligation using three peptide segments. Our synthesis approach led to the discovery of an irreversible side reaction that was successfully suppressed, a discovery, which has particular relevance to the synthesis of cysteine-rich peptides. The antimicrobial activity of full-length penaeidin and the N-terminal proline-rich domain of this isoform were compared with the corresponding peptides of penaeidin class 4 isoform 1 using a wide range of bacteria and fungi. New aspects of penaeidin function are reported that include activity against fungi of the phylum Basidiomycota (*Cryptococcus* strains), activity against fungi that are pathogenic to humans and effectiveness in the context of antibiotic resistance mechanisms (*Cryptococcus* and *Candida* spp.). The proline-rich domain of penaeidin class 4 shows the highest relative antimicrobial activity, while exhibiting no cytotoxicity to human monocytes, and therefore stands out as a potential peptide therapeutic.

## **Organic Letters**

### **Chemoselective Alkylation of N-Alkylaminoxy-Containing Peptides**

*Org. Lett.*, **8 (16)**, 3529 -3532, 2006.

Michael R. Carrasco,\* Oscar Silva, Katherine A. Rawls, Marisol S. Sweeney, and Adria A. Lombardo

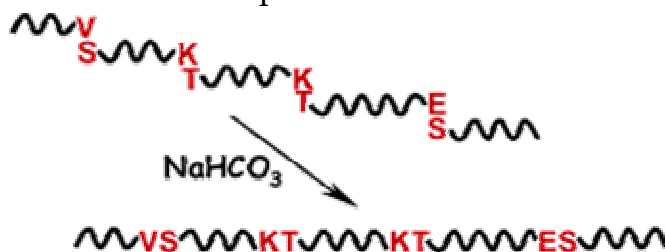
Peptides containing N-alkylaminoxy amino acids were chemoselectively alkylated with allylic, benzylic, and -carbonyl bromides, N-ethylmaleimide, and hexyl acrylate in mildly acidic aqueous/organic solutions. Alkylation at the aminoxy nitrogen proceeds in good yields with excellent to complete chemoselectivity in the presence of all common amino

acids except cysteine. This reaction complements the selective glycosylation and acylation of N-alkylaminoxy groups and provides an avenue for the synthesis of peptide arrays comprising a wide variety of neoglycopeptides and neolipopeptides.

## **Depsipeptide Methodology for Solid-Phase Peptide Synthesis: Circumventing Side Reactions and Development of an Automated Technique via Depsidipeptide Units**

*J. Org. Chem.*, 71 (16), 6171-6177, 2006.

Irene Coin,<sup>§</sup> Rudolf Dölling,<sup>|</sup> Eberhard Krause,<sup>§</sup> Michael Bienert,<sup>§</sup> Michael Beyermann,<sup>§</sup> Calin Dan Sferdean,<sup>⊥</sup> and Louis A. Carpino



The depsipeptide technique is a recently developed method for peptide synthesis which is applicable to difficult sequences when the synthetic difficulty arises because of aggregation phenomena. In the present work, application of the depsipeptide method to extremely difficult sequences has been demonstrated and a serious side reaction involving diketopiperazine formation uncovered and subsequently avoided by the appropriate use of the Bsmoc protecting group. Many other aspects of the technique have been investigated, such as the stability of the depsi units during assembly and workup procedures, the completeness of the O-acylation step, the occurrence of epimerization of the amino acid activated during O-acylation, and the nature of side products formed. In addition, the method was modified so as to allow for completely automated syntheses of long-chain depsipeptides without the need for any interruption by manual esterification procedures. Finally, the synthesis efficiency of the new depsipeptide technique was shown to be comparable to that of the well-known pseudoproline technique.

## **Science**

### **Anti-Inflammatory Activity of Immunoglobulin G Resulting from Fc Sialylation**

*Science Vol 313, Issue 5787, 670-673, 4 August 2006*

Yoshikatsu Kaneko,\* Falk Nimmerjahn,\* Jeffrey V. Ravetch

Immunoglobulin G (IgG) mediates pro- and anti-inflammatory activities through the engagement of its Fc fragment (Fc) with distinct Fcγ receptors (FcγRs). One class of Fc-FcγR interactions generates pro-inflammatory effects of immune complexes and cytotoxic antibodies. In contrast, therapeutic intravenous gamma globulin and its Fc

fragments are anti-inflammatory. We show here that these distinct properties of the IgG Fc result from differential sialylation of the Fc core polysaccharide. IgG acquires anti-inflammatory properties upon Fc sialylation, which is reduced upon the induction of an antigen-specific immune response. This differential sialylation may provide a switch from innate anti-inflammatory activity in the steady state to generating adaptive pro-inflammatory effects upon antigenic challenge.

## PNAS

### **DNA sequence-specific polyamides alleviate transcription inhibition associated with long GAA·TTC repeats in Friedreich's ataxia**

*PNAS* | August 1, 2006 | vol. 103 | no. 31 | 11497-11502

Peter B. Dervan

The DNA abnormality found in 98% of Friedreich's ataxia (FRDA) patients is the unstable hyperexpansion of a GAA·TTC triplet repeat in the first intron of the frataxin gene. Expanded GAA·TTC repeats result in decreased transcription and reduced levels of frataxin protein in affected individuals.  $\beta$ -Alanine-linked pyrrole–imidazole polyamides bind GAA·TTC tracts with high affinity and disrupt the intramolecular DNA·DNA-associated region of the sticky-DNA conformation formed by long GAA·TTC repeats. Fluorescent polyamide-Bodipy conjugates localize in the nucleus of a lymphoid cell line derived from a FRDA patient. The synthetic ligands increase transcription of the frataxin gene in cell culture, resulting in increased levels of frataxin protein. DNA microarray analyses indicate that a limited number of genes are significantly affected in FRDA cells. Polyamides may increase transcription by altering the DNA conformation of genes harboring long GAA·TTC repeats or by chromatin opening.

### **Structural basis for unique mechanisms of folding and hemoglobin binding by a malarial protease**

*PNAS* | August 1, 2006 | vol. 103 | no. 31 | 11503-11508

Falcipain-2 (FP2), the major cysteine protease of the human malaria parasite *Plasmodium falciparum*, is a hemoglobinase and promising drug target. Here we report the crystal structure of FP2 in complex with a protease inhibitor, cystatin. The FP2 structure reveals two previously undescribed cysteine protease structural motifs, designated FP2nose and FP2arm, in addition to details of the active site that will help focus inhibitor design. Unlike most cysteine proteases, FP2 does not require a prodomain but only the short FP2nose motif to correctly fold and gain catalytic activity. Our structure and mutagenesis data suggest a molecular basis for this unique mechanism by highlighting the functional role of two Tyr within FP2nose and a conserved Glu outside this motif. The FP2arm motif is required for hemoglobinase activity. The structure reveals topographic features and a negative charge cluster surrounding FP2arm that suggest it may serve as an exo-site for hemoglobin binding.

Motifs similar to FP2nose and FP2arm are found only in related plasmodial proteases, suggesting that they confer malaria-specific functions.

## **Molecular dynamics analyses of cross- $\beta$ -spine steric zipper models: $\beta$ -Sheet twisting and aggregation**

***PNAS | August 1, 2006 | vol. 103 | no. 31 | 11533-11538***

The structural characterization of amyloid fibers is one of the most investigated areas in structural biology. The structural motif, denoted as steric zipper, recently discovered for the peptide GNNQQNY [Nelson, R., Sawaya, M. R., Balbirnie, M., Madsen, A. O., Riek, C., Grothe, R. & Eisenberg, D. (2005) *Nature* 435, 773–778], is expected to exert strong influence in this field. To obtain further insights into the features of this unique structural motif, we report several molecular dynamics simulations of various GNNQQNY aggregates. Our analyses show that even pairs of  $\beta$ -sheets composed of a limited number of  $\beta$ -strands are stable in the 20-ns time interval considered, which suggests that steric zipper interactions at a  $\beta$ -sheet– $\beta$ -sheet interface strongly contribute to the stability of these aggregates. Moreover, although the basic features of side chain–side chain interactions are preserved in the simulation, the backbone structure undergoes significant variations. Upon equilibration, a significant twist of the  $\beta$ -strands that compose the  $\beta$ -sheets is observed. These results demonstrate that the occurrence of steric zipper interactions is compatible with flat and twisted  $\beta$ -sheets. Molecular dynamics simulations carried out on two pairs of  $\beta$ -sheets, separated in the crystal state by a hydrated interface, lead to interesting results. The two pairs of sheets, while twisting, associate through stable peptide–peptide interactions. These findings provide insight into the mechanism that leads to the formation of higher aggregates. On these bases, it is possible to reconcile the crystallographic and the EM data on the size of the basic GNNQQNY fiber unit.

## **Aptamers evolved from live cells as effective molecular probes for cancer study**

***PNAS | August 8, 2006 | vol. 103 | no. 32 | 11838-11843***

Using cell-based aptamer selection, we have developed a strategy to use the differences at the molecular level between any two types of cells for the identification of molecular signatures on the surface of targeted cells. A group of aptamers have been generated for the specific recognition of leukemia cells. The selected aptamers can bind to target cells with an equilibrium dissociation constant ( $K_d$ ) in the nanomolar-to-picomolar range. The cell-based selection process is simple, fast, straightforward, and reproducible, and, most importantly, can be done without prior knowledge of target molecules. The selected aptamers can specifically recognize target leukemia cells mixed with normal human bone marrow aspirates and can also identify cancer cells closely related to the target cell line in real clinical specimens. The cell-based aptamer selection holds a great promise in developing specific molecular probes for cancer diagnosis and cancer biomarker discovery.

## **Evaluation of RNA-binding specificity of aminoglycosides with DNA microarrays**

*Chi-Huey Wong PNAS | August 15, 2006 | vol. 103 | no. 33 | 12311-12316*

We have developed methods for using DNA array technology to probe the entire transcriptome to determine the RNA-binding specificity of ligands. Two methods were investigated. In the first method, the RNA-binding aminoglycoside antibiotic tobramycin was covalently linked to magnetic beads. The beads were bound to human liver mRNA and washed, and specifically bound RNA was eluted, amplified, and analyzed with DNA array technology. A small number of genes were found to bind specifically to the tobramycin beads. In the second method, the aminoglycoside ligand was added directly to the array hybridization reaction, and the signal was compared with a control experiment in the absence of ligand. The aminoglycosides were found to interfere with a small percentage of all hybridization events. These methods differ from traditional DNA array experiments in that the readout is a direct measure of the interaction between mRNA and a ligand, rather than an indirect measure of effect on expression. We expect that the results will lead to the discovery of new aminoglycoside-binding RNA motifs and may also have relevance toward understanding and overcoming the side effects observed with these antibiotics in the clinic.

## **Glycoproteomic probes for fluorescent imaging of fucosylated glycans in vivo**

*Chi-Huey Wong PNAS | August 15, 2006 | vol. 103 | no. 33 | 12371-12376*

Glycomics is emerging as a new field for the biology of complex glycoproteins and glycoconjugates. The lack of versatile glycan-labeling methods has presented a major obstacle to visualizing at the cellular level and studying glycoconjugates. To address this issue, we developed a fluorescent labeling technique based on the Cu(I)-catalyzed [3 + 2] cycloaddition, or click chemistry, which allows rapid, versatile, and specific covalent labeling of cellular glycans bearing azide groups. The method entails generating a fluorescent probe from a nonfluorescent precursor, 4-ethynyl-N-ethyl-1,8-naphthalimide, by clicking the fluorescent trigger, the alkyne at the 4 position, with an azido-modified sugar. Using this click-activated fluorescent probe, we demonstrate incorporation of an azido-containing fucose analog into glycoproteins via the fucose salvage pathway. Distinct fluorescent signals were observed by flow cytometry when cells treated with 6-azidofucose were labeled with the click-activated fluorogenic probe or biotinylated alkyne. The intracellular localization of fucosylated glycoconjugates was visualized by using fluorescence microscopy. This technique will allow dynamic imaging of cellular fucosylation and facilitate studies of fucosylated glycoproteins and glycolipids.

## **Nature designs tough collagen: Explaining the nanostructure of collagen fibrils**

Collagen is a protein material with superior mechanical properties. It consists of collagen fibrils composed of a staggered array of ultra-long tropocollagen (TC) molecules. Theoretical and molecular modeling suggests that this natural design of collagen fibrils maximizes the strength and provides large energy dissipation during deformation, thus creating a tough and robust material. We find that the mechanics of collagen fibrils can be understood quantitatively in terms of two critical molecular length scales  $\lambda_S$  and  $\lambda_R$  that characterize when (i) deformation changes from homogeneous intermolecular shear to propagation of slip pulses and when (ii) covalent bonds within TC molecules begin to fracture, leading to brittle-like failure. The ratio  $\lambda_S/\lambda_R$  indicates which mechanism dominates deformation. Our modeling rigorously links the chemical properties of individual TC molecules to the macroscopic mechanical response of fibrils. The results help to explain why collagen fibers found in nature consist of TC molecules with lengths in the proximity of 300 nm and advance the understanding how collagen diseases that change intermolecular adhesion properties influence mechanical properties.

## **The role of hydrophobic interactions in initiation and propagation of protein folding**

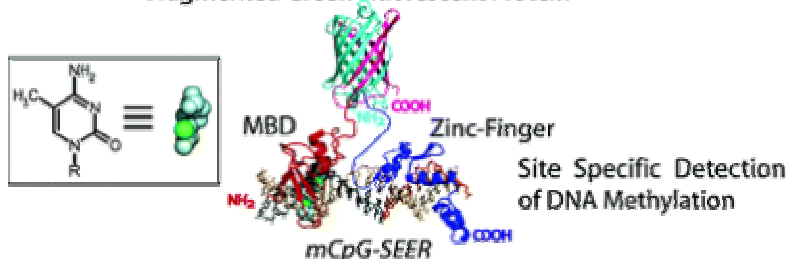
Globular proteins fold by minimizing the nonpolar surface that is exposed to water, while simultaneously providing hydrogen-bonding interactions for buried backbone groups, usually in the form of secondary structures such as  $\alpha$ -helices,  $\beta$ -sheets, and tight turns. A primary thermodynamic driving force for the formation of globular structure is thus the sequestration of nonpolar groups, but the correlation between the parts of proteins that are observed to fold first (termed folding initiation sites) and the "hydrophobicity" (as customarily defined) of the amino acids in these regions has been quite weak. It has previously been noted that many amino acid side chains contain considerable nonpolar sections, even if they also contain polar or charged groups. For example, a lysine side chain contains four methylenes, which may undergo hydrophobic interactions if the charged  $\epsilon$ -NH<sub>3</sub><sup>+</sup> group is salt-bridged or hydrogen-bonded. Folding initiation sites might therefore contain not only accepted "hydrophobic" amino acids, but also larger charged side chains. Recent experiments on the folding of mutant apomyoglobins provides corroboration for models based on the hypothesis that folding initiation sites arise from hydrophobic interactions. A near-perfect correlation was observed between the areas of the molecule that are present in the burst-phase kinetic intermediate and both the free energy of formation of hydrophobic initiation sites and the parameter "average area buried upon folding," which pinpoints large side chains, even those containing charged or polar portions. These results provide a putative mechanism for the control of protein-folding initiation and growth by polar/nonpolar sequence propensity alone.

# Journal of the American Chemical Society

## Site-Specific Detection of DNA Methylation Utilizing mCpG-SEER

*J. Am. Chem. Soc.*, 2006, 128 (30), 9761 -9765

Cliff I. Stains,<sup>†</sup> Jennifer L. Furman,<sup>†</sup> David J. Segal,<sup>‡</sup> and Indraneel Ghosh\*  
Fragmented Green Fluorescent Protein

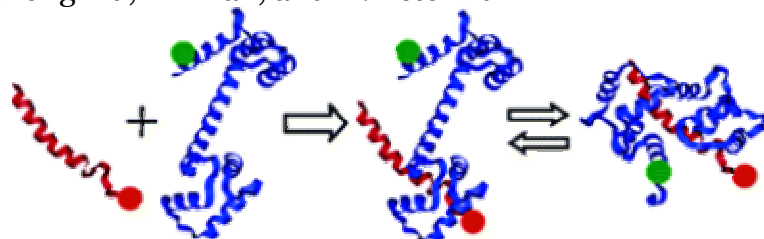


Currently there are no direct methods for the sequence-specific detection of DNA-methylation at CpG dinucleotides, which provide a possible diagnostic marker for cancer. Toward this goal, we present a methodology termed mCpG-Sequence Enabled Reassembly (mCpG-SEER) of proteins utilizing a split green fluorescent protein (GFP) tethered to specific DNA recognition elements. Our system, mCpG-SEER, employs a zinc-finger attached to one-half of GFP to target a specific sequence of dsDNA, while a methyl-CpG binding domain protein attached to the complementary half of GFP targets an adjacent methylated CpG dinucleotide site. We demonstrate that the presence of both DNA sites is necessary for the reassembly and concomitant fluorescence of the reassembled GFP. We further show that the GFP-dependent fluorescence reaches a maximum when the methyl-CpG and zinc-finger sites are separated by two base pairs and the fluorescence signal is linear to 5 pmol of methylated target DNA. Finally, the specificity of this reporter system, mCpG-SEER, was found to be >40-fold between a methylated versus a nonmethylated CpG target site.

## Revealing Two-State Protein-Protein Interactions of Calmodulin by Single-Molecule Spectroscopy

*J. Am. Chem. Soc.*, 2006, 128 (31), 10034 -10042

Ruchuan Liu, Dehong Hu, Xin Tan, and H. Peter Lu



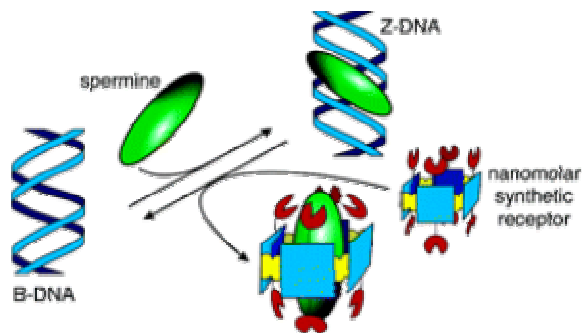
We report a single-molecule fluorescence resonance energy transfer (FRET) and polarization study of conformational dynamics of calmodulin (CaM) interacting with a target peptide, C28W of a 28 amino acid oligomer. The C28W peptide represents the essential binding sequence domain of the Ca-ATPase protein interacting with CaM, which is important in cellular signaling for the regulation of energy in metabolism.

However, the mechanism of the CaM/C28W recognition complex formation is still unclear. The amino-terminal (N-terminal) domain of the CaM was labeled with a fluorescein-based arsenical hairpin binder (FAsH) that enables our unambiguous probing of the CaM N-terminal target-binding domain motions on a millisecond time scale without convolution of the probe-dye random motions. By analyzing the distribution of FRET efficiency between FAsH labeled CaM and Texas Red labeled C28W and the polarization fluctuation dynamics and distributions of the CaM N-terminal domain, we reveal binding-unbinding motions of the N-terminal domain of the CaM in CaM/C28W complexes, which is strong evidence of a two-state binding interaction of CaM-mediated cell signaling.

## Controlling the Biological Effects of Spermine Using a Synthetic Receptor

*J. Am. Chem. Soc.*, 2006, 128 (31), 10253 -10257.

Laurent Vial,<sup>†</sup> R. Frederick Ludlow, Julien Leclaire,<sup>‡</sup> Ruth Pérez-Fernández, and Sijbren Otto



Polyamines play an important role in biology, yet their exact function in many processes is poorly understood. Artificial host molecules capable of sequestering polyamines could be useful tools for studying their cellular function. However, designing synthetic receptors with affinities sufficient to compete with biological polyamine receptors remains a huge challenge. Binding affinities of synthetic hosts are typically separated by a gap of several orders of magnitude from those of biomolecules. We now report that a dynamic combinatorial selection approach can deliver a synthetic receptor that bridges this gap. The selected receptor binds spermine with a dissociation constant of 22 nM, sufficient to remove it from its natural host DNA and reverse some of the biological effects of spermine on the nucleic acid. In low concentrations, spermine induces the formation of left-handed DNA, but upon addition of our receptor, the DNA reverts back to its right-handed form. NMR studies and computer simulations suggest that the spermine complex has the form of a pseudo-rotaxane. The spermine receptor is a promising lead for the development of therapeutics or molecular probes for elucidating spermine's role in cell biology.

## Synthesis and Application of Quantum Dots FRET-Based Protease Sensors

*J. Am. Chem. Soc.*, 2006, 128 (32), 10378 -10379

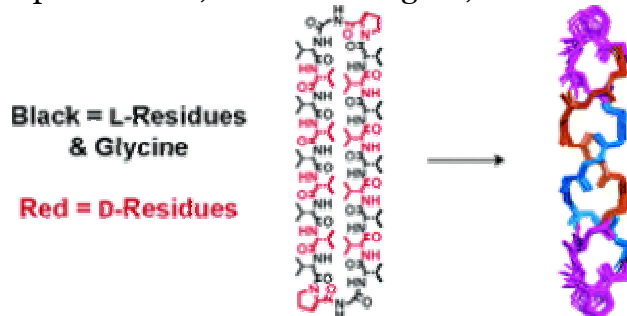
Lifang Shi, Vania De Paoli, Nitsa Rosenzweig, and Zeev Rosenzweig

Preparation of FRET-based quantum dots as protease sensors-RGDC peptide molecules are bound to the surface of CdSe/ZnS quantum dots. The peptide molecules are then labeled with rhodamine dye molecules. The emission color of the quantum dots change from green to orange due to fluorescence resonance energy transfer (FRET) between the quantum dots and the bound rhodamine molecules. Cleavage of the peptide by selective proteases releases the rhodamine molecules from the quantum dots surface, which results in decreasing FRET efficiency between the quantum dots and the rhodamine molecules. The emission color of the quantum dots changes back to green.

## Cyclic Peptide Helices: A Hybrid $^3_1$ -Hairpin/ $^3_1$ -Helical Supersecondary Structure

*J. Am. Chem. Soc.*, 128 (33), 10650 -10651, 2006.

Mallika Sastry,<sup>†</sup> Christopher Brown,<sup>‡</sup> Gerhard Wagner,<sup>†</sup> and Thomas D. Clark

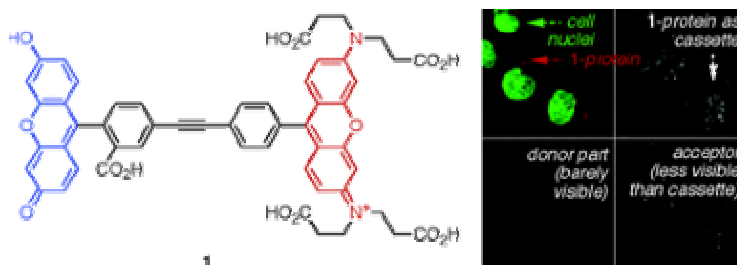


This communication describes the design and characterization of a new peptide motif that is a hybrid of  $^3_1$ -hairpin and  $^3_1$ -helical structures. This motif was supported by CD and IR spectroscopies in nonpolar organic solvents and was confirmed by NMR spectroscopy and structure calculations using NMR-derived restraints. The  $^3_1$ -hairpin/ $^3_1$ -helical motif represents a potentially attractive new structural template for a variety of possible applications in biomolecular design, such as ligands for macromolecular targets and building blocks for new protein architectures.

## Water-Soluble Through-Bond Energy Transfer Cassettes for Intracellular Imaging

*J. Am. Chem. Soc.*, 128 (33), 10688 -10689, 2006.

Rakeshwar Bandichhor,<sup>†</sup> Anca D. Petrescu,<sup>‡</sup> Aude Vespa,<sup>‡</sup> Ann B. Kier,<sup>‡</sup> Friedhelm Schroeder,<sup>‡</sup> and Kevin Burgess

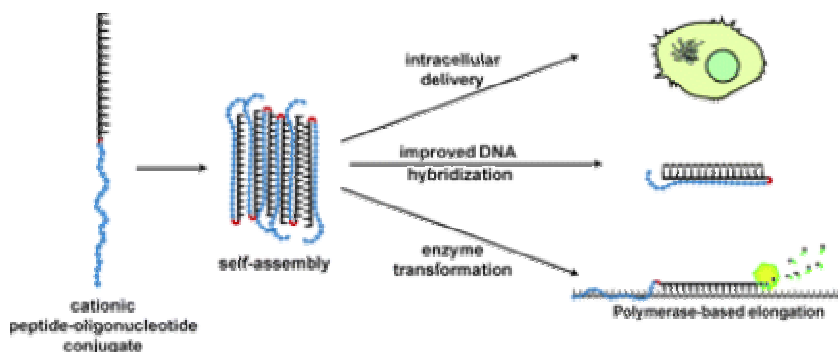


A special, water-soluble, fluorescent probe **1** was designed. This consisted of a fluorescein-based component to harvest irradiation at 488 nm and a rhodamine-based part designed to emit it at a significantly longer wavelength. This cassette was used to label an illustrative protein called ACBP. Evidence was accumulated to support the assertion that ACBP-1 bound its native ligand with a binding constant similar to that of the unlabeled protein, and retained its secondary structure (CD). ACBP-1 was imported into cells using the Chariot peptide. Confocal images proved that some ACBP-1 localized into the nucleus (as expected) and, most significantly, it could be visualized more effectively by irradiating at the donor (fluorescein-like) part of the cassette, than the acceptor (rhodamine-like) part. Overall, this study demonstrates that cassettes of this kind can label a protein without significantly perturbing its function or secondary structure and they can be visualized effectively via irradiation of the donor and observation of the acceptor fluorescence.

## Cationic Oligonucleotide-Peptide Conjugates with Aggregating Properties Enter Efficiently into Cells while Maintaining Hybridization Properties and Enzymatic Recognition

*J. Am. Chem. Soc.*, **128** (33), 10763-10771, 2006.

Andrew W. Fraley,† Bénédicte Pons, Deniz Dalkara, Gérard Nullans, Jean-Paul Behr, and Guy Zuber



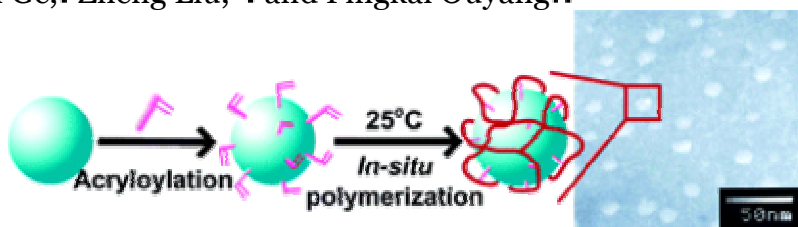
Oligonucleotide delivery is a crucial issue for therapeutical purposes and is often addressed by conjugation to short cationic peptides although with controversial results. To further examine this mechanism, a 15-mer anionic oligonucleotide was conjugated to a cationic peptide in order to obtain a diblock compound with an overall positive charge with aggregation properties. These microaggregates were efficiently internalized in cells via the expeditious pathway used by commercial gene delivery systems. Moreover, stability of the duplex formed with the complementary sequence increased without inhibiting oligonucleotide enzyme recognition as shown by the properties of the

conjugate to prime chain elongation by Taq DNA polymerase in a linear amplification/sequencing process.

## Encapsulation of Single Enzyme in Nanogel with Enhanced Biocatalytic Activity and Stability

*J. Am. Chem. Soc.*, 128 (34), 11008 -11009, 2006.

Ming Yan,<sup>†</sup> Jun Ge,<sup>†</sup> Zheng Liu,<sup>\*†</sup> and Pingkai Ouyang<sup>†‡</sup>

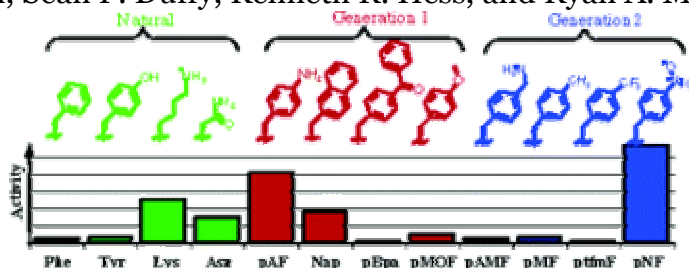


Single protein encapsulated into nanogels with uniformed size and controllable shell thickness were prepared by surface acryloylation of a protein molecule followed by aqueous in situ polymerization. Compared to its free counterpart, the encapsulated protein exhibits similar biocatalytic behavior and significantly improved stability at high temperature and in the presence of organic solvent.

## Improving Nature's Enzyme Active Site with Genetically Encoded Unnatural Amino Acids

*J. Am. Chem. Soc.*, 128 (34), 11124 -11127, 2006.

Jennifer C. Jackson, Sean P. Duffy, Kenneth R. Hess, and Ryan A. Mehl\*



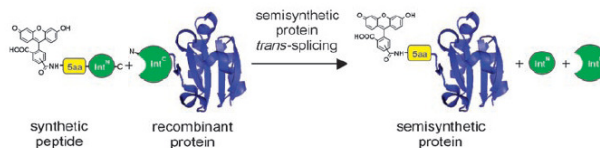
The ability to site-specifically incorporate a diverse set of unnatural amino acids (>30) into proteins and quickly add new structures of interest has recently changed our approach to protein use and study. One important question yet unaddressed with unnatural amino acids (UAAs) is whether they can improve the activity of an enzyme beyond that available from the natural 20 amino acids. Herein, we report the >30-fold improvement of prodrug activator nitroreductase activity with an UAA over that of the native active site and a >2.3-fold improvement over the best possible natural amino acid. Because immense structural and electrostatic diversity at a single location can be sampled very quickly, UAAs can be implemented to improve enzyme active sites and tune a site to multiple substrates.

## Angewandte Chemie

## Protein Modifications

C. Ludwig, M. Pfeiff, U. Linne,  
H. D. Mootz\* \_\_\_\_\_ 5218–5221

Ligation of a Synthetic Peptide to the N  
Terminus of a Recombinant Protein Using  
Semisynthetic Protein *trans*-Splicing



**Splice it!** An active intein can be reconstituted from a recombinant C-terminal and a short synthetic N-terminal fragment. This allows ligation of a synthetic peptide to the N terminus of a recombi-

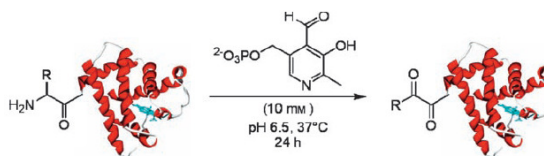
nant protein of interest by protein *trans*-splicing and circumvents the requirement for a C-terminal thioester or other reactive groups common to chemical ligation approaches.

## Volume 45, Issue 32, Pages 5227-5395 (August 11, 2006)

### Bioorganic Chemistry

J. M. Gilmore, R. A. Scheck,  
A. P. Esser-Kahn, N. S. Joshi,  
M. B. Francis\* \_\_\_\_\_ 5307–5311

N-Terminal Protein Modification through  
a Biomimetic Transamination Reaction



**One hit wonder:** A biomimetic transamination reaction has been developed that employs pyridoxal-5-phosphate to modify the N terminus of proteins and peptides under mild conditions. This technique introduces a uniquely reactive carbonyl

group in a single location (see scheme), thus allowing further elaboration through oxime or hydrazone formation. This modification strategy is also compatible with proteins containing a free cysteine residue.

## Journal of Biological Chemistry

### Characterization of the HIV N-terminal Fusion Peptide-containing Region in Context of Key gp41 Fusion Conformations

*J. Biol. Chem.*, Vol. 281, Issue 31, 21755-21762, August 4, 2006

Kelly Sackett, Yael Wexler-Cohen, and Yechiel Shai

Central to our understanding of human immunodeficiency virus-induced fusion is the high resolution structure of fragments of the gp41 fusion protein folded in a low energy core conformation. However, regions fundamental to fusion, like the fusion peptide (FP), have yet to be characterized in the context of the cognate protein regardless of its conformation. Based on conformation-specific monoclonal antibody recognition, we identified the polar region consecutive to the N36 fragment as a stabilizer of trimeric coiled-coil assembly, thereby enhancing inhibitory potency. This tertiary organization is retained in the context of the hydrophobic FP (N70 fragment). Our data indicate that the N70 fragment recapitulates the expected organization of this region in the viral fusion intermediate (N-terminal half of the pre-hairpin intermediate (N-PHI)), which happens to be the prime target for fusion inhibitors. Regarding the low energy conformation, we show for the first time core formation in the context of the FP (N70 core). The  $\alpha$ -helical and coiled-coil stabilizing polar region confers substantial thermal stability to the core, whereas the hydrophobic FP does not add further stability. For the two key fusion conformations, N-PHI and N70 core, we find that the FP adopts a

nonhelical structure and directs higher order assembly (assembly of coiled coils in N-PHI and assembly of bundles in the N70 core). This supra-molecular organization of coiled coils or folded cores is seen only in the context of the FP. This study is the first to characterize the FP region in the context of the folded core and provides a basic understanding of the role of the elusive FP for key gp41 fusion conformations.