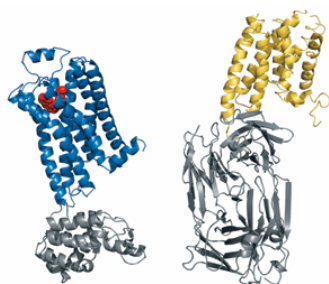


# Chmielewski Group Literature Abstracts

CHEMISTRY

BIOLOGY

## November 2007



Although they are one of the most important families of proteins in the human body and a common target of drugs, GPCRs have been almost impossible to analyze structurally. Only one crystal GPCR structure, that of the light-sensitive protein rhodopsin, had been obtained. This year, two groups obtained the second GPCR structure, that of the human  $\beta$ 2 adrenergic receptor ( $\beta$ 2AR), by two different approaches.

Brian K. Kobilka of Stanford University School of Medicine and coworkers stabilized the protein for structural analysis by binding an antibody fragment to GPCR (*Nature* **2007**, 450, 383; *Nat. Methods* **2007**, 4, 927). And Kobilka, Raymond C. Stevens of Scripps, and coworkers enhanced  $\beta$ 2AR's stability by linking the enzyme T4 lysozyme to it (*Science* **2007**, 318, 1258 and 1266). Researchers will now try to adapt these approaches to solve the structures of other GPCRs more easily than has been possible before. Such a capability would have major implications for drug discovery and could lead to a better understanding of receptor biology.

### **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

## Crystal structure of the human beta2 adrenergic G-protein-coupled receptor

*Nature* 450, 383-387 (15 November 2007)

Søren G. F. Rasmussen<sup>1,6</sup>, Hee-Jung Choi<sup>1,2,6</sup>, Daniel M. Rosenbaum<sup>1,6</sup>, Tong Sun Kobilka<sup>1</sup>, Foon Sun Thian<sup>1</sup>, Patricia C. Edwards<sup>3</sup>, Manfred Burghammer<sup>4</sup>, Venkata R. P. Ratnala<sup>1</sup>, Ruslan Sanishvili<sup>5</sup>, Robert F. Fischetti<sup>5</sup>, Gebhard F. X. Schertler<sup>3</sup>, William I. Weis<sup>1,2</sup> & Brian K. Kobilka

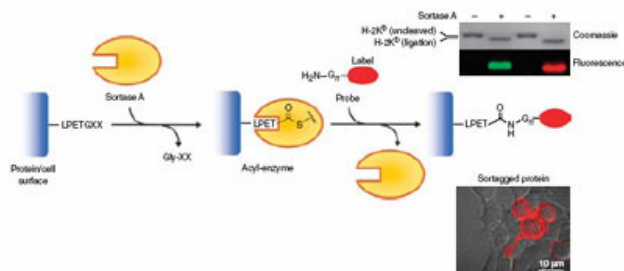
Structural analysis of G-protein-coupled receptors (GPCRs) for hormones and neurotransmitters has been hindered by their low natural abundance, inherent structural flexibility, and instability in detergent solutions. Here we report a structure of the human beta2 adrenoceptor (beta2AR), which was crystallized in a lipid environment when bound to an inverse agonist and in complex with a Fab that binds to the third intracellular loop. Diffraction data were obtained by high-brilliance microcrystallography and the structure determined at 3.4 Å/3.7 Å resolution. The cytoplasmic ends of the beta2AR transmembrane segments and the connecting loops are well resolved, whereas the extracellular regions of the beta2AR are not seen. The beta2AR structure differs from rhodopsin in having weaker interactions between the cytoplasmic ends of transmembrane (TM)<sub>3</sub> and TM<sub>6</sub>, involving the conserved E/DRY sequences. These differences may be responsible for the relatively high basal activity and structural instability of the beta2AR, and contribute to the challenges in obtaining diffraction-quality crystals of non-rhodopsin GPCRs.

## Nature Chemical Biology

### Sortagging: a versatile method for protein labeling

*Nature Chemical Biology* 3, 707-708 (2007)

Maximilian W Popp<sup>1,2</sup>, John M Antos<sup>1</sup>, Gijsbert M Grotenbreg<sup>1</sup>, Eric Spooner<sup>1</sup> & Hidde L Ploegh



Genetically encoded reporter constructs that yield fluorescently labeled fusion proteins are a powerful tool for observing cell biological phenomena, but they have limitations. Sortagging (sortase-mediated transpeptidation) is a versatile chemoenzymatic system

for site-specific labeling of proteins with small (<2 kDa) probes. Sortagging combines the precision of a genetically encoded tag with the specificity of an enzymatic reaction and the ease and chemical versatility of peptide synthesis. Here we apply this technique to proteins in vitro and on the surface of living cells.

## **Nature Nanotechnology**

### **Direct imaging of single-walled carbon nanotubes in cells**

*Nature Nanotechnology* **2**, 713 - 717 (2007)

Alexandra E. Porter<sup>1</sup>, Mhairi Gass<sup>2</sup>, Karin Muller<sup>3</sup>, Jeremy N. Skepper<sup>3</sup>, Paul A. Midgley & Mark Welland

The development of single-walled carbon nanotubes for various biomedical applications is an area of great promise. However, the contradictory data on the toxic effects of single-walled carbon nanotubes<sup>1, 2, 3, 4, 5, 6, 7, 8, 9, 10</sup> highlight the need for alternative ways to study their uptake and cytotoxic effects in cells. Single-walled carbon nanotubes have been shown to be acutely toxic<sup>1, 2, 3</sup> in a number of types of cells, but the direct observation of cellular uptake of single-walled carbon nanotubes has not been demonstrated previously due to difficulties in discriminating carbon-based nanotubes from carbon-rich cell structures. Here we use transmission electron microscopy and confocal microscopy to image the translocation of single-walled carbon nanotubes into cells in both stained and unstained human cells. The nanotubes were seen to enter the cytoplasm and localize within the cell nucleus, causing cell mortality in a dose-dependent manner.

### **Sequence-specific detection of individual DNA polymerase complexes in real time using a nanopore**

*Nature Nanotechnology* **2**, 718 - 724 (2007)

Seico Benner<sup>1</sup>, Roger J. A. Chen<sup>2</sup>, Noah A. Wilson<sup>3</sup>, Robin Abu-Shumays<sup>1</sup>, Nicholas Hurt<sup>2</sup>, Kate R. Lieberman<sup>1</sup>, David W. Deamer<sup>1,2</sup>, William B. Dunbar<sup>1,3</sup> & Mark Akeson

Nanoscale pores have potential to be used as biosensors and are an established tool for analysing the structure and composition of single DNA or RNA molecules<sup>1, 2, 3</sup>. Recently, nanopores have been used to measure the binding of enzymes to their DNA substrates<sup>4, 5</sup>. In this technique, a polynucleotide bound to an enzyme is drawn into the nanopore by an applied voltage. The force exerted on the charged backbone of the polynucleotide by the electric field is used to examine the enzyme–polynucleotide interactions. Here we show that a nanopore sensor can accurately identify DNA templates bound in the catalytic site of individual DNA polymerase molecules. Discrimination among unbound DNA, binary DNA/polymerase complexes, and ternary DNA/polymerase/deoxynucleotide triphosphate complexes was achieved in real time using finite state machine logic. This technique is applicable to numerous enzymes that bind or modify DNA or RNA including exonucleases, kinases and other polymerases.

## Science

### **A Predictably Selective Aliphatic C–H Oxidation Reaction for Complex Molecule Synthesis**

**Vol. 318. no. 5851, pp. 783 - 787**

Mark S. Chen and M. Christina White

Realizing the extraordinary potential of unactivated sp<sup>3</sup> C–H bond oxidation in organic synthesis requires the discovery of catalysts that are both highly reactive and predictably selective. We report an iron (Fe)–based small molecule catalyst that uses hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) to oxidize a broad range of substrates. Predictable selectivity is achieved solely on the basis of the electronic and steric properties of the C–H bonds, without the need for directing groups. Additionally, carboxylate directing groups may be used to furnish five-membered ring lactone products. We demonstrate that these three modes of selectivity enable the predictable oxidation of complex natural products and their derivatives at specific C–H bonds with preparatively useful yields. This type of general and predictable reactivity stands to enable aliphatic C–H oxidation as a method for streamlining complex molecule synthesis.

### **Engineering Entropy-Driven Reactions and Networks Catalyzed by DNA**

**Vol. 318. no. 5853, pp. 1121 - 1125**

David Yu Zhang,<sup>1</sup>{dagger} Andrew J. Turberfield,<sup>2</sup> Bernard Yurke, Erik Winfree

Artificial biochemical circuits are likely to play as large a role in biological engineering as electrical circuits have played in the engineering of electromechanical devices. Toward that end, nucleic acids provide a designable substrate for the regulation of biochemical reactions. However, it has been difficult to incorporate signal amplification components. We introduce a design strategy that allows a specified input oligonucleotide to catalyze the release of a specified output oligonucleotide, which in turn can serve as a catalyst for other reactions. This reaction, which is driven forward by the configurational entropy of the released molecule, provides an amplifying circuit element that is simple, fast, modular, composable, and robust. We have constructed and characterized several circuits that amplify nucleic acid signals, including a feedforward cascade with quadratic kinetics and a positive feedback circuit with exponential growth kinetics.

### **High-Resolution Crystal Structure of an Engineered Human $\beta$ -Adrenergic G Protein–Coupled Receptor**

**Vol. 318. no. 5854, pp. 1258 - 1265**

Vadim Cherezov,<sup>1\*</sup> Daniel M. Rosenbaum,<sup>2\*</sup> Michael A. Hanson,<sup>1</sup> Søren G. F. Rasmussen,<sup>2</sup> Foon Sun Thian,<sup>2</sup> Tong Sun Kobilka,<sup>2</sup> Hee-Jung Choi,<sup>2,3</sup> Peter Kuhn,<sup>4</sup> William I. Weis, Brian K. Kobilka, Raymond C. Stevens

Heterotrimeric guanine nucleotide-binding protein (G protein)-coupled receptors constitute the largest family of eukaryotic signal transduction proteins that communicate across the membrane. We report the crystal structure of a human  $\beta$ 2-adrenergic receptor-T4 lysozyme fusion protein bound to the partial inverse agonist carazolol at 2.4 angstrom resolution. The structure provides a high-resolution view of a human G protein-coupled receptor bound to a diffusible ligand. Ligand-binding site accessibility is enabled by the second extracellular loop, which is held out of the binding cavity by a pair of closely spaced disulfide bridges and a short helical segment within the loop. Cholesterol, a necessary component for crystallization, mediates an intriguing parallel association of receptor molecules in the crystal lattice. Although the location of carazolol in the  $\beta$ 2-adrenergic receptor is very similar to that of retinal in rhodopsin, structural differences in the ligand-binding site and other regions highlight the challenges in using rhodopsin as a template model for this large receptor family.

## PNAS

### **Designer enediynes generate DNA breaks, interstrand cross-links, or both, with concomitant changes in the regulation of DNA damage responses**

*PNAS* | **November 6, 2007** | *vol. 104* | *no. 45* | **17632-17637**  
Daniel R. Kennedy\*, Jianhua Ju, Ben Shen , , and Terry A. Beerman

The ability of the radiomimetic anticancer enediyne C-1027 to induce ataxia-telangiectasia mutated (ATM) and ATM and Rad3-related (ATR)-independent damage responses was discovered to reside in its unique ability to concurrently generate robust amounts of double-strand breaks (DSBs) and interstrand cross-links (ICLs) in cellular DNA. Furthermore, a single substitution to the chromophore's benzoxazolate moiety shifted DNA damage to primarily ICLs and an ATR- but not ATM-dependent damage response. In contrast, single substitutions of the chromophore's -amino acid component shifted DNA damage to primarily DSBs, consistent with its induction of conventional ATM-dependent damage responses of the type generated by ionizing radiation and other radiomimetics. Thus, phosphatidylinositol 3-kinase-like protein kinase regulation of DNA damage responses is dictated by the relative proportions of DSBs and ICLs.

### **Effect of flexibility and cis residues in single-molecule FRET studies of polyproline**

*PNAS* | **November 27, 2007** | *vol. 104* | *no. 48* | **18964-18969**  
Robert B. Best\*, , Kusai A. Merchant\*, Irina V. Gopich\*, Benjamin Schuler\*, , Ad Bax\*, and William A. Eaton

Polyproline has recently been used as a spacer between donor and acceptor chromophores to help establish the accuracy of distances determined from single-molecule Förster resonance energy transfer (FRET) measurements. This work showed

that the FRET efficiency in water is higher than expected for a rigid spacer and was attributed to the flexibility of the polypeptide. Here, we investigate this issue further, using a combination of single-molecule fluorescence intensity and lifetime measurements, NMR, theory, and molecular dynamics simulations of polyproline-20 that include the dyes and their linkers to the polypeptide. NMR shows that in water 30% of the molecules contain internal cis prolines, whereas none are detectable in trifluoroethanol. Simulations suggest that the all-trans form of polyproline is relatively stiff, with persistence lengths of 9–13 nm using different established force fields, and that the kinks arising from internal cis prolines are primarily responsible for the higher mean FRET efficiency in water. We show that the observed efficiency histograms and distributions of donor fluorescence lifetimes are explained by the presence of multiple species with efficiencies consistent with the simulations and populations determined by NMR. In calculating FRET efficiencies from the simulation, we find that the fluctuations of the chromophores, attached to long flexible linkers, also play an important role. A similar simulation approach suggests that the flexibility of the chromophore linkers is largely responsible for the previously unexplained high value of  $R_0$  required to fit the data in the classic study of Stryer and Haugland.

### **Molecular architecture of human prion protein amyloid: A parallel, in-register $\beta$ -structure**

*PNAS* | November 27, 2007 | vol. 104 | no. 48 | 18946-18951

Nathan J. Cobb\*, Frank D. Sönnichsen\*, Hassane Mchaourab, and Witold K. Surewicz

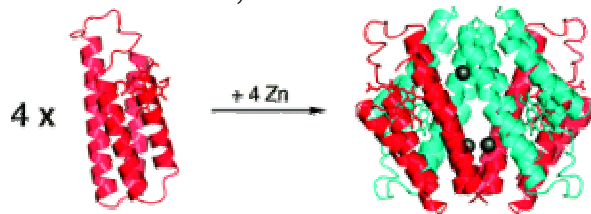
Transmissible spongiform encephalopathies (TSEs) represent a group of fatal neurodegenerative diseases that are associated with conformational conversion of the normally monomeric and  $\alpha$ -helical prion protein, PrPC, to the  $\beta$ -sheet-rich PrPSc. This latter conformer is believed to constitute the main component of the infectious TSE agent. In contrast to high-resolution data for the PrPC monomer, structures of the pathogenic PrPSc or synthetic PrPSc-like aggregates remain elusive. Here we have used site-directed spin labeling and EPR spectroscopy to probe the molecular architecture of the recombinant PrP amyloid, a misfolded form recently reported to induce transmissible disease in mice overexpressing an N-terminally truncated form of PrPC. Our data show that, in contrast to earlier, largely theoretical models, the conformational conversion of PrPC involves major refolding of the C-terminal  $\alpha$ -helical region. The core of the amyloid maps to C-terminal residues from 160–220, and these residues form single-molecule layers that stack on top of one another with parallel, in-register alignment of  $\beta$ -strands. This structural insight has important implications for understanding the molecular basis of prion propagation, as well as hereditary prion diseases, most of which are associated with point mutations in the region found to undergo a refolding to  $\beta$ -structure.

## **Journal of the American Chemical Society**

### **Controlling Protein-Protein Interactions through Metal Coordination: Assembly of a 16-Helix Bundle Protein**

*J. Am. Chem. Soc.*, 2007, 129 (44), 13374 -13375

Eric N. Salgado, Jasmin Faraone-Mennella, and F. Akif Tezcan

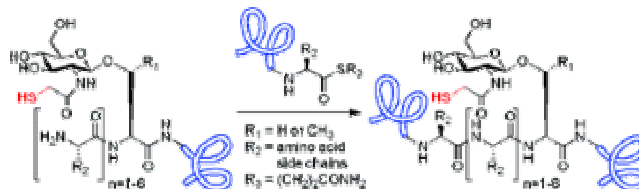


The prediction, design, and control of protein-protein interactions (PPIs) remain great challenges despite recent advances. Here we describe the chemical control of PPIs through the use of metal coordination, which circumvents the requirement of PPIs for an extensive set of weak interactions spread over a large surface. A non-self-associating four-bundle protein, cytochrome cb562, with appropriately engineered metal-binding motifs self-assembles to a 16-helix quaternary structure upon addition of equimolar Zn. The crystal structure of the assembly, combined with PFG diffusion NMR and sedimentation velocity experiments, indicates that the oligomerization properties of cytochrome cb562 are governed entirely by metal coordination without significant thermodynamic bias from specific PPIs.

## Extended Sugar-Assisted Glycopeptide Ligations: Development, Scope, and Applications

*J. Am. Chem. Soc.*, 2007, 129 (44), 13527 -13536

Richard J. Payne,<sup>†</sup> Simon Ficht,<sup>†</sup> Sishi Tang,<sup>‡</sup> Ashraf Brik,<sup>†#</sup> Yu-Ying Yang,<sup>†</sup> David A. Case,<sup>‡</sup> and Chi-Huey Wong



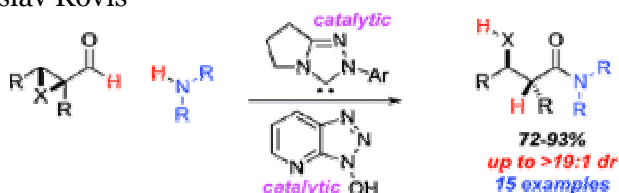
Recently, we reported the development of sugar-assisted ligation (SAL), a novel peptide ligation method for the synthesis of glycopeptides. After screening a large number of glycoprotein sequences in a glycoprotein database, it became evident that a large proportion (approximately 53%) of O-glycosylation sites contain amino acid residues that will not undergo SAL reactions. To overcome these inherent limitations and broaden the scope of the method we report here the development of an extended SAL method. Glycopeptides containing up to six amino acid extensions N-terminal to the glycosylated residue were shown to facilitate ligation reactions with peptide thioesters, and these products were isolated in good yields. Kinetic analysis was used to show that as glycopeptides were extended by further amino acid residues, ligation reactions became slower. This finding was rationalized by molecular dynamics simulations using AMBER9. These studies suggested a general trend whereby the proximal distance between the reactive sites of the thioester intermediate (the N-terminal amine and the carbonyl carbon of the thioester) increased as glycopeptides were extended, thus slowing down the ligation rate. Each of the extended SAL methods showed broad tolerance to a number of different amino acid combinations at the ligation junction. Re-evaluation of the glycoprotein database suggested that 95% of the O-linked glycosylation

sites can now be utilized to facilitate SAL or extended SAL reactions. As such, this method represents an extremely valuable tool for the synthesis of naturally occurring glycopeptides and glycoproteins. To demonstrate the applicability of the method, extended SAL was successfully implemented in the synthesis of the starting unit of the cancer-associated MUC1 glycoprotein.

## Nucleophilic Carbene and HOAt Relay Catalysis in an Amide Bond Coupling: An Orthogonal Peptide Bond Forming Reaction

*J. Am. Chem. Soc.*, 2007, 129 (45), 13796 -13797

Harit U. Vora and Tomislav Rovis

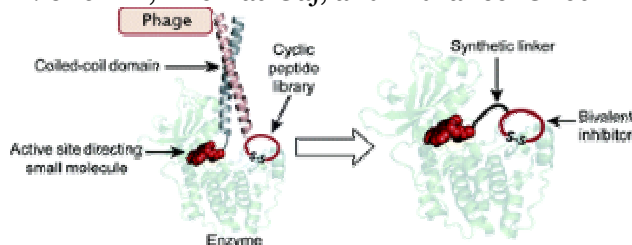


A catalyzed internal redox process provides a route from  $\alpha$ -reducible aldehydes and amines to  $\alpha$ -reduced amides. The chemistry is catalyzed by nucleophilic carbenes and common peptide cocatalysts such as HOBT and HOAt in a relay fashion. The transformation proceeds in excellent yields using a variety of primary and secondary alkyl and aryl amines. The aldehyde component may be varied from haloaldehydes to epoxy and aziridino aldehydes as well as enals. The latter three substrates provide for a waste-free amide bond forming reaction.

## Tethering Small Molecules to a Phage Display Library: Discovery of a Selective Bivalent Inhibitor of Protein Kinase A

*J. Am. Chem. Soc.*, 2007, 129 (45), 13812 -13813

Scott C. Meyer, Carolyn D. Shomin, Thomas Gaj, and Indraneel Ghosh



We report a noncovalent tethering methodology for the fragment-based selection of bivalent ligands targeting protein kinases. In this approach, a small-molecule warhead, staurosporine, directs a phage display cyclic peptide library to the active site of cAMP-dependent protein kinase (PKA), allowing for targeted library enrichment. A cyclic peptide discovered through this selection, when covalently attached to a staurosporine derivative, displayed a 90-fold increase in affinity for PKA. Moreover, the bivalent inhibitor was shown to be significantly more selective than the starting warhead when tested against a small panel of kinases. Thus our general methodology allows for covalent linkage of known small-molecule ligands to biological libraries for discovering potent bivalent inhibitors of biological targets.

## Self-Assembled Templates for Polypeptide Synthesis

*J. Am. Chem. Soc.*, 2007, 129 (45), 14074 -14081

Maxim G. Ryadnov\*† and Derek N. Woolfson

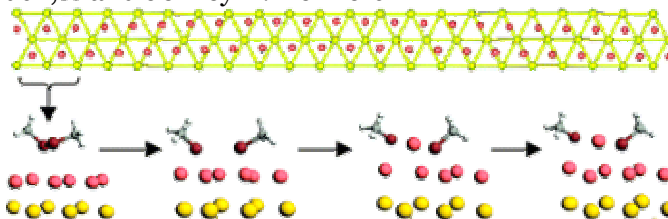


The chemical synthesis of polypeptide chains >50 amino acids with prescribed sequences is challenging. In one approach, native chemical ligation (NCL), short, unprotected peptides are connected through peptide bonds to render proteins in water. Here we combine chemical ligation with peptide self-assembly to deliver extremely long polypeptide chains with stipulated, repeated sequences. We use a self-assembling fiber (SAF) system to form structures tens of micrometers long. In these assemblies, tens of thousands of peptides align with their N- and C-termini abutting. This arrangement facilitates chemical ligation without the usual requirement for a catalytic cysteine residue at the reactive N-terminus. We introduced peptides with C-terminal thioester moieties into the SAFs. Subsequent ligation and disassembly of the noncovalent components produced extended chains  $\geq 10 \mu\text{m}$  long and estimated at  $\geq 3$  MDa in mass. These extremely long molecules were characterized by a combination of biophysical, hydrodynamic, and microscopic measurements.

## Formation of Gold-Methanethiyl Self-Assembled Monolayers

*J. Am. Chem. Soc.*, 129 (47), 14532 -14533, 2007.

Yun Wang,† Noel S. Hush,†† and Jeffrey R. Reimers

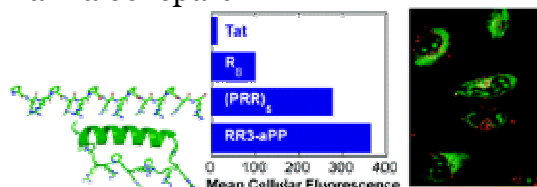


The energetics of formation of thiol-gold self-assembled monolayers is investigated using density-functional theory simulations. It is found that the chemisorption of dimethyl disulfide on the reconstructed Au(111) ( $22 \times \sqrt{3}$ ) surface is most favored at the fcc reconstruction stripe, with initial physisorption leading to disulfide dissociation, adatom/vacancy-pair formation, and then, at a coverage of 7.8% sulfur atoms per gold atom, surface reconstruction lifting. At higher coverages, monolayer formation proceeds similarly on the unreconstructed surface, leading to surface pitting. Formation of the analogous adatom/vacancy-pair bound dissociated adsorbate complex on exposure of the clean unreconstructed surface to methanethiol is shown to be endothermic, however.

## Intrinsically Cell-Permeable Miniature Proteins Based on a Minimal Cationic PPII Motif

*J. Am. Chem. Soc.*, 129 (47), 14578 -14579, 2007.

Douglas S. Daniels<sup>†</sup> and Alanna Schepartz

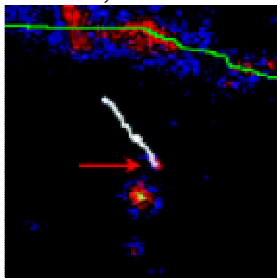


Cell-penetrating peptides (CPPs) provide promising tools for the cellular delivery of molecular cargos ranging in size from small molecules and peptides to proteins and quantum dots. CPPs are typically cationic and/or amphipathic sequences that are unstructured or  $\alpha$ -helical. We expand the repertoire of cell-penetrating motifs by designing encodable CPPs possessing type-II polyproline (PPII) helical structure. These motifs surpass the uptake efficiency of existing CPPs and are not cytotoxic at concentrations 100 times greater than that necessary for delivery. By replacing the PPII helix of a miniature protein, the motif can endow

## Imaging and Tracking of Tat Peptide-Conjugated Quantum Dots in Living Cells: New Insights into Nanoparticle Uptake, Intracellular Transport, and Vesicle Shedding

*J. Am. Chem. Soc.*, **129** (47), 14759-14766, 2007.

Gang Ruan,<sup>†</sup> Amit Agrawal,<sup>†</sup> Adam I. Marcus,<sup>†</sup> and Shuming Nie

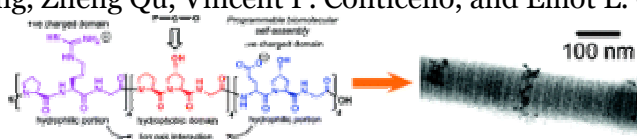


We report the use of Tat peptide-conjugated quantum dots (Tat-QDs) to examine the complex behavior of nanoparticle probes in live cells, a topic that is of considerable current interest in developing advanced nanoparticle agents for molecular and cellular imaging. Dynamic confocal imaging studies indicate that the peptide-conjugated QDs are internalized by macropinocytosis, a fluid-phase endocytosis process triggered by Tat-QD binding to negatively charged cell membranes. The internalized Tat-QDs are tethered to the inner vesicle surfaces and are trapped in cytoplasmic organelles. The QD loaded vesicles are found to be actively transported by molecular machines (such as dyneins) along microtubule tracks. The destination of this active transport is an asymmetric perinuclear region (outside the cell nucleus) known as the microtubule organizing center (MTOC). We also find that Tat-QDs strongly bind to cellular membrane structures such as filopodia and that large QD-containing vesicles are released from the tips of filopodia by vesicle shedding. These results provide new insights into the mechanisms of Tat peptide-mediated delivery as well as toward the design of functionalized nanoparticles for molecular imaging and targeted therapy.

## D-Periodic Collagen-Mimetic Microfibers

**J. Am. Chem. Soc., 129 (47), 14780 -14787, 2007.**

Shyam Rele, Yuhua Song, Zheng Qu, Vincent P. Conticello, and Elliot L. Chaikof

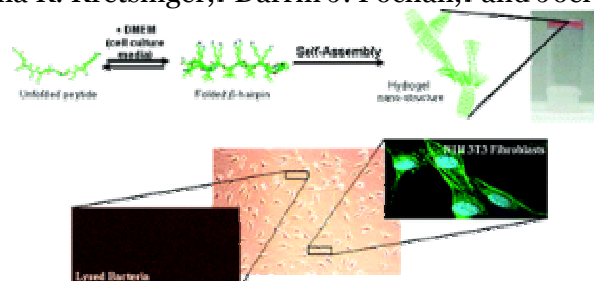


Self-assembling peptides have been previously designed that assemble into macroscopic membranes, nanotapes, and filaments through electrostatic interactions. However, the formation of highly ordered collagen-like fibrils, which display D-periodic features, has yet to be achieved. In this report, we describe for the first time a synthetic peptide system that self-assembles into a fibrous structure with well-defined periodicity that can be visualized by transmission electron microscopy (TEM). Specifically, we designed and synthesized a peptide that utilizes charged amino acids within the ubiquitous Xaa-Yaa-Gly triad sequence to bias the self-assembly into collagen-like homotrimeric helices that are capable of fibrillogenesis with the production of D-periodic microfibers. Potential molecular mechanisms for peptide assembly into triple-helical protomers and their subsequent organization into structurally defined, linear assemblies were explored through molecular dynamics (MD) simulations. The formation of thermodynamically stable complexes was attributed to the presence of strong electrostatic and hydrogen bond interactions at staggered positions along the linear assembly. This unexpected mimicry of native collagen structure using a relatively simple oligopeptide sequence establishes new opportunities for engineering linear assemblies with highly ordered nano- and microscale periodic features. In turn, the capacity to precisely design periodic elements into an assembly that faithfully reproduces these features over large length scales may facilitate the fabrication of ordered two- and three-dimensional fiber networks containing oriented biologically, chemically, or optically active elements.

## Inherent Antibacterial Activity of a Peptide-Based $\beta$ -Hairpin Hydrogel

**J. Am. Chem. Soc., 129 (47), 14793 -14799, 2007.**

Daphne A. Salick,<sup>†</sup> Juliana K. Kretsinger,<sup>†</sup> Darrin J. Pochan,<sup>‡</sup> and Joel P. Schneider



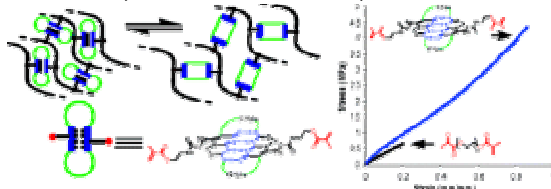
Among several important considerations for implantation of a biomaterial, a main concern is the introduction of infection. We have designed a hydrogel scaffold from the self-assembling peptide, MAX1, for tissue regeneration applications whose surface exhibits inherent antibacterial activity. In experiments where MAX1 gels are challenged with bacterial solutions ranging in concentrations from  $2 \times 10^3$  colony forming units (CFUs)/dm<sup>2</sup> to  $2 \times 10^9$  CFUs/dm<sup>2</sup>, gel surfaces exhibit broad-spectrum antibacterial activity. Results show that the hydrogel surface is active against Gram-positive (*Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Streptococcus pyogenes*) and Gram-negative (*Klebsiella pneumoniae* and *Escherichia coli*) bacteria, all prevalent in

hospital settings. Live-dead assays employing laser scanning confocal microscopy show that bacteria are killed when they engage the surface. In addition, the surface of MAX1 hydrogels was shown to cause inner and outer membrane disruption in experiments that monitor the release of  $\beta$ -galactosidase from the cytoplasm of lactose permease-deficient *E. coli* ML-35. These data suggest a mechanism of antibacterial action that involves membrane disruption that leads to cell death upon cellular contact with the gel surface. Although the hydrogel surface exhibits bactericidal activity, co-culture experiments indicate hydrogel surfaces show selective toxicity to bacterial versus mammalian cells. Additionally, gel surfaces are nonhemolytic toward human erythrocytes, which maintain healthy morphologies when in contact with the surface. These material attributes make MAX1 gels attractive candidates for use in tissue regeneration, even in nonsterile environments.

### **Biomimetic Design of Reversibly Unfolding Cross-Linker to Enhance Mechanical Properties of 3D Network Polymers**

*J. Am. Chem. Soc.*, **129** (46), 14110 -14111, 2007.

Aaron M. Kushner, Vahe Gabuchian, Evan G. Johnson, and Zhibin Guan

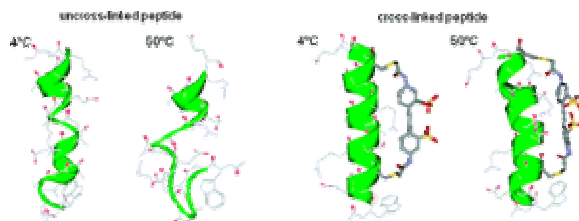


We report here a biomimetic design of a reversibly unfolding modular cross-linker to enhance mechanical properties of 3D network polymers. The inspiration comes from the modular biopolymers observed in nature. A cyclic modular cross-linker based on the quadruple hydrogen bonding 4-ureido-2-pyrimidone (UPy) motif was synthesized via multistep organic synthesis. The modular cross-linker was incorporated into poly(*n*-butyl acrylate) by free radical polymerization. Stress-strain measurements show that the samples containing our modular cross-linker exhibit significantly enhanced mechanical properties over the control samples. Most strikingly, with increasing cross-linker density, both modulus and tensile strength are significantly improved without sacrificing extensibility. The enhanced tensile properties are attributed to the increased energy dissipating ability of the reversibly unfolding cross-linker. This introduces a novel biomimetic concept to enhance network mechanical properties through design of molecularly engineered cross-linkers.

### **Stabilization of Folded Peptide and Protein Structures via Distance Matching with a Long, Rigid Cross-Linker**

*J. Am. Chem. Soc.*, **129** (46)

Fuzhong Zhang, Oleg Sadovski, Steven J. Xin, and G. Andrew Woolley

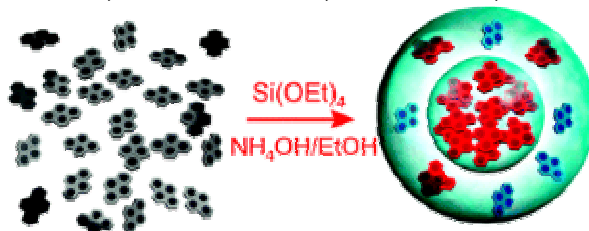


Intramolecular cross-linking is predicted to stabilize the folded state of peptides and proteins most effectively if the cross-linker provides a rigid link that is well-matched in end-to-end distance with attachment sites in the peptide or protein. We describe a thiol-reactive sulfonated alkyne-based cross-linker that is demonstrably more effective than more flexible counterparts. Exceptional stabilization

## Self-Organizing Core-Shell Nanostructures: Spontaneous Accumulation of Dye in the Core of Doped Silica Nanoparticles

*J. Am. Chem. Soc.*, 129 (46), 14251 -14256, 2007.

Enrico Rampazzo, Sara Bonacchi, Marco Montalti,\* Luca Prodi, and Nelsi Zaccheroni

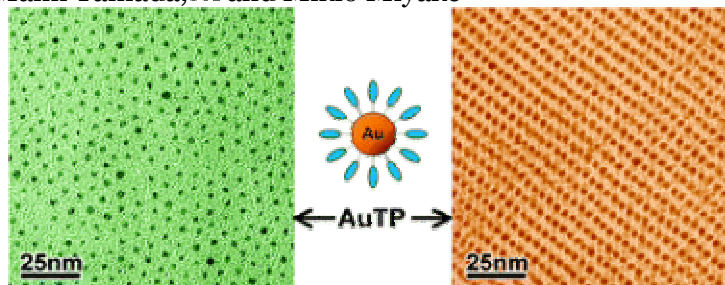


The process of formation of silica nanoparticles doped with a newly synthesized pyrene derivative has been investigated by means of fluorescence steady-state and time-resolved spectroscopy. The changes in the photophysical properties of the fluorophore were correlated to the increase of the nanoparticles hydrodynamic volume measured via dynamic light scattering (DLS) allowing us to determine the radial profile of the concentration of the dye. Experiments performed at a "low" degree of doping show that the fluorophore is almost completely included considerably before the end of the nanoparticles growth, allowing us to identify a self-organizing core-shell substructure. A strong enhancement of the fluorescence of the dye and a corresponding increase of its excited-state lifetime was observed upon its inclusion as a result of the shielding effect from molecular oxygen due to the silica matrix, a situation confirmed by the absence of the oxygen singlet emission in the near-infrared luminescence spectra. In the case of "high" loading, on the other hand, a heavily doped core showing an excimeric-like emission is first formed. Further growth leads to the formation of layers where the concentration of dye gradually decreases and the monomeric emission becomes relevant. The effect of the degree of doping on the kinetics of growth is also reported. At both concentration regimes, ultrafiltration experiments revealed the complete inclusion of the dye molecules. The average number of dye molecule per nanoparticles was also determined.

## Control of Stripelike and Hexagonal Self-Assembly of Gold Nanoparticles by the Tuning of Interactions between Triphenylene Ligands

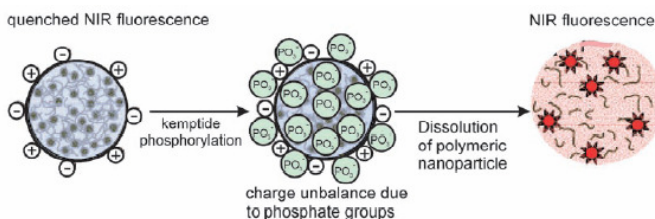
*J. Am. Chem. Soc.*, 129 (46), 14271–14280, 2007.

Zhongrong Shen,<sup>†</sup> Mami Yamada,<sup>††</sup> and Mikio Miyake



We describe the self-assembly of gold nanoparticles (Au NPs) protected with newly synthesized discotic liquid crystalline molecules of hexaalkoxy-substituted triphenylene (TP) in mixed toluene/methanol solvent. The stripelike (i.e., 2D consisting of linear 1D in stripe) self-assembly is realized successfully by the aid of  $\pi$ - $\pi$  stacking of TP ligand on Au NPs. The smaller Au NPs with TP (AuTP) or the longer alkyl chain between TP and the gold core provide more free spaces among TP moieties. These spaces allow easy insertion of TP on adjacent AuTPs to lead an interparticle  $\pi$ - $\pi$  interaction to form the stripelike arrangement. The solvent hydrophilicity can also serve as a controlled index to tune arrangement among stripelike, hexagonal close packed (hcp), or disorder. We have changed the solvent hydrophilicity by changing the ratio of methanol to toluene, which affects the balance of solution of AuTP (in toluene) and deposition (in methanol). The larger space between TPs and appropriate solvent hydrophilicity realize stripelike self-assembly caused by a strong  $\pi$ - $\pi$  interaction between TPs, which was characterized by TEM, as well as fluorescence, dynamic light scattering, and <sup>1</sup>H NMR spectra.

## Angewandte Chemie



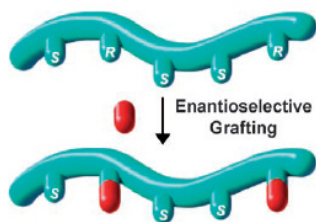
**Cell-permeable, biocompatible nanoparticles** consisting of a positively charged polymer, which is conjugated to a near-infrared (NIR) fluorochrome and a protein-kinase-specific peptide, and a negatively charged polymer constitute innova-

tive probes for measuring protein kinase activity in living cells. Protein kinase activity selectively induces dissolution of the nanoparticles and is accompanied by a strong NIR fluorescence signal (see scheme).

### Imaging Agents

B. Schuster\* \_\_\_\_\_ 8744–8746

Polymeric Nanoparticles as Imaging Probes for Protein Kinase Activity in Cells

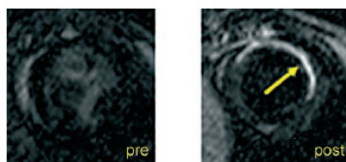


**A programmed response:** Enzyme-responsive materials have been prepared from enzymatically synthesized, enantiomerically pure monomers. The extent of the material's response is encoded within its chiral makeup. This code can be effectively read out by an enzymatic process, which leads to a change in the thermal properties of the material. The picture shows esterification of chiral alcohol groups on a polymer backbone (green) with vinyl acetate (red).

#### Enzyme Catalysis

C. J. Duxbury, I. Hilker,  
S. M. A. de Wildeman,  
A. Heise\* \_\_\_\_\_ 8452–8454

Enzyme-Responsive Materials: Chirality to Program Polymer Reactivity

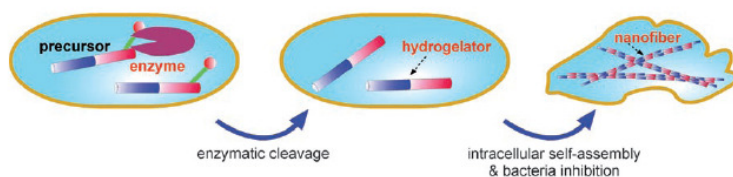


**A cyclic peptide** specific for type I collagen is derivatized with three {Gd(dtpa)} moieties to create a molecular MRI contrast agent for fibrosis imaging. In a mouse model of myocardial infarction (heart attack), collagen levels are elevated in the infarct zone. MRI after injection of the contrast agent selectively enhances and delineates the infarct zone (see preinjection and postinjection images); dtpa = diethylenetriaminepentaacetate.

#### Contrast Agents

P. Caravan,\* B. Das, S. Dumas,  
F. H. Epstein, P. A. Helm, V. Jacques,  
S. Koerner, A. Kolodziej, L. Shen,  
W.-C. Sun, Z. Zhang \_\_\_\_\_ 8171–8173

Collagen-Targeted MRI Contrast Agent for Molecular Imaging of Fibrosis



**Control from within:** Supramolecular hydrogelation to form nanostructures intracellularly (see picture) has been developed as a new methodology to control the fate of cells. Enzyme-regulated self-assembly of small molecules inside

cells could lead to a new paradigm for managing cellular processes, understanding cellular functions, and developing new therapeutics through supramolecular chemistry.

#### Bacterial Inhibition

Z. Yang, G. Liang, Z. Guo, Z. Guo,  
B. Xu\* \_\_\_\_\_ 8216–8219

Intracellular Hydrogelation of Small Molecules Inhibits Bacterial Growth

## ACS Chemical Biology

### Design and Implementation of Cell-Based Assays To Model Human Disease

*ACS Chem. Biol.*, 2 (11), 718–724

Jeremy O. Jones and Marc I. Diamond

Cell-based assays, if appropriately designed, can be used to rapidly identify molecular mechanisms of human disease and develop novel therapeutics. In the last 20 years, many genes that cause or contribute to diverse disorders, including cancer and neurodegenerative disease, have been identified. With such genes in hand, scientists have created numerous model systems to dissect the molecular mechanisms of basic cellular and developmental biology. Meanwhile, techniques for high-throughput screening that use large chemical libraries have been developed, as have cDNA and RNA

interference libraries that cover the entire human genome. By combining cell-based assays with chemical and genetic screens, we now have vastly improved our ability to dissect molecular mechanisms of disease and to identify therapeutic targets and therapeutic lead compounds. However, cell-based screening systems have yet to yield many fundamental insights into disease pathogenesis, and the development of therapeutic leads is frustratingly slow. This may be due to a failure of such assays to accurately reflect key aspects of pathogenesis. This Review attempts to guide the design of productive cellular models of human disease that may be used in high-throughput chemical and genetic screens. We emphasize two points: (i) model systems should use quantifiable molecular indicators of a pathogenic process, and (ii) small chemical libraries that include molecules with known biological activity and/or acceptable safety profiles are very useful.

## **Chemistry and Biology**

### **Inhibition of the Pathogenically Related Morphologic Transition in *Candida albicans* by Disrupting Cdc42 Binding to Its Effectors**

*Volume 14, Issue 11, 26 November 2007, Pages 1273-1282*

Zhengding Su, Hongjian Li, Yang Li and Feng Ni

Morphologic transition from the yeast to the hyphal state in *Candida albicans* is associated with pathogenicity of this human pathogen. Such invasive transition of *C. albicans* cells is regulated by numerous cell signal transduction pathways, one of which involves a small GTPase, the *C. albicans* Cdc42 (CaCdc42), with specific binding to downstream effectors, e.g., CaCla4 and Cst20, containing CRIB domains. Here, we report that *in vivo* inhibition of CaCdc42 by peptide-mediated transduction of the CRIB polypeptides can inactivate and even reverse the pathogenically related morphologic transition of *C. albicans*. The current work provides a promising strategy for disease intervention through disrupting protein-protein interactions in signal transduction pathways and brings the concept of signal transduction therapy into the front line of antifungal design as well as therapy for other signal transduction-related diseases.

### **Structural Proof of a Dimeric Positive Modulator Bridging Two Identical AMPA Receptor-Binding Sites**

*Volume 14, Issue 11, 26 November 2007, Pages 1294-1303*

Birgitte H. Kaae, Kasper Harpsøe, Jette S. Kastrup, Alberto Contreras Sanz, Darryl S. Pickering, Bjørn Metzler, Rasmus P. Clausen, Michael Gajhede, Per Sauerberg, Tommy Liljefors and Ulf Madsen

Dimeric positive allosteric modulators of ionotropic glutamate receptors were designed, synthesized, and characterized pharmacologically in electrophysiological experiments. The designed compounds are dimers of arylpropylsulfonamides and have been constructed without a linker. The monomeric arylpropylsulfonamides were derived from known modulators and target the cyclothiazide-binding site at the AMPA receptors. The three stereoisomers—R,R, meso, and S,S—of the two constructed dimers were prepared,

and in vitro testing showed the R,R forms to be the most potent stereoisomers. The biarylpropylsulfonamides have dramatically increased potencies, more than three orders of magnitude higher than the corresponding monomers. Dimer (R,R)-2a was cocrystallized with the GluR2-S1S2J construct, and an X-ray crystallographic analysis showed (R,R)-2a to bridge two identical binding pockets on two neighboring GluR2 subunits. Thus, this is biostructural evidence of a homomeric dimer bridging two identical receptor-binding sites.

## Chemical Biology and Drug Design

### Anticancer Activity of Selected Phenolic

*Chemical Biology & Drug Design* 70 (5), 424–436.

Sisir Nandi<sup>1</sup>, Marjan Vracko<sup>2</sup> and Manish C. Bagchi

Phenol and its congeners are known to induce caspase-mediated apoptosis activity and cytotoxicity on various cancer cell lines. Apoptosis, scavenging of radicals, antioxidant, and pro-oxidant characteristics are primarily responsible for the antitumor activities of phenolic compounds. Quantitative structure–activity relationship studies on the cellular apoptosis and cytotoxicity of phenolic compounds have been investigated recently by Selassie and colleagues (*J Med Chem*;48:7234, 2005) wherein models were developed for various carcinogenic cell lines. These quantitative structure–activity relationship models are based on few experimentally obtained physicochemical parameters such as Verloop’s sterimol descriptor, hydrophobicity, Hammett electronic parameter, and octanol/water partition coefficient. The paper deals with structure–activity relationships of phenols and its derivatives for the development of predictive models from the standpoint of theoretical structural parameters and ridge regression methodology. The quantitative structure–activity relationship studies developed here for the caspase-mediated apoptosis activity and cytotoxicity on murine leukemia cell line (L1210), human promyelocytic cell line (HL-60), human breast cancer cell line (MCF-7), parenteral human acute lymphoblastic cells (CCRF-CEM), and multidrug-resistant subline of CCRF-resistant to vinblastine (CEM/VLB) cells utilize physicochemical molecular descriptors calculated solely from the structure of phenolic compounds under investigation along with the descriptors used by Selassie and group. It is seen that such quantitative structure–activity relationships can provide a better quality predictive model for the phenolic compounds. The biological activities of the nine sets of phenolic compounds have been calculated based on ridge regression analysis that clearly gives a better significant correlation compared to the activities predicted by Selassie and co-workers. Counter-propagation artificial neural network studies have been introduced in the present investigation for a better understanding of multidimensional rational patterns in more complex data sets. The counter-propagation artificial neural network studies were performed on the same data set and with the same descriptors as have been carried out in developing ridge regression models and the result of counter-propagation neural network models produces very interesting findings in terms of leave-one-out test. Finally, an attempt has been made for a comparative study of the relative effectiveness of linear statistical methods versus nonlinear techniques, such as counter-propagation neural networks in modeling structure–activity studies of the phenolic compounds.

## **Combining Anticholinergic and Anti-inflammatory Activities into a Single Moiety: A Novel Approach to Reduce Gastrointestinal Toxicity of Ibuprofen and Ketoprofen**

*Chemical Biology & Drug Design* 70 (5), 450-455.

Parmeshwari K. Halen, Kewal K. Chagti, Rajani Giridhar and Mange R. Yadav

With the aim of reducing the local gastric irritation associated with non-steroidal anti-inflammatory drugs, a series of N,N-disubstituted aminoalcohol ester derivatives of ibuprofen and ketoprofen were synthesized and evaluated. The esters were specially designed to possess the anticholinergic activity in the intact form and exhibit the anti-inflammatory action after hydrolysis to the respective parent drug. The rationale being that besides blocking the acidic carboxylic group of the parent drug, the existence of the anticholinergic effect in the intact molecule would further aid in reducing the gastrointestinal mucosal damage by decreasing the gastric secretions and motility. All the ester derivatives were found to be stable in acidic and basic buffers. The synthesized derivatives, with experimentally proven good anti-inflammatory and anticholinergic activities, showed significant reduction of ulcerogenicity in the stomach. These results are attributed to the acquired anticholinergic activity with a simultaneous reduction of acidic character compared to the parent compounds. The study offers a new strategy for design and development of compounds with safer therapeutic profile for long-term treatment of inflammation-associated disorders.

## **Microwave-Assisted Solid-Phase Peptide Synthesis Utilizing N-Fmoc-Protected ( $\alpha$ -aminoacyl)benzotriazoles**

*Chemical Biology & Drug Design* 70 (5), 465-468.

Alan R. Katritzky<sup>1,\*</sup>, Niveen M. Khashab<sup>1</sup>, Megumi Yoshioka<sup>1</sup>, Danniebelle N. Haase<sup>1</sup>, Krista R. Wilson<sup>2</sup>, Jodie V. Johnson<sup>3</sup>, Alfred Chung<sup>4</sup> and Carrie Haskell-Luevano

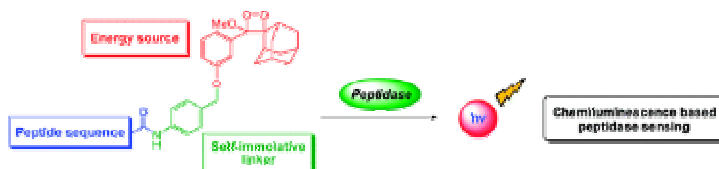
A novel microwave-assisted solid-phase peptide synthesis utilizing N-Fmoc-protected( $\alpha$ -aminoacyl)benzotriazoles was applied in the preparation of tri-, tetra-, penta-, hexa-, and heptapeptides in 71% average crude yield.

## **Organic Letters**

### **Chemiluminescent Probe for the in Vitro Detection of Protease Activity**

*Org. Lett.*, 9 (23), 4853-4855, 2007

Jean-Alexandre Richard,<sup>††</sup> Ludovic Jean,<sup>‡</sup> Anthony Romieu,<sup>\*†</sup> Marc Massonneau,<sup>‡</sup> Pauline Noack-Fraissignes,<sup>‡</sup> and Pierre-Yves Renard

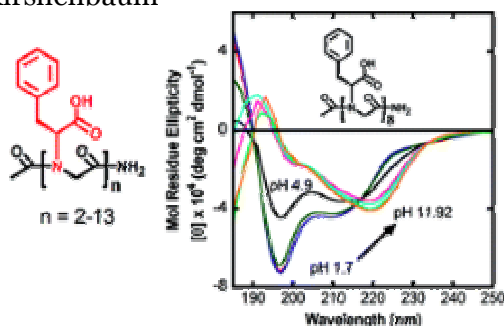


A strategy involving the use of a self-immolative linker has been investigated for the chemiluminescent sensing of proteases. The reactive linker enabled the release of a 1,2-dioxetane light precursor. As a proof of principle, caspase-3, a key peptidase involved in apoptosis has been targeted. An *in vitro* assay has been carried out and proved the decomposition of the linker and the selectivity for caspase-3.

## Conformational Rearrangements by Water-Soluble Peptoid Foldamers

*Org. Lett.*, 9 (24), 5003 -5006, 2007.

Sung Bin Y. Shin and Kent Kirshenbaum



Peptoids are a family of N-substituted glycine oligomers that are capable of forming stable helical structures. We seek peptoid monomers that can establish a strong folding propensity in aqueous conditions. Here we utilize L-phenylalanine tert-butyl ester as a readily available reagent for the synthesis of (S)-N-(1-carboxy-2-phenylethyl)glycine oligomers. The products form stable secondary structures in aqueous solution in which the conformation is dramatically responsive to variations in pH and solvent composition.