

Chmielewski Group Literature Abstracts

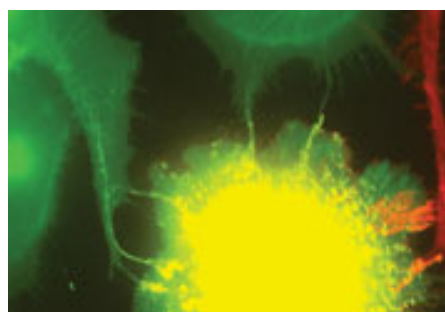
CHEMISTRY

BIOLOGY

Highlight of the Month

Nature Cell Biol. doi:10.1038/ncb1544 (2007)

Videos showing how retroviruses invade cells have revealed that some use an unexpected tactic: they can establish 'bridges' to cross from one cell to an uninfected neighbour. Walther Mothes of Yale University in New Haven, Connecticut, and his colleagues fluorescently labelled three retroviruses, including HIV, and tracked their movement between cells. Compressing a video of the process into a single picture reveals the tracks of viral particles (which appear green in the image) crossing cytoplasmic bridges. Interactions between a viral envelope protein and proteins on the uninfected cell surface seem to stabilize the bridges. The experiments were performed in cell culture, but if the findings hold in vivo they could suggest new therapies to limit retroviral spread.



January 2007

Contributing Editors:

Stefan Hershberger (*Science*)

Marcos Pires (*Nature and Nature subdivisions*)

Brandon Gaddis/Iris Geisler (*JACS*)

Jee Yeon Lee (*PNAS*)

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

Dave Przybyla (*Angewandte Chemie*)

Hilda Namanja (*ACS Chemical Biology*)

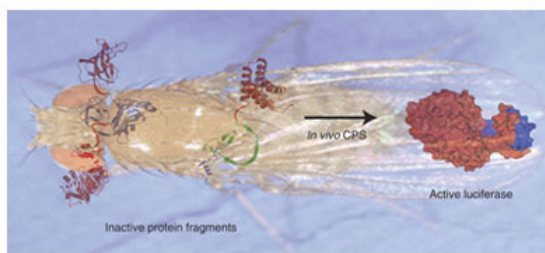
Nicole O'Neil (*Org Lett*)

Nature Chemical Biology

Post-translational enzyme activation in an animal via optimized conditional protein splicing

Nature Chemical Biology 3, 50-54 (2006)

Edmund C Schwartz, Lino Saez, Michael W Young and Tom W Muir



Control over the timing, location and level of protein activity in vivo is crucial to understanding biological function¹. Living systems are able to respond to external and internal stimuli rapidly and in a graded fashion by maintaining a pool of proteins whose activities are altered through post-translational modifications². Here we show that the process of protein trans-splicing³ can be used to modulate enzymatic activity both in cultured cells and in *Drosophila melanogaster*. We used an optimized conditional protein splicing⁴ system to rapidly trigger the in vivo ligation of two inactive fragments of firefly luciferase in a tunable manner. This technique provides a means of controlling enzymatic function with greater speed and precision than with standard genetic techniques and is a useful tool for probing biological processes.

Nature Materials

Polyarginine segments in block copolypeptides drive both vesicular assembly and intracellular delivery

Nature Materials 6, 52-57 (2007)

Eric P. Holowka, Victor Z. Sun, Daniel T. Kamei And Timothy J. Deming

Polymeric vesicles are a relatively new class of nanoscale self-assembled materials that show great promise as robust encapsulants. Compared with liposomes, use of polymeric building blocks for membrane formation allows increased stability, stimuli responsiveness and chemical diversity, which may prove advantageous for drug-delivery applications¹. A major drawback of most polymeric vesicles is the lack of biofunctionality, which restricts their ability to interact with cells and tissues. We have prepared vesicles composed of polyarginine and polyleucine segments that are stable in media, can entrap water soluble species, and can be processed to different sizes and prepared in large quantities. The remarkable feature of these materials is that the polyarginine segments both direct structure for vesicle formation and provide functionality for efficient intracellular delivery of the vesicles. This unique synergy

between nanoscale self-assembly and inherent peptide functionality provides a new approach for design of multifunctional materials for drug delivery.

Nature Nanotechnology

In vivo biodistribution and highly efficient tumour targeting of carbon nanotubes in mice

Nature Nanotechnology **2**, 47 - 52 (2006)

Zhuang Liu, Weibo Cai, Lina He, Nozomi Nakayama¹, Kai Chen, Xiaoming Sun, Xiaoyuan Chen and Hongjie Dai

Single-walled carbon nanotubes (SWNTs) exhibit unique size, shape and physical properties^{1, 2, 3} that make them promising candidates for biological applications. Here, we investigate the biodistribution of radio-labelled SWNTs in mice by in vivo positron emission tomography (PET), ex vivo biodistribution and Raman spectroscopy. It is found that SWNTs that are functionalized with phospholipids bearing polyethylene-glycol (PEG) are surprisingly stable in vivo. The effect of PEG chain length on the biodistribution and circulation of the SWNTs is studied. Effectively PEGylated SWNTs exhibit relatively long blood circulation times and low uptake by the reticuloendothelial system (RES). Efficient targeting of integrin positive tumour in mice is achieved with SWNTs coated with PEG chains linked to an arginine-glycine-aspartic acid (RGD) peptide. A high tumour accumulation is attributed to the multivalent effect of the SWNTs. The Raman signatures of SWNTs are used to directly probe the presence of nanotubes in mice tissues and confirm the radio-label-based results.

Nature Structural & Molecular Biology

An arginine ladder in OprP mediates phosphate-specific transfer across the outer membrane

Nature Structural & Molecular Biology - **14**, 85 - 87 (2007)

Trevor F Moraes¹, Manjeet Bains², Robert E W Hancock & Natalie C J Strynadka

The outer membrane protein OprP mediates the transport of essential phosphate anions into the pathogenic bacterium *Pseudomonas aeruginosa*. Here we report the crystallographic structure of trimeric OprP at 1.9-Å resolution, revealing an unprecedented 9-residue arginine 'ladder' that spans from the extracellular surface down through a constriction zone where phosphate is coordinated. Lysine residues coat the inner periplasmic surface, creating an 'electropositive sink' that pulls the phosphates through the eyelet and into the cell.

Science

An Inward-Facing Conformation of a Putative Metal-Chelate-Type ABC Transporter

Science Vol 315, Issue 5810, 373-377, 19 January 2007

H. W. Pinkett,¹ A. T. Lee,¹ P. Lum, K. P. Locher, D. C. Rees

The crystal structure of a putative metal-chelate-type adenosine triphosphate (ATP)-binding cassette (ABC) transporter encoded by genes HI1470 and HI1471 of *Haemophilus influenzae* has been solved at 2.4 angstrom resolution. The permeation pathway exhibits an inward-facing conformation, in contrast to the outward-facing state previously observed for the homologous vitamin B12 importer BtuCD. Although the structures of both HI1470/1 and BtuCD have been solved in nucleotide-free states, the pairs of ABC subunits in these two structures differ by a translational shift in the plane of the membrane that coincides with a repositioning of the membrane-spanning subunits. The differences observed between these ABC transporters involve relatively modest rearrangements and may serve as structural models for inward- and outward-facing conformations relevant to the alternating access mechanism of substrate translocation.

PNAS

Small-molecule agonists for the glucagon-like peptide 1 receptor

PNAS | January 16, 2007 | vol. 104 | no. 3 | 937-942

Madsen, Preben H. Olesen, Jacob S. Petersen*, Fritz Poulsen*, Ulla G. Sidelmann , Jeppe Sturis , Larry Truesdale||, John May , and Jesper Lau

The peptide hormone glucagon-like peptide (GLP)-1 has important actions resulting in glucose lowering along with weight loss in patients with type 2 diabetes. As a peptide hormone, GLP-1 has to be administered by injection. Only a few small-molecule agonists to peptide hormone receptors have been described and none in the B family of the G protein coupled receptors to which the GLP-1 receptor belongs. We have discovered a series of small molecules known as ago-allosteric modulators selective for the human GLP-1 receptor. These compounds act as both allosteric activators of the receptor and independent agonists. Potency of GLP-1 was not changed by the allosteric agonists, but affinity of GLP-1 for the receptor was increased. The most potent compound identified stimulates glucose-dependent insulin release from normal mouse islets but, importantly, not from GLP-1 receptor knockout mice. Also, the compound stimulates insulin release from perfused rat pancreas in a manner additive with GLP-1 itself. These compounds may lead to the identification or design of orally active GLP-1 agonists.

B-Sheet core of human prion protein amyloid fibrils as determined by hydrogen/deuterium exchange

PNAS | January 30, 2007 | vol. 104 | no. 5 | 1510-1515

Xiaojun Lu*, , Patrick L. Wintrobe*, and Witold K. Surewicz

Propagation of transmissible spongiform encephalopathies is associated with the conversion of normal prion protein, PrPC, into a misfolded, oligomeric form, PrPSc. Although the high-resolution structure of the PrPC is well characterized, the structural properties of PrPSc remain elusive. Here we used MS analysis of H/D backbone amide exchange to examine the structure of amyloid fibrils formed by the recombinant human PrP corresponding to residues 90–231 (PrP90–231), a misfolded form recently reported to be infectious in transgenic mice overexpressing PrPC. Analysis of H/D exchange data allowed us to map the systematically H-bonded β -sheet core of PrP amyloid to the C-terminal region (starting at residue 169) that in the native structure of PrP monomer corresponds to β -helix 2, a major part of β -helix 3, and the loop between these two helices. No extensive hydrogen bonding (as indicated by the lack of significant protection of amide hydrogens) was detected in the N-terminal part of PrP90–231 fibrils, arguing against the involvement of residues within this region in stable β -structure. These data provide long-sought experimentally derived constraints for high-resolution structural models of PrP amyloid fibrils.

Journal of the American Chemical Society

Kinase-Catalyzed Biotinylation for Phosphoprotein Detection

J. Am. Chem. Soc., 2007, 129 (1), 10 -11

Keith D. Green and Mary Kay H. Pflum

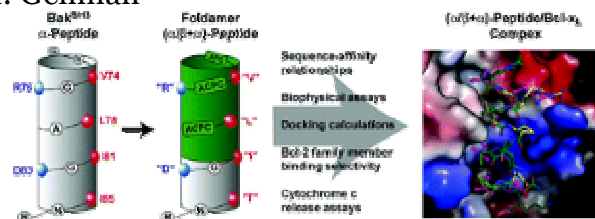


Protein phosphorylation plays a critical role in a variety of cellular functions. As a result, the monitoring of phosphoproteins in cells represents an important goal for proteomics research. To facilitate phosphoprotein detection, the first enzymatic phosphorylation-dependent biotinylation reaction of proteins is described. Specifically, kinase enzymes were coupled with an ATP-biotin conjugate to efficiently biotinylate substrate peptides and proteins after phosphate transfer. The kinase-mediated biotinylation reaction enables efficient detection of phosphoproteins in cell lysates or phosphopeptides after trypsin proteolysis, demonstrating its utility for proteomics research. Importantly, the studies reveal the cosubstrate promiscuity of kinase enzymes, laying the foundation for development of new chemical tools targeting the phosphoproteome.

$(\alpha/\beta + \gamma)$ -Peptide Antagonists of BH3 Domain/Bcl-xL Recognition: Toward General Strategies for Foldamer-Based Inhibition of Protein-Protein Interactions

J. Am. Chem. Soc., 2007, 129 (1), 139 -154

Jack D. Sadowsky,[†] W. Douglas Fairlie, Erik B. Hadley, Hee-Seung Lee, Naoki Umezawa, Zaneta Nikolovska-Coleska, Shaomeng Wang, David C. S. Huang, York Tomita, and Samuel H. Gellman

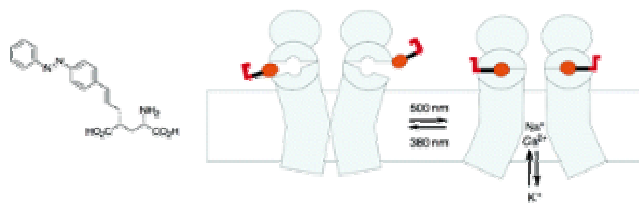


The development of molecules that bind to specific protein surface sites and inhibit protein-protein interactions is a fundamental challenge in molecular recognition. New strategies for approaching this challenge could have important long-term ramifications in biology and medicine. We are exploring the concept that unnatural oligomers with well-defined conformations ("foldamers") can mimic protein secondary structural elements and thereby block specific protein-protein interactions. Here, we describe the identification and analysis of helical peptide-based foldamers that bind to a specific cleft on the anti-apoptotic protein Bcl-xL by mimicking an α -helical BH3 domain. Initial studies, employing a fluorescence polarization (FP) competition assay, revealed that among several α/β - and β -peptide foldamer backbones only α/β -peptides intended to adopt 14/15-helical secondary structure display significant binding to Bcl-xL. The most tightly binding Bcl-xL ligands are chimeric oligomers in which an N-terminal α/β -peptide segment is fused to a C-terminal α -peptide segment ($(\alpha/\beta + \alpha)$ -peptides). Sequence-affinity relationships were probed via standard and nonstandard techniques (alanine scanning and hydrophile scanning, respectively), and the results allowed us to construct a computational model of the ligand/Bcl-xL complex. Analytical ultracentrifugation with a high-affinity $(\alpha/\beta + \alpha)$ -peptide established 1:1 ligand:Bcl-xL stoichiometry under FP assay conditions. Binding selectivity studies with the most potent $(\alpha/\beta + \alpha)$ -peptide, conducted via surface plasmon resonance measurements, revealed that this ligand binds tightly to Bcl-w as well as to Bcl-xL, while binding to Bcl-2 is somewhat weaker. No binding could be detected with Mcl-1. We show that our most potent $(\alpha/\beta + \alpha)$ -peptide can induce cytochrome C release from mitochondria, an early step in apoptosis, in cell lysates, and that this activity is dependent upon inhibition of protein-protein interactions involving Bcl-xL.

Reversibly Caged Glutamate: A Photochromic Agonist of Ionotropic Glutamate Receptors

J. Am. Chem. Soc., 2007, 129 (2), 260 -261

Matthew Volgraf,[†] Pau Gorostiza,[‡] Stephanie Szobota,[§] Max R. Helix,[†] Ehud Y. Isacoff,^{*†#} and Dirk Trauner

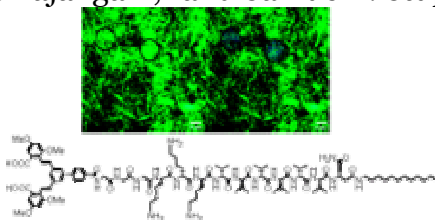


The design, synthesis, and biological evaluation of a photochromic glutamate analogue is described. The molecule functions as a reversibly caged neurotransmitter and can be used to influence neural activity with light.

Modulation of Fluorescence through Coassembly of Molecules in Organic Nanostructures

J. Am. Chem. Soc., 2007, 129 (2), 321 -327

Heather A. Behanna,[†] Kanya Rajangam,[‡] and Samuel I. Stupp

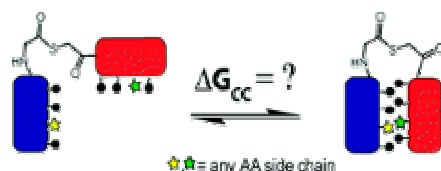


This paper describes the fluorescence of bimolecular coassemblies that form one-dimensional nanostructures. One molecule is a fluorescent peptide amphiphile containing its branched stilbene chromophore covalently linked to the hydrophilic end of the amphiphile, and the second molecule is a shorter, nonfluorescent peptide amphiphile of complementary charge. Using circular dichroism we observe that mixing both molecules results in coassemblies that exhibit a β -sheet signature in the peptide region indicative of these types of nanostructures. The nature of the coassembly is dependent on the molar ratio of each component, and the changing CD spectra suggest the formation of domains along the length of the nanofibers with decreasing concentrations of the fluorescent component. In coassemblies with dilute concentrations of the fluorophore, we observe an increase in fluorescence intensity and quantum yield, as well as chiral transfer to the achiral segment of the fluorescent peptide amphiphile. The coassemblies studied containing a fluorescent component at a low molar ratio exhibit fluorescence resonance energy transfer to fluorescent acceptors in solution. When the nonfluorescent peptide amphiphile component is designed to bind the important bioactive polysaccharide heparin, a selective transfer of energy is observed between fluorescein-tagged heparin and the coassemblies in both dilute solution and in macroscopic gels.

An Antiparallel α -Helical Coiled-Coil Model System for Rapid Assessment of Side-Chain Recognition at the Hydrophobic Interface

Erik B. Hadley and Samuel H. Gellman

J. Am. Chem. Soc., 128 (51), 16444 -16445, 2006.

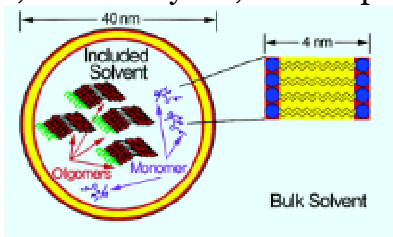


Both parallel and antiparallel α -helical coiled-coil dimers are common among proteins; however, biophysical scrutiny has focused almost entirely on parallel dimers. We describe the development of a model system that enables efficient and systematic analysis of hydrophobic packing between antiparallel α -helices. Our findings reveal significant differences in packing preferences between parallel and antiparallel coiled-coils.

Encapsulation and NMR on an Aggregating Peptide before Fibrillogenesis

J. Am. Chem. Soc., **128** (51), 16460 -16461, 2006

Kristi L. Lazar, Josh W. Kurutz,[†] Robert Tycko,[|] and Stephen C. Meredith



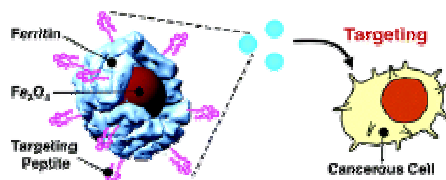
The early stages of peptide and protein aggregation include the formation of soluble oligomers, some of which may be cytotoxic. There is a paucity of structural information on these oligomers, however, because they are temporally unstable and tend to aggregate further into insoluble protofibrils and fibrils. To obtain structural information on soluble oligomers, we have developed a procedure for encapsulating a fibril-forming peptide, Peptide 1 (NH₂-SDDYYYGFGSNKFGPRDD-COOH), in 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine single bilayer vesicles (POPC SBVs). We also encapsulated a non-fibril forming peptide, Peptide 2 (NH₂-EEWEE-COOH), in POPC SBVs. The nominal concentration of Peptide 1 in the resulting 40 nm diameter SBVs was 2.4 ± 0.1 mM, well above the concentration at which Peptide 1 forms fibrils. We demonstrated that these peptides had indeed been encapsulated by measuring longitudinal relaxation times (T₁) in the presence and absence of a paramagnetic substance, 1 mM Gd-EDTA, by NMR spectroscopy. When the peptides were free in solution, they showed the expected shortening of T₁ times and broadening of NMR peaks. In contrast, peptide encapsulated in POPC SBVs were shielded from the effects of Gd-EDTA and showed preservation of T₁ values and NMR line widths. To demonstrate that encapsulation inhibits fibril formation, we measured one-dimensional proton (1D-1H) NMR spectra of the peptides in solution, and of the encapsulated peptides immediately after encapsulation, and 4 days after encapsulation, because Peptide 1 forms fibrils within 1 day. A 2.8 mM solution of Peptide 1 shows the loss of NMR signal expected for a fibrillizing peptide. In contrast, the 1D-1H spectra of encapsulated Peptide 1 measured immediately after encapsulation and 4 days after encapsulation were essentially identical, with preservation of line width at 4 days, i.e., well within the time frame of most high-resolution NMR experiments. Encapsulation may provide a means to obtain high-resolution NMR data on unstable

soluble oligomers of peptides implicated in amyloidoses such as Alzheimer's Disease and provide the first detailed structural information about these possibly cytotoxic species that have hitherto been inaccessible to analysis.

Targeting of Cancer Cells with Ferrimagnetic Ferritin Cage Nanoparticles

J. Am. Chem. Soc., **128** (51), 16626 -16633, 2006.

Masaki Uchida, Michelle L. Flenniken, Mark Allen, Deborah A. Willits, Bridgid E. Crowley, Susan Brumfield, Ann F. Willis, Larissa Jackiw, Mark Jutila, Mark J. Young, and Trevor Douglas

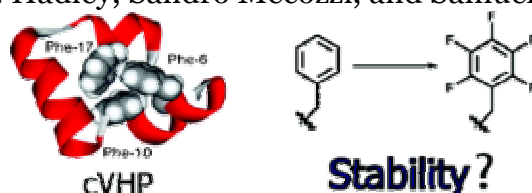


Protein cage architectures such as virus capsids and ferritins are versatile nanoscale platforms amenable to both genetic and chemical modification. Incorporation of multiple functionalities within these nanometer-sized protein architectures demonstrate their potential to serve as functional nanomaterials with applications in medical imaging and therapy. In the present study, we synthesized an iron oxide (magnetite) nanoparticle within the interior cavity of a genetically engineered human H-chain ferritin (HF_n). A cell-specific targeting peptide, RGD-4C which binds $\alpha_v\beta_3$ integrins upregulated on tumor vasculature, was genetically incorporated on the exterior surface of HF_n. Both magnetite-containing and fluorescently labeled RGD4C-F_n cages bound C32 melanoma cells in vitro. Together these results demonstrate the capability of a genetically modified protein cage architecture to serve as a multifunctional nanoscale container for simultaneous iron oxide loading and cell-specific targeting.

Stabilizing and Destabilizing Effects of Phenylalanine →F5-Phenylalanine Mutations on the Folding of a Small Protein

J. Am. Chem. Soc., **128** (50), 15932 -15933, 2006.

Matthew G. Woll, Erik B. Hadley, Sandro Mecozzi, and Samuel H. Gellman



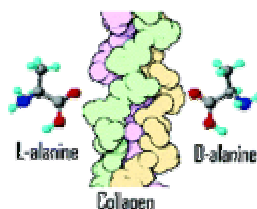
We report a systematic evaluation of phenylalanine-to-pentafluorophenylalanine (Phe → F5-Phe) mutants for the 35-residue chicken villin headpiece subdomain (c-VHP), the hydrophobic core of which features a cluster of three Phe side chains (residues 6, 10, and 17). Phe → F5-Phe mutations are interesting because aryl-perfluoroaryl interactions of optimal geometry are intrinsically more favorable than aryl-aryl interactions and because perfluoroaryl units are more hydrophobic than are analogous aryl units. One

mutant, Phe-10 →F5-Phe, provides enhanced tertiary structural stability relative to the native sequence. The other six mutants analyzed caused a decrease in stability.

Collagen Fibers as a Chiral Agent: A Demonstration of Stereochemistry Effects

J. Am. Chem. Soc., **128** (50), 15956 -15957, 2006.

Uzi Eliav* and Gil Navon

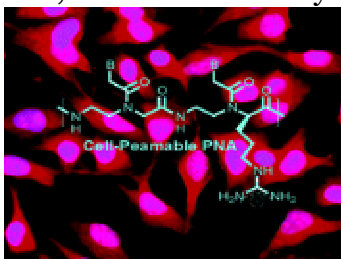


The collagen is the most common protein in mammals. Thus its interaction with small molecules and particularly amino acids is of interest. Owing to the high degree of order of collagen fibers in a tendon, the 1H-1H and 1H-13C dipolar interactions and the 2H quadrupolar interaction of small molecules interacting with it do not average to zero. In the present work we report that these residual interactions for alanine in intact tendons are significantly different for the L and D enantiomers meaning that the collagen in its native state acts as a chiral agent. The different L/D ratios for each of the residual interactions along the different vectors in the alanine molecule and the similarly transferred NOE from the collagen to the L and D enantiomers indicate that the main source of the different residual dipolar and quadrupolar interactions is the stereochemistry of the binding and not the amounts of bound molecules.

Cell-Permeable Peptide Nucleic Acid Designed to Bind to the 5'-Untranslated Region of E-cadherin Transcript Induces Potent and Sequence-Specific Antisense Effects

J. Am. Chem. Soc., **128** (50), 16104 -16112, 2006.

Anca Dragulescu-Andrasi,[†] Srinivas Rapireddy,[†] Gaofei He,[†] Birendra Bhattacharya, Jens J. Hyldig-Nielsen,[‡] Gerald Zon,[‡] and Danith H. Ly



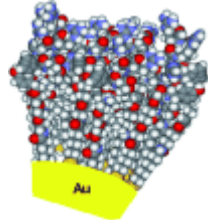
Establishing a general and effective method for regulating gene expression in mammalian systems is important for many aspects of biological and biomedical research. Herein we report the antisense activities of a cell-permeable, guanidine-based peptide nucleic acid (PNA) called GPNA. We show that a GPNA oligomer designed to bind to the transcriptional start-site of human E-cadherin gene induces potent and sequence-specific antisense effects and is less toxic to the cells than the corresponding PNA-polyarginine conjugate. GPNA confers its silencing effect by blocking protein

translation. The findings reported in this study provide a molecular framework for designing the next generation cell-permeable nucleic acid mimics for regulating gene expression in live cells and intact organisms.

Angewandte Chemie

Substrate Modulation of the Activity of an Artificial Nanoesterase Made of Peptide-Functionalized Gold Nanoparticles

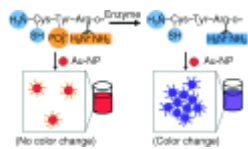
(p 400-404) *Paolo Pengo, Lars Baltzer, Lucia Pasquato, Paolo Scrimin*



Nanozymes with a heart of gold: A functional artificial protein has been prepared by grafting a dodecapeptide onto the surface of gold nanoparticles (see picture). The system catalyzes the hydrolysis of carboxylate esters and features enzyme-like properties.

Sensing Phosphatase Activity by Using Gold Nanoparticles

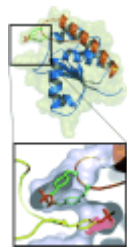
(p 707-709) *Yongdoo Choi, Nan-Hui Ho, Ching-Hsuan Tung*



To change your colors: A colorimetric enzyme assay based on peptide-induced aggregation of gold nanoparticles (Au-NPs) has been developed to sense phosphatase activity. Once the phosphate group is eliminated by alkaline phosphatase, peptide-induced Au-NP aggregation is triggered, resulting in a significant change in absorbance at 650 nm.

Linking Chemistry and Biology for the Study of Protein Function

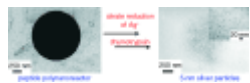
(p 826-829) *Daniel Rauh, Herbert Waldmann*



Chop and change: Techniques that combine chemistry and biology for the modification of proteins have proved themselves to be a good means to study protein function. Expressed protein ligation (EPL), with the help of preparative amounts of phospholated tyrosinephosphatases, prenylated variants of Rab-GTPase Ypt1, biochemically characterized the influence of phosphorylation on the function of phosphatases (see picture; blue: phosphatase; orange: synthetic peptide).

A Self-Assembling Peptide Polyanoreactor

(p 969-972) Maxim G. Ryadnov



What goes on within: A mesoscopic nanoporous dendrimer-like assembly of peptides hosts numerous nanometer-sized cavities that function as encapsulating sites. As an example, the assembly amplifies a single silver nanoparticle into many discrete nanoparticles of uniform size, and thus it acts as a polyanoreactor (see picture).

ACS Chemical Biology

Small-Molecule Screening: Advances in Microarraying and Cell-Imaging Technologies

ACS Chem. Biol. **2** (1), 24–30

Rebecca L. Nicholson[†], Martin Welch[‡], Mark Ladlow[§], and David R. Spring

Cell-permeable small molecules can be used to modulate protein function selectively, rapidly, reversibly, and conditionally with temporal and quantitative control in biological systems. The identification of these chemical probes can require the screening of large numbers of small molecules. With the advent of new technologies, small-molecule high-throughput screening is widely available. This Review focuses on the emerging technologies of microarray screening platforms and high-content screening formats.

Chemical Tools for Biomolecular Imaging

ACS Chem. Biol. **2** (1), 31–38

Nils Johnsson^{†,*} and Kai Johnsson

The visualization of biologically relevant molecules and activities inside living cells continues to transform cell biology into a truly quantitative science. However, despite the spectacular achievements in some areas of cell biology, the majority of cellular processes still operate invisibly, not illuminated by even our brightest laser beams. Further progress therefore will depend not only on improvements in instrumentation but also increasingly on the development of new fluorophores and fluorescent sensors to target these activities. In the following, we review some of the recent approaches to generating such sensors, the methods to attach them to selected biomolecules, and their applications to various biological problems.

Chemistry and Biology

Electron Hole Flow Patterns through the RNA-Cleaving 8-17 Deoxyribozyme Yield Unusual Information about Its Structure and Folding

Chemistry & Biology 14, 41–51, January 2007

Edward K.Y. Leung and Dipankar Sen

DNA double helices have been shown to conduct electron holes over significant distances. Here, we report on the hole flow patterns within a more intricately folded DNA complex, the 8-17 deoxyribozyme bound to a DNA pseudosubstrate, incorporating three helical elements and two catalytically relevant loops. The observed hole flow patterns within the complex permitted a quantitative assessment of the stacking preferences of the three constituent helices and provided evidence for significant transitions within the complex's global geometry. The patterns further suggested varying levels of solvent exposure of the complex's constituent parts, and revealed that a catalytically relevant cytosine within the folded complex exists in an unusual structural/electronic environment. Our data suggest that the study of charge flow may provide novel perspectives on the structure and folding of intricately folded DNAs and RNAs.

The Binding Avidity of a Nanoparticle-Based Multivalent Targeted Drug Delivery Platform

Chemistry & Biology 14, 107–115, January 2007

Seungpyo Hong, Pascale R. Leroueil, István J. Majoros, Bradford G. Orr, Jr., James R. Baker and Mark M. Banaszak Holl

Dendrimer-based anticancer nanotherapeutics containing 5 folate molecules have shown in vitro and in vivo efficacy in cancer cell targeting. Multivalent interactions have been inferred from observed targeting efficacy, but have not been experimentally proven. This study provides quantitative and systematic evidence for multivalent interactions between these nanodevices and folate-binding protein (FBP). A series of the nanodevices were synthesized by conjugation with different amounts of folate. Dissociation constants (KD) between the nanodevices and FBP measured by SPR are dramatically enhanced through multivalency (2,500- to 170,000-fold). Qualitative evidence is also provided for a multivalent targeting effect to KB cells using flow cytometry. These data support the hypothesis that multivalent enhancement of KD, not an enhanced rate of endocytosis, is the key factor resulting in the improved biological targeting by these drug delivery platforms.

Chemical Biology and Drug Design

Binding Pathways of Ligands to HIV-1 Protease: Coarse-grained and Atomistic Simulations

Chemical Biology & Drug Design 69 (1), 5–13

Chia-En A. Chang, Joanna Trylska, Valentina Tozzini, J. Andrew McCammon

Multiscale simulations (coarse-grained Brownian dynamics simulations and all-atom molecular dynamics simulations in implicit solvent) were applied to reveal the binding processes of ligands as they enter the binding site of the HIV-1 protease. The initial structures used for the molecular dynamics simulations were generated based on the Brownian dynamics trajectories, and this is the first molecular dynamics simulation of modeling the association of a ligand with the protease. We found that a protease substrate successfully binds to the protein when the flaps are fully open. Surprisingly, a smaller cyclic urea inhibitor (XK263) can reach the binding site when the flaps are not fully open. However, if the flaps are nearly closed, the inhibitor must rearrange or binding can fail because the inhibitor cannot attain proper conformations to enter the binding site. Both the peptide substrate and XK263 can also affect the protein's internal motion, which may help the flaps to open. Simulations allow us to efficiently study the ligand binding processes and may help those who study drug discovery to find optimal association pathways and to design those ligands with the best binding kinetics.

Site-specific Fluorescent Labeling of Poly-histidine Sequences Using a Metal-chelating Cysteine

Chemical Biology & Drug Design 69 (1), 31–40

Beena Krishnan, Aneta Szymanska, Lila M. Gierasch

Coupling genetically encoded target sequences with specific and selective labeling strategies has made it possible to utilize fluorescence spectroscopy in complex mixtures to investigate the structure, function, and dynamics of proteins. Thus, there is a growing need for a repertoire of such labeling approaches to deploy based on a given application and to utilize in combination with one another by orthogonal reactivity. We have developed a simple approach to synthesize a fluorescent probe that binds to a poly-histidine sequence. The amino group of cysteine was converted into nitrilotriacetate to create a metal-chelating cysteine molecule, Cys-nitrilotriacetate. Two Cys-nitrilotriacetate molecules were then cross-linked using dibromobimane to generate a fluorophore capable of binding a His-tag on a protein, NTA2-BM. NTA2-BM is a potential fluorophore for selective tagging of proteins in vivo.

Molecular Conceptor™ for Training in Medicinal Chemistry, Drug Design, and Cheminformatics

Chemical Biology & Drug Design 69 (1), 75–82

Claude Cohen, Ouri Fischel, Elie Cohen

Current emphasis on structure-based design and other computational methods have encouraged medicinal chemists to learn traditionally 'expert' techniques of molecular modeling, computer-aided drug design, and cheminformatics. Molecular Conceptor™ (Synergix Ltd) is a multimedia software for teaching three-dimensional drug design principles. It present techniques and strategies used in drug design and cheminformatics with general guidelines for their successful application. Discovery of lead compounds and concepts are illustrated with manipulatable views of molecules,

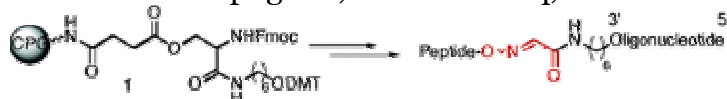
pharmacophores, and protein–ligand complexes. It is a unique teaching and learning aid for medicinal chemists, instructors, students, and others who need in-depth knowledge of these important techniques, as well as a valuable refresher course for professional modelers.

Organic Letters

New Solid Support for the Synthesis of 3'-Oligonucleotide Conjugates through Glyoxylic Oxime Bond Formation

Organic Letters - 9, 219 -222 (2007)

Nicolas Spinelli, Om Prakash Edupuganti, Eric Defrancq, & Pascal Dumy

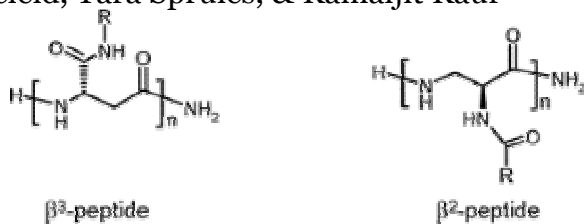


A novel solid support 1 was synthesized to incorporate glyoxylic aldehyde functionality at the oligonucleotide 3'-terminus. 6-mer and 11-mer oligonucleotide sequences containing 3'-glyoxylic aldehyde functionality were prepared by using this support. These modified oligonucleotides were coupled to reporters containing an aminoxy group to prepare oligonucleotide 3'-conjugates through glyoxylic oxime bond formation. The hydrolytic stability of a glyoxylic oxime linkage was also investigated.

Solid-Phase Synthesis and CD Spectroscopic Investigations of Novel β^3 -Peptides from L-Aspartic Acid and β^2 -Amino-L-alanine

Organic Letters - 9, 25 -28 (2007)

Sahar Ahmed, Reem Beleid, Tara Sprules, & Kamaljit Kaur



A solid-phase synthesis method for the preparation of novel β^3 - and β^2 -peptides derived from L-aspartic acid and β^2 -amino-L-alanine, respectively, is described. The methodology allows independent buildup of the β^3 -peptide backbone and the introduction of sequential side chain substitutions. Representative peptides from the two classes, an amino-substituted β^3 -hexapeptide and an acyl-substituted β^2 -hexapeptide, have been prepared, and their solution conformation is studied by circular dichroism (CD) spectroscopy.