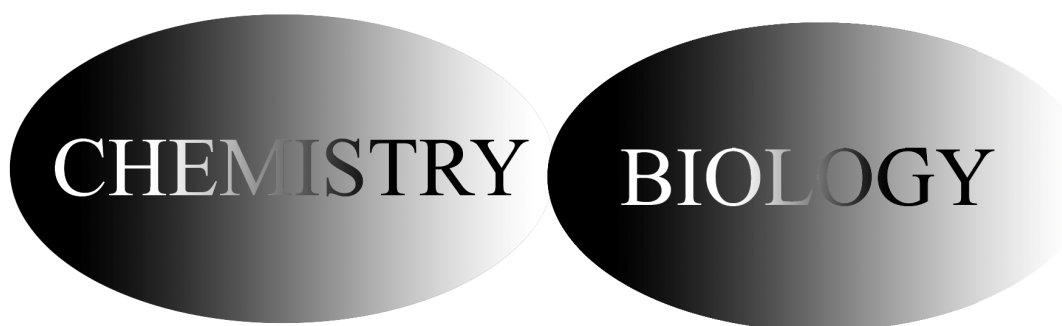


# Chmielewski Group Literature Abstracts



**August 2007**

***Contributing Editors:***

Stefan Hershberger (*Science*)

Marcos Pires (*Nature and Nature subdivisions*)

Brandon Gaddis/Iris Geisler (*JACS*)

Jee Yeon Lee (*PNAS*)

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

Dave Przybyla (*Angewandte Chemie*)

Hilda Namanja (*Chem & Bio*)

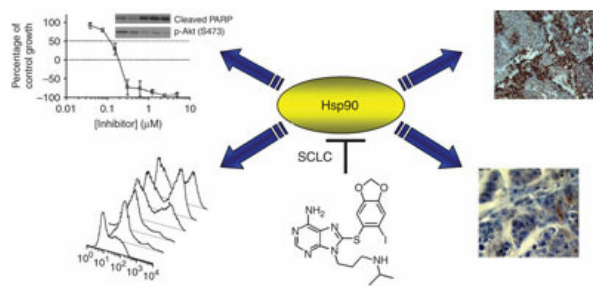
Nicole O'Neil (*Org Lett*)

## Nature Chemical Biology

### Selective compounds define Hsp90 as a major inhibitor of apoptosis in small-cell lung cancer

*Nature Chemical Biology* 3, 498-507 (2007)

Sara Felts<sup>4</sup>, Peter Wipf<sup>5</sup>, Joan Massague<sup>2</sup>, Xuejun Jiang<sup>6</sup>, Jeffrey L Brodsky<sup>7</sup>, Geoffrey W Krystal<sup>3</sup> & Gabriela Chiosis



The heat shock protein 90 (Hsp90) has a critical role in malignant transformation. Whereas its ability to maintain the functional conformations of mutant and aberrant oncoproteins is established, a transformation-specific regulation of the antiapoptotic phenotype by Hsp90 is poorly understood. By using selective compounds, we have discovered that small-cell lung carcinoma is a distinctive cellular system in which apoptosis is mainly regulated by Hsp90. Unlike the well-characterized antiapoptotic chaperone Hsp70, Hsp90 is not a general inhibitor of apoptosis, but it assumes this role in systems such as small-cell lung carcinoma, in which apoptosis is uniquely dependent on and effected through the intrinsic pathway, without involvement of caspase elements upstream of mitochondria or alternate pathways that are not apoptosome-channeled. These results provide important evidence for a transformation-specific interplay between chaperones in regulating apoptosis in malignant cells.

## Nature Materials

### Synthetic pores with reactive signal amplifiers as artificial tongues

*Nature Materials* 6, 576 - 580 (2007)

Svetlana Litvinchuk<sup>1</sup>, Hiroyuki Tanaka<sup>1</sup>, Tomohiro Miyatake<sup>1,2</sup>, Dario Pasini<sup>1,3</sup>, Takatsugu Tanaka<sup>1,4</sup>, Guillaume Bollot<sup>1</sup>, Jiri Mareda<sup>1</sup> & Stefan Matile

The sensation of taste is mediated by activation or deactivation of transmembrane pores. Artificial stimulus-responsive pores are enormously appealing as sensor components because changes in their activity are readily detectable in many different ways. However, the detection of multiple components in complex matrices (such as foods) with one pore sensor has so far remained elusive because the specificity necessary for sensing a target compound in complex mixtures is incompatible with the broad applicability needed for the detection of multiple components. Here, we present synthetic pores that, like our tongues, can sense flavours in food and in addition make

them visibly detectable. Differential sensing and pattern recognition are solutions based on empirical and biomimetic approaches. They have been explored with synthetic receptor arrays and electronic tongues. In contrast, our approach is non-empirical as it exploits reactive amplifiers that covalently capture elusive analytes after enzymatic signal generation and drag them into synthetic pores for blockage. Reactive amplification proved to be highly sensitive and adaptable to various analytes and pores. Moreover, it can be combined with reactive filtration for minimizing interference. The system was tested on real food samples for detection of sucrose, acetate, citrate and glutamate to demonstrate the feasibility of these synthetic pores as universal sensors.

## Science

### Forced Unfolding of Proteins Within Cells

*Science Vol 317, Issue 5838, 663-666, 3 August 2007*

Colin P. Johnson Hsin-Yao Tang, Christine Carag, David W. Speicher, Dennis E. Discher

To identify cytoskeletal proteins that change conformation or assembly within stressed cells, in situ labeling of sterically shielded cysteines with fluorophores was analyzed by fluorescence imaging, quantitative mass spectrometry, and sequential two-dye labeling. Within red blood cells, shotgun labeling showed that shielded cysteines in the two isoforms of the cytoskeletal protein spectrin were increasingly labeled as a function of shear stress and time, indicative of forced unfolding of specific domains. Within mesenchymal stem cells—as a prototypical adherent cell—nonmuscle myosin IIA and vimentin are just two of the cytoskeletal proteins identified that show differential labeling in tensed versus drug-relaxed cells. Cysteine labeling of proteins within live cells can thus be used to fluorescently map out sites of molecular-scale deformation, and the results also suggest means to colocalize signaling events such as phosphorylation with forced unfolding.

### Direct Synthesis of Amides from Alcohols and Amines with Liberation of H<sub>2</sub>

*Science Vol 317, Issue 5839, 790-792, 10 August 2007*

Chidambaram Gunanathan, Yehoshoa Ben-David, David Milstein

Given the widespread importance of amides in biochemical and chemical systems, an efficient synthesis that avoids wasteful use of stoichiometric coupling reagents or corrosive acidic and basic media is highly desirable. We report a reaction in which primary amines are directly acylated by equimolar amounts of alcohols to produce amides and molecular hydrogen (the only products) in high yields and high turnover numbers. This reaction is catalyzed by a ruthenium complex based on a dearomatized PNN-type ligand [where PNN is 2-(di-tert-butylphosphinomethyl)-6-(diethylaminomethyl)pyridine], and no base or acid promoters are required. Use of primary diamines in the reaction leads to bis-amides, whereas with a mixed primary-secondary amine substrate, chemoselective acylation of the primary amine group takes

place. The proposed mechanism involves dehydrogenation of hemiaminal intermediates formed by the reaction of an aldehyde intermediate with the amine.

## **Immunization by Avian H5 Influenza Hemagglutinin Mutants with Altered Receptor Binding Specificity**

*Science Vol 317, Issue 5839, 825-828, 10 August 2007*

Zhi-Yong Yang,<sup>1\*</sup> Chih-Jen Wei,<sup>1\*</sup> Wing-Pui Kong,<sup>1</sup> Lan Wu,<sup>1</sup> Ling Xu,<sup>1</sup> David F. Smith,<sup>2</sup> Gary J. Nabel

Influenza virus entry is mediated by the receptor binding domain (RBD) of its spike, the hemagglutinin (HA). Adaptation of avian viruses to humans is associated with HA specificity for  $\alpha$ 2,6- rather than  $\alpha$ 2,3-linked sialic acid (SA) receptors. Here, we define mutations in influenza A subtype H5N1 (avian) HA that alter its specificity for SA either by decreasing  $\alpha$ 2,3- or increasing  $\alpha$ 2,6-SA recognition. RBD mutants were used to develop vaccines and monoclonal antibodies that neutralized new variants. Structure-based modification of HA specificity can guide the development of preemptive vaccines and therapeutic monoclonal antibodies that can be evaluated before the emergence of human-adapted H5N1 strains.

## **Structure and Function of an Essential Component of the Outer Membrane Protein Assembly Machine**

*Science Vol 317, Issue 5840, 961-964, 17 August 2007*

Seokhee Kim,<sup>1</sup> Juliana C. Malinverni,<sup>2</sup> Piotr Sliz,<sup>3,4</sup> Thomas J. Silhavy,<sup>2</sup> Stephen C. Harrison,<sup>3,4</sup> Daniel Kahne

Integral  $\beta$ -barrel proteins are found in the outer membranes of mitochondria, chloroplasts, and Gram-negative bacteria. The machine that assembles these proteins contains an integral membrane protein, called YaeT in *Escherichia coli*, which has one or more polypeptide transport-associated (POTRA) domains. The crystal structure of a periplasmic fragment of YaeT reveals the POTRA domain fold and suggests a model for how POTRA domains can bind different peptide sequences, as required for a machine that handles numerous  $\beta$ -barrel protein precursors. Analysis of POTRA domain deletions shows which are essential and provides a view of the spatial organization of this assembly machine.

## **PNAS**

## **HIV protease inhibitors block the zinc metalloproteinase ZMPSTE24 and lead to an accumulation of prelamin A in cells**

*PNAS | August 14, 2007 | vol. 104 | no. 33 | 13432-13437*

Catherine Coffinier\*,<sup>†</sup> Sarah E. Hudon<sup>‡</sup>, Emily A. Farber\*, Sandy Y. Chang\*, Christine A. Hrycyna<sup>†,‡</sup>, Stephen G. Young\*,<sup>†</sup> and Loren G. Fong

HIV protease inhibitors (HIV-PIs) target the HIV aspartyl protease, which cleaves the HIV gag-pol polyprotein into shorter proteins required for the production of new

virions. HIV-PIs are a cornerstone of treatment for HIV but have been associated with lipodystrophy and other side effects. In both human and mouse fibroblasts, we show that HIV-PIs caused an accumulation of prelamins A. The prelamins A in HIV-PI-treated fibroblasts migrated more rapidly than nonfarnesylated prelamins A, comigrating with the farnesylated form of prelamins A that accumulates in ZMPSTE24-deficient fibroblasts. The accumulation of farnesyl-prelamins A in response to HIV-PI treatment was exaggerated in fibroblasts heterozygous for Zmpste24 deficiency. HIV-PIs inhibited the endoproteolytic processing of a GFP-prelamins A fusion protein. The HIV-PIs did not affect the farnesylation of HDJ-2, nor did they inhibit protein farnesyltransferase in vitro. HIV-PIs also did not inhibit the activities of the isoprenyl-cysteine carboxyl methyltransferase ICMT or the prenylprotein endoprotease RCE1 in vitro, but they did inhibit ZMPSTE24 (IC<sub>50</sub>: lopinavir, 18.4 ± 4.6 μM; tipranavir, 1.2 ± 0.4 μM). We conclude that the HIV-PIs inhibit ZMPSTE24, leading to an accumulation of farnesyl-prelamins A. The inhibition of ZMPSTE24 by HIV-PIs could play a role in the side effects of these drugs.

## Targeting amyloid-β in glaucoma treatment

***PNAS* | August 14, 2007 | vol. 104 | no. 33 | 13444-13449**

Li Guo\*, Thomas E. Salt†, Vy Luong‡, Nicholas Wood\*, William Cheung\*, Annelie Maass\*, Giulio Ferrari\*,‡, Françoise Russo-Marie§,¶, Adam M. Sillito†, Michael E. Cheetham||, Stephen E. Moss§, Frederick W. Fitzke†, and M. Francesca Cordeiro

The development of the devastating neurodegenerative condition, Alzheimer's disease, is strongly associated with amyloid-β (Aβ) deposition, neuronal apoptosis, and cell loss. Here, we provide evidence that implicates these same mechanisms in the retinal disease glaucoma, a major cause of irreversible blindness worldwide, previously associated simply with the effects of intraocular pressure. We show that Aβ colocalizes with apoptotic retinal ganglion cells (RGC) in experimental glaucoma and induces significant RGC apoptosis in vivo in a dose- and time-dependent manner. We demonstrate that targeting different components of the Aβ formation and aggregation pathway can effectively reduce glaucomatous RGC apoptosis in vivo, and finally, that combining treatments (triple therapy) is more effective than monotherapy. Our work suggests that targeting the Aβ pathway provides a therapeutic avenue in glaucoma management. Furthermore, our work demonstrates that the combination of agents affecting multiple stages in the Aβ pathway may be the most effective strategy in Aβ-related diseases.

## Evolution of a fluorinated green fluorescent protein

***PNAS* | August 28, 2007 | vol. 104 | no. 35 | 13887-13890**

Tae Hyeon Yoo, A. James Link, and David A. Tirrell

The fluorescence of bacterial cells expressing a variant (GFPm) of the green fluorescent protein (GFP) was reduced to background levels by global replacement of the leucine residues of GFPm by 5,5,5-trifluoroleucine. Eleven rounds of random mutagenesis and screening via fluorescence-activated cell sorting yielded a GFP mutant containing 20 amino acid substitutions. The mutant protein in fluorinated form showed improved

folding efficiency both in vivo and in vitro, and the median fluorescence of cells expressing the fluorinated protein was improved  $\approx 650$ -fold in comparison to that of cells expressing fluorinated GFPm. The success of this approach demonstrates the feasibility of engineering functional proteins containing many copies of abiological amino acid constituents.

## High-throughput fluorescent-based optimization of eukaryotic membrane protein overexpression and purification in *Saccharomyces cerevisiae*

*PNAS* | August 28, 2007 | vol. 104 | no. 35 | 13936-13941

Simon Newstead\*, Hyun Kim†, Gunnar von Heijne†, So Iwata\*,‡,¶,‡, and David Drew

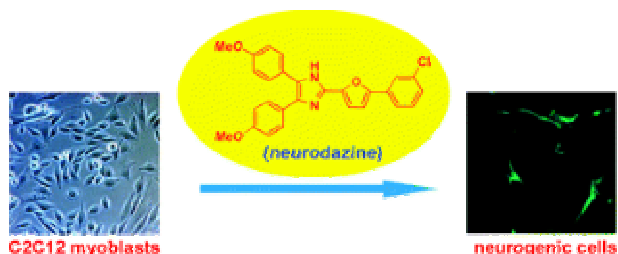
Eukaryotic membrane proteins are often difficult to produce in large quantities, which is a significant obstacle for further structural and biochemical investigation. Based on the analysis of 43 eukaryotic membrane proteins, we present a cost-effective high-throughput approach for rapidly screening membrane proteins that can be overproduced to levels of >1 mg per liter in *Saccharomyces cerevisiae*. We find that 70% of the well expressed membrane proteins tested in this system are stable, targeted to the correct organelle, and monodisperse in either Fos-choline 12 (FC-12) or n-dodecyl- $\beta$ -D-maltoside. We illustrate the advantage of such an approach, with the purification of monodisperse human and yeast nucleotide-sugar transporters to unprecedented levels. We estimate that our approach should be able to provide milligram quantities for at least one-quarter of all membrane proteins from both yeast and higher eukaryotic organisms.

## Journal of the American Chemical Society

### Synthetic Small Molecules that Induce Neurogenesis in Skeletal Muscle

*J. Am. Chem. Soc.*, 2007, 129 (30), 9258 -9259

Darren R. Williams, Myung-Ryul Lee, Young-Ah Song, Sung-Kyun Ko, Gun-Hee Kim, and Injae Shin



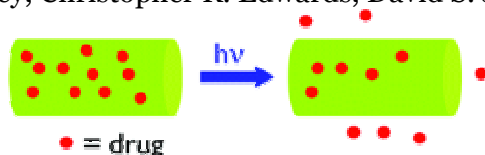
Neurons are not regenerated effectively, and their injury causes neurodegenerative diseases. These diseases may be treated by the transplantation of neural stem cells. However, ethical and technical issues restrict cell therapies using neural stem cells. A more convenient and attractive approach is the use of small molecules with the capacity to induce neurogenesis from easily available cells or tissues. Such small molecules have the potential to allow tight controls over the timing and speed of cell differentiation.

Herein, we describe the discovery of the first such molecule, neurodazine, identified by screening an imidazole library with C2C12 myoblasts. Further analyses show that neurodazine promotes the expression of neuron-specific markers in treated C2C12 cells. In addition, the use of neurodazines in conjunction with a microtubule-destabilizing agent allows neurogenic conversion of both differentiated immature myotubes and mature skeletal muscle.

## Light-Triggered Molecule-Scale Drug Dosing Devices

*J. Am. Chem. Soc.*, 2007, 129 (31), 9572 -9573

Colin P. McCoy,\* Clare Rooney, Christopher R. Edwards, David S. Jones, and Sean P. Gorman

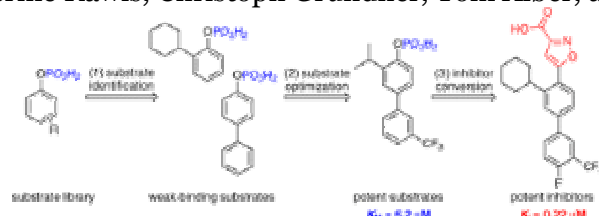


We have synthesized a range of photolabile conjugates of 3,5-dimethoxybenzoin with acetyl salicylic acid, ibuprofen, and ketoprofen. These conjugates can be precisely kinetically controlled to liberate free drug following exposure to 365 nm light for defined periods. Incorporation of the conjugates into limited-porosity hydrogels, comprising a hydrated copolymer of 2-(hydroxyethyl) methacrylate and methyl methacrylate, crosslinked with ethylene glycol dimethacrylate, leads to dosing devices functional at the molecular level, which release precise doses of drug in response to applied light. A paradigm for the controlled, light-triggered release of "stepped" doses of drugs from polymeric scaffolds is thus demonstrated.

## Fragment-Based Substrate Activity Screening Method for the Identification of Potent Inhibitors of the Mycobacterium tuberculosis Phosphatase PtpB

*J. Am. Chem. Soc.*, 2007, 129 (31), 9613 -9615

Matthew Soellner, Katherine Rawls, Christoph Grundner, Tom Alber, and Jonathan A. Ellman

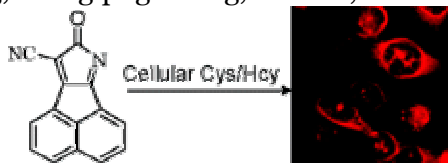


A new substrate-based fragment approach for the identification of novel PTP inhibitors is presented. This method was applied to Mycobacterium tuberculosis PtpB, a promising new target for the treatment of tuberculosis. This resulted in the development of the most potent PtpB inhibitor reported to date (0.22  $\mu$ M) with low molecular weight and good selectivity against a panel of other protein tyrosine phosphatases.

## A Highly Selective Fluorescence Turn-on Sensor for Cysteine/Homocysteine and Its Application in Bioimaging

*J. Am. Chem. Soc.*, 129 (34), 10322 -10323, 2007.

Meng Zhang,<sup>†</sup> Mengxiao Yu,<sup>†</sup> Fuyou Li,<sup>\*†</sup> Minwei Zhu,<sup>‡</sup> Manyu Li,<sup>§</sup> Yanhong Gao,<sup>⊥</sup> Lei Li,<sup>†</sup> Zhiqiang Liu,<sup>†</sup> Jianping Zhang,<sup>§</sup> Dengqing Zhang,<sup>†</sup> Tao Yi,<sup>†</sup> and Chunhui Huang

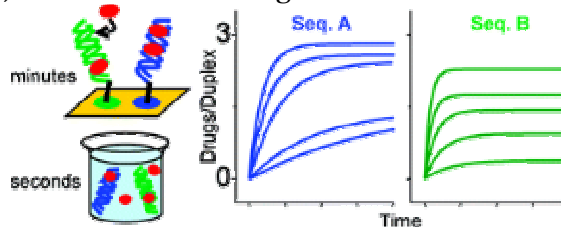


We demonstrated a highly electron-deficient system **1** as a turn-on fluorescence sensor for intracellular imaging of Cys/Hcy in biological samples. On the basis of the specific nucleophilic ability of thiols of Cys and Hcy, **1** exhibited high selectivity and sensitivity for Cys/Hcy over other amino acids and thiol biomolecules. This probe is the first Cys/Hcy sensor with excitation in the visible region and turn-on (75-fold) fluorescence emission and also gives a significant increase in two-photon excited fluorescence for sensing Cys/Hcy. Moreover, confocal laser scanning microscopy and two-photon laser scanning microscopy experiments further established that **1** can be used for sensing Cys/Hcy within biological samples.

## Kinetic Discrimination of Sequence-Specific DNA-Drug Binding Measured by Surface Plasmon Resonance Imaging and Comparison to Solution-Phase Measurements

*J. Am. Chem. Soc.*, **129** (34), 10503-10511, 2007.

Lauren K. Wolf, Yang Gao, and Rosina M. Georgiadis

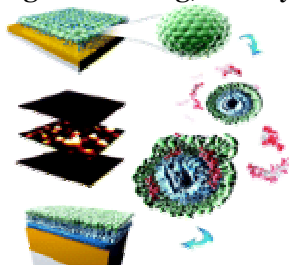


We demonstrate the use of surface plasmon resonance (SPR) imaging for direct detection of small-molecule binding to surface-bound DNA probes. Using a carefully designed array surface, we quantitatively discriminate between the interactions of a model drug with different immobilized DNA binding sites. Specifically, we measure the association and dissociation intercalation rates of actinomycin-D (ACTD) to and from double-stranded 5'-TGCT-3' and 5'-GGCA-3' binding sites. The rates measured provide mechanistic information about the DNA-ACTD interaction; ACTD initially binds nonspecifically to DNA but exerts its activity by dissociating slowly from strong affinity sites. We observe a slow dissociation time of  $k_{d-1} = 3300 \pm 100$  s for ACTD bound to the strong affinity site 5'-TGCT-3' and a much faster dissociation time ( $210 \pm 15$  s) for ACTD bound weakly to the site 5'-GGCA-3'. These dissociation rates, which differ by an order of magnitude, determine the binding affinity for each site ( $8.8 \times 10^6$  and  $1.0 \times 10^6$  M<sup>-1</sup>, respectively). We assess the effect the surface environment has on these biosensor measurements by determining kinetic and thermodynamic constants for the same DNA-ACTD interactions in solution. The surface suppresses binding affinities  $\sim 4$ -fold for both binding sites. This suppression suggests a barrier to DNA-drug association; ACTD binding to duplex DNA is  $\sim 100$  times slower on the surface than in solution.

## Employing an Amphipathic Viral Peptide to Create a Lipid Bilayer on Au and TiO<sub>2</sub>

*J. Am. Chem. Soc.*, **129** (33), 10050 -10051, 2007.

Nam-Joon Cho, Sang-Joon Cho, Kwang Ho Cheong, Jeffrey S. Glenn, and Curtis W. Frank

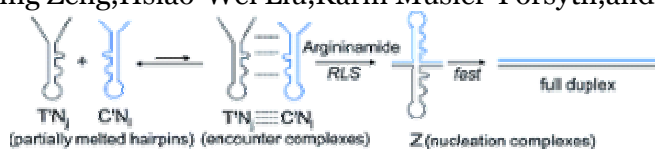


Supported lipid bilayers formed by the fusion of small unilamellar vesicles onto SiO<sub>2</sub> or organic film-modified surfaces serve as model membranes in both scientific research and practical applications. Here, we describe the use of an amphipathic  $\alpha$ -helical viral peptide derived from the hepatitis C virus NS5A protein (AH peptide) to destabilize the vesicles leading to lipid bilayer formation on gold and TiO<sub>2</sub> solid substrates. Whereas previous researchers have been limited in their selection of surface materials for lipid biomembranes, the use of such peptides as destabilizing agents will allow the freedom to choose a broader variety of solid substrates to support planar bilayers. In particular, the favorable electrical properties of gold and the beneficial biocompatibility of TiO<sub>2</sub> make these substrates attractive. The formation of model lipid bilayers supported on gold and TiO<sub>2</sub> substrates can be utilized in many membrane-associated biological, physiological, or electrochemical applications with the advantages provided by both of these supporting solid surfaces.

## Single-Molecule Study of the Inhibition of HIV-1 Transactivation Response Region DNA/DNA Annealing by Argininamide

*J. Am. Chem. Soc.*, **129** (33), 10181 -10188, 2007.

Christy F. Landes, Yining Zeng, Hsiao-Wei Liu, Karin Musier-Forsyth, and Paul F. Barbara



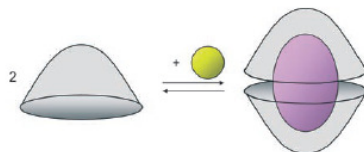
Single-molecule spectroscopy was used to examine how a model inhibitor of HIV-1, argininamide, modulates the nucleic acid chaperone activity of the nucleocapsid protein (NC) in the minus-strand transfer step of HIV-1 reverse transcription, *in vitro*. In minus-strand transfer, the transactivation response region (TAR) RNA of the genome is annealed to the complementary "TAR DNA" generated during minus-strand strong-stop DNA synthesis. Argininamide and its analogs are known to bind to the hairpin bulge region of TAR RNA as well as to various DNA loop structures, but its ability to inhibit the strand transfer process has only been implied. Here, we explore how argininamide modulates the annealing kinetics and secondary structure of TAR DNA. The studies reveal that the argininamide inhibitory mechanism involves a shift of the secondary structure of TAR, away from the NC-induced "Y" form, an intermediate in reverse transcription, and toward the free closed or "C" form. In addition, more potent

inhibition of the loop-mediated annealing pathway than stem-mediated annealing is observed. Taken together, these data suggest a molecular mechanism wherein argininamide inhibits NC-facilitated TAR RNA/DNA annealing in vitro by interfering with the formation of key annealing intermediates.

## Angewandte Chemie

### Highlights

**Molecular hospitality:** Self-assembled molecular containers provide a very specific geometric as well as chemical environment for a bound guest, which allows for selective guest binding or the stabilization of unstable molecules or unstable conformations within the capsule (see scheme). The geometric constraints imposed by the container can even change the outcome of a chemical reaction.

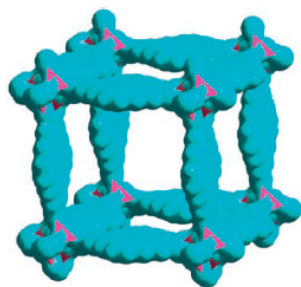


#### Supramolecular Chemistry

C. Schmuck\* ————— 5830 – 5833

Guest Encapsulation within Self-Assembled Molecular Containers

**Quick on the uptake:** The metal–organic framework Mg-MOF-C30 (see picture) contains  $\text{Mg}_4\text{O}(\text{CO}_2)_6$  building units (pink) and aromatic organic linkers containing 30 carbon atoms (teal). This material leads to 8.08 wt%  $\text{H}_2$  uptake at 77 K and 20 bar, the highest among investigated MOF structures.



#### Hydrogen Storage

S. S. Han, W.-Q. Deng,  
W. A. Goddard, III\* ————— 6289 – 6292

Improved Designs of Metal–Organic Frameworks for Hydrogen Storage

## ACS Chemical Biology

### Synthetic Estrogen Derivatives Demonstrate the Functionality of Intracellular GPR30

*ACS Chem. Biol.*, 2 (8), 536–544

Chetana M. Revankar, Hugh D. Mitchell, Angela S. Field, Ritwik Burai, Cesear Corona, Chinnasamy Ramesh, Larry A. Sklar, Jeffrey B. Arterburn, and Eric R. Prossnitz

Estrogen mediates its effects through multiple cellular receptors. In addition to the classical nuclear estrogen receptors ( $\text{ER}\alpha$  and  $\text{ER}\beta$ ), estrogen also signals through the seven-transmembrane G-protein-coupled receptor (GPCR) GPR30. Although estrogen is a cell-permeable ligand, it is often assumed that all GPCRs function solely as cell surface receptors. Our previous results showed that GPR30 appeared to be expressed predominantly in the endoplasmic reticulum. A critical question that arises is whether this localization represents the site of functional receptor. To address this question, we synthesized a collection of cell-permeable and cell-impermeable estrogen derivatives. We hypothesized that if functional GPR30 were expressed at the cell surface, both

permeable and impermeable derivatives would show activity. However, if functional GPR30 were predominantly intracellular, like ER $\alpha$ , only the permeable ligands should show activity. Cell permeability was assessed using cells expressing ER $\alpha$  as a model intracellular estrogen-binding receptor. Our results reveal that despite exhibiting similar binding affinities for GPR30, only the cell-permeable ligands are capable of stimulating rapid calcium mobilization and phosphoinositide 3-kinase (PI3K) activation. We conclude that GPR30 expressed intracellularly is capable of initiating cellular signaling and that there is insufficient GPR30 expressed on the cell surface to initiate signaling in response to impermeable ligands in the cell lines examined. To our knowledge, this is the first definitive demonstration of a functional intracellular transmembrane estrogen receptor.

### **A Steric Block in Translation Caused by the Antibiotic Spectinomycin**

*ACS Chem. Biol.*, **2 (8)**, 545–552

Maria A. Borovinskaya, Shinichiro Shoji, James M. Holton, Kurt Fredrick, and Jamie H. D. Cate

The widely used antibiotic spectinomycin inhibits bacterial protein synthesis by blocking translocation of messenger RNA and transfer RNAs on the ribosome. Here, we show that in crystals of the Escherichia coli 70S ribosome spectinomycin binding traps a distinct swiveling state of the head domain of the small ribosomal subunit. Spectinomycin also alters the rate and completeness of reverse translocation in vitro. These structural and biochemical data indicate that in solution spectinomycin sterically blocks swiveling of the head domain of the small ribosomal subunit and thereby disrupts the translocation cycle.

## **Chemistry and Biology**

### **Improved Mutasynthetic Approaches for the Production of Modified Aminocoumarin Antibiotics**

*Volume 14, Issue 8, 24 August 2007, Pages 955-967*

Christine Anderle, usanne Hennig, Bernd Kammerer, Shu-Ming Li, Ludger Wessjohann, Bertolt Gust and Lutz Heide

This study reports improved mutasynthetic approaches for the production of aminocoumarin antibiotics. Previously, the mutasynthetic production of aminocoumarins with differently substituted benzoyl moieties was limited by the substrate specificity of the amide synthetase CloL. We expressed two amide synthetases with different substrate specificity, CouL and SimL, in appropriately engineered producer strains. After feeding of precursor analogs that were not accepted by CloL, but by SimL or CouL, a range of aminocoumarins, unattainable in our previous experiments, was produced and isolated in preparative amounts. Further, we developed a two-stage mutasynthesis procedure for the production of hybrid antibiotics that showed the substitution pattern of novobiocin in the aminocoumarin moiety and that of

clorobiocin in the deoxysugar moiety. The substitution pattern of the benzoyl moiety was determined by external addition of an appropriate precursor. Twenty-five aminocoumarin compounds were prepared by these methods, and their structures were elucidated with mass and <sup>1</sup>H-NMR spectroscopy.

## Chemical Biology and Drug Design

### The Antimicrobial Peptide PR-39 has a Protective Effect Against HeLa Cell Apoptosis

*Chem Biol Drug Des* 2007; 70: 154–157

Christopher R. Rossi<sup>1</sup>, Giovanni Ricevuti<sup>2</sup> and Anna Ivana Scovassi

PR-39 is a cathelicidin with antimicrobial properties, which acts as a suppressor of inflammation and exerts a number of additional activities. We investigated for the first time the effect of PR-39 on human cells, by addressing the possible interference with HeLa cell metabolism. We observed that the continuous administration of PR-39 to HeLa cell cultures was not cytotoxic and did not interfere with DNA synthesis. When used in combination with a panel of drugs inducing apoptosis through different mechanisms of action, i.e. etoposide, bleomycin, tert-butylhydroperoxide and 2-deoxy-d-ribose, PR-39 attenuated the apoptotic response of HeLa cells.

### Fully 2'-Deoxy-2'-Fluoro Substituted Nucleic Acids Induce RNA Interference in Mammalian Cell Culture

*Chem Biol Drug Des* 2007; 70: 113–122

Richard A. Blidner<sup>1</sup>, Robert P. Hammer<sup>2</sup>, Mandi J. Lopez<sup>3</sup>, Sandra O. Robinson<sup>3</sup> and W. Todd Monroe

RNA interference is a phenomenon in which RNA molecules elicit potent and sequence-specific post-transcriptional gene silencing. Recent studies have shown that small interfering RNA containing pyrimidine 2'-fluoro modifications elicit RNAi. In this study, we demonstrate that fully-2'-fluorinated nucleic acids can be generated for RNAi studies through either custom solid-phase synthesis or in vitro transcription using a mutated polymerase and fluorinated nucleoside triphosphates. Single-stranded and hybridized fully-2'-fluorinated nucleic acids were subjected to a ribonuclease to assess their resistance to digestion. Duplex siFNA and antisense fully-2'-fluorinated nucleic acids were evaluated for their ability to knockdown green fluorescent protein expression in mammalian cell culture. Based on the results, fully-2'-fluorinated nucleic acids can be successfully generated, and fully-2'-fluorinated nucleic acids products show superior resistance to digestion over native RNA. Melt curve analysis suggests that transcribed fully-2'-fluorinated nucleic acids may contain base miscoding errors or early termination products. Small interfering fluoronucleic acid can induce RNAi and the silencing efficiency is nearly equivalent to the unmodified small interfering RNA species. Silencing from antisense fully-2'-fluorinated nucleic acids was greatly reduced relative to the duplex form. The lack of silencing activity from single-stranded fully-2'-fluorinated nucleic acids, combined with reverse transcription polymerase chain

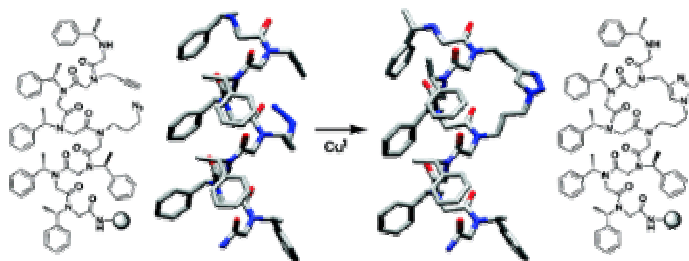
reaction data showing that mRNA decreases following siFNA treatment, suggests that knockdown from siFNA is likely enzymatically driven as opposed to simple translational arrest.

## Organic Letters

### Fit To Be Tied: Conformation-Directed Macrocyclization of Peptoid Foldamers

*Org. Lett.*, 9 (17), 3275 -3278, 2007.

Justin M. Holub,<sup>†</sup> Hangjun Jang, and Kent Kirshenbaum

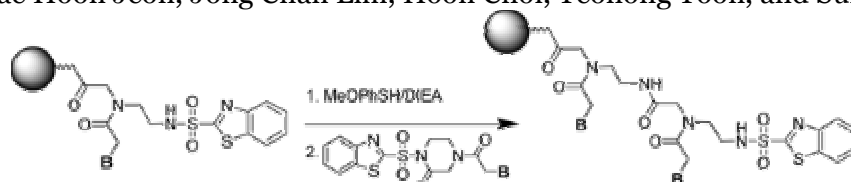


Covalent macrocyclic constraints can be readily installed on N-substituted glycine "peptoid" oligomer substrates. Cu(I)-catalyzed [3+2] cycloaddition reactions were conducted on solid support to ligate peptoid side chain azide and alkyne functionalities. Intramolecular macrocycle formation is facilitated by preorganizing the reactive groups across one turn of the helical secondary structure. These results confirm that conformational ordering can be exploited to assist the macrocyclization of folded oligomers.

### Peptide Nucleic Acid Synthesis by Novel Amide Formation

*Org. Lett.*, 9 (17), 3291 -3293, 2007.

Hyunil Lee,<sup>\*</sup> Jae Hoon Jeon, Jong Chan Lim, Hoon Choi, Yeohong Yoon, and Sung Kee Kim



Synthesis of self-activated peptide nucleic acid (PNA) monomers and an efficient method for PNA synthesis using a benzothiazole-2-sulfonyl (Bts) group as an amine-protecting group as well as an acid-activating group are reported. Couplings were complete within 120 min, and the deprotection was performed in 10 min. This Bts strategy provides a high purity PNA oligomer and is appropriate for large-scale synthesis. The results of the 15-mer PNA oligomer are described.