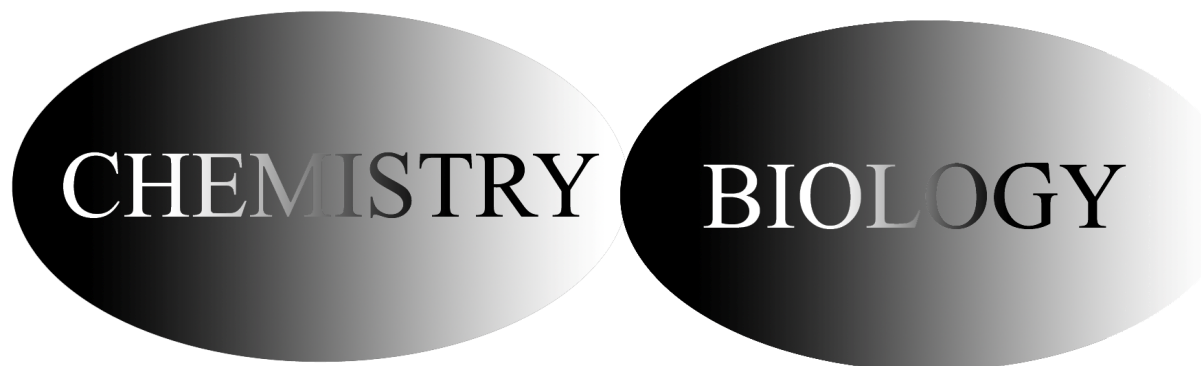


Chmielewski Group Literature Abstracts



December 2006

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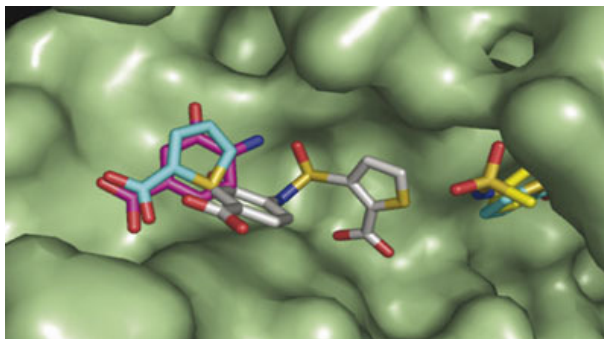
Dave Przybyla (*Org Lett*)

Nature Chemical Biology

Deconstructing fragment-based inhibitor discovery

Nature Chemical Biology 2, 720-723 (2006)

Kerim Babaoğlu¹ and Brian K Shoichet¹



Fragment-based screens test multiple low-molecular weight molecules for binding to a target^{1, 2, 3, 4}. Fragments often bind with low affinities but typically have better ligand efficiencies ($\Delta G_{\text{bind}}/\text{heavy atom count}$) than traditional screening hits⁵. This efficiency, combined with accompanying atomic-resolution structures, has made fragments popular starting points for drug discovery programs^{2, 6, 7, 8, 9, 10, 11, 12, 13}. Fragment-based design adopts a constructive strategy: affinity is enhanced either by cycles of functional-group addition or by joining two independent fragments together. The final inhibitor is expected to adopt the same geometry as the original fragment hit. Here we consider whether the inverse, deconstructive logic also applies—can one always parse a higher-affinity inhibitor into fragments that recapitulate the binding geometry of the larger molecule? Cocrystal structures of fragments deconstructed from a known beta-lactamase inhibitor suggest that this is not always the case.

Nature Methods

A highly sensitive protein-protein interaction assay based on *Gaussia* luciferase

Nature Methods - 3, 977 - 979 (2006)

Ingrid Remy & Stephen W Michnick

Protein-fragment complementation assays (PCAs) provide a general strategy to study the dynamics of protein-protein interactions *in vivo* and *in vitro*. The full potential of PCA requires assays that are fully reversible and sensitive at subendogenous protein expression levels. We describe a new assay that meets these criteria, based on the *Gaussia* princeps luciferase enzyme, demonstrating chemical reversal, and induction and inhibition of a key interaction linking insulin and TGFβ signaling.

Protein interaction screening by quantitative immunoprecipitation combined with knockdown (QUICK)

Nature Methods - 3, 981 - 983 (2006)

Matthias Selbach & Matthias Mann

Present screening methods for protein-protein interactions (PPIs) rely on the overexpression of artificial fusion proteins, making it difficult to assess in vivo relevance. Here we combine stable isotope labeling with amino acids in cell culture (SILAC), RNA interference (RNAi), coimmunoprecipitation and quantitative mass-spectrometry analysis to detect cellular interaction partners of endogenous proteins in mammalian cells with very high confidence. We used this screen to identify interaction partners of beta-catenin and Cbl.

Monitoring regulated protein-protein interactions using split TEV

Nature Methods - 3, 985 - 993 (2006)

Michael C Wehr^{1, 3}, Rico Laage^{2, 3}, Ulrike Bolz², Tobias M Fischer¹, Sylvia

Signaling cascades integrate extracellular stimuli primarily through regulated protein-protein interactions (PPIs). Intracellular signal transduction strictly depends on PPIs occurring at the membrane and in the cytosol. To monitor constitutive and regulated protein interactions within living mammalian cells, we have developed a biological assay termed split TEV. We engineered inactive fragments of the NIa protease from the tobacco etch virus (TEV protease) that regain activity only when coexpressed as fusion constructs with interacting proteins. Functional reconstitution of TEV protease fragments can be monitored with 'proteolysis-only' reporters, which can be previously silent fluorescent and luminescent reporter proteins. Additionally, proteolytically cleavable inactive transcription factors can be combined with any downstream reporter gene of choice to yield 'transcription-coupled' reporter systems. Thus, split TEV combines the advantages of split enzyme- and reporter gene-mediated assays, and provides full flexibility with regard to the final readout. In a first biological application, we monitored neuregulin-induced ErbB2/ErbB4 receptor tyrosine kinase heterodimerization.

Direct observation of individual endogenous protein complexes in situ by proximity ligation

Nature Methods - 3, 995 - 1000 (2006)

Ola Söderberg^{1, 3}, Mats Gullberg^{1, 3}, Malin Jarvius^{1, 3}, Karin Ridderstråle², Karl-Johan Leuchowius¹, Jonas Jarvius¹, Kenneth Wester¹, Per Hydbring², Fuad Bahram², Lars-Gunnar Larsson² & Ulf Landegren¹

Cellular processes can only be understood as the dynamic interplay of molecules. There is a need for techniques to monitor interactions of endogenous proteins directly in individual cells and tissues to reveal the cellular and molecular architecture and its responses to perturbations. Here we report our adaptation of the recently developed

proximity ligation method to examine the subcellular localization of protein-protein interactions at single-molecule resolution. Proximity probes—oligonucleotides attached to antibodies against the two target proteins—guided the formation of circular DNA strands when bound in close proximity. The DNA circles in turn served as templates for localized rolling-circle amplification (RCA), allowing individual interacting pairs of protein molecules to be visualized and counted in human cell lines and clinical specimens. We used this method to show specific regulation of protein-protein interactions between endogenous Myc and Max oncogenic transcription factors in response to interferon- γ (IFN- γ) signaling and low-molecular-weight inhibitors.

A rigorous experimental framework for detecting protein oligomerization using bioluminescence resonance energy transfer

Nature Methods - **3**, 1001 - 1006 (2006)

John R James, Marta I Oliveira, Alexandre M Carmo, Andrea Iaboni¹ & Simon J Davis

Correspondence should be addressed to Simon J Davis simon.davis@ndm.ox.ac.uk
Bioluminescence resonance energy transfer (BRET), which relies on nonradiative energy transfer between luciferase-coupled donors and GFP-coupled acceptors, is emerging as a useful tool for analyzing the quaternary structures of cell-surface molecules. Conventional BRET analyses are generally done at maximal expression levels and single acceptor/donor ratios. We show that under these conditions substantial energy transfer arises from random interactions within the membrane. The dependence of BRET efficiency on acceptor/donor ratio at fixed surface density, or expression level at a defined acceptor/donor ratio, can nevertheless be used to correctly distinguish between well-characterized monomeric and oligomeric proteins, including a very weak dimer. The pitfalls associated with the nonrigorous treatment of BRET data are illustrated for the case of G protein-coupled receptors (GPCRs) proposed to form homophilic and/or mixed oligomers on the basis of previous, conventional BRET experiments.

ACS Chemical Biology

HIV-1 Reverse Transcriptase Structure with RNase H Inhibitor Dihydroxy Benzoyl Naphthyl Hydrazone Bound at a Novel Site

ACS Chem. Biol. **1** (11), 702–712

Hossain, Kessler McCoy-Simandle, Tatiana Ilina, Arthur D. Clark Jr., Jennifer L. Knight[§], John G. Julias, Patrick K. Clark, Karsten Krogh-Jespersen[§], Ronald M. Levy, Stephen H. Hughes, Michael A. Parniak, and Eddy Arnold

The rapid emergence of drug-resistant variants of human immunodeficiency virus, type 1 (HIV-1), has limited the efficacy of anti-acquired immune deficiency syndrome (AIDS) treatments, and new lead compounds that target novel binding sites are needed. We

have determined the 3.15 Å resolution crystal structure of HIV-1 reverse transcriptase (RT) complexed with dihydroxy benzoyl naphthyl hydrazone (DHBNH), an HIV-1 RT RNase H (RNH) inhibitor (RNHI). DHBNH is effective against a variety of drug-resistant HIV-1 RT mutants. While DHBNH has little effect on most aspects of RT-catalyzed DNA synthesis, at relatively high concentrations it does inhibit the initiation of RNA-primed DNA synthesis. Although primarily an RNHI, DHBNH binds >50 Å away from the RNH active site, at a novel site near both the polymerase active site and the non-nucleoside RT inhibitor (NNRTI) binding pocket. When DHBNH binds, both Tyr181 and Tyr188 remain in the conformations seen in unliganded HIV-1 RT. DHBNH interacts with conserved residues (Asp186, Trp229) and has substantial interactions with the backbones of several less well-conserved residues. On the basis of this structure, we designed substituted DHBNH derivatives that interact with the NNRTI-binding pocket. These compounds inhibit both the polymerase and RNH activities of RT.

Chemistry and Biology

β Strand Peptidomimetics as Potent PDZ Domain Ligands

Chemistry & Biology 13, 1247–1251, December 2006

Ming C. Hammond¹, Baruch Z. Harris², Wendell A. Lim² and Paul A. Bartlett¹

The search for general strategies for inhibiting protein-protein interactions has been stimulated by recognition of the key role they play in virtually every process of living systems. Multiprotein complex assembly and localization by PDZ domain-containing proteins exemplify processes critical to cell physiology and function that are mediated by β strand association. Here we describe the development of substituted “@-tides,” protease-resistant peptidomimetics incorporating conformationally restricted amino acid surrogates that reproduce the hydrogen-bonding pattern and side-chain functionality of a β strand. The synthetic flexibility and generality of the substituted @-tide design was demonstrated by the synthesis of a panel of ligands for the α1-syntrophin PDZ domain. The rational design of a small molecule of unprecedented affinity for the PDZ domain suggests that these peptidomimetics may provide a general method for inhibiting protein-protein interactions involving extended peptide chains.

Structural and Antitumor Properties of the YSNSG Cyclopeptide Derived from Tumstatin

Chemistry & Biology 13, 1307–1315, December 2006

Jessica Thevenard, Nicolas Floquet, Laurent Ramont, Elise Prost³Jean-Marc Nuzillard^{3, 6}, Manuel Dauchez^{1, 4}, Hocine Yezid, Alain J.P. Alix, François-Xavier Maquart^{1, 4}, Jean-Claude Monboisse^{1, 4} and Sylvie Brassart-Pasco

We previously demonstrated that the NC1[α3(IV)185–191] CNYYSNS peptide inhibited in vivo tumor progression. The YSNS motif formed a β turn crucial for biological activity. The aim of the present study was to design a YSNSG cyclopeptide with a

constrained β turn on the YSNS residues more stable than CNYYSNS. By nuclear magnetic resonance and molecular modeling, we demonstrated that the YSNSG cyclopeptide actually adopted the expected β -turn conformation. It promoted melanoma cell adhesion and prevented their adhesion to the native peptide. It inhibited in vitro cell proliferation and migration through Matrigel by downregulating proteolytic cascades. Moreover, intraperitoneal administration of the YSNSG cyclopeptide inhibited melanoma progression far more efficiently than the native peptide. The increased solubility and stability at low pH of the YSNSG cyclopeptide suggest this peptide as a potent antitumor therapeutic agent.

Chemical Biology and Drug Design

Influence of Generation 2–5 of PAMAM Dendrimer on the Inhibition of Tat Peptide/TAR RNA Binding in HIV-1 Transcription

Chem Biol Drug Des 2006; 68: 314–318

Wei Wang, Zhenpeng Guo, Yi Chen, Tao Liu and Long Jiang

The special binding of Tat protein to TAR RNA leads to the transcription of HIV-1 virus. In this study, the influence of 2–5 generation of PAMAM dendrimers on the inhibition of Tat protein/TAR RNA binding has been investigated. The adsorption of PAMAM dendrimers on TAR RNA, fixed on a gold substrate through an avidin–biotin connection, was carried out by using a quartz crystal microbalance. Experimental result shows a Langmuir-type isotherm could be used to describe this kind of binding, implying a specific and monolayer adsorption existed. The combination coefficient (K-1D) s can be calculated according to Langmuir Equation, having the order of $G_3 > G_4 > G_5 > \text{Tat} > G_2$, indicating that PAMAM G₃, G₄ and G₅ having the possibility to be the inhibitors of HIV-1 transcription. The migration time (T_{migra}) of capillary electrophoretic technique has the same sequence as (K-1D) s. These two parameters could be used as simple and quantitative criteria for the selection of possible drugs from numerous candidates for HIV therapy in vitro.

Peptides by Extension at the N- or C-termini of Lysine

Chem Biol Drug Des 2006; 68: 326–333

Alan R. Katritzky*, Geeta Meher and Parul Angrish

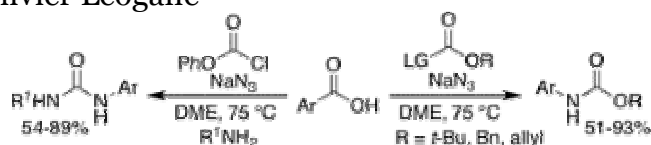
Dipeptides 3a–g, (3a + 3a ϕ), (3d + 3d ϕ), (3l + 3l ϕ)a and tripeptides 6a–e, (6b + 6b ϕ), (6e + 6e ϕ) incorporating Z₋-Lys were prepared in high yields (70–95%) and enantiopurity (\approx 97%) in partially aqueous acetonitrile solution by coupling using (i) Z₋-Lys with N-(Z- and Fmoc-aminoacyl)benzotriazoles 1a–g, (ii) Z₋-Lys with N-Z-dipeptidoylbenzotriazoles 5a–c, and (iii) N-Fmoca-Z₋-L-Lys-Bt 1h and amino acids 2a,c–e. Unnatural dipeptides 3h–j, (3h + 3h ϕ) and tripeptides 6f were similarly prepared from Za-Lys. Retention of chirality was demonstrated by parallel experiments involving L-Ala, DL-Ala, L-Met, and DL-Met by NMR and HPLC analysis.

Organic Letters

Curtius Rearrangement of Aromatic Carboxylic Acids to Access Protected Anilines and Aromatic Ureas

Org. Lett., **8** (25), 5717-5720, 2006

Hélène Lebel* and Olivier Leogane

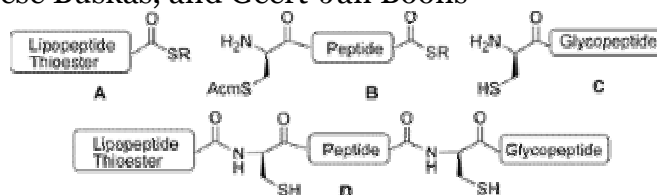


The reaction of a chloroformate or di-tert-butyl dicarbonate and sodium azide with an aromatic carboxylic acid produces the corresponding acyl azide, presumably through the formation of an azidoformate. The acyl azide undergoes a Curtius rearrangement to form an isocyanate derivative which is trapped either by an alkoxide or by an amine to form the aromatic carbamate or urea. The reaction conditions are compatible with a variety of functional groups and allow the synthesis of a number of aniline derivatives containing alkyl, halide, nitro, ketone, ether, and thioether substituents.

Synthesis of Glyco(lipo)peptides by Liposome-Mediated Native Chemical Ligation

Org. Lett., **8** (25), 5785-5788, 2006.

Sampat Ingale, Therese Buskas, and Geert-Jan Boons

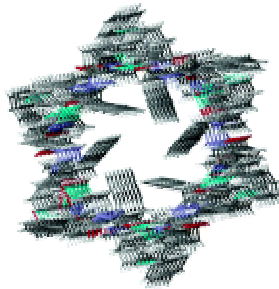


Although native chemical ligation (NCL) is emerging as a powerful method for the assembly of (glyco)peptide building blocks, its applicability is reduced when peptide segments are poorly soluble in aqueous buffer. We have found that incorporating reactants in liposomes allows NCL of lipophilic peptides and lipopeptides. Furthermore, the reaction rates of liposome-mediated NCL are higher than traditional reaction conditions resulting in improved yields

A Helical, Aromatic, Peptide Nanotube

Org. Lett., **8** (26), 6091-6094, 2006.

Marco Crisma,* Claudio Toniolo, Soledad Royo, Ana I. Jiménez, and Carlos Cativiela



The self-assembly in the crystal state of the terminally protected, linear dipeptide Boc-(S,S)c₃diPhe-(R,R)c₃diPhe-NHiPr (1) through intermolecular hydrogen bonds leads to the formation of a supramolecular helix of large diameter (18 Å), internally decorated with phenyl rings. As a result, a hollow helical channel large enough to accommodate guest molecules is observed. This supramolecular structure differs from previous examples of peptide nanotubes. Compound 1 incorporates a highly restricted cyclopropane phenylalanine analogue (c₃diPhe) with remarkable conformational properties.

Science

Enzyme-Free Nucleic Acid Logic Circuits

Science Vol 314, Issue 5805, 1585-1588, 8 December 2006

Georg Seelig,¹ David Soloveichik,² David Yu Zhang,² Erik Winfree

Biological organisms perform complex information processing and control tasks using sophisticated biochemical circuits, yet the engineering of such circuits remains ineffective compared with that of electronic circuits. To systematically create complex yet reliable circuits, electrical engineers use digital logic, wherein gates and subcircuits are composed modularly and signal restoration prevents signal degradation. We report the design and experimental implementation of DNA-based digital logic circuits. We demonstrate AND, OR, and NOT gates, signal restoration, amplification, feedback, and cascading. Gate design and circuit construction is modular. The gates use single-stranded nucleic acids as inputs and outputs, and the mechanism relies exclusively on sequence recognition and strand displacement. Biological nucleic acids such as microRNAs can serve as inputs, suggesting applications in biotechnology and bioengineering.

Relating Three-Dimensional Structures to Protein Networks Provides Evolutionary Insights

Science Vol 314, Issue 5807, 1938-1941, 22 December 2006

Philip M. Kim,¹ Long J. Lu,¹ Yu Xia,^{4,5} Mark B. Gerstein

Most studies of protein networks operate on a high level of abstraction, neglecting structural and chemical aspects of each interaction. Here, we characterize interactions by using atomic-resolution information from three-dimensional protein structures. We find that some previously recognized relationships between network topology and genomic features (e.g., hubs tending to

be essential proteins) are actually more reflective of a structural quantity, the number of distinct binding interfaces. Subdividing hubs with respect to this quantity provides insight into their evolutionary rate and indicates that additional mechanisms of network growth are active in evolution (beyond effective preferential attachment through gene duplication).

PNAS

Self-renewal of embryonic stem cells by a small molecule

***PNAS* | November 14, 2006 | vol. 103 | no. 46 | 17266-17271**

Shuibing Chen*, Jeong Tae Do†, Qisheng Zhang*, Shuyuan Yao*, Feng Yan‡, Eric C. Peters†, Hans R. Schöler†, Peter G. Schultz* and Sheng Ding

A cell-based screen of chemical libraries was carried out to identify small molecules that control the self-renewal of ES cells. A previously uncharacterized heterocycle, SC1, was discovered that allows one to propagate murine ES cells in an undifferentiated, pluripotent state under chemically defined conditions in the absence of feeder cells, serum, and leukemia inhibitory factor. Long-term SC1-expanded murine ES cells can be differentiated into cells of the three primary germ layers in vitro and also can generate chimeric mice and contribute to the germ line in vivo. Biochemical and cellular experiments suggest that SC1 works through dual inhibition of RasGAP and ERK1. Molecules of this kind may not only facilitate practical applications of stem cells in research and therapy, but also provide previously undescribed insights into the complex biology of stem cells.

Cooperative deformation of mineral and collagen in bone at the nanoscale

***PNAS* | November 21, 2006 | vol. 103 | no. 47 | 17741-17746**

Jong Seto, Wolfgang Wagermaier, Paul Zaslansky, Peter Boesecke, and Peter Fratzl

In biomineralized tissues such as bone, the recurring structural motif at the supramolecular level is an anisotropic stiff inorganic component reinforcing the soft organic matrix. The high toughness and defect tolerance of natural biomineralized composites is believed to arise from these nanometer scale structural motifs. Specifically, load transfer in bone has been proposed to occur by a transfer of tensile strains between the stiff inorganic (mineral apatite) particles via shearing in the intervening soft organic (collagen) layers. This raises the question as to how and to what extent do the mineral particles and fibrils deform concurrently in response to tissue deformation. Here we show that both mineral nanoparticles and the enclosing mineralized fibril deform initially elastically, but to different degrees. Using in situ tensile testing with combined high brilliance synchrotron X-ray diffraction and scattering on the same sample, we show that tissue, fibrils, and mineral particles take up successively lower levels of strain, in a ratio of 12:5:2. The maximum strain seen in mineral nanoparticles (≈ 0.15 – 0.20%) can reach up to twice the fracture strain calculated for bulk apatite. The results are consistent with a staggered model of load

transfer in bone matrix, exemplifying the hierarchical nature of bone deformation. We believe this process results in a mechanism of fibril–matrix decoupling for protecting the brittle mineral phase in bone, while effectively redistributing the strain energy within the bone tissue.

Secondary structure provides a template for the folding of nearby polypeptides

PNAS | November 21, 2006 | vol. 103 | no. 47 | 17765-17770

Tomoshi Kameda*,†, and Shoji Takada

Although protein structures are primarily encoded by their sequences, they are also critically dependent on environmental factors such as solvents and interactions with other molecules. Here we investigate how the folding-energy landscape of a short peptide is altered by interactions with another peptide, by performing atomistic replica-exchange molecular dynamics simulations of polyalanines in various environments. We analyzed the free-energy landscapes of Ala7 and Ala8 in isolation, near an α -helix template, and near a β -strand template. The isolated Ala7 and Ala8 at 270 K were mainly in polyproline II helix conformations and in equilibrium between the α -helix and polyproline II helix, respectively, in harmony with the experiment. Interestingly, we found remarkably strong secondary-structure "templating"; namely, the α -helix template enhanced α -helix conformation and the β -strand template induced β -strand conformation in the simulated Ala8. The α -helix template lowered the nearby dielectric constant, which strengthened hydrogen bonds in the simulated Ala8, leading to α -helix stabilization. The β -strand template provided hydrogen bond positions to the simulated Ala8, sharply inducing β -strand structure. With or without templates, the energy landscape of Ala8 is always funnel-like and centered at the α -helix conformation, whereas entropic contribution disfavors the α -helix, leading to subtle competition. Secondary-structure templating may play a critical role in protein conformation dynamics in the cellular environment.

Journal of the American Chemical Society

Measuring Protein Concentrations by NMR Spectroscopy

J. Am. Chem. Soc., 2006, 128 (8), 2571 -2576

Gerhard Wider* and Lars Dreier



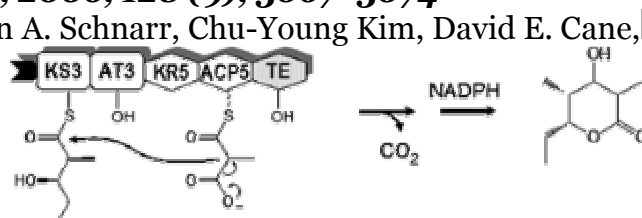
In applications of NMR to biological macromolecules in solution, the concentration of the NMR sample is an important parameter describing the sample and providing information for the selection and planning of experiments. Although concentrations can be measured directly by NMR spectroscopy, other methods are usually preferred to measure the concentration of macromolecules in NMR samples. The reasons are the difficulties in the correlation of the sample of interest with the signal intensity

We report unrestrained, all-atom molecular dynamics simulations of HIV-1 protease (HIV-PR) with a continuum solvent model that reproducibly sample closing of the active site flaps following manual placement of a cyclic urea inhibitor into the substrate binding site of the open protease. The open form was obtained from the unbound, semi-open HIV-PR crystal structure, which we recently reported (Hornak, V.; et al. Proc. Natl. Acad. Sci. U.S.A. 2006, 103, 915-920.) to have spontaneously opened during unrestrained dynamics. In those simulations, the transiently open flaps always returned to the semi-open form that is observed in all crystal structures of the free protease. Here, we show that manual docking of the inhibitor reproducibly induces spontaneous conversion to the closed form as seen in all inhibitor-bound HIV-PR crystal structures. These simulations reproduced not only the greater degree of flap closure, but also the striking difference in flap "handedness" between bound and free enzyme. In most of the simulations, the final structures were highly accurate. Root-mean-square deviations (RMSD) from the crystal structure of the complex were ~ 1.5 Å (averaged over the last 100 ps) for the inhibitor and each flap despite initial RMSD of 2-5 Å for the inhibitors and 6-11 Å for the flaps. Key hydrogen bonds were formed between the flap tips and between flaps and inhibitor that match those seen in the crystal structure. The results demonstrate that all-atom simulations have the ability to significantly improve poorly docked ligand conformations and reproduce large-scale receptor conformational changes that occur upon binding.

Extender Unit and Acyl Carrier Protein Specificity of Ketosynthase Domains of the 6-Deoxyerythronolide B Synthase

J. Am. Chem. Soc., 2006, 128 (9), 3067-3074

Alice Y. Chen, Nathan A. Schnarr, Chu-Young Kim, David E. Cane, and Chaitan Khosla



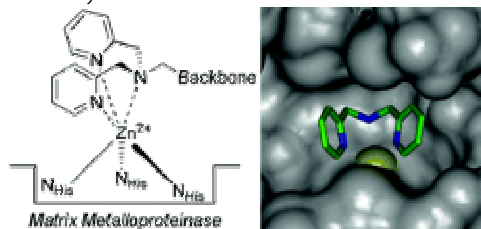
Polyketide synthases (PKSs) catalyze the production of numerous biologically important natural products via repeated decarboxylative condensation reactions. Modular PKSs, such as the 6-deoxyerythronolide B synthase (DEBS), consist of multiple catalytic modules, each containing a unique set of covalently linked catalytic domains. To better understand the engineering opportunities of these assembly lines, the extender unit and acyl carrier protein (ACP) specificity of keto synthase (KS) domains from modules 3 and 6 of DEBS were analyzed. These studies were undertaken with a newly developed didomain [KS][AT] construct, which lacks its own ACP domain and can therefore be interrogated with homologous or heterologous ACP or acyl-ACP substrates. By substituting the natural methylmalonyl extender unit with a malonyl group, a modest role was demonstrated for the KS in recognition of the nucleophilic substrate. The KS domain from module 3 of DEBS was found to exhibit a distinct ACP-recognition profile from the KS domain of module 6. On the basis of the above kinetic insights, a hybrid module was constructed ([KS₃][AT₃][KR₅][ACP₅][TE]) which displayed substrate recognition and elongation capabilities consistent with the natural module 3 protein. Unlike module 3, however, which lacks a ketoreductase (KR) domain, the hybrid module

was able to catalyze reduction of the β -keto thioester product of chain elongation. The high expression level and functionality of this hybrid protein demonstrates the usefulness of kinetic analysis for hybrid module design.

A New Role for Old Ligands: Discerning Chelators for Zinc Metalloproteinases

J. Am. Chem. Soc., 2006, 128 (10), 3156-3157

Faith E. Jacobsen, Jana A. Lewis, and Seth M. Cohen

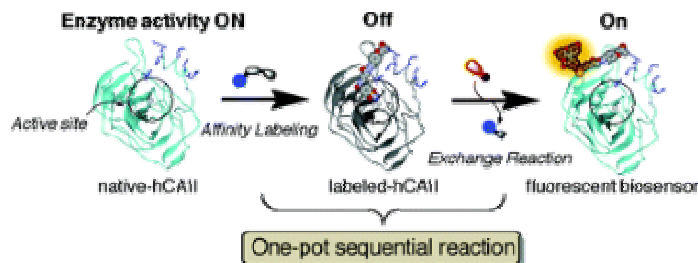


In an effort to identify promising non-hydroxamate inhibitors of matrix metalloproteinases (MMPs) several new zinc-binding groups (ZBGs) based on pyridine-derived or aza-macrocyclic chelators have been examined. Fluorescence-based enzyme assays have been used to determine the IC₅₀ values for these ZBGs against MMP-1, MMP-3, and anthrax lethal factor (LF). Many of these ligands were found to be remarkably potent, with IC₅₀ values as much as 185-fold lower than that found for acetohydroxamic acid. These ligands are proposed to be more selective "warheads" for the inhibition of metalloenzymes that contain Zn²⁺ versus other metal ions at their active site.

One-Pot and Sequential Organic Chemistry on an Enzyme Surface to Tether a Fluorescent Probe at the Proximity of the Active Site with Restoring Enzyme Activity

J. Am. Chem. Soc., 2006, 128 (10), 3273-3280

Yousuke Takaoka,[§] Hiroshi Tsutsumi,[‡] Noriyuki Kasagi,[§] Eiji Nakata,[†] and Itaru Hamachi



A new and simple method to tether a functional molecule at the proximity of the active site of an enzyme has been successfully developed without any activity loss. The one-pot sequential reaction was conducted on a surface of human carbonic anhydrase II (hCAII) based on the affinity labeling and the subsequent hydrazone/oxime exchange reaction. The reaction proceeds in a greater than 90% yield in the overall steps under mild conditions. The enzymatic activity assay demonstrated that the release of the affinity

ligand from the active site of hCAII concurrently occurred with the replacement by the aminoxy derivatives, so that it restored the enzymatic activity from the completely suppressed state of the labeled hCAII. Such restoring of the activity upon the sequential modification is quite unique compared to conventional affinity labeling methods. The peptide mapping experiment revealed that the labeling reaction was selectively directed to His-3 or His-4, located on a protein surface proximal to the active site. When the fluorescent probe was tethered using the present sequential chemistry, the engineered hCAII can act as a fluorescent biosensor toward the hCAII inhibitors. This clearly indicates the two advantages of this method, that is (i) the modification is directed to the proximity of the active site and (ii) the sequential reaction re-opens the active site cavity of the target enzyme.

An Antiparallel alpha-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

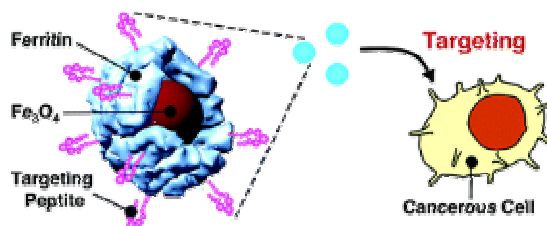


Both parallel and antiparallel alpha-helical coiled-coil dimers are common among proteins; however, biophysical scrutiny has focused almost entirely on parallel dimers. We describe the development of a model system that enables efficient and systematic analysis of hydrophobic packing between antiparallel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

Targeting of Cancer Cells with Ferrimagnetic Ferritin Cage Nanoparticles

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

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



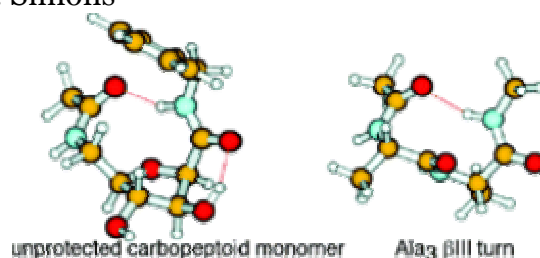
Protein cage architectures such as virus capsids and ferritins are versatile nanoscale platforms amenable to both genetic and chemical modification. Incorporation of multiple functionalities within these nanometer-sized protein architectures demonstrate

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.

Carbohydrate Amino Acids: The Intrinsic Conformational Preference for a β -Turn-Type Structure in a Carbopeptoid Building Block

J. Am. Chem. Soc., **128** (51), 16771 -16777, 2006.

Rebecca A. Jockusch,*^{†‡} Francis O. Talbot,[†] Paul S. Rogers,[‡] Michela I. Simone,[‡] George W. J. Fleet,[‡] and John P. Simons

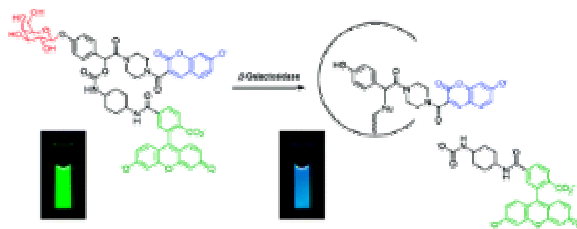


Infrared ion-dip spectroscopy coupled with DFT and ab initio calculations are used to establish the intrinsic conformational preference of the basic structural unit of a peptide mimic, a cis-tetrahydrofuran-based "carbopeptoid" (amide-sugar-amide), isolated at low temperature in the gas phase. The carbopeptoid units form a β -turn-type structure, stabilized by an intramolecular NH \rightarrow O=C hydrogen bond across the sugar ring, thus forming a 10-membered, C10 β -turn structure. Despite the clear preference for C10 β -turn structures in the basic unit, however, the presence of multiple hydrogen-bond donating and accepting groups also generates a rich conformational landscape, and alternative structures may be populated in related molecules. Calculations on an extended, carbopeptoid dimer unit, which includes an alternating amide-sugar-amide-sugar-amide chain, identify conformers exhibiting alternative hydrogen-bonding arrangements that are somewhat more stable than the lowest-energy double β -turn forming conformer.

Design and Synthesis of an Enzyme Activity-Based Labeling Molecule with Fluorescence Spectral Change

J. Am. Chem. Soc., **128** (50), 15946 -15947, 2006.

Toru Komatsu,[‡] Kazuya Kikuchi,[‡] Hideo Takakusa,[‡] Kenjiro Hanaoka,[†] Tasuku Ueno,[†] Mako Kamiya,[‡] Yasuteru Urano,[†] and Tetsuo Nagano

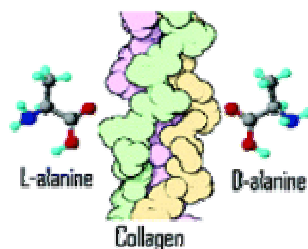


Methods of covalent labeling of a specific tag protein with small-molecular dyes play an important role in studying dynamic behaviors of proteins in living cells. On the basis of quinone methide chemistry, we designed and synthesized a β -galactosidase labeling probe, CMF^β-gal, which shows a fluorescence wavelength change accompanying the labeling reaction, owing to fluorescence resonance energy transfer (FRET). Since the FRET efficiency changes accompanying the labeling reaction, fluorescence of labeled protein can be observed separately from that of the unreacted probe, so immediate detection of the target protein is possible. This is the first report of a protein labeling probe which features a change of fluorescence wavelength upon reaction, allowing the labeled protein to be detected even in the presence of unreacted probe.

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

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

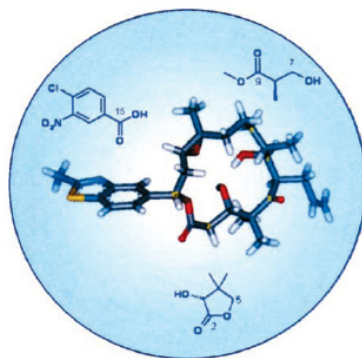
Uzi Eliav* and Gil Navon



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.

Angewandte Chemie

Going to trial: From about 350 active epothilone analogues synthesized by a highly convergent synthesis, one (ZK-EPO, see picture) has been chosen for clinical development on the basis of its outstanding preclinical data. This compound exhibits higher activity and efficacy than taxanes (e.g. paclitaxel) and second-generation epothilones, a fast and efficient cellular uptake, no recognition by efflux mechanisms, and an improved therapeutic window.

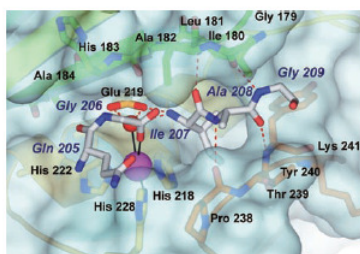


Natural Products



U. Klar,* B. Buchmann, W. Schwede,
W. Skuballa, J. Hoffmann,
R. B. Lichtner _____ 7942–7948

Total Synthesis and Antitumor Activity of
ZK-EPO: The First Fully Synthetic
Epothilone in Clinical Development



The series of events that occur in the catalytic cycle of matrix metalloproteinases were modeled on the basis of X-ray crystal structures of the active, uninhibited enzymes and of the same enzymes following the hydrolysis of a peptide substrate. After the peptide bond has been broken, both peptide fragments remain bound to the protein initially (see structure of the active-site cavity of the enzyme MMP-12 immediately after substrate hydrolysis).

Enzyme Mechanisms

I. Bertini,* V. Calderone, M. Fragai,
C. Luchinat, M. Maletta,
K. J. Yeo _____ 7952–7955

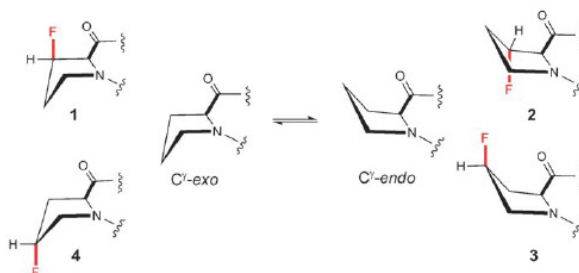
Snapshots of the Reaction Mechanism of
Matrix Metalloproteinases



Protein Engineering

W. Kim, K. I. Hardcastle,
V. P. Conticello* _____ 8141–8145

Fluoroproline Flip-Flop: Regiochemical
Reversal of a Stereoelectronic Effect on
Peptide and Protein Structures



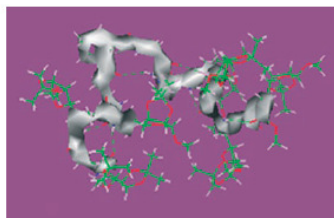
Pucker up! Structural analysis of the epimeric *N*-acetyl-(2*R*,3*R*)- and *N*-acetyl-(2*R*,3*S*)-3-fluoroproline methyl esters (**1** and **2**; see scheme) reveals opposing ring puckers that invert the conformational preferences of the corresponding 4-fluor-

oprolines derivatives **3** and **4**. Substitution of these fluoroprolines into proteins provides a method for controlling local conformation through stereoelectronic and steric effects.

Foldamers

G. V. M. Sharma,* V. Subash,
K. Narsimulu, A. R. Sankar,
A. C. Kunwar* _____ 8207–8210

De Novo Design and Synthesis of Helix–
Turn–Helix Structure from Short and
Robust Mixed Helices Derived from
C-Linked Carbo- β -Amino Acids



In a twist: Tethering of short peptides with robust 10/12-mixed helices, derived from C-linked carbo- β -amino acids, to the turn-inducing motif, β -hGly-D-Pro-Gly- β -hGly, permitted a de novo design of the helix–turn–helix motif in the foldamer domain.