Chmielewski Group
Literature Abstracts

CHEMISTRY BIOLOGY

December 2007

Highlight of the Month

Device can spot cancer cells in blood

Microchip could help doctors tailor patient treatment, researchers say

In cancer patients, circulating tumor cells (CTCs) in the blood are the hallmark of the spread of the disease. But these cells are difficult to isolate because they are a tiny fraction of the total number of cells in blood. Medical engineer Mehmet Toner and coworkers at Massachusetts General Hospital and Harvard Medical School have now come up with a microfluidic device that isolates CTCs from whole blood samples (Nature 2007, 450, 1235). The CTC chip consists of an array of micrometer-scale posts coated with antibodies against a molecule found on the surface of epithelial cells from various organs but not on blood cells. The researchers used the device to capture CTCs in blood samples from patients with lung, prostate, pancreatic, breast, or colon cancer. They were able to distinguish CTCs from blood cells and further analyze the captured CTCs for tumor-specific markers, such as prostate-specific antigen. In follow-up samples, the CTC measurements correlated with patients' clinical response to treatment. The microfluidic device could become a tool for monitoring cancer treatment, for detecting relapses, and eventually for early detection, Toner says.

Contributing Editors:

Stefan Hershberger (*Science*)
Marcos Pires (*Nature and Nature subdivisions*)
Brandon Gaddis/Iris Geisler (*JACS*)
Jee Yeon Lee (*PNAS*)
Dawn Ernenwein (*ACS Chemical Biology/Chem Biol & Drug Design*)
Dave Przybyla (*Angewandte Chemie)*
Hilda Namanja (*Chem & Bio*)
Nicole O'Neil (*Org Lett*)

Nature

Crystal structure of the plasma membrane proton pump

Nature 450, 1111-1114 (13 December 2007)
Bjørn P. Pedersen1,2,4, Morten J. Buch-Pedersen1,2,3,4, J. Preben Morth1,2, Michael G. Palmgren1,3 & Poul Nissen

A prerequisite for life is the ability to maintain electrochemical imbalances across biomembranes. In all eukaryotes the plasma membrane potential and secondary transport systems are energized by the activity of P-type ATPase membrane proteins: H+-ATPase (the proton pump) in plants and fungi1, 2, 3, and Na+,K+-ATPase (the sodium–potassium pump) in animals4. The name P-type derives from the fact that these proteins exploit a phosphorylated reaction cycle intermediate of ATP hydrolysis5. The plasma membrane proton pumps belong to the type III P-type ATPase subfamily, whereas Na+,K+-ATPase and Ca2+-ATPase are type II6. Electron microscopy has revealed the overall shape of proton pumps7, however, an atomic structure has been lacking. Here we present the first structure of a P-type proton pump determined by X-ray crystallography. Ten transmembrane helices and three cytoplasmic domains define the functional unit of ATP-coupled proton transport across the plasma membrane, and the structure is locked in a functional state not previously observed in P-type ATPases. The transmembrane domain reveals a large cavity, which is likely to be filled with water, located near the middle of the membrane plane where it is lined by conserved hydrophilic and charged residues. Proton transport against a high membrane potential is readily explained by this structural arrangement.

Isolation of rare circulating tumour cells in cancer patients by microchip technology

Nature 450, 1235-1239 (20 December 2007)
Irimia1, Lindsey Ulkus2, Matthew R. Smith2, Eunice L. Kwak2, Subba Digumarthy2, Alona Muzikansky2, Paula Ryan2, Ulysses J. Balis1,4, Ronald G. Tompkins1, Daniel A. Haber2 & Mehmet Toner

Viable tumour-derived epithelial cells (circulating tumour cells or CTCs) have been identified in peripheral blood from cancer patients and are probably the origin of intractable metastatic disease1, 2, 3, 4. Although extremely rare, CTCs represent a potential alternative to invasive biopsies as a source of tumour tissue for the detection, characterization and monitoring of non-haematologic cancers5, 6, 7, 8. The ability to identify, isolate, propagate and molecularly characterize CTC subpopulations could further the discovery of cancer stem cell biomarkers and expand the understanding of the biology of metastasis. Current strategies for isolating CTCs are limited to complex analytic approaches that generate very low yield and purity9. Here we describe the development of a unique microfluidic platform (the 'CTC-chip') capable of efficient and selective separation of viable CTCs from peripheral whole blood samples, mediated by the interaction of target CTCs with antibody (EpCAM)-coated microposts under precisely controlled laminar flow conditions, and without requisite pre-labelling or processing of samples. The CTC-chip successfully identified CTCs in the peripheral blood of patients with metastatic lung, prostate, pancreatic, breast and colon cancer in 115 of 116 (99%) samples, with a range of

5–1,281 CTCs per ml and approximately 50% purity. In addition, CTCs were isolated in 7/7 patients with early-stage prostate cancer. Given the high sensitivity and specificity of the CTC-chip, we tested its potential utility in monitoring response to anti-cancer therapy. In a small cohort of patients with metastatic cancer undergoing systemic treatment, temporal changes in CTC numbers correlated reasonably well with the clinical course of disease as measured by standard radiographic methods. Thus, the CTC-chip provides a new and effective tool for accurate identification and measurement of CTCs in patients with cancer. It has broad implications in advancing both cancer biology research and clinical cancer management, including the detection, diagnosis and monitoring of cancer[10].

Nature Biotechnology

Redirecting lipoic acid ligase for cell surface protein labeling with small-molecule probes

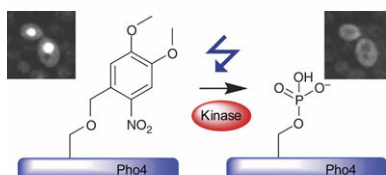
***Nature Biotechnology* 25, 1483 - 1487 (2007)**
Marta Fernández-Suárez[1], Hemanta Baruah[1], Laura Martínez-Hernández[1], Kathleen T Xie[1], Jeremy M Baskin[2], Carolyn R Bertozzi[2,3,4,5] & Alice Y Ting

Live cell imaging is a powerful method to study protein dynamics at the cell surface, but conventional imaging probes are bulky, or interfere with protein function[1, 2], or dissociate from proteins after internalization[3, 4]. Here, we report technology for covalent, specific tagging of cellular proteins with chemical probes. Through rational design, we redirected a microbial lipoic acid ligase (LplA)[5] to specifically attach an alkyl azide onto an engineered LplA acceptor peptide (LAP). The alkyl azide was then selectively derivatized with cyclo-octyne[6] conjugates to various probes. We labeled LAP fusion proteins expressed in living mammalian cells with Cy3, Alexa Fluor 568 and biotin. We also combined LplA labeling with our previous biotin ligase labeling[7, 8], to simultaneously image the dynamics of two different receptors, coexpressed in the same cell. Our methodology should provide general access to biochemical and imaging studies of cell surface proteins, using small fluorophores introduced via a short peptide tag.

Nature Chemical Biology

Control of protein phosphorylation with a genetically encoded photocaged amino acid

***Nature Chemical Biology* 3, 769-772 (2007)**
Edward A Lemke[1,3], Daniel Summerer[1,3], Bernhard H Geierstanger[2], Scott M Brittain[2] & Peter G Schultz

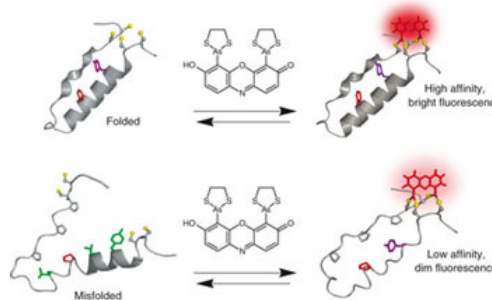


We genetically encoded the photocaged amino acid 4,5-dimethoxy-2-nitrobenzylserine (DMNB-Ser, 1) in Saccharomyces cerevisiae in response to the amber nonsense codon

TAG. This amino acid was converted to serine in living cells by irradiation with relatively low-energy blue light and was used to noninvasively photoactivate phosphorylation of the transcription factor Pho4, which controls the cellular response to inorganic phosphate1. When substituted at phosphoserine sites that control nuclear export of Pho4, 1 blocks phosphorylation and subsequent export by the receptor Msn5 (ref. 2). We triggered phosphorylation of individual serine residues with a visible laser pulse and monitored nuclear export of Pho4-GFP fusion constructs in real time. We observed distinct export kinetics for differentially phosphorylated Pho4 mutants, which demonstrates dynamic regulation of Pho4 function. This methodology should also facilitate the analysis of other cellular processes involving free serine residues, including catalysis, biomolecular recognition and ion transport.

Surveying polypeptide and protein domain conformation and association with FlAsH and ReAsH

Nature Chemical Biology 3, 779-784 (2007)
Nathan W Luedtke1,3, Rachel J Dexter1, Daniel B Fried1 & Alanna Schepartz



Recombinant polypeptides and protein domains containing two cysteine pairs located distal in primary sequence but proximal in the native folded or assembled state are labeled selectively in vitro and in mammalian cells using the profluorescent biarsenical reagents FlAsH-EDT2 and ReAsH-EDT2. This strategy, termed bipartite tetracysteine display, enables the detection of protein-protein interactions and alternative protein conformations in live cells. As proof of principle, we show that the equilibrium stability and fluorescence intensity of polypeptide–biarsenical complexes correlates with the thermodynamic stability of the protein fold or assembly. Destabilized protein variants form less stable and less bright biarsenical complexes, which allows discrimination of live cells expressing folded polypeptide and protein domains from those containing disruptive point mutations. Bipartite tetracysteine display may provide a means to detect early protein misfolding events associated with Alzheimer's disease, Parkinson's disease and cystic fibrosis; it may also enable high-throughput screening of compounds that stabilize discrete protein folds.

Nature Nanotechnology

Nanomechanical analysis of cells from cancer patients

Nature Nanotechnology 2, 780 - 783 (2007)
Sarah E. Cross1,2,4, Yu-Sheng Jin3,4, Jianyu Rao3,4 & James K. Gimzewski

Change in cell stiffness is a new characteristic of cancer cells that affects the way they spread1, 2. Despite several studies on architectural changes in cultured cell lines1, 3, no ex

vivo mechanical analyses of cancer cells obtained from patients have been reported. Using atomic force microscopy, we report the stiffness of live metastatic cancer cells taken from the body (pleural) fluids of patients with suspected lung, breast and pancreas cancer. Within the same sample, we find that the cell stiffness of metastatic cancer cells is more than 70% softer, with a standard deviation over five times narrower, than the benign cells that line the body cavity. Different cancer types were found to display a common stiffness. Our work shows that mechanical analysis can distinguish cancerous cells from normal ones even when they show similar shapes. These results show that nanomechanical analysis correlates well with immunohistochemical testing currently used for detecting cancer.

Nature Structural & Molecular Biology

Evidence of fibril-like bold beta-sheet structures in a neurotoxic amyloid intermediate of Alzheimer's bold beta-amyloid

Nature Structural & Molecular Biology 14, 1157 - 1164 (2007)
Sandra Chimon1, Medhat A Shaibat1, Christopher R Jones1, Diana C Calero1, Buzulagu Aizezi1 & Yoshitaka Ishii

Diffusible subfibrillar aggregates of amyloid proteins are potent neurotoxins and primary suspects in amyloid diseases including Alzheimer's disease. Despite widespread interest, the molecular structures of the amyloid intermediates and the conformational conversions in amyloid misfolding are poorly understood. Here we present a molecular-level examination of sequence-specific secondary structures and supramolecular structures of a neurotoxic amyloid intermediate of the 40-residue beta-amyloid (Abeta) peptide involved in Alzheimer's disease. Using solid-state NMR and electron microscopy, we show that, before fibrillization, natively unstructured monomeric Abeta is subject to large conformational changes into a spherical amyloid intermediate of 15–35 nm diameter, which has predominantly parallel beta-sheet structures. Structural comparison with Abeta fibrils demonstrates that formation of this beta-sheet intermediate (Ibeta) largely defines conformational transitions in amyloid misfolding. Neurotoxicity assays on PC12 cells show that Ibeta shows higher toxicity than the fibril, indicating that the beta-sheet formation may trigger neurotoxicity.

Science

Stabilizing Isopeptide Bonds Revealed in Gram-Positive Bacterial Pilus Structure

Science Vol 318, Issue 5856, 1625-1628, 7 December 2007
Hae Joo Kang,1,2 Fasséli Coulibaly,1,2 Fiona Clow,1,3 Thomas Proft,1,3* Edward N. Baker

Many bacterial pathogens have long, slender pili through which they adhere to host cells. The crystal structure of the major pilin subunit from the Gram-positive human pathogen Streptococcus pyogenes at 2.2 angstroms resolution reveals an extended structure comprising two all-β domains. The molecules associate in columns through the crystal, with each carboxyl terminus adjacent to a conserved lysine of the next molecule. This lysine forms the isopeptide bonds that link the subunits in native pili, validating the

relevance of the crystal assembly. Each subunit contains two lysine-asparagine isopeptide bonds generated by an intramolecular reaction, and we find evidence for similar isopeptide bonds in other cell surface proteins of Gram-positive bacteria. The present structure explains the strength and stability of such Gram-positive pili and could facilitate vaccine development.

Combinatorial Synthesis of Peptide Arrays onto a Microchip

Science Vol 318, Issue 5858, 1888, 21 December 2007
Fernandez,1 Klaus Leibe,1 Gloria Torralba,2 Michael Hausmann,2 Ulrich Trunk,2 Volker Lindenstruth,2† F. Ralf Bischoff,1†‡ Volker Stadler,1†‡ Frank Breitling

Arrays promise to advance biology through parallel screening for binding partners. We show the combinatorial in situ synthesis of 40,000 peptide spots per square centimeter on a microchip. Our variant Merrifield synthesis immobilizes activated amino acids as monomers within particles, which are successively attracted by electric fields generated on each pixel electrode of the chip. With all different amino acids addressed, particles are melted at once to initiate coupling. Repetitive coupling cycles should allow for the translation of whole proteomes into arrays of overlapping peptides that could be used for proteome research and antibody profiling.

Role of Intermolecular Forces in Defining Material Properties of Protein Nanofibrils

Science Vol 318, Issue 5858, 1900-1903, 21 December 2007
Tuomas P. Knowles,1,2* Anthony W. Fitzpatrick,2* Sarah Meehan,3 Helen R. Mott,4 Michele Vendruscolo,3 Christopher M. Dobson,3† Mark E. Welland

Protein molecules have the ability to form a rich variety of natural and artificial structures and materials. We show that amyloid fibrils, ordered supramolecular nanostructures that are self-assembled from a wide range of polypeptide molecules, have rigidities varying over four orders of magnitude, and constitute a class of high-performance biomaterials. We elucidate the molecular origin of fibril material properties and show that the major contribution to their rigidity stems from a generic interbackbone hydrogen-bonding network that is modulated by variable side-chain interactions.

PNAS

Directed selection of a conformational antibody domain that prevents mature amyloid fibril formation by stabilizing Aß protofibrils

PNAS | December 4, 2007 | vol. 104 | no. 49 | 19232-19237
Gernot Habicht*, Christian Haupt,, Ralf P. Friedrich, Peter Hortschansky*, Carsten Sachse, Jessica Meinhardt, Karin Wieligmann, Gerald P. Gellermann,, Michael Brodhun¶, Jürgen Götz||, Karl-Jürgen Halbhuber**, Christoph Röcken, Uwe Horn*, and Marcus Fändrich

The formation of amyloid fibrils is a common biochemical characteristic that occurs in Alzheimer's disease and several other amyloidoses. The unifying structural feature of amyloid fibrils is their specific type of ß-sheet conformation that differentiates these fibrils

from the products of normal protein folding reactions. Here we describe the generation of an antibody domain, termed B10, that recognizes an amyloid-specific and conformationally defined epitope. This antibody domain was selected by phage-display from a recombinant library of camelid antibody domains. Surface plasmon resonance, immunoblots, and immunohistochemistry show that this antibody domain distinguishes Aß amyloid fibrils from disaggregated Aß peptide as well as from specific Aß oligomers. The antibody domain possesses functional activity in preventing the formation of mature amyloid fibrils by stabilizing Aß protofibrils. These data suggest possible applications of B10 in the detection of amyloid fibrils or in the modulation of their formation.

Protein–protein interaction inhibition (2P2I) combining high throughput and virtual screening: Application to the HIV-1 Nef protein

PNAS | December 4, 2007 | vol. 104 | no. 49 | 19256-19261
Stéphane Betzi*, Audrey Restouin,,, Sandrine Opi,,, Stefan T. Arold¶, Isabelle Parrot||, Françoise Guerlesquin*, Xavier Morelli, and Yves Collette

Protein–protein recognition is the cornerstone of multiple cellular and pathological functions. Therefore, protein–protein interaction inhibition (2P2I) is endowed with great therapeutic potential despite the initial belief that 2P2I was refractory to small-molecule intervention. Improved knowledge of complex molecular binding surfaces has recently stimulated renewed interest for 2P2I, especially after identification of "hot spots" and first inhibitory compounds. However, the combination of target complexity and lack of starting compound has thwarted experimental results and created intellectual barriers. Here we combined virtual and experimental screening when no previously known inhibitors can be used as starting point in a structure-based research program that targets an SH3 binding surface of the HIV type I Nef protein. High-throughput docking and application of a pharmacophoric filter on one hand and search for analogy on the other hand identified drug-like compounds that were further confirmed to bind Nef in the micromolar range (isothermal titration calorimetry), to target the Nef SH3 binding surface (NMR experiments), and to efficiently compete for Nef–SH3 interactions (cell-based assay, GST pull-down). Initial identification of these compounds by virtual screening was validated by screening of the very same library of compounds in the cell-based assay, demonstrating that a significant enrichment factor was attained by the in silico screening. To our knowledge, our results identify the first set of drug-like compounds that functionally target the HIV-1 Nef SH3 binding surface and provide the basis for a powerful discovery process that should help to speed up 2P2I strategies and open avenues for new class of antiviral molecules.

Small molecule obatoclax (GX15-070) antagonizes MCL-1 and overcomes MCL-1-mediated resistance to apoptosis

PNAS | December 4, 2007 | vol. 104 | no. 49 | 19512-19517
Mai Nguyen*, Richard C. Marcellus, Anne Roulston, Mark Watson, Lucile Serfass, S. R. Murthy Madiraju, Daniel Goulet, Jean Viallet, Laurent Bélec, Xavier Billot, Stephane Acoca*,, Enrico Purisima*,, Adrian Wiegmans, Leonie Cluse, Ricky W. Johnstone, Pierre Beauparlant,¶, and Gordon C. Shore

Elevated expression of members of the BCL-2 pro-survival family of proteins can confer resistance to apoptosis in cancer cells. Small molecule obatoclax (GX15-070), which is predicted to occupy a hydrophobic pocket within the BH3 binding groove of BCL-2, antagonizes these members and induces apoptosis, dependent on BAX and BAK. Reconstitution in yeast confirmed that obatoclax acts on the pathway and overcomes BCL-2-, BCL-XL-, BCL-w-, and MCL-1-mediated resistance to BAX or BAK. The compound potently interfered with the direct interaction between MCL-1 and BAK in intact mitochondrial outer membrane and inhibited the association between MCL-1 and BAK in intact cells. MCL-1 has been shown to confer resistance to the BCL-2/BCL-XL/BCL-w-selective antagonist ABT-737 and to the proteasome inhibitor bortezomib. In both cases, this resistance was overcome by obatoclax. These findings support a rational clinical development opportunity for the compound in cancer indications or treatments where MCL-1 contributes to resistance to cell killing.

A chemical approach to unraveling the biological function of the glycosylphosphatidylinositol anchor

PNAS | December 18, 2007 | vol. 104 | no. 51 | 20332-20337
Margot G. Paulick*, Martin B. Forstner*, Jay T. Groves*,, and Carolyn R. Bertozzi

The glycosylphosphatidylinositol (GPI) anchor is a C-terminal posttranslational modification found on many eukaryotic proteins that reside in the outer leaflet of the cell membrane. The complex and diverse structures of GPI anchors suggest a rich spectrum of biological functions, but few have been confirmed experimentally because of the lack of appropriate techniques that allow for structural perturbation in a cellular context. We previously synthesized a series of GPI anchor analogs with systematic deletions within the glycan core and coupled them to the GFP by a combination of expressed protein ligation and native chemical ligation [Paulick MG, Wise AR, Forstner MB, Groves JT, Bertozzi CR (2007) J Am Chem Soc 129:11543–11550]. Here we investigate the behavior of these GPI-protein analogs in living cells. These modified proteins integrated into the plasma membranes of a variety of mammalian cells and were internalized and directed to recycling endosomes similarly to GFP bearing a native GPI anchor. The GPI-protein analogs also diffused freely in cellular membranes. However, changes in the glycan structure significantly affected membrane mobility, with the loss of monosaccharide units correlating to decreased diffusion. Thus, this cellular system provides a platform for dissecting the contributions of various GPI anchor components to their biological function.

Chemical mimicry of viral capsid self-assembly

PNAS | December 26, 2007 | vol. 104 | no. 52 | 20731-20736
Arthur J. Olson,, Yunfeng H. E. Hu, and Ehud Keinan

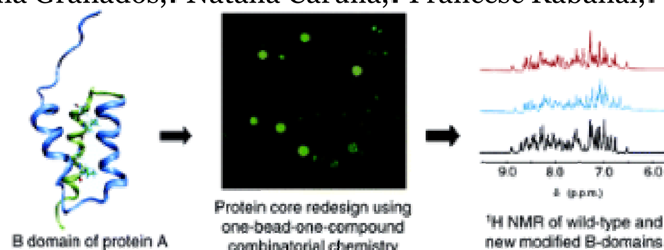
Stable structures of icosahedral symmetry can serve numerous functional roles, including chemical microencapsulation and delivery of drugs and biomolecules, epitope presentation to allow for an efficient immunization process, synthesis of nanoparticles of uniform size, observation of encapsulated reactive intermediates, formation of structural elements for supramolecular constructs, and molecular computing. By examining physical models of spherical virus assembly we have arrived at a general synthetic strategy for producing chemical capsids at size scales between fullerenes and spherical viruses. Such capsids can

be formed by self-assembly from a class of molecules developed from a symmetric pentagonal core. By designing chemical complementarity into the five interface edges of the molecule, we can produce self-assembling stable structures of icosahedral symmetry. We considered three different binding mechanisms: hydrogen bonding, metal binding, and formation of disulfide bonds. These structures can be designed to assemble and disassemble under controlled environmental conditions. We have conducted molecular dynamics simulation on a class of corannulene-based molecules to demonstrate the characteristics of self-assembly and to aid in the design of the molecular subunits. The edge complementarities can be of diverse structure, and they need not reflect the fivefold symmetry of the molecular core. Thus, self-assembling capsids formed from coded subunits can serve as addressable nanocontainers or custom-made structural elements.

Journal of the American Chemical Society

Redesign of Protein Domains Using One-Bead-One-Compound Combinatorial Chemistry

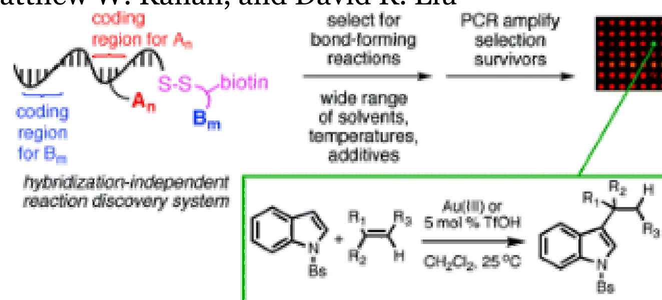
J. Am. Chem. Soc., 2007, 129 (48), 14922 -14932
Jose J. Pastor,† Giovanna Granados,† Natàlia Carulla,† Francesc Rabanal,‡ and Ernest Giralt



A novel combinatorial strategy for the redesign of proteins based on the strength and specificity of intra- and interprotein interactions is described. The strategy has been used to redesign the hydrophobic core of the B domain of protein A. Using one-bead-one-compound combinatorial chemistry, 300 analogues of the C-terminal α-helix of the B domain, H3x, have been synthesized using a biocompatible resin and the HMFS linker, allowing the screening to occur while the peptides were bound to the resin. The screening was based on the ability of the H3x analogues to interact with the N-terminal helices of the B domain, H1-H2, and retain the native B domain activity, that is binding to IgG. Eight different analogues containing some nonconservative mutations were obtained from the library, the two most frequent of which, H3P1 and H3P2, were studied in detail. CD analysis revealed that the active analogues interact with H1-H2. To validate the redesign strategy the covalent modified domains H1-H2-H3P1 and H1-H2-H3P2 were synthesized and characterized. CD and NMR analysis revealed that they had a unique, stable, and well-defined three-dimensional structure similar to that for the wild-type B domain. This combinatorial strategy allows us to select for redesigned proteins with the desired activity or the desired physicochemical properties provided the right screening test is used. Furthermore, it is rich in potential for the chemical modification of proteins overcoming the drawbacks associated with the total synthesis of large protein domains.

Development and Initial Application of a Hybridization-Independent, DNA-Encoded Reaction Discovery System Compatible with Organic Solvents

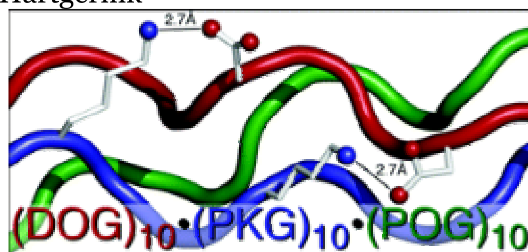
J. Am. Chem. Soc., 2007, 129 (48), 14933 -14938
Mary M. Rozenman, Matthew W. Kanan, and David R. Liu



We have developed and applied an approach to reaction discovery that takes advantage of DNA encoding, DNA-programmed assembly of substrate pairs, in vitro selection, and PCR amplification, yet does not require reaction conditions that support DNA hybridization. This system allows the simultaneous evaluation of >200 potential bond-forming combinations of substrates in a single experiment and can be applied in a range of solvent and temperature conditions. In an initial application, we applied this system to explore Au(III)-mediated chemistry and uncovered a simple, mild method for the selective Markovnikov-type hydroarylation of vinyl arenes and trisubstituted olefins with indoles.

Surprisingly High Stability of Collagen ABC Heterotrimer: Evaluation of Side Chain Charge Pairs

J. Am. Chem. Soc., 2007, 129 (48), 15034 -15041
Varun Gauba and Jeffrey D. Hartgerink

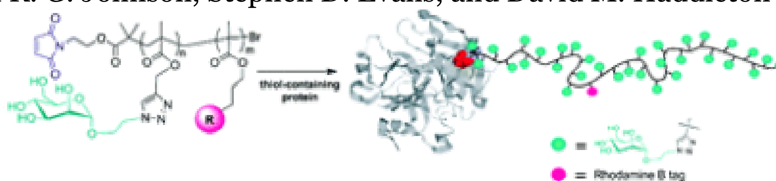


Type I collagen is a major component of skin, tendon, and ligament and forms more than 90% of bone mass. It is an AAB heterotrimer assembled from two identical α1 and one α2 chains. However, the majority of studies on the effects of amino acid substitution on triple helix stability have been performed on collagen homotrimeric helices. In a homotrimer, it is impossible to determine whether the contribution to stability is from the polyproline II helix propensity of the amino acids or from interhelix amino acid interactions. The presence of amino acids in all three chains further exaggerates their contribution. In contrast, in a heterotrimer, the individual chains may be tailored in order to have the substitution in one, two, or all three chains. Therefore, a heterotrimer can divulge specific information about any interaction based upon the substitutions in individual chains. In this paper, we evaluate the contribution of electrostatic interactions between side chain charge pairs on the stability of heterotrimers. We synthesize and analyze the stability of four AAB and four ABC heterotrimers including a surprisingly stable ABC heterotrimer composed of (DOG)10, (PKG)10, and (POG)10 chains (O = hydroxyproline). This heterotrimer has a stability comparable to that of a (POG)10 homotrimer even though D and K occur 20 times in the heterotrimeric helix and have been previously shown to significantly destabilize the triple helix compared to the P and O imino acids. These results

show that the stability of heterotrimers cannot be directly determined from the analysis of charge pairs in homotrimers. Because collagen heterotrimers can be designed to have substitution in one, two, or three chains, it gives us the ability to decode cross-strand interactions in collagen in a similar fashion to α-helical coiled-coil interactions and DNA duplex hydrogen bonding.

Site-Directed Conjugation of "Clicked" Glycopolymers To Form Glycoprotein Mimics: Binding to Mammalian Lectin and Induction of Immunological Function

J. Am. Chem. Soc., 2007, 129 (49), 15156 -15163
Jin Geng, Giuseppe Mantovani, Lei Tao, Julien Nicolas, Gaojian Chen, Russell Wallis, Daniel A. Mitchell, Benjamin R. G. Johnson, Stephen D. Evans, and David M. Haddleton



Synthesis of well-defined neoglycopolymer-protein biohybrid materials and a preliminary study focused on their ability of binding mammalian lectins and inducing immunological function is reported. Crucial intermediates for their preparation are well-defined maleimide-terminated neoglycopolymers (Mn = 8-30 kDa; Mw/Mn = 1.20-1.28) presenting multiple copies of mannose epitope units, obtained by combination of transition-metal-mediated living radical polymerization (TMM LRP) and Huisgen [2+3] cycloaddition. Bovine serum albumin (BSA) was employed as single thiol-containing model protein, and the resulting bioconjugates were purified following two independent protocols and characterized by circular dichroism (CD) spectroscopy, SDS PAGE, and SEC HPLC. The versatility of the synthetic strategy presented in this work was demonstrated by preparing a small library of conjugating glycopolymers that only differ from each other for their relative epitope density were prepared by coclicking of appropriate mixtures of mannopyranoside and galactopyranoside azides to the same polyalkyne scaffold intermediate. Surface plasmon resonance binding studies carried out using recombinant rat mannose-binding lectin (MBL) showed clear and dose-dependent MBL binding to glycopolymer-conjugated BSA. In addition, enzyme-linked immunosorbent assay (ELISA) revealed that the neoglycopolymer-protein materials described in this work possess significantly enhanced capacity to activate complement via the lectin pathway when compared with native unmodified BSA.

Non-enzymatic Covalent Protein Labeling Using a Reactive Tag

J. Am. Chem. Soc., 129 (51), 15777 -15779, 2007.
Hiroshi Nonaka, Shinya Tsukiji, Akio Ojida, and Itaru Hamachi*

We describe herein a new method for covalent labeling of proteins using a complementary recognition pair of peptide tag and synthetic molecular probe. The rapid and specific covalent labeling of a tag-fused protein was achieved by the reaction on the tag site with the probe through their selective molecular recognition. The advantages of this method involve the facile functional modification and the high labeling specificity of the tag-fused

protein, which are demonstrated in the labeling experiments in various conditions even inside cells.

The Reversal by Sulfate of the Denaturant Activity of Guanidinium

J. Am. Chem. Soc., 129 (51), 15895 -15902, 2007.
Christopher E. Dempsey,* Philip E. Mason, John W. Brady, and George W. Neilson

Guanidinium (Gdm+) chloride is a powerful protein denaturant, whereas the sulfate dianion (SO42-) is a strong stabilizer of folded protein states; Gdm2SO4 is effectively neutral in its effects on protein stability. While the "neutralizing" effects of protein-stabilizing solutes on the activity of denaturants can be broadly interpreted in terms of additive effects of the solutes, recent experimental and simulation studies support a role for hetero-ion interactions in the effect of sulfate on Gdm+ denaturation [Mason, P. E.; et al. J. Phys. Chem. B 2005, 109, 24185-24196]. Here we describe an experimental strategy for testing this mechanism that involves spectroscopic analysis of the separate effects of alkali metal sulfates (Na2SO4, Rb2SO4), GdmCl, and Gdm2SO4 on the folded populations of several peptides chosen to dissect specific noncovalent contributions to the conformational stability of proteins [alanine-based helical peptides stabilized by hydrogen bonds, tryptophan zipper (trpzip) peptides stabilized largely by cross-strand indole-indole interactions]. While the trpzip peptides are highly sensitive to GdmCl denaturation, they are unaffected by NaCl, Na2SO4, or Gdm2SO4, indicating that the reversal of the denaturant activity of Gdm+ by sulfate in this case is not due to competing stabilizing (sulfate) and destabilizing (Gdm+) interactions. Gdm2SO4 was found to retain considerable denaturant activity against alanine-based -helical peptides. The differences in the effects of Gdm2SO4 on the two peptide types can be understood in terms of the different mechanisms of Gdm+ denaturation of trpzip peptides and helical peptides, respectively, and the specific nature of Gdm+ and SO42- ionic "clustering" that differentially affects the ability of Gdm+ to make the molecular interactions with the peptides that underlie its denaturant activity.

Chemosensory Performance of Molecularly Imprinted Fluorescent Conjugated Polymer Materials

J. Am. Chem. Soc., 129 (51), 15911 -15918, 2007.
Jiahui Li, Claire E. Kendig, and Evgueni E. Nesterov

Fluorescent conjugated polymers are an attractive basis for the design of low detection limit sensing devices owing to their intrinsic signal amplification capability. A simple and universal method to rationally control or fine-tune the chemodetection selectivity of conjugated polymer materials toward a desired analytical target would further benefit their applications. In a quest of such a method we investigated a general approach to cross-linked molecularly imprinted fluorescent conjugated polymer (MICP) materials that possess an intrinsic capability for signal transduction and have potential to enhance selectivity and sensitivity of sensor devices based on conjugated polymers. To study these capabilities, we prepared an MICP material for the detection of 2,4,6-trinitrotoluene and related nitroaromatic compounds. We found the imprinting effect in this material to be based on analyte shape/size recognition being substantial and generally overcoming other competing thermodynamically determined trends. The described molecularly imprinted

fluorescent conjugated polymers show remarkable air stability and photostability, high fluorescence quantum yield, and reversible analyte binding and therefore are advantageous for sensing applications due to the ability to "preprogram" their detection selectivity through a choice of an imprinted template.

Nano-Flares: Probes for Transfection and mRNA Detection in Living Cells

J. Am. Chem. Soc., 129 (50), 15477 -15479, 2007.
David A. Giljohann, Haley D. Hill, Andrew E. Prigodich, and Chad A. Mirkin*

We demonstrate that novel oligonucleotide-modified gold nanoparticle probes hybridized to fluorophore-labeled complements can be used as both transfection agents and cellular "nano-flares" for detecting mRNA in living cells. Nano-flares take advantage of the highly efficient fluorescence quenching properties of gold, cellular uptake of oligonucleotide nanoparticle conjugates without the use of transfection agents, and the enzymatic stability of such conjugates, thus overcoming many of the challenges to creating sensitive and effective intracellular probes. Nano-flares exhibit high signaling, have low background fluorescence, and are sensitive to changes in the number of RNA transcripts present in cells.

Stepwise Noncovalent Synthesis Leading to Dendrimer-Based Assemblies in Water

J. Am. Chem. Soc., 129 (50), 15631 -15638, 2007.
Mezari, Ellen N. M. Van Leeuwen, Matthijn R. J. Vos, Pieter C. M. M. Magusin, Peter A. J. Hilbers, Marcel H. P. Van Genderen, Nico A. J. M. Sommerdijk, George Fytas, and E.W. Meijer

We provide detailed insight into complex supramolecular assembly processes by fully characterizing a multicomponent model system using dynamic light scattering, cryogenic transmission electron microscopy, atomic force microscopy, and various NMR techniques. First, a preassembly of a host molecule (the fifth-generation urea-adamantyl poly(propylene imine) dendrimer) and 32 guest molecules (a water- and chloroform-soluble ureidoacetic acid guest) was made in chloroform. The association constant in chloroform is concealed by guest self-association and is therefore higher than 103 M-1. Via the neat state the single-host complex was transferred to water, where larger dendrimer-based assemblies were formed. The core of these assemblies, consisting of multiple host molecules (on average three), is kinetically trapped upon dissolution in water, and its size is constant irrespective of the concentration. The guest molecules forming the corona of the assemblies, however, stay dynamic since they are still in rapid exchange on the NMR time scale, as they were in chloroform. A stepwise noncovalent synthesis provides a means to obtain metastable dynamic supramolecular assemblies in water, structures that cannot be formed in one step.

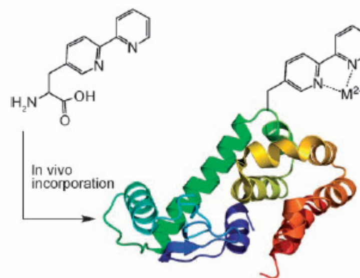
Angewandte Chemie

Nonnatural Amino Acids

J. Xie, W. Liu, P. G. Schultz* **9239–9242**

A Genetically Encoded Bidentate, Metal-Binding Amino Acid

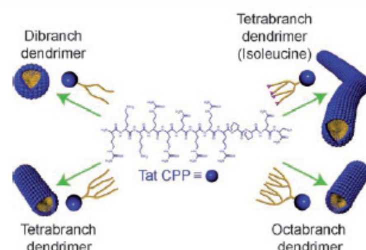
A two-ring binder: To facilitate the design of metalloproteins, the bidentate, metal-binding amino acid bipyridylalanine (BpyAla) was genetically encoded in *E. coli* in response to the amber nonsense codon with high fidelity and yield. The incorporation of BpyAla requires a BpyAla-specific aminoacyl-tRNA synthetase, which was evolved in a stepwise fashion. The structural basis of selective recognition of BpyAla by this synthetase was also determined.



Peptide-Coated Nanostructures

Y.-b. Lim, E. Lee, M. Lee* — **9011–9014**

Controlled Bioactive Nanostructures from Self-Assembly of Peptide Building Blocks



Dendrimers wearing functional coats: Dendrimerization of hydrophobic lipid segments in supramolecular building blocks comprising lipid dendrimers and Tat cell-penetrating peptide (Tat CPP; see schematic representation) enables the morphology, size, and aggregation strength of peptide-coated functional nanostructures to be controlled.

ACS Chemical Biology

An Oxazole-Based Small-Molecule Stat3 Inhibitor Modulates Stat3 Stability and Processing and Induces Antitumor Cell Effects

ACS Chem. Biol., 2 (12), 787–798

Khandaker A. Z. Siddiquee, Patrick T. Gunning, Matthew Glenn§, William P. Katt, Shumin Zhang, Christopher Schroeck, Sebti, Richard Jove, Andrew D. Hamilton§, and James Turkson

Stat3 is hyperactivated in many human tumors and represents a valid target for anticancer drug design. We present a novel small-molecule Stat3 inhibitor, S3I-M2001, and describe the dynamics of intracellular processing of activated Stat3 within the context of the biochemical and biological effects of the Stat3 inhibitor. S3I-M2001 is an oxazole-based peptidomimetic of the Stat3 Src homology (SH) 2 domain-binding phosphotyrosine peptide that selectively disrupts active Stat3:Stat3 dimers. Consequently, hyperactivated Stat3, which hitherto occurs as "dotlike" structures of nuclear bodies, undergoes an early aggregation into nonfunctional perinuclear aggresomes and a late-phase proteasome-mediated degradation in malignant cells treated with S3I-M2001. Thus, S3I-M2001 inhibited Stat3-dependent transcriptional regulation of tumor survival genes, such as Bcl-xL. Furthermore, Stat3-dependent malignant transformation, survival, and migration and invasion of mouse and human cancer cells harboring persistently activated Stat3 were inhibited by S3I-M2001. Finally, S3I-M2001 inhibited growth of human breast tumor xenografts. The study identifies a novel Stat3 inhibitor, S3I-M2001, with antitumor cell effects mediated in part through a biphasic loss of functional Stat3. The study represents the first on intracellular Stat3 stability and processing following inhibition by a small molecule that has significant antitumor activity.

Chemistry and Biology

Arabidopsis P-Glycoprotein19 Participates in the Inhibition of Gravitropism by Gravacin

Volume 14, Issue 12, 26 December 2007, Pages 1366-1376
Yan Cheng, Sean Cuttler, Wendy A. Peer, Angus S. Murphy and Natasha V. Raikhel

ATP-binding cassette (ABC) transporters have been implicated in a multitude of biological pathways. In plants, some ABC transporters are involved in the polar transport of the plant hormone auxin and the gravitropic response. We previously identified Gravacin as a potent inhibitor of gravitropism in Arabidopsis thaliana. We demonstrate that P-glycoprotein19 (PGP19) is a target for Gravacin and participates in its inhibition of gravitropism. Gravacin inhibited the auxin transport activity of PGP19 and PGP19-PIN complexes. Furthermore, we identified E1174 as an important residue for PGP19 activity and its ability to form active transport complexes with PIN1. Gravacin is an auxin transport inhibitor that inhibits PGPs, particularly PGP19, which can be used to further dissect the role of PGP19 without the inhibition of other auxin transporters, namely PIN proteins.

Emetine Regulates the Alternative Splicing of Bcl-x through a Protein Phosphatase 1-Dependent Mechanism

Volume14, Issue12, 26 December 2007, Pages 1386-1392
Kritsanapol Boon-Unge, Qingming Yu, Tie Zou, An Zhou, Piyarat Govitrapong and Jianhua Zhou

Exon 2 of the Bcl-x gene undergoes alternative splicing in which the Bcl-xS splice variant promotes apoptosis in contrast to the anti-apoptotic splice variant Bcl-xL. In this study, the regulation of the alternative splicing of pre-mRNA of Bcl-x was examined in response to emetine. Treatment of different types of cancer cells with emetine dihydrochloride downregulated the level of Bcl-xL mRNA with a concomitant increase in the mRNA level of Bcl-xS in a dose- and time-dependent manner. Pretreatment with calyculin A, an inhibitor of protein phosphatase 1 (PP1) and protein phosphatase 2A (PP2A), blocked emetine-induced alternative splicing in contrast to okadaic acid, a specific inhibitor of PP2A in cells, demonstrating a PP1-mediated mechanism. Our finding on the regulation of RNA splicing of members of the Bcl-2 family in response to emetine presents a potential target for cancer treatment

Chemical Biology and Drug Design

The Use of Rigid, Fibrillar Congo Red Nanostructures for Scaffolding Protein Assemblies and Inducing the Formation of Amyloid-like Arrangement of Molecules

Chem Biol Drug Des 2007; 70: 491–501
Konieczny[1], Janina Rybarska[1], Marcin Kro´ l[2], Irena Roterman[2], Barbara Urbanowicz[3] and Janina Zie˛ba-Palus

The ordered amyloid-like organization of protein aggregates was obtained using for their formation the rigid fibrillar nanostructures of Congo red as the scaffolding. The higher rigidity of used dye nanoparticles resulted from the stronger stacking of molecules at low

pH (near the pK of the dye amino group) because of the decreased charge repulsion. The polylysine, human globin, and immunoglobulin L chain were arranged in this way to form deposits of amyloid properties. The scaffolding was introduced simply by mixing the dye and proteins at a low pH or the dye was used in the preorganized form by maintaining it in the electric field before and during protein addition. The polarization and electron microscopy studies confirmed the unidirectional organization of the complex. The precipitate of the complex was used for studies directly or after the partial or complete removal of the dye. The results suggest that the process of formation of amyloid-like deposits may bypass the nucleation step. It is possible if the protein aggregation occurs in unidirectionally organized (because of scaffolding) assembly of molecules, arranged prior to self-association. The recognition of the structure of amphoteric Congo red nanoparticles used for the scaffolding was based on the molecular dynamics simulation.

Selectivity-determining Residues in Plk1

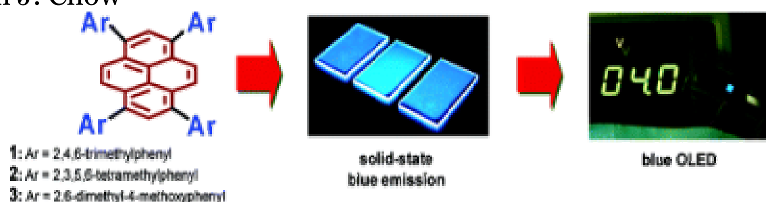
Chem Biol Drug Des 2007; 70: 540–54
Michael Kothe, Darcy Kohls, Simon Low, Rocco Coli, Glen R. Rennie, Frederic Feru, Cyrille Kuhn and Yuan-Hua Ding

Polo-like kinase 1 is an important regulator of cell cycle progression whose over-expression is often associated with oncogenesis. Polo-like kinase 1 hence represents an attractive target for cancer intervention. BI 2536 (Boehringer Ingelheim, Ingelheim, Germany), a Polo-like kinase 1 inhibitor currently in clinical trials, exhibits nanomolar potency against Polo-like kinase isoforms and high selectivity against other kinases. We have previously published the crystal structures of the Polo-like kinase 1 domain in complex with AMPPNP and an Aurora A inhibitor. In this work, we present the co-crystal structure of Polo-like kinase 1 with BI 2536. The structure, in combination with selectivity data for BI 2536 and related compounds, illustrates important features for potency and selectivity. In particular, we show that the methoxy group of BI 2536 is an important specificity determinant against non-Polo-like kinases by taking advantage of a small pocket generated by Leu 132 in the hinge region of Polo-like kinase 1. The work presented here provides a framework for structure-based drug design of Polo-like kinase 1-specific inhibitors.

Organic Letters

Steric Inhibition of π-Stacking: 1,3,6,8-Tetraarylpyrenes as Efficient Blue Emitters in Organic Light Emitting Diodes (OLEDs)

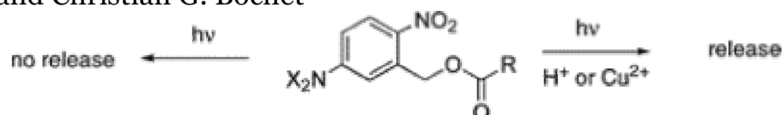
Org. Lett., 9 (25), 5215 -5218, 2007.
Jarugu Narasimha Moorthy,*† Palani Natarajan,† Parthasarathy Venkatakrishnan,† Duo-Fong Huang,‡ and Tahsin J. Chow



The sterically congested tetraarylpyrenes 1-3, which can be readily accessed by Suzuki coupling, exhibit no-aggregation (π-stacking) behavior in both solution and solid states. The indisposed tendency of 1-3 toward crystallization and their moderate molecular dimensions permit exploitation as blue light emitting materials in OLEDs with respectable device performances.

New Safety-Catch Photolabile Protecting Group

***Org. Lett.*, 9 (26), 5453 -5456, 2007.**
Emmanuel Riguet and Christian G. Bochet



Photolabile protecting groups have proven their usefulness on many occasions. Their versions as linkers are however less attractive, as robustness and real orthogonality become critical issues. Safety-catch systems, where a preliminary activation phase is necessary, circumvent the problem of premature cleavage. In this work, we introduce a new safety-catch photolabile protecting group, whose cleavage requires the simultaneous presence of light and a chemical promoter.