

Chmielewski Group Literature Abstracts

CHEMISTRY

BIOLOGY

April 2008

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Nature

A nuclear receptor-like pathway regulating multidrug resistance in fungi

***Nature* 452, 604-609 (3 April 2008)**

Jitendra K. Thakur¹, Haribabu Arthanari, Fajun Yang, Shih-Jung Pan, Xiaochun Fan, Julia Breger, Kevin Struhl, W. Scott Moye-Rowley, Brendan P. Cormack, Gerhard Wagner & Anders M. Näär

Multidrug resistance (MDR) is a serious complication during treatment of opportunistic fungal infections that frequently afflict immunocompromised individuals, such as transplant recipients and cancer patients undergoing cytotoxic chemotherapy. Improved knowledge of the molecular pathways controlling MDR in pathogenic fungi should facilitate the development of novel therapies to combat these intransigent infections. MDR is often caused by upregulation of drug efflux pumps by members of the fungal zinc-cluster transcription-factor family (for example Pdr1p orthologues). However, the molecular mechanisms are poorly understood. Here we show that Pdr1p family members in *Saccharomyces cerevisiae* and the human pathogen *Candida glabrata* directly bind to structurally diverse drugs and xenobiotics, resulting in stimulated expression of drug efflux pumps and induction of MDR. Notably, this is mechanistically similar to regulation of MDR in vertebrates by the PXR nuclear receptor, revealing an unexpected functional analogy of fungal and metazoan regulators of MDR. We have also uncovered a critical and specific role of the Gal11p/MED15 subunit of the Mediator co-activator and its activator-targeted KIX domain in antifungal/xenobiotic-dependent regulation of MDR. This detailed mechanistic understanding of a fungal nuclear receptor-like gene regulatory pathway provides novel therapeutic targets for the treatment of multidrug-resistant fungal infections.

Nature Biotechnology

Resolution of liver cirrhosis using vitamin A-coupled liposomes to deliver siRNA against a collagen-specific chaperone

***Nature Biotechnology* 26, 431 - 442 (2008)**

Yasushi Sato, Kazuyuki Murase, Junji Kato, Masayoshi Kobune, Sato, Yutaka Kawano, Rishu Takimoto, Kouichi Takada, Koji Miyanishi, Takuya Matsunaga, Tetsuji Takayama & Yoshiro Niitsu

There are currently no approved antifibrotic therapies for liver cirrhosis. We used vitamin A-coupled liposomes to deliver small interfering RNA (siRNA) against gp46, the rat homolog of human heat shock protein 47, to hepatic stellate cells. Our approach exploits the key roles of these cells in both fibrogenesis as well as uptake and storage of vitamin A. Five treatments with the siRNA-bearing vitamin A-coupled liposomes almost completely resolved liver fibrosis and prolonged survival in rats with otherwise lethal dimethylnitrosamine-induced liver cirrhosis in a dose- and duration-dependent manner. Rescue was not related to off-target effects or associated with recruitment of innate immunity. Receptor-specific siRNA delivery was similarly effective in suppressing collagen secretion and treating fibrosis induced by CCl₄ or bile duct ligation. The efficacy of the

approach using both acute and chronic models of liver fibrosis suggests its therapeutic potential for reversing human liver cirrhosis.

Science

Efficient Inhibition of the Alzheimer's Disease β -Secretase by Membrane Targeting

Science 25 April 2008:Vol. 320. no. 5875, pp. 520 - 523

Lawrence Rajendran, Anja Schneider, Sebastian Weidlich, Jonas Ries, Tobias Braxmeier, Cornelia Schroeder, Mikael Simons, Gary Jennings, Hans-Joachim Knölker, Kai Simons

β -Secretase plays a critical role in β -amyloid formation and thus provides a therapeutic target for Alzheimer's disease. Inhibitor design has usually focused on active-site binding, neglecting the subcellular localization of active enzyme. We have addressed this issue by synthesizing a membrane-anchored version of a β -secretase transition-state inhibitor by linking it to a sterol moiety. Thus, we targeted the inhibitor to active β -secretase found in endosomes and also reduced the dimensionality of the inhibitor, increasing its local membrane concentration. This inhibitor reduced enzyme activity much more efficiently than did the free inhibitor in cultured cells and in vivo. In addition to effectively targeting β -secretase, this strategy could also be used in designing potent drugs against other membrane protein targets.

PNAS

Rare steroid receptor-negative basal-like tumorigenic cells in luminal subtype human breast cancer xenografts

PNAS | April 15, 2008 | vol. 105 | no. 15 | 5774-5779

Kathryn B. Horwitz, Wendy W. Dye, Joshua Chuck Harrell, Peter Kabos, and Carol A. Sartorius

There are two major subtypes of human breast cancers: the luminal, estrogen, and progesterone receptor-positive, cytokeratin 18-positive (ER+PR+CK18+) subtype, and the basal ER-PR-CK18-CK5+ subtype. Tumor-initiating cells (CD44+) have been described for human breast cancers; whether these are common to the two subtypes is unknown. We have identified a rare population of cells that are both CD44+ and ER-PR-CK5+ in luminal-like ER+PR+ T47D human breast tumor xenografts. The tumor-isolated CD44+ cell fraction was highly enriched for clonogenic (in vitro culture) and tumorigenic (in vivo reimplantation) cells compared with the CD44- cell fraction. Rare ER-PR-CK5+ cells were present within CD44+-derived colonies. Tumor-isolated cells placed in minimal media also contained rare ER-PR-CK5+ cells at early time points (<10 cells); however, this population did not expand with increasing colony size. The number of ER+PR+CK5- cells, conversely, increased linearly with colony growth. Similarly, tumors originating in vivo from CD44+ cells contained a rare static ER-PR-CK5+ population, an intermediate ER-PR-CK5- population, and an expanding ER+PR+CK5- population. Putative ER+PR+CK5+ transitional cells could be seen only in colonies or tumors treated with a progestin. We propose that luminal ER+PR+ breast tumors contain a minor ER-PR-CK5+ population that has the capacity to generate the majority of ER+PR+CK18+CK5- cells.

Luminal breast cancers are treated with endocrine therapies that target ER. The rare ER–PR–CK5+ progenitor cells would escape such treatments and survive to repopulate the tumor.

Artificial miRNAs mitigate shRNA-mediated toxicity in the brain: Implications for the therapeutic development of RNAi

PNAS | **April 15, 2008** | *vol. 105* | *no. 15* | **5868-5873**

Jodi L. McBride, Ryan L. Boudreau, Scott Q. Harper, Patrick D. Staber, Alex Mas Monteys, Inês Martins, Brian L. Gilmore, Haim Burstein, Richard W. Peluso, Barry Polisky, Barrie J. Carter, and Beverly L. Davidson

Huntington's disease (HD) is a fatal, dominant neurodegenerative disease caused by a polyglutamine repeat expansion in exon 1 of the HD gene, which encodes the huntingtin protein. We and others have shown that RNAi is a candidate therapy for HD because expression of inhibitory RNAs targeting mutant human HD transgenes improved neuropathology and behavioral deficits in HD mouse models. Here, we developed shRNAs targeting conserved sequences in human HD and mouse HD homolog (HDh) mRNAs to initiate preclinical testing in a knockin mouse model of HD. We screened 35 shRNAs in vitro and subsequently narrowed our focus to three candidates for in vivo testing. Unexpectedly, two active shRNAs induced significant neurotoxicity in mouse striatum, although HDh mRNA expression was reduced to similar levels by all three. Additionally, a control shRNA containing mismatches also induced toxicity, although it did not reduce HDh mRNA expression. Interestingly, the toxic shRNAs generated higher antisense RNA levels, compared with the nontoxic shRNA. These results demonstrate that the robust levels of antisense RNAs emerging from shRNA expression systems can be problematic in the mouse brain. Importantly, when sequences that were toxic in the context of shRNAs were placed into artificial microRNA (miRNA) expression systems, molecular and neuropathological readouts of neurotoxicity were significantly attenuated without compromising mouse HDh silencing efficacy. Thus, miRNA-based approaches may provide more appropriate biological tools for expressing inhibitory RNAs in the brain, the implications of which are crucial to the development of RNAi for both basic biological and therapeutic applications.

A mutation of the H-loop selectively affects rhodamine transport by the yeast multidrug ABC transporter Pdr5

PNAS | **April 1, 2008** | *vol. 105* | *no. 13* | **5069-5074**

Robert Ernst, Petra Kueppers, Cornelia M. Klein, Tobias Schwarzmüller, Karl Kuchler, and Lutz Schmitt

The yeast ABC transporter Pdr5 plays a major role in drug resistance against a large number of structurally unrelated compounds. Although Pdr5 has been extensively studied, many important aspects regarding its molecular mechanisms remain unresolved. For example, a striking degeneration of conserved amino acid residues exists in the nucleotide binding domains (NBDs), but their functional relevance is unknown. Here, we performed in vivo and in vitro experiments to address the functional asymmetry of NBDs. It became evident by ATPase activity and drug transport studies that catalysis at only one of the two NBD composite sites is crucial for protein function. Furthermore, mutations of the

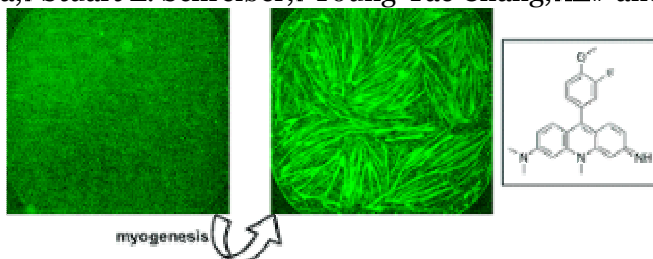
proposed "catalytic carboxylate" (E1036) and the "catalytic dyad histidine" (H1068) were characterized. Although a mutation of the glutamate abolished ATPase activity and substrate transport, mutation of H1068 had no influence on ATP consumption. However, the H1068A mutation abolished rhodamine transport in vivo and in vitro, while leaving the transport of other substrates unaffected. By contrast to mammalian P-glycoprotein (P-gp), the ATPase activity of yeast Pdr5 is not stimulated by the addition of substrates, indicating that Pdr5 is an uncoupled ABC transporter that constantly hydrolyses ATP to ensure active substrate transport. Taken together, our data provide important insights into the molecular mechanism of Pdr5 and suggest that not solely the transmembrane domains dictate substrate selection.

Journal of the American Chemical Society

Small-Molecule Fluorophores To Detect Cell-State Switching in the Context of High-Throughput Screening

J. Am. Chem. Soc., 2008, 130 (13), 4208-4209

Bridget K. Wagner,*† Hyman A. Carrinski,† Young-Hoon Ahn,‡ Yun Kyung Kim,§¶# Tamara J. Gilbert,† Dina A. Fomina,† Stuart L. Schreiber,† Young-Tae Chang,§¶# and Paul A. Clemons

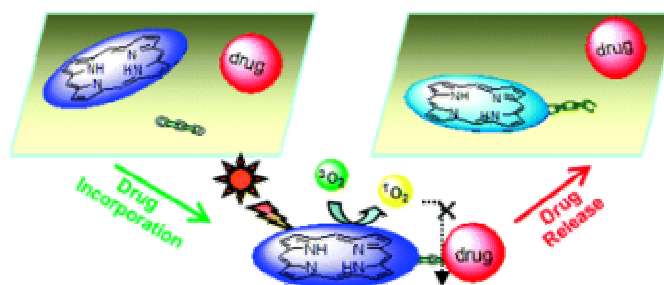


A small molecule capable of distinguishing the distinct states resulting from cellular differentiation would be of enormous value, for example, in efforts aimed at regenerative medicine. We screened a collection of fluorescent small molecules for the ability to distinguish the differentiated state of a mouse skeletal muscle cell line. High-throughput fluorescence-based screening of C2C12 myoblasts and myotubes resulted in the identification of six compounds with the desired selectivity, which was confirmed by high-content screening in the same cell states. The compound that resulted in the greatest fluorescence intensity difference between the cell states was used as the screening agent in a pilot screen of 84 kinase inhibitors, each present in four doses, for inhibition of myogenesis. Of the kinase inhibitors, 17 resulted in reduction of fluorescence at one or more concentrations; among the "hits" included known inhibitors of myogenesis, confirming that this compound is capable of detecting the differentiated myotube state. We suggest that the strategy of screening for screening agents reported here may be extended more broadly in the future.

Site-Specific Prodrug Release Using Visible Light

J. Am. Chem. Soc., 2008, 130 (13), 4236-4237

Michael Y. Jiang and David Dolphin

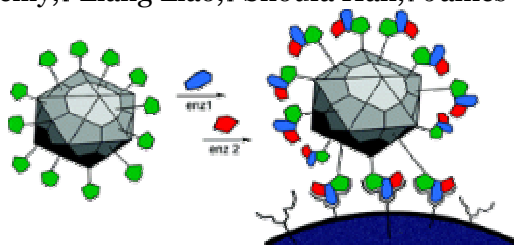


Using a photosensitization-singlet oxygenation-dioxetane cleavage strategy, a photodynamic prodrug system has been developed, whereby drugs bearing carbonyl groups can first be attached to a photosensitizer to give a photosensitizer-drug complex and then released from the complex upon visible light irradiation. Visible light, which has good penetration through tissue, generates singlet oxygen via the photosensitizer, which then releases the prodrug when and where required. With this system, drug mimics and methyl esters of NSAIDs have been successfully incorporated with photosensitizers related to verteporfin and then released by visible light illumination in high to quantitative yields within minutes.

On-Virus Construction of Polyvalent Glycan Ligands for Cell-Surface Receptors

J. Am. Chem. Soc., 2008, 130 (14), 4578-4579

Eiton Kaltgrad,[†] Mary K. O'Reilly,[‡] Liang Liao,[‡] Shoufa Han,[‡] James C. Paulson,^{*‡} and M. G. Finn

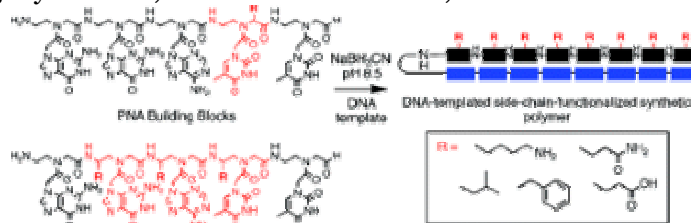


Glycans arrayed on the exterior of virus particles were used as substrates for glycosyltransferase reactions to build di- and trisaccharides from the virus surface. The resulting particles exhibited tight and specific associations with cognate receptors on beads and cells, in one example defeating in cis cell-surface interactions in a manner characteristic of polyvalent binding. Combined with the ability of viruses to provide structurally well-defined attachment points, the methodology provides a convenient and powerful way to prepare complex carbohydrate ligands for clustered receptors.

DNA-Templated Polymerization of Side-Chain-Functionalized Peptide Nucleic Acid Aldehydes

J. Am. Chem. Soc., 2008, 130 (14), 4646-4659

Ralph E. Kleiner, Yevgeny Brudno, Michael E. Birnbaum, and David R. Liu

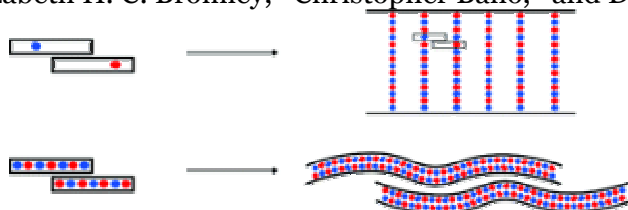


The DNA-templated polymerization of synthetic building blocks provides a potential route to the laboratory evolution of sequence-defined polymers with structures and properties not necessarily limited to those of natural biopolymers. We previously reported the efficient and sequence-specific DNA-templated polymerization of peptide nucleic acid (PNA) aldehydes. Here, we report the enzyme-free, DNA-templated polymerization of side-chain-functionalized PNA tetramer and pentamer aldehydes. We observed that polymerization of tetramer and pentamer PNA building blocks with a single lysine-based side chain at various positions in the building block could proceed efficiently and sequence specifically. In addition, DNA-templated polymerization also proceeded efficiently and in a sequence-specific manner with pentamer PNA aldehydes containing two or three lysine side chains in a single building block to generate more densely functionalized polymers. To further our understanding of side-chain compatibility and expand the capabilities of this system, we also examined the polymerization efficiencies of 20 pentamer building blocks each containing one of five different side-chain groups and four different side-chain regio- and stereochemistries. Polymerization reactions were efficient for all five different side-chain groups and for three of the four combinations of side-chain regio- and stereochemistries. Differences in the efficiency and initial rate of polymerization correlate with the apparent melting temperature of each building block, which is dependent on side-chain regio- and stereochemistry but relatively insensitive to side-chain structure among the substrates tested. Our findings represent a significant step toward the evolution of sequence-defined synthetic polymers and also demonstrate that enzyme-free nucleic acid-templated polymerization can occur efficiently using substrates with a wide range of side-chain structures, functionalization positions within each building block, and functionalization densities.

Electrostatic Control of Thickness and Stiffness in a Designed Protein Fiber

J. Am. Chem. Soc., 2008, 130 (15), 5124–5130

David Papapostolou, Elizabeth H. C. Bromley,[†] Christopher Bano,[†] and Derek N. Woolfson



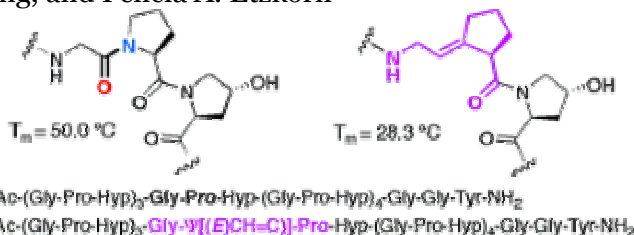
Attempts to design peptide-based fibers from first principles test our understanding of protein folding and assembly, and potentially provide routes to new biomaterials. Several groups have presented such designs based on α -helical and β -strand building blocks. A key issue in this area now is engineering and controlling fiber morphology and related properties. Previously, we have reported the design and characterization of a self-assembling peptide fiber (SAF) system based on α -helical coiled-coil building blocks. With preceding designs, the SAFs are thickened, highly ordered structures in which many coiled coils are tightly bundled. As a result, the fibers behave as rigid rods. Here we report successful attempts to design new fibers that are thinner and more flexible by further programming at the amino-acid sequence level. This was done by introducing extended, or “smeared”, electrostatic networks of arginine and glutamate residues to the surfaces of the coiled-coil building blocks. Furthermore, using arginine⁺ rather than lysine⁺ in these

networks plays a major role in the fiber assembly, presumably by facilitating multidentate intra and intercoiled-coil salt bridges.

The Effect of a Trans-Locked Gly-Pro Alkene Isostere on Collagen Triple Helix Stability

J. Am. Chem. Soc., **130** (16), 5396–5397, 2008.

Nan Dai, Xiaodong J. Wang, and Felicia A. Etzkorn



An alkene isostere of Gly-trans-Pro was synthesized and incorporated into a host Ac-(Gly-Pro-Hyp)₈-Gly-Gly-Tyr-NH₂ peptide to investigate the effect of locking a proline amide bond. Proline amide bond isomerization is the slow step in collagen folding. By locking the amide, we hypothesized an increase in stability of the collagen triple helix. The substitution instead destabilized the collagen host peptide. The T_m value of the host control peptide was 50.0 °C, while the peptide containing the isostere, Ac-(Gly-Pro-Hyp)₃-Gly-Ψ[(E)CHC]-Pro-Hyp-(Gly-Pro-Hyp)₄-Gly-Gly-Tyr-NH₂, had a T_m value of 28.3 °C. There are clearly factors that contribute to collagen stability and folding that we do not yet understand.

Disassembly of Noncovalent Amphiphilic Polymers with Proteins and Utility in Pattern Sensing

J. Am. Chem. Soc., **130** (16), 5416–5417, 2008.

Elamprakash N. Savariar, Suhrit Ghosh, Daniella C. González, and S. Thayumanavan

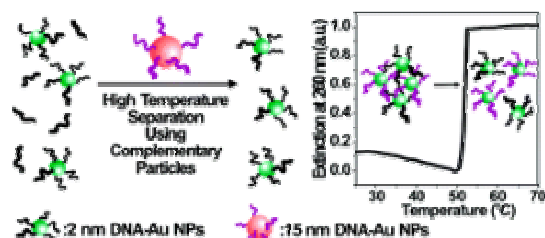


A simple strategy for pattern recognition of proteins through micellar disassembly is introduced. Five different noncovalently assembled receptors have been generated, and the disassembly was studied by monitoring the encapsulated dye release in response to five different proteins. The disassembly induced fluorescence change of the guest molecule produces protein-specific patterns.

Thermodynamically Controlled Separation of Polyvalent 2-nm Gold Nanoparticle-Oligonucleotide Conjugates

J. Am. Chem. Soc., **130** (16), 5430–5431, 2008.

Jae-Seung Lee, Dwight S. Seferos, David A. Giljohann, and Chad A. Mirkin

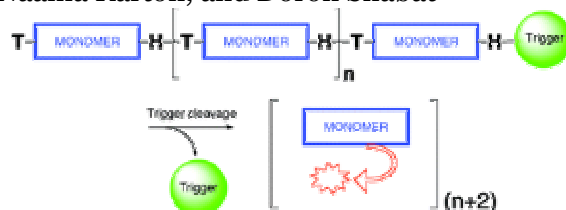


We describe the synthesis of small (2-nm diameter) gold nanoparticles densely functionalized with thiolated DNA (DNA-Au NPs) and a method to separate these particles from excess free DNA after synthesis. The separation method utilizes the thermodynamically enhanced binding properties of 2-nm DNA-Au NPs, compared to free excess DNA, to selectively hybridize these small particles to larger (15-nm diameter) DNA-Au NPs and form aggregates that can be isolated by simple centrifugation. These 2-nm DNA-Au NPs are obtained in a 46% overall yield, have a high surface coverage of DNA (64.8 \pm 6.4 pmol/cm²), and as a result, exhibit increased melting temperatures and cooperative melting properties.

Self-Immolative Polymers

J. Am. Chem. Soc., **130** (16), 5434–5435, 2008.

Amit Sagi, Roy Weinstein, Naama Karton, and Doron Shabat

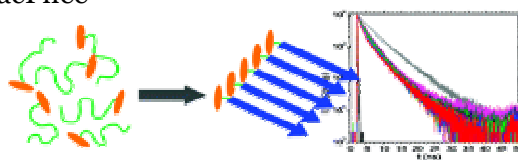


Smart polymers are special kinds of polymeric molecules that respond to external stimuli. We have developed a novel smart polymer designed to sequentially disassemble into its building blocks upon initiation by a triggering event at the polymer head. The polymer structure is based on a polyurethane backbone that disassembles through a domino-like, 1,6-elimination and decarboxylation reactions. We synthesized a self-immolative polymer that amplifies a single cleavage reaction into multiple release of fluorogenic molecules and confirmed the head-to-tail disassembly concept. These polymers can be used to prepare highly sensitive molecular sensors with large signal-to-noise ratios. The sensors should be useful for the detection of a wide range of biological and chemical activities through use of the appropriate trigger at the polymer head.

Modification of Fluorophore Photophysics through Peptide-Driven Self-Assembly

J. Am. Chem. Soc., **130** (16), 5487–5491, 2008.

Kevin J. Channon,[†] Glyn L. Devlin,[‡] Steven W. Magennis,[§] Chris E. Finlayson,[†] Anna K. Tickler,[†] Carlos Silva,[□] and Cait E. MacPhee

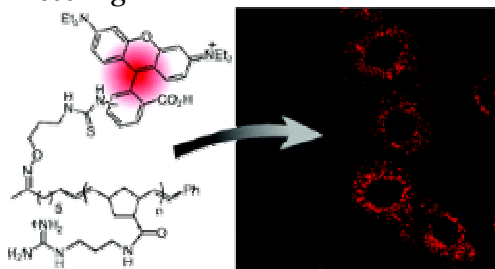


We describe the formation of self-assembling nanoscale fibrillar aggregates from a hybrid system comprising a short polypeptide conjugated to the fluorophore fluorene. The fibrils are typically unbranched, ~7 nm in diameter, and many microns in length. A range of techniques are used to demonstrate that the spectroscopic nature of the fluorophore is significantly altered in the fibrillar environment. Time-resolved fluorescence spectroscopy reveals changes in the guest fluorophore, consistent with energy migration and excimer formation within the fibrils. We thus demonstrate the use of self-assembling peptides to drive the assembly of a guest moiety, in which novel characteristics are observed as a consequence. We suggest that this method could be used to drive the assembly of a wide range of guests, offering the development of a variety of useful, smart nanomaterials that are able to self-assemble in a controllable and robust fashion.

A Polymeric Domain That Promotes Cellular Internalization

J. Am. Chem. Soc., **130** (17), 5626–5627, 2008.

Erin M. Kolonko and Laura L. Kiessling

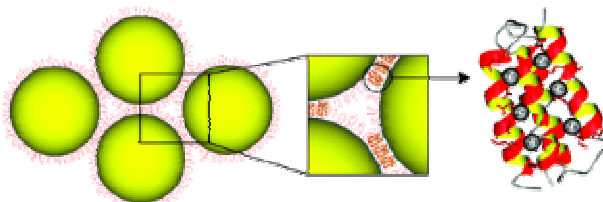


Polymers have emerged as powerful biological tools; however, their ability to gain access to the intracellular environment is limited. To expand the biological utility of polymer scaffolds, we have synthesized an internalization domain using the ring-opening metathesis polymerization (ROMP). A polymer functionalized with guanidinium groups is effectively internalized by cells and localized in both vesicles and the cytoplasm. Because the synthesis of such materials is modular, we anticipate that compounds of this type can be fashioned that facilitate the delivery of cargo via end-cap derivatization or block copolymer synthesis.

Folding Induced Assembly of Polypeptide Decorated Gold Nanoparticles

J. Am. Chem. Soc., **130** (17), 5780–5788, 2008.

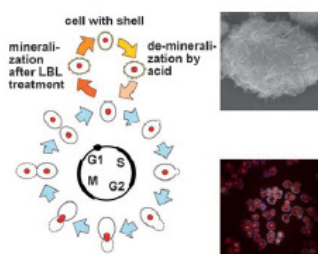
Daniel Aili,[‡] Karin Enander,[‡] Johan Rydberg,[‡] Irina Nesterenko,[‡] Fredrik Björefors,[‡] Lars Baltzer,[‡] and Bo Liedberg



Reversible assembly of gold nanoparticles controlled by the homodimerization and folding of an immobilized de novo designed synthetic polypeptide is described. In solution at neutral pH, the polypeptide folds into a helix–loop–helix four-helix bundle in the presence of zinc ions. When immobilized on gold nanoparticles, the addition of zinc ions induces dimerization and folding between peptide monomers located on separate particles, resulting in rapid particle aggregation. The particles can be completely redispersed by

removal of the zinc ions from the peptide upon addition of EDTA. Calcium ions, which do not induce folding in solution, have no effect on the stability of the peptide decorated particles. The contribution from folding on particle assembly was further determined utilizing a reference peptide with the same primary sequence but containing both D and L amino acids. Particles functionalized with the reference peptide do not aggregate, as the peptides are unable to fold. The two peptides, linked to the nanoparticle surface via a cysteine residue located in the loop region, form submonolayers on planar gold with comparable properties regarding surface density, orientation, and ability to interact with zinc ions. These results demonstrate that nanoparticle assembly can be induced, controlled, and to some extent tuned, by exploiting specific molecular interactions involved in polypeptide folding.

Angewandte Chemie



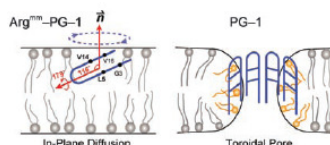
Inspired by eggshells in nature, living yeast cells were conferred with an artificial mineral coat by using a combination of the layer-by-layer (LBL) treatment with functional polymers and in situ biomimetic mineralization. The resulting hard inorganic shells have a tremendous effect on the storage, protection, delivery, and modification of the cells.

Cells with Mineral Shells

B. Wang, P. Liu, W. Jiang, H. Pan, X. Xu, R. Tang* _____ 3560–3564

Yeast Cells with an Artificial Mineral Shell: Protection and Modification of Living Cells by Biomimetic Mineralization

Barreling through: Guanidinium–phosphate hydrogen bonding significantly affects the structure and activity of the antimicrobial peptide PG-1. Solid-state NMR data show that a mutant of PG-1, having dimethylated Arg residues, adopts an in-plane orientation, interfacial location, and fast uniaxial motion around the membrane normal (see scheme). The less active mutant thus disrupts the membrane by in-plane diffusion, in contrast to the more active wild-type PG-1, which forms immobile transmembrane β -barrels to cause toroidal-pore membrane defects.

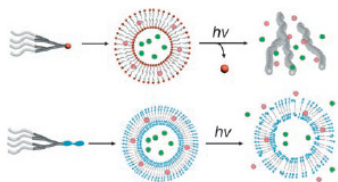


Transmembrane peptides

M. Tang, A. J. Waring, R. I. Lehrer, M. Hong* _____ 3202–3205

Effects of Guanidinium–Phosphate Hydrogen Bonding on the Membrane-Bound Structure and Activity of an Arginine-Rich Membrane Peptide from Solid-State NMR Spectroscopy

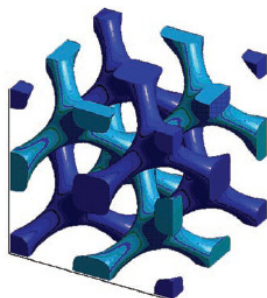
The missing link: Supramolecular transformation from a vesicle to a fibrous nanostructure has been achieved by photolytic cleavage and supramolecular reorganization of a dendritic building block containing a 2-nitrobenzyl group (see picture). Furthermore, supramolecular aggregates of an amide dendron with a photoisomerizable azobenzene unit exhibit controlled release of encapsulated molecules upon photoirradiation in the aqueous phase.



Self-Assembly

C. Park, J. Lim, M. Yun, C. Kim* _____ 2959–2963

Photoinduced Release of Guest Molecules by Supramolecular Transformation of Self-Assembled Aggregates Derived from Dendrons



Pore performance: The TAT protein of HIV can cross cell membranes with remarkable efficiency. By applying ideas from coordination chemistry, soft-condensed-matter physics, and differential geometry, it has been shown that TAT induces saddle-splay curvature in cell membranes, a process that is required for pore formation (see picture of two nonintersecting networks of pores). The results have potential implications for the design of cell-penetrating peptides.

Cell-Penetrating Peptides

A. Mishra, V. D. Gordon, L. Yang, R. Coridan, G. C. L. Wong* – 2986 – 2989

HIV TAT Forms Pores in Membranes by Inducing Saddle-Splay Curvature: Potential Role of Bidentate Hydrogen Bonding

Biomacromolecules

Collagen-Based Biomimetic Nanofibrous Scaffolds: Preparation and Characterization of Collagen/Silk Fibroin Bicomponent Nanofibrous Structures

Biomacromolecules, 9 (4), 1106–1116, 2008.

In-Sung Yeo,[†]| Ju-Eun Oh,[†]| Lim Jeong,[‡] Taek Seung Lee,[‡] Seung Jin Lee,[§] Won Ho Park,^{‡,*} and Byung-Moo Min

Electrospinning of collagen (COL)/silk fibroin (SF) blend solutions in 1,1,1,3,3,3-hexafluoro-2-propanol was investigated for fabrication of a biocompatible and biomimetic nanostructured scaffold for tissue engineering. The morphology of the electrospun COL/SF blend nanofibers was observed by scanning electron microscopy. The average diameters of COL/SF blend fibers ranged from 320 to 360 nm, irrespective of SF content in the blends. Both COL and SF components in the as-spun COL/SF blend matrices were stabilized by glutaraldehyde and water vapor, respectively, under the saturated glutaraldehyde aqueous solution at 25 °C. The glutaraldehyde vapor chemically stabilized the COL component via cross-linking, whereas the water vapor physically stabilized the SF component via crystallization to the β -sheet structure. These structural changes of after-treated COL/SF blend matrices were examined using ATR-IR and CP/MAS ^{13}C NMR spectroscopy. To assay the cytocompatibility and cellular behavior of the COL/SF blend nanofibrous scaffolds, cell attachment and the spreading of normal human epidermal keratinocytes (NHEK) and fibroblasts (NHEF) seeded on the scaffolds were studied. In addition, both morphological changes and cellular responses of COL/SF blend nanofibrous matrices were also compared with COL/SF hybrid nanofibrous matrices. Generally similar levels of cell attachment and spreading of NHEF were shown in the COL/SF blend nanofibrous matrix compared with those of the pure COL and pure SF matrices; the cellular responses of NHEK were, however, markedly decreased in the COL/SF blend nanofibrous matrix as compared to the pure matrices. In contrast, cell attachment and spreading of NHEK on the COL/SF hybrid nanofibrous matrix were significantly higher than that of the COL/SF blend nanofibrous matrix. Our results indicate that a COL/SF hybrid nanofibrous matrix may be a better candidate than a COL/SF blend nanofibrous matrix for biomedical applications such as wound dressing and scaffolds for tissue engineering.

Polyaspartylhydrazide Copolymer-Based Supramolecular Vesicular Aggregates as Delivery Devices for Anticancer Drugs

Biomacromolecules, 9 (4), 1117–1130, 2008.

D. Paolino,[†] D. Cosco,[†] M. Licciardi,[‡] G. Giammona,[‡] M. Fresta,[†] and G. Cavallaro

In this paper we report on three different hydrophilic copolymers based on α,β -polyaspartylhydrazide (PAHy) bearing butyric groups in the side chain (C4) (PAHy-C4) or a combination of butyric groups and positive charged residues ((carboxypropyl)trimethylammonium chloride, CPTACl) (PAHy-C4-CPTA) that were synthesized and used for the preparation of new supramolecular vesicular aggregates (SVAs) containing gemcitabine as an antitumor drug. Gemcitabine-loaded SVAs containing synthesized PAHy derivatives were characterized from the physicochemical and technological point of view and the in vitro toxicity and anticancer activity on two different human cancer cell lines, i.e., CaCo-2 (human colon carcinoma) and ARO (human anaplastic thyroid carcinoma) cells, were also evaluated. Moreover, considering that carrier–cell interaction is an important factor to achieve an improvement of anticancer drug activity, confocal laser scanning microscopy and flow cytometric experiments were carried out on the two different cancer cell lines.

Porous Devices Derived from Co-Continuous Polymer Blends as a Route for Controlled Drug Release

Biomacromolecules, 9 (4), 1131–1138

Pouneh Salehi, Pierre Sarazin, and Basil D. Favis

In this study we examine the release profile of bovine serum albumin (BSA) from a porous polymer matrix derived from a co-continuous polymer blend. The porosity is generated through the selective extraction of one of the continuous phases. This is the first study to examine the approach of using morphologically tailored co-continuous polymer blends as a template for generating porous polymer materials for use in controlled release. A method for the preparation of polymeric capsules is introduced, and the effect of matrix pore size and surface area on the BSA release profile is investigated. Furthermore, the effect of surface charge on release is examined by surface modification of the porous substrate using layer-by-layer deposition techniques. Synthetic, nonerodible polymer, high-density polyethylene (HDPE), was used as a model substrate prepared by melt blending with two different styrene–ethylene–butylene copolymers. Blends with HDPE allow for the preparation of porous substrates with small pore sizes (300 and 600 nm). A blend of polylactide (PLA) and polystyrene was also used to prepare porous PLA with a larger pore size (1.5 μm). The extents of interconnectivity, surface area, and pore dimension of the prepared porous substrates were examined via gravimetric solvent extraction, BET nitrogen adsorption, mercury porosimetry, and image analysis of scanning electron microscopy micrographs. With a loading protocol into the porous HDPE and PLA involving the alternate application of pressure and vacuum, it is shown that virtually the entire porous network was accessible to BSA loading, and loading efficiencies of between 80% and 96% were obtained depending on the pore size of the carrier and the applied pressure. The release profile of BSA from the microporous structure was monitored by UV spectrophotometry. The influence of pore size, surface area, surface charge, and number of deposited layers is demonstrated. It is shown that an effective closed-cell structure in porous PLA can be prepared, effectively eliminating all short-term BSA release.

Biodegradable and pH-Sensitive Hydrogels for Cell Encapsulation and Controlled Drug Release

Biomacromolecules, **9 (4)**, 1155–1162, 2008

De-Qun Wu, Yun-Xia Sun, Xiao-Ding Xu, Si-Xue Cheng, Xian-Zheng Zhang,* and Ren-Xi Zhuo

Hydrogels with pH-sensitive poly(acrylic acid) (PAAc) chains and biodegradable acryloyl–poly(ϵ -caprolactone)–2-hydroxyethyl methacrylate (AC-PCL-HEMA) chains were designed and synthesized. The morphology of hydrogel was observed by scanning electron microscopy. The degradation of the hydrogel in the presence of *Pseudomonas* lipase was studied. The in vitro release of bovine serum albumin from the hydrogel was investigated. Cytotoxicity study shows that the AC-PCL-HEMA/AAC copolymer exhibits good biocompatibility. Cell adhesion and migration into the hydrogel networks were evaluated by using different cell lines. The hydrogel with a lower cross-linking density and a larger pore size exhibited a better performance for cells migration.

ACS Chemistry & Biology

Ribosomal Synthesis of Peptidase-Resistant Peptides Closed by a Nonreducible Inter-Side-Chain Bond

ACS Chemical Biology, **3**, 241–249 (2008)

Yusuke Sako, Yuki Goto, Hiroshi Murakami, and Hiroaki Suga

Here we report a new enabling technology for the synthesis of peptidase-resistant cyclic peptide by means of genetic code reprogramming involving the flexizyme (a tRNA acylation ribozyme) and PURE (a reconstituted cellfree translation) systems. In this work, we have developed a new nonproteinogenic amino acid bearing a chloroacetyl group in the side chain, which forms a physiologically stable thioether bond by intramolecular reaction with the sulfhydryl group of a Cys residue in the peptide chain upon translation. Significantly, this chemistry takes place spontaneously in situ of the translation solution, giving the corresponding cyclic peptides independent of ring sizes. We have used this method to convert human urotensin II, known as a potent vasoconstrictor, to its analogue containing a thioether bond, showing that this new analogue retains biological activity. Moreover, this peptide exhibits remarkable resistance against peptidases under reducing conditions. Thus, this technology offers a new means to accelerate the discovery of therapeutic peptidic drugs.

Chemistry & Biology

Inhibition of Polo-like Kinase 1 by Blocking Polo-Box Domain-Dependent Protein-Protein Interactions

Volume 15, Issue 5, 19 May 2008, Pages 459-466

Wolfgang Reindl, Juping Yuan, Andrea Krämer, Klaus Strebhardt, and Thorsten Berg

The serine/threonine kinase Polo-like kinase 1 (Plk1) is overexpressed in many types of human cancers, and has been implicated as an adverse prognostic marker for cancer

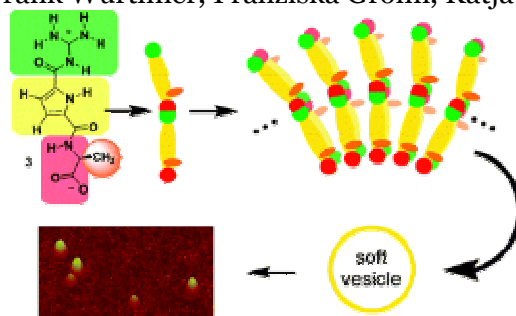
patients. Plk1 localizes to its intracellular anchoring sites via its polo-box domain (PBD). Here we show that Plk1 can be inhibited by small molecules which interfere with its intracellular localization by inhibiting the function of the PBD. We report the natural product thymoquinone and, especially, the synthetic thymoquinone derivative Poloxin as inhibitors of the Plk1 PBD. Both compounds inhibit the function of the Plk1 PBD in vitro, and cause Plk1 mislocalization, chromosome congression defects, mitotic arrest, and apoptosis in HeLa cells. Our data validate the Plk1 PBD as an anticancer target and provide a rationale for developing thymoquinone derivatives as anticancer drugs.

Organic Letters

A New Type of Soft Vesicle-Forming Molecule: An Amino Acid Derived Guanidiniocarbonyl Pyrrole Carboxylate Zwitterion

Org. Lett., 10 (7), 1469 -1472, 2008.

Thomas Rehm,, Xin Zhang, Frank Würthner, Franziska Gröhn, Katja Klein, and Carsten Schmuck

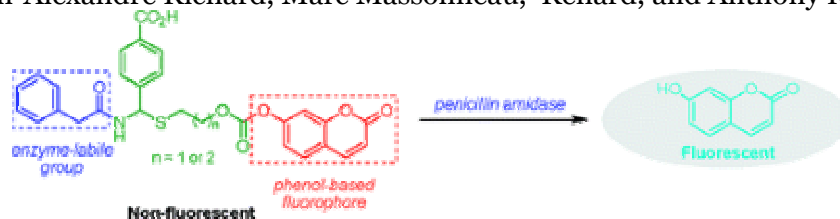


The self-assembly of the L-alanine derived zwitterion 3 leads to the formation of soft vesicles in solution even though this surprisingly small molecule does not possess the classical amphiphilic features of other vesicle-forming monomers.

Development of a New Nonpeptidic Self-Immolative Spacer. Application to the Design of Protease Sensing Fluorogenic Probes

Org. Lett., 10 (8), 1517 -1520, 2008.

Yves Meyer, Jean-Alexandre Richard, Marc Massonneau, Renard, and Anthony Romieu

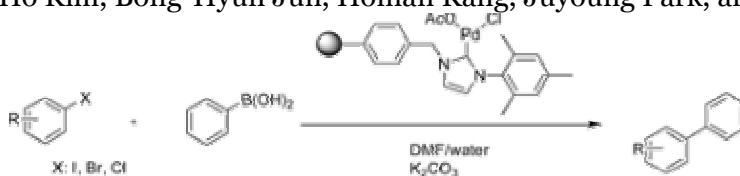


The design and synthesis of novel self-immolative spacer systems aiming at the release of phenol-containing compounds are described. The newly designed traceless linkers proved to be conveniently stable under physiological conditions and operate through spontaneous decomposition of an hemithioaminal intermediate under neutral aqueous conditions. Their utility was then illustrated by the preparation of original fluorogenic substrates of penicillin amidase whose strong fluorescence is unveiled through enzyme-initiated domino reactions.

Macroporous Polystyrene-Supported Palladium Catalyst Containing a Bulky N-Heterocyclic Carbene Ligand for Suzuki Reaction of Aryl Chlorides

Org. Lett., 10 (8), 1609 -1612, 2008.

Dong-Ho Lee, Jong-Ho Kim, Bong-Hyun Jun, Homan Kang, Juyoung Park, and Yoon-Sik Lee



Macroporous polystyrene (MPS)-supported 1-mesitylimidazolium chloride resin was prepared by reacting macroporous chloromethyl polystyrene with 1-mesitylimidazole as a supported N-heterocyclic carbene (NHC) precursor for the immobilization of a palladium catalyst. This MPS-supported NHC precursor readily formed a stable complex with Pd(OAc)₂, which effectively catalyzed the Suzuki reaction of aryl iodide and bromides at room temperature and even aryl chlorides at elevated temperatures (100 °C). This catalyst showed reusability in the Suzuki reaction of aryl bromide.