

Chmielewski Group Literature Abstracts

CHEMISTRY

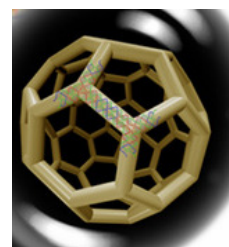
BIOLOGY

March 2008

Highlight of the Month

**Hierarchical self-assembly of DNA into
symmetric supramolecular polyhedra**

Nature 452, 198-201 (13 March 2008)
Chengde Mao et al.



Contributing Editors:

Marcos Pires (*Nature*)
Brandon Gaddis/Iris Geisler (*JACS*)
Jee Yeon Lee (*Science*)
Dawn Ernenwein (*Biomacro*)
Dave Przybyla (*Angewandte Chemie*)
Hilda Namanja (*PNAS*)
Nicole O'Neil (*Nature subdivisions*)
Joseph Chaney (*Chem & Bio*)
Aditya Kulkarni (*ACS Chem Bio/CBDD*)
Charles Rubert (*Org Lett*)

Nature

The inflammasome recognizes cytosolic microbial and host DNA and triggers an innate immune response

Nature 452, 103-107 (6 March 2008)

Daniel A. Muruve^{1,5}, Virginie Pétrilli^{3,5}, Anne K. Zaiss², Lindsay R. White¹, Sharon A. Clark¹, P. Joel Ross⁴, Robin J. Parks & Jurg Tschopp

The innate immune system recognizes nucleic acids during infection and tissue damage. Whereas viral RNA is detected by endosomal toll-like receptors (TLR3, TLR7, TLR8) and cytoplasmic RIG-I and MDA5, endosomal TLR9 and cytoplasmic DAI bind DNA_i, resulting in the activation of nuclear factor- κ B and interferon regulatory factor transcription factors. However, viruses also trigger pro-inflammatory responses², which remain poorly defined. Here we show that internalized adenoviral DNA induces maturation of pro-interleukin-1 β in macrophages, which is dependent on NALP3 and ASC, components of the innate cytosolic molecular complex termed the inflammasome. Correspondingly, NALP3- and ASC-deficient mice display reduced innate inflammatory responses to adenovirus particles. Inflammasome activation also occurs as a result of transfected cytosolic bacterial, viral and mammalian (host) DNA, but in this case sensing is dependent on ASC but not NALP3. The DNA-sensing pro-inflammatory pathway functions independently of TLRs and interferon regulatory factors. Thus, in addition to viral and bacterial components or danger signals in general, inflammasomes sense potentially dangerous cytoplasmic DNA, strengthening their central role in innate immunity.

Multi-membrane hydrogels

Nature 452, 76-79 (6 March 2008)

Sébastien Ladet¹, Laurent David¹ & Alain Domard

Polysaccharide-based hydrogels are useful for numerous applications, from food¹ and cosmetic processing to drug delivery and tissue engineering^{2, 3}. The formation of hydrogels from polyelectrolyte solutions is complex, involving a variety of molecular interactions. The physical gelation of polysaccharides can be achieved by balancing solvophobic and solvophilic interactions⁴. Polymer chain reorganization can be obtained by solvent exchange, one of the processing routes forming a simple hydrogel assembly. Nevertheless, many studies on hydrogel formation are empirical with a limited understanding of the mechanisms involved, delaying the processing of more complex structures. Here we use a multi-step interrupted gelation process in controlled physico-chemical conditions to generate complex hydrogels with multi-membrane 'onion-like' architectures. Our approach greatly simplifies the processing of gels with complex shapes and a multi-membrane organization. In contrast with existing assemblies described in the literature, our method allows the formation of free 'inter-membrane' spaces well suited for cell or drug introduction. These architectures, potentially useful in biomedical applications, open interesting perspectives by taking advantage of tailor-made three-dimensional multi-membrane tubular or spherical structures.

Hierarchical self-assembly of DNA into symmetric supramolecular polyhedra

Nature 452, 198-201 (13 March 2008)

Yu He¹, Tao Ye¹, Min Su², Chuan Zhang¹, Alexander E. Ribbe¹, Wen Jiang & Chengde Mao

DNA is renowned for its double helix structure and the base pairing that enables the recognition and highly selective binding of complementary DNA strands. These features, and the ability to create DNA strands with any desired sequence of bases, have led to the use of DNA rationally to design various nanostructures and even execute molecular computations. Of the wide range of self-assembled DNA nanostructures reported, most are one- or two-dimensional. Examples of three-dimensional DNA structures include cubes¹⁰, truncated octahedra¹¹, octohedra¹² and tetrahedra^{13, 14}, which are all comprised of many different DNA strands with unique sequences. When aiming for large structures, the need to synthesize large numbers (hundreds) of unique DNA strands poses a challenging design problem^{9, 15}. Here, we demonstrate a simple solution to this problem: the design of basic DNA building units in such a way that many copies of identical units assemble into larger three-dimensional structures. We test this hierarchical self-assembly concept with DNA molecules that form three-point-star motifs, or tiles. By controlling the flexibility and concentration of the tiles, the one-pot assembly yields tetrahedra, dodecahedra or buckyballs that are tens of nanometres in size and comprised of four, twenty or sixty individual tiles, respectively. We expect that our assembly strategy can be adapted to allow the fabrication of a range of relatively complex three-dimensional structures.

Proline-catalysed Mannich reactions of acetaldehyde

Nature 452, 453-455 (27 March 2008)

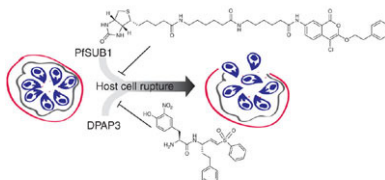
Jung Woon Yang¹, Carley Chandler¹, Michael Stadler¹, Daniela Kampen & Benjamin List

Small organic molecules recently emerged as a third class of broadly useful asymmetric catalysts that direct reactions to yield predominantly one chiral product, complementing enzymes and metal complexes¹. For instance, the amino acid proline and its derivatives are useful for the catalytic activation of carbonyl compounds via nucleophilic enamine intermediates. Several important carbon–carbon bond-forming reactions, including the Mannich reaction, have been developed using this approach², all of which are useful for making chiral, biologically relevant compounds. Remarkably, despite attempts^{3, 4}, the simplest of all nucleophiles, acetaldehyde, could not be used in this way. Here we show that acetaldehyde is a powerful nucleophile in asymmetric, proline-catalysed Mannich reactions with N-tert-butoxycarbonyl (N-Boc)-imines, yielding beta-amino aldehydes with extremely high enantioselectivities—desirable products as drug intermediates and in the synthesis of other biologically active molecules. Although acetaldehyde has been used as a nucleophile in reactions with biological catalysts such as aldolases⁵ and thiamine-dependent enzymes⁶, and has also been employed indirectly^{7, 8, 9}, its use as an inexpensive and versatile two-carbon nucleophile in asymmetric, small-molecule catalysis will find many practical applications.

Identification of proteases that regulate erythrocyte rupture by the malaria parasite *Plasmodium falciparum*

Nature Chemical Biology 4, 203 - 213 (2008)

Shirin Arastu-Kapur¹, Munira Grainger³, Carolyn I Phillips², James C Powers⁵ & Matthew Bogyo



Newly replicated *Plasmodium falciparum* parasites escape from host erythrocytes through a tightly regulated process that is mediated by multiple classes of proteolytic enzymes. However, the identification of specific proteases has been challenging. We describe here a forward chemical genetic screen using a highly focused library of more than 1,200 covalent serine and cysteine protease inhibitors to identify compounds that block host cell rupture by *P. falciparum*. Using hits from the library screen, we identified the subtilisin-family serine protease PfSU B1 and the cysteine protease dipeptidyl peptidase 3 (DPAP3) as primary regulators of this process. Inhibition of both DPAP3 and PfSUB1 caused a block in proteolytic processing of the serine repeat antigen (SERA) protein SERA5 that correlated with the observed block in rupture. Furthermore, DPAP3 inhibition reduced the levels of mature PfSUB1. These results suggest that two mechanistically distinct proteases function to regulate processing of downstream substrates required for efficient release of parasites from host red blood cells.

Nature Materials

Biofunctional polymer nanoparticles for intra-articular targeting and retention in cartilage

Nature Materials 7, 248 - 254 (2008)

Dominique A. Rothenfluh¹, Harry Bermudez^{1,2}, Conlin P. O'Neil¹ & Jeffrey A. Hubbell

The extracellular matrix of dense, avascular tissues presents a barrier to entry for polymer-based therapeutics, such as drugs encapsulated within polymeric particles. Here, we present an approach by which polymer nanoparticles, sufficiently small to enter the matrix of the targeted tissue, here articular cartilage, are further modified with a biomolecular ligand for matrix binding. This combination of ultrasmall size and biomolecular binding converts the matrix from a barrier into a reservoir, resisting rapid release of the nanoparticles and clearance from the tissue site. Phage display of a peptide library was used to discover appropriate targeting ligands by biopanning on denuded cartilage. The ligand WYRGRL was selected in 94 of 96 clones sequenced after five rounds of biopanning and was demonstrated to bind to collagen II ¹. Peptide-functionalized nanoparticles targeted articular cartilage up to 72-fold more than nanoparticles displaying a scrambled peptide sequence following intra-articular injection in the mouse.

Science

Synthesis of Macrocyclic Copolymer Brushes and Their Self-Assembly into Supramolecular Tubes

Science 14 March 2008 Vol. 319, no. 5869, pp. 1512 - 1515

Michel Schappacher and Alain Deffieux

We report on an efficient route to design large macrocyclic polymers of controlled molar mass and narrow dispersity. The strategy is based on the synthesis of a triblock copolymer ABC, in which the long central block B is extended by two short A and C sequences bearing reactive antagonist functions. When reacted under highly dilute conditions, this precursor produces the corresponding macrocycle by intramolecular coupling of the A and C blocks. Chloroethyl vinyl ether was selected as the monomer for the central block B, because it can be readily derivatized into brushlike polymers by a grafting process. The corresponding macrocyclic brushes were decorated with polystyrene or randomly distributed polystyrene and polyisoprene branches. In a selective solvent for the polyisoprene branches, the macrocyclic brushes self-assemble into cylindrical tubes of up to 700 nanometers.

Self-Assembly of Large and Small Molecules into Hierarchically Ordered Sacs and Membranes

Science 28 March 2008 Vol. 319, no. 5871, pp. 1812 - 1816

Ramille M. Capito, Helena S. Azevedo, Yuri S. Velichko,³ Alvaro Mata,¹ Samuel I. Stupp

We report here the self-assembly of macroscopic sacs and membranes at the interface between two aqueous solutions, one containing a megadalton polymer and the other, small self-assembling molecules bearing opposite charge. The resulting structures have a highly ordered architecture in which nanofiber bundles align and reorient by nearly 90° as the membrane grows. The formation of a diffusion barrier upon contact between the two liquids prevents their chaotic mixing. We hypothesize that growth of the membrane is then driven by a dynamic synergy between osmotic pressure of ions and static self-assembly. These robust, self-sealing macroscopic structures offer opportunities in many areas, including the formation of privileged environments for cells, immune barriers, new biological assays, and self-assembly of ordered thick membranes for diverse applications.

PNAS

Antisense transcripts from immunoglobulin heavy-chain locus V(D)J and switch regions

PNAS | March 11, 2008 | vol. 105 | no. 10 | 3843-3848

Thomas Perlot, Gang Li, and Frederick W. Alt

Activation-induced cytosine deaminase (AID) is essential for both somatic hypermutation (SHM) and class switch recombination (CSR), two processes involved in antibody diversification. Previously, various groups showed both in vitro and in vivo that AID initiates SHM and CSR by deaminating cytosines in DNA in a transcription-dependent

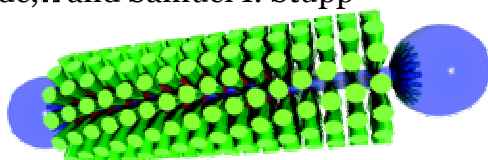
manner. Although in vivo both DNA strands are equally targeted by AID, many in vitro and bacterial experiments found that AID almost exclusively targets the nontemplate strand of a transcribed substrate. Here, we report the detection of antisense transcripts in assembled Ig heavy chain (IgH) variable region exons and their immediate downstream region, as well as in switch regions, sequences that, respectively, are targets for SHM and CSR in vivo. In contrast, we did not detect antisense transcripts from the C_μ constant region exons, which lie between the IgH variable region exons and downstream S regions and which are not normally an AID target. Expression of the antisense variable region/flanking region and the S-region transcripts were found in all lymphocytes that transcribe these sequences in the sense direction. Steady-state levels of antisense transcripts appeared very low, and start sites potentially appeared heterogeneous. We discuss the potential implications of antisense IgH locus transcription for AID targeting or other processes.

Journal of the American Chemical Society

A Templating Approach for Monodisperse Self-Assembled Organic Nanostructures

J. Am. Chem. Soc., 2008, 130 (9), 2742 -2743

Steve R. Bull,[†] Liam C. Palmer,[†] Nathaniel J. Fry,[†] Megan A. Greenfield,[‡] Benjamin W. Messmore,[†] Thomas J. Meade,[‡] and Samuel I. Stupp

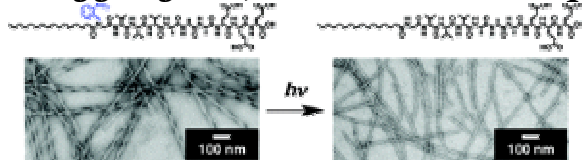


The precise structural control is known for self-assembly into closed spherical structures (e.g., micelles), but similar control of open structures is much more challenging. Inspired by natural tobacco mosaic virus, we present the use of a rigid-rod template to control the size of a one-dimensional self-assembly. We believe that this strategy is novel for organic self-assembly and should provide a general approach to controlling size and dimension.

Quadruple Helix Formation of a Photoresponsive Peptide Amphiphile and Its Light-Triggered Dissociation into Single Fibers

J. Am. Chem. Soc., 2008 130 (10), 2946 -2947

Takahiro Muraoka,[†] Honggang Cui,[‡] and Samuel I. Stupp

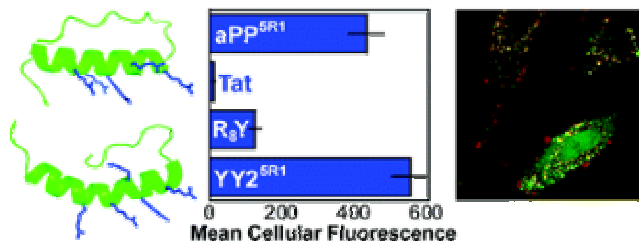


Using a peptide amphiphile having a bulky photolabile 2-nitrobenzyl group between the alkyl chain and the peptide segment, we demonstrated quadruple helical fiber formation and its dissociation into single fibrils in response to light. Putting the bulky group close to the core of a fibril is thought to induce a distortion of the alignment of molecules, which can in turn lead to quadruple helices. Photoirradiation to cleave the bulky group transforms the helices into single fibrils.

Minimally Cationic Cell-Permeable Miniature Proteins via α -Helical Arginine Display

J. Am. Chem. Soc., 2008 130 (10), 2948 -2949

Betsy A. Smith,[†] Douglas S. Daniels,[†] Abigail E. Coplin, Gregory E. Jordan, Lynn M. McGregor, and Alanna Schepartz

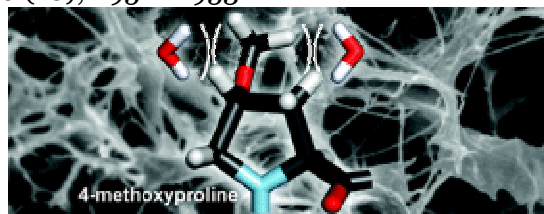


Protein therapeutics are a blossoming industry, with revenues exceeding \$51 billion in 2005 and a growth rate nearly three times that of the overall pharmaceutical industry. Although it has been known for decades that cationic polymers can transport molecular cargos across the plasma membrane, inefficient cellular delivery continues to impede the development of protein drugs. Our lab recently reported that small, folded proteins containing a minimal cationic motif embedded within a type II polyproline (PPII) helix efficiently cross the plasma membrane of eukaryotic cells. Here we demonstrate that an even smaller cationic motif can be embedded within the α -helix of a small, folded protein to generate molecules that penetrate cells significantly more efficiently than arginine-rich sequences or Tat. Our results suggest that the function of cell permeability can be encoded by judicious placement of as few as 2-3 additional arginine residues on a protein α -helix.

Stabilization of the Collagen Triple Helix by O-Methylation of Hydroxyproline Residues

Frank W. Kotch,[‡] Ilia A. Guzei,[†] and Ronald T. Raines

J. Am. Chem. Soc., 2008 130 (10), 2952 -2953



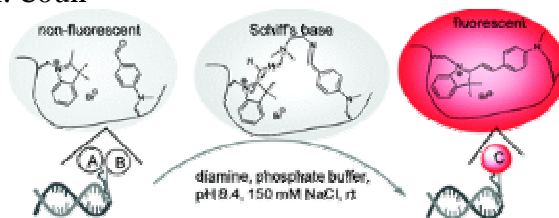
Collagen is the most abundant protein in animals, including humans. The prevalent (2S,4R)-4-hydroxyproline (Hyp) residues of collagen are known to confer great stability upon its triple-helical conformation. The basis for that stability has been attributed to hydration of the pendant hydroxyl groups. Here, that attribution is shown to be incorrect. Replacement of the natural Hyp residues with synthetic (2S,4R)-4-methoxyproline (Mop) residues is shown by circular dichroism spectroscopy and differential scanning calorimetry to increase the conformational stability of the collagen triple helix. The thermodynamic parameters indicate that, as expected, O-methylation decreases the hydration of the triple helix. Apparently, hydration of Hyp residues is deleterious, rather than advantageous, to the collagen triple helix. The crystal structure of Ac-Mop-OMe reveals the manifestation of two stereoelectronic effects: a gauche effect and an $n \rightarrow \pi$ interaction, which preorganize the main-chain atoms properly for triple-helix formation. Thus, the conformational stability conferred upon the collagen triple helix by O-methylation provides strong evidence that

the hydroxyl group of Hyp acts primarily through stereoelectronic effects and that its hydration provides no benefit. This information could have practical utility, as Mop could be prepared in situ by the O-methylation of Hyp residues in natural collagen. Such a semisynthetic collagen could have superior properties as a biomaterial.

Diamine Catalyzed Hemicyanine Dye Formation from Nonfluorescent Precursors through DNA Programmed Chemistry

J. Am. Chem. Soc., 130 (11), 3238 -3239, 2008.

Yumei Huang and James M. Coull

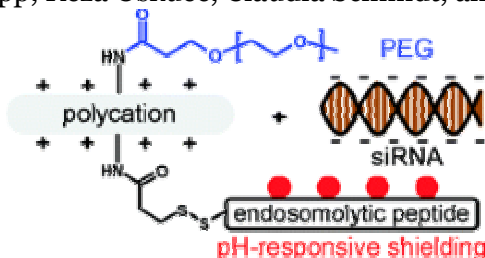


A hemicyanine fluorescent dye was generated by a diamine-catalyzed, DNA-templated, aldol-type condensation from nonfluorescent precursors. Studies of reaction rate and yield as a function of catalyst structure indicated the diamine catalyst operates in a concerted mechanism on both reaction components. Our findings expand the scope of reactions that can be performed by DPC and demonstrate that de novo chemical synthesis of labels can be coupled to biological recognition events in a homogeneous format with essentially no background.

Breathing Life into Polycations: Functionalization with pH-Responsive Endosomolytic Peptides and Polyethylene Glycol Enables siRNA Delivery

J. Am. Chem. Soc., 130 (11), 3272 -3273, 2008.

Martin Meyer, Alexander Philipp, Reza Oskuee, Claudia Schmidt, and Ernst Wagner

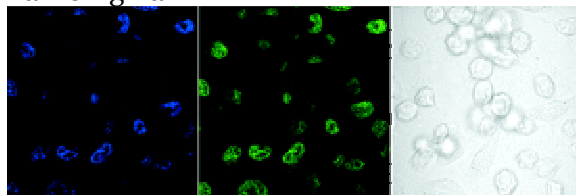


The lack of efficient delivery systems is still limiting the full therapeutic potential of siRNA. For the purpose of nucleic acid transfer, among other synthetic carrier systems, polycations have been applied. Favorable characteristics of suitable polymers include nucleic acid binding, compaction, protection, and biocompatibility. However the lack of nucleic acid transfer activity in transfection-based screening often abandons promising candidates. Here we present that functionalization may turn polycations with poor delivery activity into efficient carriers: for example, polylysine, on its own lacking nucleic acid transfer activity, displayed high efficiency in siRNA delivery after modification with polyethylene glycol and a pH-responsive endosomolytic peptide. Hence these findings have implication for the selection process of polymeric carriers for siRNA.

Emissive Terbium Probe for Multiphoton in Vitro Cell Imaging

***J. Am. Chem. Soc.*, 130 (12), 3714 -3715, 2008.**

Ga-Lai Law, Ka-Leung Wong, Cornelia Wing-Yin Man, Wing-Tak Wong, Sai-Wah Tsao, Michael Hon-Wah Lam, and Paul Kwan-Sing Lam

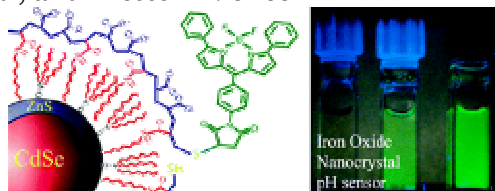


A polymeric terbium complex that can be excited by near-infrared excitation at 800 nm via multiphoton absorption processes has been synthesized. This complex has been demonstrated to show strong, observable, three-photon-induced f-f emission in cell imaging. In vitro studies carried out in three carcinoma cell lines (A549, HONE1, and HeLa) have been performed and shown to have low cytotoxicity. This complex is therefore a potential candidate for future infrared excitation imaging dyes.

Imparting Nanoparticle Function with Size-Controlled Amphiphilic Polymers

***J. Am. Chem. Soc.*, 130 (12), 3744 -3745, 2008.**

Yingchuan Chen, Rahul Thakar, and Preston T. Snee

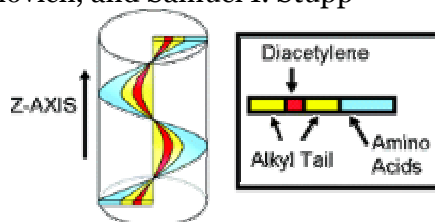


Herein we report our method of water solubilization and subsequent functionalization of a variety of nanoparticle systems with amphiphilic polymers containing build-in "chemical handles". We have used these polymers, which have narrow polydispersity indices, to impart water solubility and chemical sensitivity toward targeted species (here: pH). These material systems have high chemical conjugation efficiencies in aqueous conditions which may be used to create a variety of chemical and biological multifunctional materials.

Peptide Amphiphile Nanofibers with Conjugated Polydiacetylene Backbones in Their Core

***J. Am. Chem. Soc.*, 130 (12), 3892 -3899, 2008.**

Lorraine Hsu, Gregory L. Cvetanovich, and Samuel I. Stupp

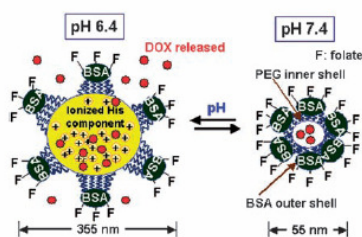


The coupling of electronic and biological functionality through self-assembly is an interesting target in supramolecular chemistry. We report here on a set of diacetylene-derivatized peptide amphiphiles (PAs) that react to form conjugated polydiacetylene backbones following self-assembly into cylindrical nanofibers. The polymerization reaction

yields highly conjugated backbones when the peptidic segment of the PAs has a linear, as opposed to a branched, architecture. Given the topotactic nature of the polymerization, these results suggest that a high degree of internal order exists in the supramolecular nanofibers formed by the linear PA. On the basis of microscopy, the formation of a polydiacetylene backbone to covalently connect the β -sheets that help form the fibers does not disrupt the fiber shape. Interestingly, we observe the appearance of a polydiacetylene (PDA) circular dichroism band at 547 nm in linear PA nanofibers suggesting the conjugated backbone in the core of the nanostructures is twisted. We believe this CD signal is due to chiral induction by the β -sheets, which are normally twisted in helical fashion. Heating and cooling shows simultaneous changes in β -sheet and conjugated backbone structure, indicating they are both correlated. At the same time, poor polymerization in nanofibers formed by branched PAs indicates that less internal order exists in these nanostructures and, as expected, then a circular dichroism signal is not observed for the conjugated backbone. The general variety of materials investigated here has the obvious potential to couple electronic properties and in vitro bioactivity. Furthermore, the polymerization of monomers in peptide amphiphile assemblies by a rigid conjugated backbone also leads to mechanical robustness and insolubility, two properties that may be important for the patterning of these materials at the cellular scale.

Angewandte Chemie

Delivering the goods: A pH-sensitive nanogel consists of a hydrophobic copolymer core and two layers of hydrophilic shell (see picture). The core is loaded with a model anticancer drug, doxorubicin (DOX). The nanogel infects tumor cells in a receptor-dependent manner, kills the cells, and migrates to neighboring cells like a virus. BSA = bovine serum albumin, F = folate, PEG = polyethylene glycol.



Drug Delivery

E. S. Lee, D. Kim, Y. S. Youn, K. T. Oh,
Y. H. Bae* _____ 2418–2421

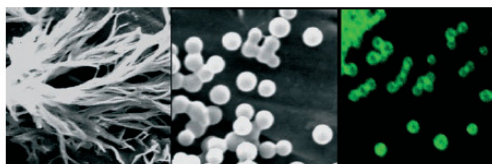
A Virus-Mimetic Nanogel Vehicle

Biotin Structures

K. B. Joshi, S. Verma* _____ 2860–2863



Dityryptophan Conjugation Triggers
Conversion of Biotin Fibers into Soft
Spherical Structures



Trigger happy: Biotin and its methyl ester form long fibers in solution, which are transformed into soft spherical structures upon simple conjugation with dityryptophan dipeptide (see picture). Such morphogenesis is not achieved in a controlled fashion by other aromatic amino acids.

Biomacromolecules

Polymer Nanoparticles Covered with Phosphorylcholine Groups and Immobilized with Antibody for High-Affinity Separation of Proteins

Biomacromolecules, 9 (3), 828–833, 2008.

Yusuke Goto, Ryosuke Matsuno, Tomohiro Konno, Madoka Takai, and Kazuhiko Ishihara

Novel polymer nanoparticles were prepared for the selective capture of a specific protein from a mixture with high effectiveness. The nanoparticle surface was covered with hydrophilic phosphorylcholine groups and active ester groups for easy immobilization of antibodies. Phospholipid polymers (PMBN) composed of 2-methacryloyloxyethyl phosphorylcholine, n-butyl methacrylate, and p-nitrophenyloxycarbonyl polyethyleneglycol methacrylate, were synthesized for the surface modification of poly(L-lactic acid) nanoparticles. Surface analysis of the nanoparticles using laser-Doppler electrophoresis and X-ray photoelectron spectroscopy revealed that the surface of nanoparticles was covered with PMBN. Protein adsorption was evaluated with regard to the nonspecific adsorption on the nanoparticles that was effectively suppressed by the phosphorylcholine groups. The immobilization of antibodies on nanoparticles was carried out under physiological conditions to ensure specific binding of antigens. The antibody immobilized on the nanoparticles exhibited high activity and strong affinity for the antigen similar to that exhibited by an antibody in a solution. The selective binding of a specific protein as an antigen from a protein mixture was relatively high compared to that observed with conventional antibody-immobilized polymer nanoparticles. In conclusion, nanoparticles having both phosphorylcholine and active ester groups for antibody immobilization have strong potential for use in highly selective separation based on the biological affinities between biomolecules.

Simultaneous Processing of Fibril Formation and Cross-Linking Improves Mechanical Properties of Collagen

Biomacromolecules, 9 (3), 879–885, 2008.

Shunji Yunoki and Takehisa Matsuda

In vitro “simultaneous processing” was investigated in which fibril formation of collagen and cross-linking occur simultaneously in the presence of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) as a cross-linking reagent. Fibril formation in simultaneous processing was monitored using turbidity. The EDC in simultaneous processing increased $T_{1/2}$ (time required for half of the plateau value in turbidity) and decreased the degree of the fibril formation dose dependently. The reduced fibril formation rate ($T_{1/2} > 60$ s) suggests the introduction of intrafibrillar cross-linking during fibril formation. The collagen gels prepared using simultaneous processing had a compressive modulus that was 6-fold higher than that using sequential processing, which is an advantage of simultaneous processing. Atomic force microscopy images acquired under water on the wet gels demonstrated that the simultaneous processing provided a unique double-network structure: intrafibrillarly cross-linked collagen fibrils among which nonfibrous collagens act as interfibrillar cross-linkages.

Self-Assembly of Nanofiber with Uniform Width from Wheel-Type Trigonal- β -Sheet-Forming Peptide

Biomacromolecules, 9 (3), 913–918, 2008

Kazuya Murasato, Kazunori Matsuura, and Nobuo Kimizuka

A novel C₃ symmetric peptide conjugate “Wheel-FKFE” consisting of three β -sheet-forming peptides with wheel-like arrangement is developed, and the morphology of self-assembled peptide conjugates in aqueous solutions is observed at various pH. The CD spectra of Wheel-FKFE show the formation of β -sheet structures in pH 6.9 phosphate buffer, whereas random structures are formed in aqueous HCl (pH 3.3) and NaOH (pH 11) solutions. In transmission electron microscopy, nanofibers with a uniform width of 3–4 nm and lengths of several micrometers are observed in pH 6.9 phosphate buffer, whereas nanorods with the width of several nanometers and the length of several tens of nanometers are observed for that of aqueous HCl (pH 3.3) and NaOH (pH 11) solutions. The uniform width (3–4 nm) of the fibers observed in neutral solution indicates formation of columnar self-assembly of Wheel-FKFEs. The fluorescence spectrum of polarity sensitive dye, sodium 8-anilino-1-naphthalenesulfonate (ANS), in the presence of Wheel-FKFE fibers revealed that the polarity inside the fibers corresponds to that of acetone, indicating that the internal space of the fibers possesses medium hydrophobic environment.

Regulation of in vitro Calcium Phosphate Mineralization by Combinatorially Selected Hydroxyapatite-Binding Peptides

Biomacromolecules, **9 (3)**, 966–973, 2008.

Hanson Fong, Il Won Kim, John Spencer Evans, Candan Tamerler, and Mehmet Sarikaya

We report selection and characterization of hydroxyapatite-binding heptapeptides from a peptide–phage library and demonstrate the effects of two peptides, with different binding affinities and structural properties, on the mineralization of calcium phosphate mineral. In vitro mineralization studies carried out using one strong- and one weak-binding peptide, HABP1 and HABP2, respectively, revealed that the former exhibited a drastic outcome on mineralization kinetics and particle morphology. Strong-binding peptide yielded significantly larger crystals, as observed by electron microscopy, in comparison to those formed in the presence of a weak-binding peptide or in the negative control. Molecular structural studies carried out by circular dichroism revealed that HABP1 and HABP2 differed in their secondary structure and conformational stability. The results indicate that sequence, structure, and molecular stability strongly influence the mineralization activity of these peptides. The implication of the research is that the combinatorially selected short-sequence peptides may be used in the restoration or regeneration of hard tissues through their control over of the formation of calcium phosphate biominerals.

Folate-Conjugated Thermoresponsive Block Copolymers: Highly Efficient Conjugation and Solution Self-Assembly

Biomacromolecules, **9 (3)**, 1064–1070, 2008.

Priyadarsi De, Sudershan R. Gondi, and Brent S. Sumerlin

A combination of controlled radical polymerization and azide–alkyne click chemistry was employed to prepare temperature-responsive block copolymer micelles conjugated with biological ligands with potential for active targeting of cancer tissues. Block copolymers of N-isopropylacrylamide (NIPAM) and N,N-dimethylacrylamide (DMA) were synthesized by reversible addition–fragmentation chain transfer (RAFT) polymerization with an azido chain transfer agent (CTA). Pseudo-first-order kinetics and linear molecular weight

dependence on conversion were observed for the RAFT polymerizations. Cu(I)-catalyzed coupling with propargyl folate resulted in folic acid residues being efficiently conjugated to the α -azido chain ends of the homo and block copolymers. Temperature-induced self-assembly resulted in aggregates capable of controlled release of a model hydrophobic drug. Cu(I)-catalyzed azide-alkyne cycloaddition has proven superior to conventional methods for conjugation of biological ligands to macromolecules, and the general strategy presented herein can potentially be extended to the preparation of folate-functionalized assemblies with other stimuli susceptibility (e.g., pH) for therapeutic and imaging applications.

Chemistry and Biology

High-Throughput Screening of Glycan-Binding Proteins Using Miniature Pig Kidney N-Glycan-Immobilized Beads

Volume 15, Issue 3, Pages 201-302 (21 March 2008)

Yun-Gon Kim, Dong-Sik Shin, Yung-Hun Yang, Geun-Cheol Gil, Chung-Gyu Park, Yusuke Mimura, David K.C. Cooper, Pauline M. Rudd, Raymond A. Dwek, Yoon-Sik Lee, Byung-Gee Kim

Glycan recognition leading to cell-cell interactions, signaling, and immune responses is mediated by various glycan-binding proteins (GBPs) showing highly diverse ligand specificities. We describe here a rapid glycan immobilization technique via 4-hydrazinobenzoic acid (HBA)-functionalized beads and its application to high-throughput screening of miniature pig kidney N-glycan-binding proteins by using a mass-spectrometric approach. Without any derivatization steps, the purified pig kidney N-glycans were directly immobilized on to HBA-functionalized beads and subsequently used to identify GBPs from human serum. This screening method showed remarkable performance for identifying potential GBPs closely involved in pig-to-human xenograft rejection mediated by human serum, including antibodies, cytokines, complement components, siglec, and CD antigens. Thus, these results demonstrate that the GBP screening method was firmly established by one-step immobilization of the N-glycans on to microsphere and highly sensitive mass-spectrometric analysis.

Chemical Biology and Drug Design

Template-directed Assembly of Signaling Proteins: A Novel Drug Screening and Research Tool

Chemical Biology and Drug Design. 71; 278-281 (2008)

Shrout, Anthony L., Esposito, Edward A. and Weis, Robert M.

A multitude of proteins reside at or near the cell membrane, which provides a unique environment for organizing and promoting assemblies of proteins that are involved in a variety of cellular signaling functions. Many of these proteins and pathways are implicated in disease. For example, strong links have been established between receptor tyrosine kinases and disease, most notably, cancer. However, a significant impediment to researchers remains: membrane-associated proteins are difficult to reconstitute and study. Template-directed assembly represents a powerful new technology that enables the assembly of membrane-associated proteins. We show that template-directed assembly

restores tyrosine kinase activity and regulation, and provides a way for researchers to build multicomponent assemblies. As an example of better enzyme regulation, the Tie2 tyrosine kinase domain exhibits (biologically relevant) autoinhibitory behavior when template assembled. Also, template-assembled insulin receptor tyrosine kinase domains exhibit significant autophosphorylation (none detected without template-directed assembly) and an eightfold increase in substrate phosphorylation (compared to best solution conditions). Thus, template-directed assembly has a demonstrated ability to effectively produce more biologically relevant results using these commercial reagents. Template-directed assembly promises to be generally applicable to the signaling networks important for human health, because these pathways frequently contain membrane-associated proteins that require the organizing influence of a membrane surface.

Organic Letters

Fluorescence Imaging of Cellular Glutathione Using a Latent Rhodamine

Org. Lett., 10 (5), 837-840, 2008.

Marcos M. Pires and Jean Chmielewski

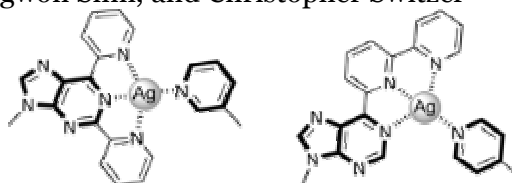


Glutathione is a crucial component of the redox homeostasis of cells, and altered levels have been linked to human pathologies. We constructed a latent fluorophore (RhoSS) that responds to cellular thiols in vitro and in cyto following intracellular reduction by glutathione to yield rhodamine 110. Importantly, RhoSS was demonstrated to respond to changing levels of glutathione in cells. This compound represents a class of rationally designed latent fluorophores with exciting potential for monitoring cellular thiols.

Two Watson-Crick-Like Metallo Base-Pairs

Org. Lett., 10 (6), 1091-1094, 2008.

Benjamin D. Heuberger, Dongwon Shin, and Christopher Switzer

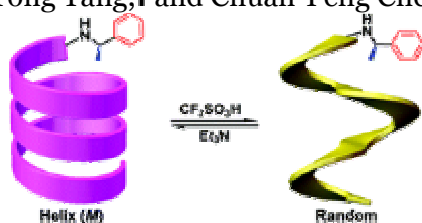


Two Watson-Crick-like metallo base-pairs are described with mutually independent geometries that have similar dimensions and stabilities to their natural, hydrogen-bonded counterparts.

Chiral Induction in Phenanthroline-Derived Oligoamide Foldamers: An Acid- and Base Controllable Switch in Helical Molecular Strands

Org. Lett., 10 (6), 1275 -1278, 2008

Hai-Yu Hu,^{††} Jun-Feng Xiang,[†] Yong Yang,[†] and Chuan-Feng Chen



A series of phenanthroline-derived oligoamides bearing a chiral (R)-phenethylamino end group were synthesized that displayed chiral helical induction and subsequently formed one-hand helical foldamers in solution. Moreover, an acid- and base-controllable switch in the helical molecular strands was observed, which has been demonstrated by NMR, UV-vis, and circular dichroism spectroscopy.