Sino-German Forum on Nanoscience and Biomedicine

Oct 12-15, Beijing

Organized by Institute of Chemistry, CAS Max-Planck-Institute of Colloids and Interfaces, MPG

Sponsored by Sino-German Center for Research Promotion (NSFC-DFG)

Aim and Topic

The interplay between nanoscience and biomedicine is the hallmark of current scientific research worldwide. The use of nanoscience may open new vistas of improving the effectiveness and efficiency of medical diagnosis and therapeutics, so called nanomedicine. An appealing example is the use of quantum dots as fluorescent labels. On the other hand, the language of biomedicine can be translated to allow formation of nanostructures with biomimetic structural and functional complexity. For instance, DNA is extensively used to direct the organization of nanoparticles. Due to the large difference between nanotechnology and biomedicine, however, there exist numbers of misunderstanding between scientists from chemistry, physics and engineering and those from biology and medicine. Accordingly, the scientific aim of the forum is to bridge the gap between nanoscience and biomedicine to establish a common understanding of how nanoscience and biomedicine can augment each other and what impact can be envisioned from this cooperation.

The past decade has seen a boom of nanoscience in China. The practical aim of the forum is to provide a chance to implement extensive communication between experts from different disciplines in China with those from Germany, Switzerland, and the US, with intent to drive further developments of nanomedicine in China.

Scientific Program

October 11th Registration

October 12th 8:30 Opening ceremony

9:00 Morning Session 1 Chair: Mingyuan Gao

Plenary lecture: 9:00-9:40 Trend and Status of Nanotechnology in China, S. Xie

Invited lectures:

9:40-10:00 Bioengineered Nanocrystals for Mega-amplified Signals, R. Renneberg 10:00-10:20 Chitosan Based Gene Delivery System and Particle Internalization, C. Gao

10:20-10:50 Coffee Break

10:50 Morning Session 2 Chair: Jochen Feldmann

Plenary lecture

10:50-11:30 Cryo-Nanobiotechnology : Preservation of Cells with Therapeutic Relevance in Microsystems and on an Nanostructured Surfaces, H. Zimmermann

Invited lecture

11:30-11:50 Novel Design Strategies for Biosensors Based on Conjugated Polymers, S. Wang

12:00-13:15 Lunch

13:15 Afternoon Session 1 Chair: Xi Zhang

Plenary Lecture:

13:15-13:55, Nanoparticles as Optical Sensors and Actuators, J. Feldmann

Invited lectures

13:55-14:15 Better Living through Nanobiodetection, C. Fan 14:15-14:35 Biofunctional Nanoparticles and Nanofibers: Synthesis and Applications, B. Xu

14:35-15:05 Coffee Break

15:05 Afternoon Session 2 Chair: Heiko Zimmermann

Plenary lecture:

15:05-15:45 Design and Creation of Bioinspired Surfaces with Special Wettability, L. Jiang

Invited lectures

15:45-16:05 Self-Organization of Nanoparticles: Surprising Analogies with Protein Superstructures, N. Kotov
16:05-16:25 Anisotropic Behavior of Cells on Mesotropic Patterned Surfaces, L. Chi
16:25-16:45 Design of Artificial Seleno-enzymes, J. Liu

18:30 Welcome Banquet

October 13th 9:00 Morning Session 1 Chair: Zuhong Lu

Plenary lecture:

9:00-9:40 Organized Collapse Structures in Lipid like Monolayer Films and in Lung Surfactants, H. J. Galla

Invited lectures:

9:40-10:00 Amyloid-like Neuronal Tau is a Toxicant and Induces Neuron Apoptosis, Q. He 10:00-10:20 Cargo Transport on Engineered Surfaces Powered by Molecular motors, C. Brunner

10:30-10:50 Coffee Break

10:50 Morning Session 2 Chair: Lei Jiang

Plenary lecture

10:50-11:30 Integration of Sample Processing and Microarray for Multiple-gene Detection, Z. Lu

Invited lecture

11:30-11:50 Using Hydrogel Microspheres to Deliver Nanocrystals, D. Wang

12:00-13:15 Lunch

14:00 Visiting Institute of Chemistry and Institute of Physics

18:30 Dinner

20:15 City Excursion

October 14th 9:00 Morning Session 1 Chair: Bing Xu

Plenary lecture:

9:00-9:40 Nanomedicines for the Advanced Delivery of Drugs Across Biological Barriers, Claus-Michael Lehr

Invited lectures:

9:40-10:00 Inorganic Nanocrystals: From Preparations to Bio-applications, M. Gao 10:00-10:20 Nanoparticles for Molecular Imaging and Targeted Therapy: Science and Fiction, U. Pison

10:20-10:50 Coffee Break

10:50 Morning Session 2 Chair: H. J. Galla

Plenary lecture

10:50-11:30 Multilayer Thin Films of Polymers: from Layered Architecture to Functional Assembly, X. Zhang

Invited lecture

11:30-11:50 Single Molecule Imaging and Tracking of Membrane Protein for Cell Signaling Study, X. Fang

12:00-13:15 Lunch

13:15 Afternoon Session 1 Chair: Claus-Michael Lehr

Plenary Lecture:

13:15-13:55, Monodisperse Nanocrystals and Nanospheres, Y. Li

Invited lectures

13:55-14:15 Nanoparticle Magnetic Resonance Imaging Contrast Agents and Issues Regarding Their Biological Effects, H. Lei 14:15-14:35 Studies on Proton Driven DNA Nanomachine, D. Liu

14:35-15:05 Coffee Break

15:05 Afternoon Session 2 Chair: Dayang Wang

Plenary lecture:

15:05-15:45 Controlling Permeability and Mechanics of Core/Shell Micro- and Nanocapsules, H. Möhwald

Invited lectures

15:45-16:05 Bio-inspired Strategies for Self-assembly of Inorganic Materials and Inorganic-organic Hybrids with Complex Form, S. Yu 16:05-16:25 Nanofibrous Scaffolds and their Biological Effects, H. Xu

16:25: Closing remarks

18:30 Farewell Dinner

October 15th Departure

Abstracts

Catalog

1.	Sishen Xie	.1
2.	Reinhard RENNEBERG	.2
3.	Changyou GAO	.4
4.	Heiko ZIMMERMANN	.7
5.	Shu WANG	.9
6.	Jochen FELDMANN	11
7.	Chunhai FAN1	2
8.	Bing XU1	3
9.	Lei JIANG1	5
10.	Nicholas A. KOTOV1	.7
11.	Lifeng CHI1	8
12.	Junqiu LIU1	9
13.	Hans-Joachim GALLA	0
14.	Qiaorong HE2	2
15.	Christian BRUNNER	23
16.	Zuhong LU2	24
17.	Dayang WANG	5
18.	Claus-Michael LEHR	26
19.	Mingyuan GAO	27
20.	Ulrich PISON	28
21.	Xi ZHANG	30
22.	Xiaohong FANG	3

23. Yadong LI	34
24. Hao LEI	
25. Dongsheng LIU	
26. Helmuth MÖHWALD	
27. Shuhong YU	41
28. Haiyan XU	42
29. Régis CARTIER	44
30. Yunhua YANG	45
31. Andrey ROGACH	
32. Yali CUI	47
33. Wensheng YANG	48
34. Fengqin HU	49

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Home Page:



In this lecture, I will talk about the trend and status of nanotechnology in China.





Bioengineered Nanocrystals for Mega-amplified Signals

Advances in nanotechnology have had significant impacts on the field of biodiagnostics. Novel applications of dissolvable, organic, and biofunctional nanocrystals for the improvement in sensitivity and limits of detection of immunoassays and the quantitative detection of a PCR product have been described. Fluorescein diacetate (FDA), a fluorogenic precursor of fluorescein, was milled in a solution of a polymeric surfactant to create a stable, nanosized colloid with an interface for coupling biomolecules. The applications of these particulate labels in a model sandwich immunoassay and for the quantitative detection of biotinylated human papillomavirus (HPV) DNA, amplified in a standard PCR procedure, were demonstrated. After the affinity reaction, the FDA molecules were dissolved and concomitantly converted into fluorescein. This approach resulted in a high selectivity, short incubation times and a sensitivity up to 147-2700 times greater than obtained from state-of-the-art, directly fluorescent-labeled biomolecules. This innovative method offers rapid detection of small amounts of nucleic acids because less target material and thus fewer PCR cycles are required. It provides high sensitivity and low limits of detection without the need for long incubation times, making it an interesting alternative in biolabel technology.

Scheme 1. Preparation of Biofunctional Fluorescent Labels^a



^a A simple two-step approach is described in the current study: (a) FDA was milled into nanometer-sized (107 nm on average) crystals in an aqueous surfactant (DSPE-PEG(2000)Amine) medium and (b) subsequently adsorbed with streptavidin molecules. The FDA conjugated streptavidin can be used to capture any biotinylated biomolecules.

Scheme 2. Amplified HPV-DNA Hybridization using Nanocrystalline FDA as Label^b



^b (a) The amplified HPV-DNA labeled with biotin was first hybridized with the immobilized probes in the microtitre plate and then (b) captured by the nanocrystalline FDA conjugated with streptavidin. (c) High signal amplification was achieved after solubilization, release and conversion of the precursor FDA into fluorescein molecules by the addition of DMSO and NaOH.

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Chitosan Based Gene Delivery System and Particle Internalization

Gene therapy shows a potential to treat and prevent a wide variety of genetic and acquired diseases. To fully utilize this potential, safer and more efficient vectors for delivery of genes are required. Vectors based on polyelectrolyte complexes of nucleic acids and synthetic cationic polymers (polyplexes) represent one of the major alternatives to viral vectors. Chitosan is a good candidate for gene delivery system because it is a biodegradable polycationic polymer with low toxicity and immunogenicity.

The chitosan–DNA complexes appeared homogenous spherical with a mean size around several hundred nanometers dependent on the composition of the complexes. The complexes were stable under storage condition after crosslinked by glutaraldehyde. The complexes can be internalized by cells in a couple of hours. We also studied the transfection efficiency of this kind of complex, and observed very different results in human fibroblasts and SMMC-7721 cell line. From CLSM observations, we found that the complexes could enter SMMC-7721 cell nucleus, yielding higher transfection efficiency (Fig. 1). In vitro fibroblast culture demonstrated the biocompatibility of this kind of complexes.

To achieve higher transfection efficiency, we synthesized N-trimethyl chitosan chloride (TMC), which has larger charge density. However, the transfection efficiency decreased with higher trimethyl substitution ratio due to higher cellular toxicity. An emerging class of such polyplexes relies on polymers capable to undergo phase transition in response to changes in environment, e.g. temperature, which shows promising approach to overcome the low efficiency and high toxicity of TMC. TMC-g-PNIPAAm graft copolymer was then synthesized by grafting poly (N-isopropylacrylamide) (PNIPAAm-COOH) to TMC. After obtaining the DNA/TMC-g-PNIPAAm complex particles, we studied the mean diameters of the DNA/TMC-g-PNIPAAm particles as a function of their composition. The morphologies of these particles were observed by TEM and the DNA-protecting abilities of TMC or TMC-g-PNIPAAm were examined by gel electrophoresis. Using HEK293 cells as the model, the thermosensitive ability of the TMC-g-PNIPAAm in gene delivery was studied in vitro. The grafting of PNIPAAm increased the transfection efficiency and lowered the cytotoxicity

notably (Fig. 2), indicating that the TMC-g-PNIPAAm could be a promising gene carrier.

Acknowledgements

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Fig.1. The transfection efficiency of chitosan/DNA complexes (upper) and their cellular distribution (lower)



Fig. 2. The transfection efficiency of TMC/DNA and TMC-g-PNIPAAm/DNA complexes

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Cryo-Nanobiotechnology : Preservation of Cells with Therapeutic Relevance in Microsystems and on an Nanostructured Surfaces

The term 'Cryo-Nanobiotechnology' combines the words 'Cryoconservation', 'Nanotechnology' and 'Biotechnology'. Cryoconservation describes the storage of 'living' cells and tissues at temperatures below -130°C. At these temperatures the vitality, functionality and differentiation of cells is maintained unchanged. Cryoconservation is currently the only method that enables animal, vegetable and in particular human cells and tissues to be kept vital for indefinite periods before reanimation on demand for therapeutic, diagnostic or scientific purposes. The required temperature range can be achieved through cooling with liquid nitrogen (nominal temperature: -196.6 °C). Nitrogen is abundant and readily available as it makes up approximately 78% of the Earth's atmosphere as an inert and harmless (in this state and concentration) gas. In addition to being a reliable coolant, liquid nitrogen is thus cost-effective, even in enormous quantities, and relatively easy to handle. In cryobanks cell suspensions, cord

blood and tissue samples are placed on special metal shelves in large double-walled steel vessels. A vacuum between the walls enables an inner container to be thermally isolated and hence the samples can be kept in the cold gas phase, or (as occasionally required) in the liquid phase of the nitrogen. Samples stored by this means can survive for years, decades or even centuries as the cells' metabolism and entire biochemistry have stopped completely.



Nature has not achieved freezing of cells at such low temperatures as the liquefaction of gases is a human invention (Carl von Linde, 1895) and as cryoconservation can only be achieved when specific stringent biophysical constraints are adhered to. For example, the conservation of living biological material is impossible without a cryoprotectant and without its entry into and / or surrounding of every cell. In addition, only certain temperature courses can be used for the freezing of biological samples as the reaction of the cells to changes in salt

concentrations of the surrounding medium is extremely sensitive. The cooling process of a biological sample is hence determined by its physical parameters such as heat capacity, sample shape / size and cooling rate. The requirement of a freezing process that allows later revival thus typically limits the characteristic lengths of conserved tissue pieces to a few millimetres.

Just as with nature, cryoconservation is also more successful with smaller sample volumes. In addition, smaller sample volumes are also financially beneficial as they require less space in cryo-tanks. The microsystem based cryo substrates have thus achieved a significant reduction in total sample volume. Their performance for the cryoconservation of Langerhans' islet was proven in a key experiment (Zimmermann et al. 2004, 2005).

Cryopreservation of cells attached on a substrate (e.g. embryonic stem cells) is complex. One problem is the detachment of cells during ice formation. Successful cryopreservation requires new cryobiological methods and new surfaces, such as nanostructures as well as a method for studying the cell-substrate interface. New method show that the distance between cell and substrate can change during cryopreservation and depends on the cell-substrate interaction and ice formation.



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Novel Design Strategies for Biosensors Based on Conjugated Polymers

Single-stranded DNA with G-rich sequences can fold into secondary structures named as G-quadruplex via intramolecular hydrogen bonding interactions. G-quadruplex DNA can further convert to duplex form in the presence of its complementary strand. These conformational changes can be detected by a homogeneous assay method based on fluorescence resonance energy transfer (FRET) using water-soluble cationic conjugated polymer (CCP). The backbone of the conjugated polymers consists of a large number of absorbing repeated segments. The excitation energy transferring to the reporter chromophore along the whole conjugated polymer backbone results in the amplification of fluorescent signals. It is possible to demonstrate novel FRET-based, homogeneous biosensors to detect DNA, protein and metal ions that couples the folding conformation of a guanine-rich oligonucleotide with the optical amplification of CCPs. Thus, the G-quartet-DNA/CCPs assembly can be used as novel platforms for biosensor applications.



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Nanoparticles as optical sensors and actuators

I will review our recent activities to use noble metal and semiconductor nanoparticles for various nanophotonic applications such as

- detection of biomolecular binding and unbinding events [1,2]
- electrical control of energy transfer processes [3] and
- surface-enhanced Raman scattering on single molecules

- 1. G. Raschke et al., Biomolecular Recognition Based on Single Gold Nanoparticle Light Scattering, *Nano Letters* **2003**, *3*, 935.
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Better Living through Nanobiodetection

The use of gold nanoparticles (AuNPs) has a long history in biology, which dates back to the application of "immunogold" in biological imaging in the 1970's. In 1994, Mirkin group and Alivisatos group independently reported that AuNPs-DNA conjugates could serve as scaffolds for nanostructures. This has motivated worldwide interest to develop AuNPs-based biodetection. Our research interests are focused on improving biodetection performance by exploiting nanoparticle-biomolecules interactions. Here I will present several examples from our group, including nanoparticle PCR (nanoPCR), electrochemical DNA detection by using AuNPs-DNA complexes and aptamer-based detection via unmodified AuNPs.

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Biofunctional Nanoparticles and Nanofibers: Synthesis and Applications

This talk will focus on the development of biofunctional nanomaterials based on magnetic nanoparticles and nanofibers formed by small therapeutic molecules.^[1,2] We will demonstrate that the applications of magnetic nanoparticles for pathogen detections (Fig. 1) and protein separations as well as the solution-phase synthesis of nanoparticle heterodimers, which act as a platform for engineering molecules within 20 nm scales. Compared to conventional used magnetic particles (with the sizes of 1-5 µm) in biological separation or drug delivery, magnetic nanoparticles, combining with specific receptor-ligand interactions, promise a sensitive and rapid protocol to detect pathogens. For example, covalently linked to vancomycin (Van), chemically stable and highly magnetic anisotropic FePt magnetic nanoparticles (3-4 nm) become water-soluble and capture vancomycin resistant enterococci (VRE) and other Gram-positive bacteria at concentration $\sim 10^1$ cfu/mL via polyvalent ligand-receptor interactions. This talk also will discuss a novel hydrogel based on vancomycin and water. In addition, this talk will discuss other hydrogels, formed by self-assembly of bioactive molecules, as the scaffolds for potential biomedical applications such as wound healing, drug delivery, and inhibitor screening. For example, two types of therapeutic agents, which have discrete yet complementary functions, self-assemble into nanofibers in water to formulate a new supramolecular hydrogel. As a self-delivery biomaterial that is topically administered on simulated uranium wounds on mice, this hydrogel reduces the toxicity of uranyl oxide at the wound sites (Fig. 2).



Fig. 1

Fig. 2



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Design and Creation of Bioinspired Surfaces with Special Wettability

Bio-inspired smart materials should be a "live" material with various functions like organism in nature, they must have three essential elements as sense, drive and control. The putting forward of bio-inspired smart interfacial materials just bases on the above mentioned elements, while the design principle can be divided into five levels: (1) bio-mimetic and intelligently design of the materials; (2) cooperate of multi-scale structure effects; (3) design for the heterogeneous interfaces; (4) cooperate with various physical properties of different materials; and (5) bi-steady state effect. Our recent studies are focused on the design and fabrication of bio-inspired surfaces with special wettability based on these ideas. The studies on lotus and rice leaves reveal that a super-hydrophobic surface with both a large CA and small sliding angle needs the cooperation of micro- and nanostructures, and the arrangement of the microstructures on this surface can influence the way a water droplet tends to move. These results from the natural world provide a guide for constructing artificial super-hydrophobic surfaces and designing surfaces with controllable wettability. Accordingly, super-hydrophobic surfaces of aligned carbon nanotube films, aligned polymer nanofibers and differently patterned aligned carbon nanotube films have been fabricated. The large scale fabrications of super-hydrophobic polymer surfaces have been developed by modification of the traditional template method, the adoption of one-step coatings and electrohydrodynamics, respectively. Considering the arrangement of the micro- and nanostructures, the surface structures of the water-strider's legs were studied in detail, indicating the relationships between super-hydrophobicity and orientation of the micro- and nano-scale composite structures, which will guide us to fabricate micro-fluid devices artificially in the near future. In further, the cooperation between surface micro- and nanostructures and surface modification of poly (N-isopropylacrylamide) gave reversible switching between superhydrophilicity and superhydrophobicity in a narrow temperature range of about 10 °C. The transition can be enhanced by depositing the polymer onto patterned silicon substrates. Additionally, UV light stimulated switcher of superhydrophobic and superhydrophilic transition by aligned ZnO film are successfully obtained. These two kinds of switcher materials are intrigue great interest in the world and were reported as Nature News and Science Editor Choice.



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Self-Organization of Nanoparticles: Surprising Analogies with Protein Superstructures

Methods of organization at nanoscale belong to the key problems that transcend different areas of nanotechnology. Self-organization phenomena can be regarded as convenient tools to obtain intricate nanoscale systems with a variety of potential functionalities, such as optical, electrical, thermal transport properties. In this presentation, I will review self-organization phenomena taking place with semiconductor nanocolloids starting with supercrystals from nearly spherical nanoparticles [1] and nanoparticle bioconjugates [2] to 1D [3] and 2D assemblies [4]. Comparison of the processes in solution of CdTe and other nanocolloids reveals a number of surprising similarities with processes in proteins. The conclusion that will be reached that this is the result of fundamental analogy in the scales between proteins and nanoparticles.

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Anisotropic Behavior of Cells on Mesotropic Patterned Surfaces

Topographically patterned surfaces are known to influence cellular behavior in a controllable manner. [1] However, the relatively large surface areas (several cm2) required for many biomaterial applications are beyond the practical limits of traditional lithography. Langmuir–Blodgett lithography (LBL), a recently developed method, was used to fabricate regularly spaced grooves of different depths (50 and 150 nm) with periodicity of sub-micrometer over several square centimeter on silicon surfaces. These topographies were transferred into polystyrene surfaces by means of nanoimprinting. Some cells show a significant anisotropic behavior to these surfaces, which can enhance cell settlement on the surface or be used to direct tissue generation on the biomaterial interface. For instance, primary osteoblasts were cultured on the patterned polymer surfaces. They were observed to align, elongate and migrate parallel to the grooves. [3] The combination of Langmuir–Blodgett lithography with nanoimprinting enables the fabrication of large, nanostructured surface areas on a wide spectrum of different biomaterials, thus providing a promising approach to surface patterning of biomaterial interfaces for implantology or tissue engineering.





Fluoresence micrographs of immunohistochemically labeled cells (here vinculin). Left colum shows cells on a smooth surface, and the right column shows cells on 150nm deep grooves. Grooves are oriented vertically.

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Design of Artificial Seleno-enzymes

Enzymes exhibit high substrate-specificity and significantly accelerate reaction rates. The nature of substrate binding and the intracomplex interactions of enzymes play important roles in strong catalytic ability. As one of a series of antioxidative selenoenzymes in living organisms, glutathione peroxidase (GPx) catalyzes the reduction of harmful hydroperoxide by glutathione and protect biological molecules from oxidative stress in cells. In recent years there were increasing interests in mimicking the functions of this important selenoenzyme, and several attempts had been made to produce synthetic selenium/tellurium compounds which mimic the properties of glutathione peroxidase(GPx).

For this purpose, a series of artificial GPx models have been designed and synthesized by using cyclodextrin, antibody, proteins and dandrmer as scarfolds (Scheme1). These GPx models

displayed significant high GPx activities. The catalytic efficiency and steady-state kinetics of GPX mimics were studied in details, revealing that the generation of substrate and correct orientation of catalytic moiety should be main factors for artificial enzyme design.



Scheme 1

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The controlled non-covalent assembly of molecular structures On the nanometer scale is the focus of our current interest. Due to their reduced size those structures are not available by conventional lithography. At the air water interface it is possible to create 2D but also 3D structures designed mainly by hydrogen bonding. With chiral substance this leads to chiral discrimination and the formation of chiral domains structures.

2D and 3D structures mainly observed in artificial surface layer systems have a direct biological correlate to the alveolar surfactant supporting the breathing cycle by a controlled reduction of the surface tension within the lung. A mixed-lipid-peptide monolayer adapts the surface tension by the reversible formation of 3D-protrusions under compression. Due to a lateral phase separation the remaining monolayer is depleted by peptides an charged lipids. The remaining non-charged lipids allow to decrease the surface tension thus allowing an econimically breathing process. The lung surfactant is another biological example where domain structures determine biological function.



Fig 1: 3-D Structures of ethyl 2-azido-4-fluoro-3-hydroxy-stearates



Fig 2: 3D-Protrusions in artificial lung surfactant material determined by chemical composition (spatially resolved TOF-SIMS) an topology (SFM)

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Amyloid-like Neuronal Tau is a Toxicant and Induces Neuron Apoptosis

Although there are many reports on methanol and formaldehyde intoxication, none pays attention to the protein misfolding that is potentially involved in the pathological way, in particular the effect of formaldehyde on protein polymerization. Damage of neuronal cells caused by misfolded protein aggregates is one of the most significant problems in both neurology and pathology. Tau is a major microtubule-binding protein that is important for the assembly and stabilization of microtubules. Misfolding of tau is related to a pathological way of neurodegeneration. In experiments we found neuronal tau was aggregated by formaldehyde under a simulated physiological condition in vitro. Changes in the fluorescence of thioflavin T show that formaldehyde induces tau to form an amyloid-like aggregate, which is further confirmed under electronic microscope. The amyloid-tau (approx, hundreds nano-mole) acts as a strong toxicant to neurotypic cell line (SY5Y), but not paired helical filament tau (PHF-tau). Cyclin-dependent kinases may play an important role in the presence of amyloid-tau during the apoptosis. Some compounds which can associate with the amyloid-like deposits, such as congo red and olomoucine (Cdk inhibitor), attenuate the positive effect of amyloid-like tau on caspase-3 activity and protect SY5Y cells from apoptosis. It suggests that the soluble amyloid-tau induces neuron apoptosis before it polymerizes into PHFs.



Figure 1. Polymerization of neuronal tau induced by formaldehyde at a low concentration observed by atomic force microscopy. Bar = 25 nm.

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Cargo Transport on Engineered Surfaces Powered by Molecular Motors

Micro- and nanoscale assembly lines with nanometer precision, where individual components are combined to produce functional and highly complex objects, are at the forefront of nanotechnological applications and would potentiate the fabrication of novel, nanoscale devices such as biosensors. While the highly parallel process of molecular self-assembly is already widely used for technological applications such as bioanalytical devices, actively driven systems that allow assembly of non-equilibrium structures with high throughput are still a matter of basic research.

A fundamental requirement for active transport on the nanoscale is the integration of molecular motors that act in concert. Biomolecular motors exceed the functionality of synthetic motors, have been proven to operate in synthetic environments, and can thus be integrated into hybrid devices. In our approach, kinesin motor proteins move microtubule filaments (molecular shuttles) along a surface. This active transport results from the conversion of chemical energy (ATP hydrolysis) into mechanical motion.

Various challenges, such as directional control over microtubule movement or specific binding of cargo to functionalized shuttles, have already been successfully addressed. In our laboratory, we are currently investigating cargo binding strategies based on biotin-streptavidin and antibody-antigen interactions and micro-scale cargo patterns to limit pick-up from defined areas. Finally, in collaboration with other research groups, a label-free microtubule visualization technique was developed for visualization of cargo pickup and transport.

The final challenge, however, will be to combine these elements into a single, fully functional device which can be used to specifically transport small quantities of material at the micro- and nanoscale. Name: Professor Zuhong LU

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Integration of Sample Processing and Microarray for Multiple-gene Detection

Many efforts have been made on the miniaturization and integration of DNA diagnostics array for multi-pathogen detection in the fields of epidemic control, food safety inspection, and anti-biological terrorism. Many studies have reported on microfluidic systems for PCR or RT-PCR. Recently, the microarray technique has been introduced into the device for high-throughput genetic detection.

We developed, a microarray-in-a-tube system shown in Fig.1, in which an oligonucleotide microarray was arranged on the inner surface of a specially designed, optically transparent, plastic Eppendorf cap. The assay system integrates PCR reaction and hybridization. The fluorescent hybridization signals are detected in an encapsulated tube, shown in Fig.2. Furthermore, we developed a unique fluorescence resonance energy transfer-based microarray platform for real-time quantification of nucleic acid targets, the principle is shown in Fig.3. Using this new approaches we successfully monitored multiple different pathogenic genomic DNAs. The advantages of both assay are low cost and simple to use, and prevents the false results associated with contamination of the PCR products.



Figure 1



Figure 3

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Using Hydrogel Microspheres to Deliver Nanocrystals

The use of inorganic nanocrystals for biomedical application is mainly blocked by their poor colloidal and chemical stability, thus leading to a highly cytotoxicity, and the lower in vivo circulation times, thus limiting the efficiency. Hydrogel microspheres have been extensively utilized as excellent biocompatible delivery vehicles of toxic drugs and biomacrolecules such as proteins. A long in vivo circulation time can be also implemented by an exquisite design of hydrogel microspheres. Consequently, the use of hydrogel microspheres to delivery nanocrystals should pave an appealing way to circumvent the challenges associated with the biomedical application of nanocrystals. This talk we will address to the current success on loading inorganic nanocrystals into hydrogel microspheres. Based on the stimuli-response swelling behavior of hydrogel microspheres various nanocrystals can be embedded within the microspheres. At the mean time, the controlled release of nanocrystals from hydrogel microspheres was demonstrated also. According to a broad size distribution of pores in hydrogel microspheres, differently sized nanocrystals can be incorporated in one gel microsphere, giving rise to multicolor fluorescence coding.

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Nanomedicines for the Advanced Delivery of Drugs Across Biological Barriers

Due to the advances of molecular biotechnology and bioinformatics, there is a strong increase of new candidate drug molecules. However, apart from their affinity to the target receptor, bioavailability and transport to the actual site of action is an important issue when it comes to developing such molecules to actual drug products. This holds in particular for macromolecular biopharmaceuticals, such as peptides, proteins, oligonucleotides or gene vectors, which normally cannot be administered orally. Instead, alternative routes of drug administration and new technologies for their controlled delivery have to be developed.

While the oral delivery of peptides and proteins still appears as a far goal, the transpulmonal delivery of peptides, such as e.g. insulin, is making good progress, and several drug products are currently being tested in advanced clinical studies. Unfortunately, there are no established in-vitro models of the blood-air barrier yet to screen compounds and to study the mechanisms of pulmonary drug transport. As a model of the alveolar mucosa, we use human alveolar epithelial cells in primary culture on permeable filters, which have a good morphological and functional resemblance to the alveolar air-blood barrier, as well as some bronchial epithelial cell lines.

In order to improve the delivery of still larger molecules, such as gene vectors, some special formulation will be required. Nanoparticles, prepared from lipids, polymers or inorganic materials may represent an alternative to viral gene vectors, especially with respect to cost and safety aspects. Still, however, their cellular binding and transport needs to be improved. In this context we are exploring the potential of lectins and lectin-like molecules by studying their interactions with cellular glyco-receptors by means of molecular modelling, surface plasmon resonance and atomic force microscopy.

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Magnetic Nanocrystals: from Preparations to Bio-applications

Over the past years, inorganic nanocrystals, especially fluorescent quantum dots (Q-dots) and magnetic nanocrystals, have been demonstrated to be potentially useful in various applications ranging from bioassays to clinical diagnosis. In this presentation, I will briefly review what we have been doing on functional inorganic nanocrystals with an emphasis on Fe_3O_4 nanocrystals from the synthesis of water-soluble, biocompatible magnetite nanocrystals and their potential applications as MRI contrast reagents for both in vitro and in vivo cancer detections.

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Nanoparticles for Molecular Imaging and Targeted Therapy: Science and Fiction

Nanoparticles are at the leading edge of the rapidly developing field of material science in nanotechnology with many potential applications in clinical medicine and research 1. Due to their unique size-dependent properties nanoparticles offer the possibility to develop both new therapeutic and diagnostic tools.

The ability to incorporate drugs into nanosystems displays a new paradigm in pharmacotherapy that could be used for cell-targeted drug delivery. Nontargeted nanosystems such as nanocarriers that are coated with polymers or albumin and solid lipid particles have been used to transport a large number of compounds. However, nowadays drugs can be coupled to nanocarriers that are specific for cells and/or organs. Thus, drugs that are either trapped within the carriers or deposited in subsurface oil layers could be specifically delivered to organs, tumors and cells. These strategies can be used to concentrate drugs in selected target tissues thus minimizing systemic side effects and toxicity. In addition to these therapeutic options, nanoparticle-based "molecular" imaging displays a field in which this new technology has set the stage for an evolutionary leap in diagnostic imaging (Figure below depicts a rat with organs imaged using multimodal nanoparticle technology).

Based on the recent progress in nanobiotechnology, nanoparticles have the potential to become useful tools as therapeutic and diagnostic tools in the near future.



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Multilayer Thin Films of Polymers: from Layered Architecture to Functional Assembly

Layer-by-Layer (LbL) assembly is a powerful method for fabricating multilayer thin films with tailored composition and architecture.^{1, 2}. Although LbL assembly has gained a rapid progress these years, there remains a challenge for fabricating functional species with single charge or without charge. In addition, this line of research also calls for a combination of layered architecture and functional assembly, leading to the fabrication of functional nanocomposites. In this presentation we will introduce different hierarchical assembling methods for fabrication of multilayer thin films, aimed to bridge the gap between layered nanoarchitecture and functional assembly.

(1) The use of LbL polyelectrolyte multilayer as a preformed matrix in chemical deposition leads to developing new approach for adjusting the morphology of Au³ or Ag nanostructures.⁴ After modifying the metal surface with alkyl-thiols to form a self-assembled monolayer (SAM), the surface has exhibited superhydrophobic property. Since the LbL assembling technique is independent of the size and shape of the substrates, we are able to fabricate the superhydrophobic coating on gold threads, which opens promising applications for biomimetic drag-reducing and quick propulsion.⁵

(2) To combine the precursor assembly of polyelectrolyte/single-charged species complex and LbL deposition allows for fabricating single-charged species into polyelectrolyte multilayer films⁶. Taking single charged dye molecule, sodium 9-anthracenepropionate (SANP) for example, we mixed cationic polyelectrolyte of poly(diallyldimethylammonium chloride) (PDDA) with anionic dye of SANP in appropriate concentrations, forming a PDDA-SANP complex by interpolyelectrolyte complexation, and then fabricated multilayer assemblies by alternate deposition of PDDA-SANP complex and poly(4-styrenesulfonate) (PSS). We have found, interestingly, that the dye molecules incorporated in the film can function as molecular templates. The release of these molecules can lead to formation imprinting sites, which allows for binding charged species with good selectivity.

(3) In order to incorporate water-insoluble molecules into the LbL assemblies, we have



developed a hierarchical assembling method that involves block copolymer micelles and LbL deposition. Namely, we incorporated water-insoluble molecule, e.g. pyrene, into the hydrophobic micellar cores of poly(styrene-b-acrylic acid) and then employed the pyrene-loaded polymer micelles as building blocks for LbL assembly.⁷ Similar concept can be applied to incorporate other water-insoluble molecules, such as azobenzene. The azobenzene incorporated into the block copolymer micelles can undergo a reversible photoisomerization under irradiation of UV and visible light. We have found, interestingly, that the photoisomerization of the azobenzene in the multilayer film is much faster than its normal solid film but very similar to that in its diluted solution, suggesting a way for enhancing the photochromic properties in the LbL films.⁸

In summary, we described several new methods for a combination of layered nanoarchitecture and functional assembly. It is anticipated greatly that this line of research will not be restricted to fundamental science, but extended to application in the areas of self-cleaning surface coating, surface molecularly imprinting, ion-permselective and photo-responsive thin films.

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Hierarchical Assembling Methods for Fabrication of Polymer Nano-composite Films

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We are interested in single molecule fluorescence imaging of membrane proteins, especially growth factor receptors, to probe their structures and dynamic behaviors for cell signaling study. For example, individual transforming growth factor- β (TGF- β) receptors have been imaged and tracked on the living cell surface by total internal reflection fluorescence microscopy¹. TGF- β receptors are transmembrane serine/threonine kinases which are involved in many cellular processes such as cell proliferation, differentiation and apoptosis². By real-time monitoring of green fluorescence protein (GFP)-labeled single TGF- β type I (T β RII) and type II (T β RII) receptors, the reduced diffusion of the receptors on TGF- β 1 activation was observed and the ligand-induced receptor oligomerization was confirmed. Moreover, the results revealed the monomer status of these important serine/threonine kinases and the effect of lipid raft on T β RI / T β RII association. This offers new information to understand the molecular interaction of the receptors and their activation process in signal transduction.



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Monodisperse Nanocrystals and Nanospheres

Monodisperse nanocrystals/nanospheres with well-designed compositions, crystal structures, sizes and surface properties are appealing to a wide range of research areas including catalysis, optics, biological label and nanodevices fields, because of their potentials in the understanding of magnetic, dielectric and transport properties in nanometer regime as well as their size-dependent properties. In this presentation, we will demonstrate the general synthesis of a broad range of nanocrystals and nanospheres, based on which, the underlying principle in size and shape control in nanoscale will be discussed.

A bubble mechanism has been demonstrated to guide the growth of monodisperse ZnSe nano/micro spheres with controllable sizes, with which as templates various oxides, sulfide and selenide semiconductors nanospheres and/or core-shell structures with tunable band gap could be readily obtained through a facile chemical conversion way. Due to their controllable optoelectric properties and self-assembly characteristics, these nanospheres may serve as ideal candidates for applications in solar cell and optoelectric nanodevices.

A green synthetic strategy has been developed to obtain natural polymer nanopsheres with biocompatible surfaces, and with these monodisperse nanoshperes as sacrificing templates, various hollow spheres of oxides or nitride semiconductors were successfully prepared. These nanospheres also show their amazing ability in embedding noble metal and/or magnetic nanocrystals to form functional core-shell structures, which have shown some interesting SERS properties and may find applications in biological fields.

A general model to get monodisperse nanocrystals has been presented to show the whole picture of the growth of functional nanocrystals with diverse crystal structures and compositions, including noble metal, semiconductors, magnetic/dielectric and fluorescence nanocrystals. The general principles in size control will be discussed. With these monodisperse nanocrystals as functional building blocks, some more unique and exciting applications could be driven from the bottom-up approach to nanoscience and nanotechnology fields.

Despite the diversity in crystals structures and properties, we have succeeded in preparing nearly all the functional nanocrystals / nanospheres with controllable size and surface properties, which show the possibilities of the final establishment of a general methodology to

low-dimensionality nanostructures and will certainly arouse some new opportunities to nano-related fields.

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Nanoparticle Magnetic Resonance Imaging Contrast Agents and Issues Regarding Their Biological Effects

With the rapid development of nanoscience and nanotechnology in recent years, growing research interests and efforts have been directed to the applications of nano-materials in the field of biomedicine and to study the biological effects of manufactured nanoparticles and substances alike. Have a wide range of applications in physics, chemistry and biomedicine, magnetic resonance imaging (MRI) and spectroscopy (MRS) are among the most important and powerful research tools currently in use, mainly because these techniques can be used in situ and noninvasively to acquire dynamic and real-time information in various samples ranging from protein solution to human brain. In this talk, the applications of nanoparticle as MRI contrast agents are introduced, especially those in cellular and molecular imaging. The use of magnetic resonance technologies in studying the biological effects of the nanoparticles is also presented.



Fig. 1 Pre- and post-contrast MR images of rat liver (a), toxoplasmic mice brain (b) and rat olfactory bulb (c) after intravenous (a and b) or intra-naris (c) application of PEG-coated iron oxide nanoparticle. The arrows in (b) indicate toxoplasmic lesions enhanced by iron oxide nanoparticle.



Fig. 2 MRI contrast agent $[Gd@C_{82}(OH)_x]_n$ nanoparticle induces rapid thrombosis In mice.



Studies on Proton Driven DNA Nanomachine

The molecular recognition properties of DNA are sufficiently well understood to enable the self-assembly of defined structures on the nanometer scale. DNA nanostructures have the potential to form the next generation of functional devices. An important challenge is to fabricate molecular components that act as machines.



Here we report a novel molecular machine driven by changes in pH. It is composed of cytosine-rich DNA that at slightly acidic pH is folded into a compact structure, called the i-motif, and can be opened in basic pH to form an expanded duplex structure. This structural change is reversible and rapid, and the by-product of each working cycle is H₂O plus salt. Multiple cycling of this machine was demonstrated. It's working ability in the solid/liquid interface has been demonstrated by scanning fluorescent microscopy. We have also shown that this molecular motor has the potential to drive a microcantilever move. In this report, we will also present our new achievement on the pH trigged DNA nanocompartment based on the proton driven DNA nanomotor.

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Controlling Permeability and Mechanics of Core/Shell Micro- and Nanocapsules

Recent advances in colloid science have enabled the formation of well-defined organic or inorganic colloids, partly with a controlled nanopore structure. Progress in interfacial science could be merged with this creating coating films with defined thickness, composition and surface chemistry. The latter was achieved by alternating coating with oppositely charged polyelectrolytes /1/ or by "grafting from" polymerization /2/. This affords a rich application potential since permeability and mechanics of the coating can be varied in a broad range and with high precision. In addition the wall can be sensitized for light absorption and temperature enabling remote release via IR or microwave radiation. Data on this will be given combining optical electron and scanning force microscopies and force measurements.

In special we will show that one may heat polyelectrolyte multilayers over a glass transition where capsule walls thicken and diameters shrink /3/. Thus defects related to the preparation process are annealed and the wall becomes impermeable also for low molecular weight compounds. The glass transition is accompanied by a change in the elastic modulus by more than two orders of magnitude. Capsules may be built with Au or Ag nanoparticles that strongly absorb IR radiation. This causes capsules to break at threshold intensities which are not yet harmful for biological cells /4/.

Initiators for surface initiated polymerization can be bound to Au or magnetite particles. This endows the particles a great selection of contact angles which is relevant for technical and biomedical applications. Later the grown polymer brushes can be crosslinked yielding again capsules. In this case starting from Au particles with 20 nm diameter capsules with inner diameter around 100 nm are formed /5/.

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Bio-inspired strategies for Sel-assembly of Inorganic Materials and Inorganic-organic Hybrids with Complex Form

New trends in the area of crystallization and morphogenesis of inorganic and inorganic-organic hybrid materials will be reviewed, including synergistic effects of crystal growth modifiers in water and in a mixed solvent, and crystallization on artificial interfaces or within matrices. Combination of a synthetic template with a normal surfactant or crystallization in a mixed solution system makes it possible to access various inorganic crystals with complex form and unique structural features. Several different morphogenesis mechanisms of crystal growth, such as selective adsorption, mesoscopic transformations, and higher order assembly, will be discussed. In addition, crystallization on artificial interfaces including monolayers, biopolymer and synthetic polymer matrices for controlled crystal growth, and emerging crystallization on foreign external templates and patterned surfaces for creation of patterned crystals will also be overviewed. Current advances indubitably emphasize that probably all inorganic crystals and inorganic-organic hybrids will be amenable to morphogenesis control by use of either flexible molecular templates or suitable self-assembly mechanisms. The latest developments in this field should provide new possibilities for rational design of various kinds of inorganic, inorganic-organic hybrid nanomaterials with ideal hierarchy and controllable length scale, which could find potential applications in various fields.

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Nanofibrous Scaffolds and their Biological Effects

This talk will introduce our investigation on nanofibrous scaffolds and their biological effects. We have fabricated single walled carbon nanotubes nonwoven (nonwoven SWNT) by chemical vapor deposition (synthesized by Beijing National Laboratory of Condensed Matter Physics, Institute of Physics, Chinese Academy of Sciences) and nanofibrous scaffolds made of carbon nanotube/polyuyrethane composite (MWNT/PU) by electrospun, respectively. The morphology and surface chemistry of both scaffolds were observed and characterized through SEM and XPS respectively before and after immersed in the culture medium (IMDM). The growth behaviors of fibroblast cells (3T3 NIH) on the scaffolds, including adhesion, proliferation, cytoskeletal development were investigated by using cell viability assay and confocal observation. The scaffolds with nanofibrous topography exhibited significant enhancement to the cells adhesion and proliferation in at least 3 weeks. Numerous and well-organized cytoskeletal structures were observed when the cells were cultured in the both scaffolds. Besides nanofibrous structure, it appeared that carbon nanotubes played an positive role in the cell growth, which might be resulted from the pure carbon composition and a huge amount of aromatic rings possessing strong charge-transfer capability. Additionally, certain substances secreted by the cells cultivated in the nanofibrous scaffolds displayed an obvious promotional influence upon the proliferation of cells growing in other substrates through cell-cell communication. The nanofibrous scaffolds are expected to be application potentials in cell amplification in large scale in vitro, tissue regeneration or guided repair, and biomedical device surface engineering.



Nonwoven SWNTs with nanotopography provided microenvironment similar to natural extra cellular matrix, which enhanced cell proliferation and skeletal development. In the above figure, A and B is optical graph and SEM image of nonwoven SWNTs respectively, C and D is actin observation of the cells growing on nonwoven SWNTs for 6 h and 72 h, respectively; E is the proliferation results of cells on the various materials by MTS measurement

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Latex-nanoparticles for Multimodal Imaging and Detection in vivo

The aim of the present work was to develop a multimodal imaging and detection approach to study the behaviour of nanoparticles in animal studies. Highly carboxylated latex nanoparticles were labelled with 68Ga for positron emission tomography, 111In for quantitative gamma scintigraphy or Gd3+ for magnetic resonance imaging. Following intravenous injection into rats, precise localization was achieved with a high spatial and temporal resolution revealing the tracer in the blood compartment with a time dependent accumulation in the liver. In addition, Rhodamine B was also incorporated to examine specific interactions with blood cells. Flow cytometry and fluorescent microscopy show uptake of nanoparticles by leucocytes and unexpectedly, thrombocytes, but not erythrocytes. Cellular internalization was an active and selective process. Further incorporation of polyethylene glycol into the nanoparticle corona could prevent uptake by thrombocytes but not macrophages or monocytes. Our data demonstrate the feasibility of a multimodal approach and its usefulness to analyse the fate of nanoparticles at the macroscopic and cellular level. It will facilitate the development of functionalized nanocarrier systems and extend their biomedical applications.

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Home Page:



Incorporating CdTe Nanocrystals intoPolystyrene Microspheres: Towards Robust Fluorescent Beads

Due to excellent optical properties such as size-tunable fluorescence and a narrow and symmetric emission profile with a broad excitation range, II–VI fluorescent quantum dots (Q-dots) have undergone intensive investigation as a new type of biolabeling material over recent years. Although Q-dots have been proven to be superior to conventional dyes in many respects, the ultrasensitivity of their fluorescence to particle surface states, and the release of highly toxic heavy-metal ions as a consequence of photodegradation are problems that remain to be solved for Q-dot-based biolabels with respect to both in vivo and in vitro application. One of the possible solutions is to coat the fluorescent Q-dots with a polymer, since effective isolation can not only suppress the photodegradation but can also impede the release of heavy-metal ions thereupon. Most importantly, the simultaneous integration of different types of fluorescent Q-dots into polymeric beads can lead to a useful encoding library for high-throughput biodetection. We report a modified miniemulsion polymerization method for preparing multiplexed optical-encoding polystyrene beads with pH-independent fluorescence and excellent antisolvent properties. We also demonstrate that the use of polystyrene (PS)as a matrix can effectively enhance the photostability of the Q-dots within the PS beads.

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Home Page:		

Polymer Microcapsules Labeled by Luminescent and Magnetic Nanocrystals as Potential Drug Delivery System

We report on the recent developments of multi-functional polymer mcirocapsules incorporating different classes of inorganic nanocrystals with a final aim on their applications as a drug delivery system [1]. The capsules were produced by the layer-by-layer adsorption of oppositely charged macromolecules on colloidal templates (latex beads or manganese carbonate particles) ranging in size from 0.5 to 10 microns. The cores were decomposed leaving behind empty capsules. The outer polymer multilayer shell remained intact providing the capsule stability from one hand and the permeability for inorganic nanocrystals from the other hand. The shells of the capsules were functionalized with highly luminescent semiconductor nanocrystals with narrow emission bands located at different wavelengths depending on the particle size. Specifically, water-soluble CdTe nanocrystals emitting in the visible and CdHgTe or HgTe nanocrystals emitting in the near-infrared spectral ranges were used. Using CdTe nanocrystals, both single-color and multicolor labeling with controlled emission intensity ratios was demonstrated, being important for detection and identification of different capsules. Simultaneous encapsulation of both luminescent semiconductor and magnetic oxide nanoparticles in polymer microcapsules was further achieved. CdTe nanocrystals served as luminescent markers, while magnetic Fe₃O₄ nanoparticles allowed for external manipulation of capsules by magnetic field gradient. Finally, incorporation of metal nanocrystals consisting of gold or dielectric core/gold shells provided a mean for controlled heating and opening of the capsules under the laser beam, which should allow for the stimuli-responsive release of encapsulated substances. The use of multifunctional microcapsules introduced in this work as a career system for the controlled release and directed drug delivery is envisaged.

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Home Page:

The Synthesis of Goldmag Nano-Particles and Their Application for Antibody Immobilization

 Fe_3O_4/Au (GoldMag) particles with core/shell structure were synthesized by reduction of Au^{3+} with hydroxylamine in the presence of Fe_3O_4 . The synthesized particles have an average size smaller than 100 nm in diameter with of superparemagnetic properties due to their Fe oxide cores. The particles show optical features with a plasmon resonance peak from 550, 570 to 590 nm correlating with increasing diameters from 50 nm, 70 nm to 100 nm.The Gold-Mag particles need only a single step for antibody immobilization and have high binding capacity for antibodies. These advantages permit improved methods of isolating and detecting biomolecules. Name: Prof. Wensheng YANG

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Home Page:

Sterically Mediated Two-Dimensional Architectures in Aggregates of Au Nanoparticles Directed by Phosphorothioate Oligonucleotide-DNA

In this presentation, we will show the possibility of constructing aggregates of Au nanoparticles with different 2D architectures by changing the length of PS poligo-DNA linkers. Due to the approximate parallel orientation of PS oligo-DNA on the particle surface, the number of helices allowed to accompany one particle in a 2D plane is determined by their length. The architectures of the nanoparticles in the aggregates can be controlled by such a steric effect related to the length of the PS oligo-DNA linkers employed. It is expected that the architectures of the nanoparticles in the aggregates can also be controlled by changing their diameters when the length of the linker molecules is fixed.

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Home Page:

Preparation of Magnetite Nanocrystals with Surface Reactive Moieties by One-pot Reaction

By one-pot reaction, through the thermal decomposition of $Fe(acac)_3$ in 2-pyrrolidone, biocompatible magnetite nanocrystals with surface reactive moieties were prepared using α,ω -dicarboxyl-terminated poly (ethylene glycol) as a surface capping agent. An EDC-mediated coupling reaction was conducted to link 9-amino acridine (9-AA) with Fe₃O₄ nanocrystals to detect the existence of the carboxylic groups on the particle surface.