

1st International Symposium on
Molecular Imaging and Nanomedicine

August 20-24, 2011
Lijiang, China

Sponsored by Institute of Chemistry, CAS
Supported by 973 Program

Background

Nanoscience and nanotechnology have gained significant momentum in recent years to interplay with biology and medical science, leading to the emergence of a new interdisciplinary field called and “nanomedicine”, which have widely penetrated into the fields of life science and medical science, ranging from in vitro bioassays, to in vivo imaging and diagnosis, and drug delivery. Based on the current research achievements, nanomedicine is believed to offer powerful and multifunctional tools for the bio-detection, bioimaging, targeted drug delivery and therapeutics in the near future, which may greatly change the current status of clinical diagnosis and treatment of malignant tumors.

Aims

The aim of this symposium is to provide a platform for exchanging ideas, to share new results and achievements in the related fields, and to strengthen the existing collaborations and meanwhile promote new collaborations among all participants in the future.

Topics

- Design and fabrication of novel molecular probes and nanoprobe;
- Applications of nanomaterials and technology in biology and medicine;
- Molecular mechanism and clinical diagnostics of tumors;
- In vivo imaging of tumors.

Scientific Program for
1st International Symposium on
Molecular Imaging and Nanomedicine

August 20-24, 2011, Lijiang, China

Sponsored by Institute of Chemistry, CAS

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Symposium Chair:

Prof. Mingyuan Gao

Institute of Chemistry, the Chinese Academy of Sciences

Organizing Committee:

Prof. Zhifang Chai

Prof. Mingyuan Gao

Prof. Jiacong Shen

Prof. Guojun Zhang

Venue

Guanfang Hotel Lijiang, Shangri-la Road, 674100 Lijiang, Yunnan, China

August 20, 2011

14:00-22:00 Registration

August 21, 2011

Morning

08:45-09:00 Opening Remarks

Section 1 Chairman: Prof. Yan Zhang

09:00-09:30 Colloidal nano- and microparticles towards sensing applications

Prof. Wolfgang Parak, AG Biophotonik, University of Marburg

09:30-10:00 Optical Molecular Imaging in Anti-Cancer Drug Discovery

Prof. Guojun Zhang, Cancer hospital of Shantou University medical college

10:00-10:20 Coffee break, Gather together for symposium photo

Section 2 Chairman: Prof. Hans-Joachim Galla

10:20-10:50 Imaging Biological Functions Using Split-Reporter Reconstitution Analyses

Prof. Takeaki Ozawa, department of Chemistry, Tokyo University

10:50-11:20 New Therapy Strategy Based on Gold Nanoparticles

Prof. Xingyu Jiang, National Center and Nanoscience and technology

11:20-11:50 MRI Cancer Molecular Imaging

Prof. Fabao Gao, West China Hospital, Sichuan University

12:00-13:00 Lunch

Afternoon

Section 1 Chairman: Prof. Xingyu Jiang

14:00-14:30 Novel Functional Bioimaging Techniques with Rationally designed fluorescence probes

Prof. Yasuteru Urano, Laboratory of Chemical Biology and Molecular Imaging, Graduate School of Medicine, The University of Tokyo

14:30-15:00 Conjugated Polymer-based Fluorescence Resonance energy Transfer (FRET) Technique for Biosensing and Cell Imaging

Prof. Shu Wang, Institute of Chemistry, CAS

15:00-15:20 Coffee Break

Section 2 Chairman: Prof. Yasuteru Urano

15:20-16:00 Flashtalk for Poster Presentations

16:00-18:00 Poster discussion

18:30 Welcome banquet

August 22, 2011

Morning

Section 1 Chairman: Prof. Guojun Zhang

09:00-09:30 Aerogels from Metal- and Semiconductor Nanocrystals

Prof. Alexander Eychmüller, Department of Chemistry and Food Chemistry, TU Dresden

09:30-10:00 Hepatic Ischemia/reperfusion Injury Promotes Lung Metastasis after Major Hepatectomy by Mobilization of Circulating Endothelial Progenitor Cells (EPCs) – Application of Optical Imaging System in Rat and Mouse Models with Othotopic Liver Cancer

Prof. Kwan Man, Department of Surgery, LKS Faculty of Medicine, The University of Hong Kong

10:00-10:30 Spectroscopic Probes and Labeling Analysis

Prof. Huimin Ma, Institute of Chemistry, CAS

10:30-10:50 Coffee Break

Section 2 Chairman: Prof. Wolfgang Parak

10:50-11:20 Development of MRI Contrast Agents for Diagnosis of Tumor and Brain Lesion

Prof. Hao Lei, Wuhan Institute of Physics and Mathematics

11:20-11:50 Engineering Interfacial Nanomaterials toward Capture of Cancer Cell

Prof. Shutao Wang, Institute of Chemistry, CAS

12:00-13:00 Lunch

Afternoon

14:00-17:30 Free Discussion

18:30 Dinner in the old city of Lijiang

20:00 Watch the show of Naxi Ancient Music show

August 23, 2011

Morning

Section 1 Chairman: Prof. Alexander Eychmüller

- 09:00-09:30 How to breath: Analysis of Surface Topology and Chemical Composition of Nanostructured Artificial lung Surfactant Films
Prof. Hans-Joachim Galla, Institute of Biochemistry, University of Münster
- 09:30-10:00 Non-cadmium quantum dots emitters covering visible to NIR
Prof. Renguo Xie, College of Chemistry, Jilin University
- 10:00-10:30 In vitro/in vivo Imaging of PtII-based Anticancer Drugs
Prof. Weijiang He, School of Chemistry&Chemical Engineering, Nanjing University
- 10:30-10:50 Coffee Break

Section 2 Chairman: Prof. Takeaki Ozawa

- 10:50-11:20 Water Soluble NaLnF₄ Nanoparticle for Highly Multiplexed Bioassays
Dr. Yi Hou, Institute of Chemistry, CAS
- 11:20-11:40 Receptor-mediated Delivery of Magnetic Nanoparticles across the Blood-Brain Barrier
Ruirui Qiao, Institute of Chemistry, CAS
- 11:40-11:50 Closing remarks
Prof. Mingyuan Gao, Institute of Chemistry, CAS
- 12:00-13:00 Lunch

Afternoon

- 14:00-17:30 Free Discussion
- 18:30 Conference dinner

August 24, 2011

Departure

Or Sightseeing to the Jade Dragon Snow Mountain, Impression Lijiang Show directed by Yimo Zhang, and Village & Baisha Murals tour.

Abstracts


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Colloidal Nano- and Microparticles towards Sensing Applications	


Nanomedicine nowadays is a popular key word in the media, though everyone seems to associate it with different visions, hopes, and even fears. From the point of view of a materials scientist it will be pointed out what new materials will be possible, how they will be designed, and which properties they could offer for diagnosis and treatment. It will be critically discussed that though sophisticated materials with advanced novel properties will be available in the future, they do not automatically match the requirements and demands of clinicians. The discussion is centred around one example, multifunctional polyelectrolyte capsules which might act as a "nano-submarine" for in vivo sensing and delivery, which is used to highlight promising interfaces between both disciplines^[1, 2, 3].

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Optical Molecular Imaging in Anti-Cancer Drug Discovery	

The drug discovery process has greatly benefited from a wealth of novel druggable targets following the sequencing of the human genome and the parallel development of combinatorial chemistry and high-throughput screening technologies. The large number of drug candidates generated by this combined approach requires an evolution and refinement of *in vivo* measurement methodologies and animal models. Meanwhile, drug makers are looking for translational biomarkers that could facilitate the clinical evaluation of the most promising molecules. Imaging technologies are particularly well suited to help address these various challenges. In this conference, I will focus our interest on optical molecular imaging technology and present how this technology is being integrated in various therapeutic areas and how it has started to impact the preclinical drug discovery process. Finally, the potential clinical applications of optical molecular imaging will be discussed.

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Imaging Biological Functions Using Split-Reporter Reconstitution Analyses	

A current focus of biological research is to quantify and image cellular processes in living subjects. To detect such cellular processes, genetically-encoded reporters have been extensively used. The most common reporters are firefly luciferase, renilla luciferase, green fluorescent protein (GFP) and its variants with various spectral properties. Herein, novel design of split GFP and split luciferase will be described; the principle is based on reconstitution of the split-reporter fragments (Figure 1-(1)); The basic strategy of the reconstitution is to split a reporter protein into two non-active fragments that are fused to a pair of interacting proteins. The interaction between the two proteins brings the two fragments into close proximity, allowing reconstitution of an intact reporter protein. To demonstrate the usefulness of the reconstitution technology, we have applied the reporters for developing a genetic method to identify mitochondrial proteins and their localization (Figure 1-(2)), and imaging dynamics of endogenous mRNA in single living cells (Figure 1-(3)). We have recently used split-luciferase reporters with different spectral characteristics for GPCR- β -arrestin interactions and Smads in living subjects. We have developed another design of reporter proteins; a cyclic luciferase by protein splicing to monitor protease activities in living cells and mice (Figure 1-(4)). Herein, we will focus on recent advances in the imaging technologies and discuss the advantages and disadvantages of the use of the reconstitution analysis of fluorescent and bioluminescent proteins.

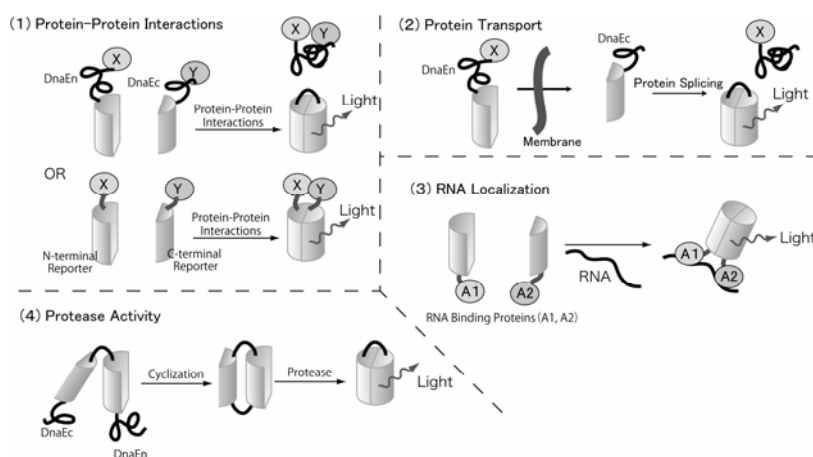


Figure 1. Protein reconstitution methods for visualizing intracellular processes in living cells.

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New Therapy Strategy Based on Gold Nanoparticles	

Gram-negative bacteria developed resistance severely to induce the dearth of antibiotics. We report an antibacterial strategy by presenting commercially available pharmaceutical precursors on nanoparticles (NPs). Our results show that these modified NPs are very effective against both type Gram-negative strains and multi-drug resistant strains. Compared with commercial antibiotics, these NPs are harder to induce bacterial resistance. We further investigate the possible antibacterial mechanism, which includes at least two factors: destabilization of cell membranes and interaction with cytoplasmic contents. These NPs with high concentrations cannot affect the proliferation of primary human cells. These nanoantibacterial agents could be applied in clinic treatments.


We are interested in the design new diagnosis system. We developed a method for the analysis of HIV infection, by indirectly detecting copper ions, using an approach based on azide-/alkyne-functionalized AuNPs and “click chemistry”. CuO NPs as a label was modified on the antibody and can be dissolved to release copper ions in acidic solutions; the copper ions can be in turn detected with high sensitivity and specificity via “click chemistry” where copper acts as a catalyst, inducing aggregation AuNPs. Since the aggregation of AuNPs can be monitored with the naked eye alone, no instruments will be necessary for the readout. We successfully detected a real blood serum of HIV-1-infected patients by this method. We expect our approach to have wide-ranging applications from basic biochemical analysis, clinical chemistry to biodefense-related assays.

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Tracking Tumor Cells in Lymphatics in Mice Xenograft Model by MR Imaging	

Purpose Lymphatic metastasis is responsible directly or indirectly for death of patients with solid tumor. However, despite the importance of lymphatic metastasis, the interaction between invading tumor cells and host lymphatic system has been insufficiently investigated. To enrich our understanding on the mechanism of tumor lymphatic metastases, we developed a model system for tracking metastatic tumor cells in lymphatic system with MR cellular imaging in live mice and test and verify our hypotheses that by observing the interaction between tumor cells and lymphatic system, we could dig into the fundamental mechanical aspects of tumor lymphatic metastasis.


Methods Human colorectal cancer LOVO cells were labeled with superparamagnetic iron oxide (SPIO)/ polyethylenimine (PEI) nanoparticles. The labeling efficiency was evaluated by Prussian blue staining. Thirty-six nude mice were divided into 6 groups, and then inoculated subcutaneously with labeled and unlabeled LOVO cells (3×10^8 cells/0.1ml) in foot pad, groin or axillary area, respectively. Serial 7.0T MR imaging of the tumors and surrounding regions was performed in following 4 weeks. After imaging, tumor tissues and regional lymph nodes were collected and subjected to immunohistologic analysis, which include hematoxylin and eosin (H&E) staining, Prussian blue (PB) staining, CD68 staining and lymphatic vessel endothelial hyaluronan receptor (LYVE-1) staining, CD31 staining, and VEGF-C staining.

Results MR T2/ T2*- weighted images showed the primary tumor growth and the draining lymphatic architecture, as well as the SPIO labeled tumor cells metastasized into regional lymph node at 8 days P.I.. MRIs also revealed information on sentinel lymph node mapping with high-resolution anatomical information. Histological finding confirmed in vivo MR imaging results and revealed lymphangiogenesis, angiogenesis, infiltration of macrophage, and expression of VEGF-C in tumor and sentinel lymph nodes as well.

Conclusion This technology provides a powerful tool for tracking SPIO-labeled cancer cells in the lymphatics by MR cellular imaging. There was a close relationship between tumor lymphatic metastasis with lymphangiogenesis.

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Novel Functional Bioimaging Techniques with Rationally Designed Fluorescence Probes	

Fluorescence imaging is one of the most powerful techniques currently available for continuous observation of dynamic intracellular processes in living cells. Suitable fluorescence probes are naturally of critical importance for these functional bioimaging. For highly efficient development of novel fluorescence probes, we have established several rational strategies for controlling the properties of visible light to NIR-excitable fluorophores based on the intramolecular photoinduced electron transfer and spirocyclization. These strategies are quite powerful and versatile, and indeed, based on these strategies, we have succeeded to develop a series of fluorescence probes for reactive oxygen species, ions, and enzymatic activities including glutathione S-transferase (GST), beta-galactosidase, aminopeptidases. All these probes are membrane permeable and can be loaded into living cells for continuous visualization of target functions.


Further, we have achieved highly specific *in vivo* cancer visualization by employing a cancer-targeting monoclonal antibody tagged with newly designed acidic pH-activatable fluorescence probes. This agent is activated after endocytotic internalization by sensing the pH change in the lysosome. As a proof of concept, we tried *in vivo* tumor imaging by injecting the probe into lung cancer model mice. The probe was highly specific for tumors with minimal background signal, and we have succeeded in visualizing tiny tumors less than 1 mm in living mice by using fluorescence endoscopy.

In this symposium, the details of representative fluorescence probes will be provided including their pros and cons as well as important notes when applying to living cells and animals to visualize target molecules of interest in real time.

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Conjugated Polymer-based Fluorescence Resonance Energy Transfer (FRET) Technique for Biosensing and Cell Imaging	

Conjugated polyelectrolytes provide a unique platform for chemical and biological sensors in view of their optical signal amplification effect. Our recent studies showed that the conjugated polyelectrolytes could be used for detecting gene modifications, such as single nucleotide polymorphisms (SNPs) and DNA methylation. Genotyping large number of SNPs will take a deep insight into understanding and clinically diagnosing the complex diseases. DNA methyltransferases catalyze the covalent addition of methyl group to adenine and cytosine residues in DNA. The resulting DNA methylation plays a key role in control of gene expression, genomic integrity maintenance and cancer origin. We use conjugated polyelectrolytes/DNA complexes combining with fluorescence resonance energy transfer (FRET) processes to assay SNP genotyping and DNA methylation, thus offering new assay strategies based on conjugated polyelectrolytes. We also showed that the complexes of CPs/enzymatic substrates can be utilized as probes for continuous and sensitive fluorescence assays for disease-related enzymes, such as nuclease, phosphatase, protease, acetylcholinesterase and kinase. The technique also provides a promising application in drug screening based on the inhibition of the cleavage reactions. Beyond biosensing, conjugated polyelectrolytes are also ideal platforms for cell imaging and disease therapy.

Reference:

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Aerogels from Metal- and Semiconductor Nanocrystals	

We report on the synthesis and characterization of non-supported nanoparticle based noble metal¹ and semiconductor aerogels².

These exhibit an average density three orders of magnitude lower than the materials in their bulk states. Their primary structural units match the size range of single nanoparticles (5–20 nm), which is an order of magnitude smaller than that of self-assembled supraspheres (cf. Fig.1). No chemical cross-linkers are involved in the self-assembly process. The formation of such noble-metal nanoparticle-based mesoporous mono-, bi- and multimetallic aerogels is an important step towards self-supported monoliths with potentially high catalytically active surfaces.

Considering that metal nanoparticles possess very specific optical properties owing to their pronounced surface plasmon resonance, aerogels from metal nanoparticles may also find future applications in nanophotonics, for example, as advanced optical sensors and ultrasensitive detectors.

Latest developments in the direction of nanoparticle based aerogels refer to combinations of semiconductor and metal nanocrystals within a common superstructure³ (cf. Fig. 2) with implications as to the emissions of the semiconductor nanoparticles and the conduction properties of the aerogels.

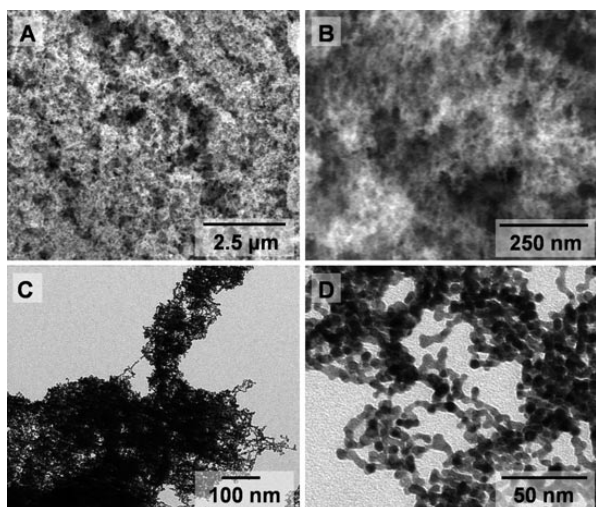


Fig.1 A, B) SEM and C,D) TEM images of aerogels from platinum nanoparticles destabilized from solution by the addition of ethanol.

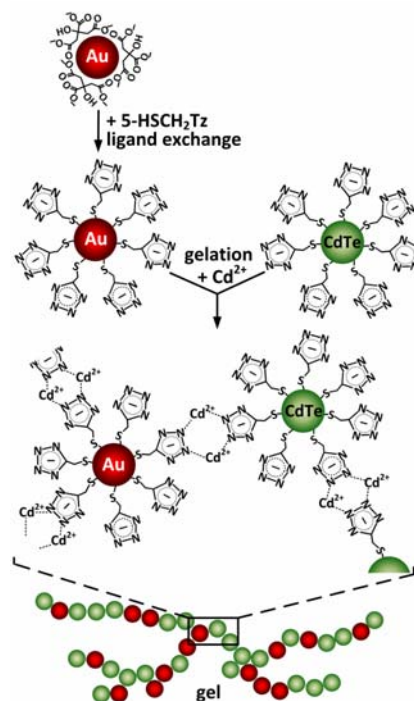



Fig.2 Illustration of the gelation process of CdTe and Au nanocrystals.

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Hepatic Ischemia/reperfusion Injury Promotes Lung Metastasis after Major Hepatectomy by Mobilization of Circulating Endothelial Progenitor Cells (EPCs) – Application of Optical Imaging System in Rat and Mouse Models with Othotopic Liver Cancer	

Objective

We aim to explore the precise mechanism of tumor metastasis under surgical stress by investigating the impact of hepatic I/R injury on mobilization of circulating endothelial progenitor cells and regulatory T cells.

Methods

Orthotopic rat liver tumor model was established in male Buffalo rats with cirrhotic liver. Major hepatectomy was performed at 3 weeks after tumor implantation in the left lobe with (I/R injury group) or without (Control group) partial hepatic ischemia/reperfusion (I/R injury - 20/20 minutes duration on right and median lobes). The tumor growth and metastases were longitudinally monitored by Xenogen in vivo imaging system (IVIS) in live animals by detection of luminance signals from tumor cells, which stably labeled with luciferase gene. Blood samples were taken at day0, 3, 7, 17, 21 and 28 after hepatectomy for detection of circulating endothelial progenitor cells (CD133+CD34+CXCR4+). Intra hepatic and circulating gene/protein markers linking to I/R injury and lung metastasis were identified. To further validate the role of EPCs with inflammatory chemokine IP10 treatment on liver tumor growth and metastasis, the EPCs with or without IP10 treatment from a rat model with green fluorescence protein (GFP) labeling were further injected into an orthotopic nude mice liver cancer model. The liver tumor growth and metastasis in nude mice were longitudinally monitored by IVIS system. The EPCs were also traced with their GFP signals.

Results

Significant high incidence of lung metastasis was present in I/R injury group (50%, 16/32) compared to the control group (10%, 2/20; $p=0.000$) at 4 weeks after major hepatectomy. The early occurrence of lung metastasis was found in I/R injury group at 2 weeks after operation detected by

IVIS. Significant higher levels of IP10/CXCR4/VEGF induced by hepatic I/R injury subsequently mobilized more bone marrow derived endothelial progenitor cells (CD133+CD34+CXCR4+) to circulation compared to the control group (day14: 12.21% vs 7.73%, $p=0.002$; day21: 12.25% vs 4.47%, $p=0.004$; day28: 9.64% vs 2.27%, $p=0.004$). The circulating protein marker linking to hemopoietic progenitor cell – myelin basic protein (MBP) was also over-expressed in I/R injury group. IP10 treated EPCs has greater potential to promote liver tumor growth and metastasis via increasing angiogenesis in the orthotopic nude mice liver tumor model.

Conclusion

Hepatic ischemia/reperfusion injury promoted lung metastasis after major hepatectomy by mobilization of circulating endothelial progenitor cells.

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Spectroscopic Probes and Labeling Analysis		


Spectroscopic probes may be described as the molecules that can react with analytes (targets), accompanying the changes in their spectroscopic (e.g., chromogenic, fluorescent, or chemiluminescent) properties, and based on such changes the targets could thus be determined [1,2]. Spectroscopic probes have been extensively investigated and used widely in many fields because of their powerful ability to improve analytical sensitivity, to offer greater temporal and spatial sampling capability, and in some cases to make it possible to visualize a molecule event by naked eye. At present, the hot topics in this area may be: a) developing in situ spectroscopic traps/sensors to achieve real-time analysis; b) synthesizing the probes with long-wavelength features to reduce background signal and damage to biological species; c) designing chiral spectroscopic probes for analysis of biological chiral compounds; d) developing site-specific labeling methods to acquire local information of biomacromolecules, etc. For near two decades, our lab has been engaged in this field, and several new spectroscopic probes and labeling methods for biologically active species have been developed by virtue of suitably chemical functional reactions [2-5]. These include: water-soluble polymeric chromogenic reagents; chemiluminescent traps for singlet oxygen; polarity- and viscosity-sensitive fluorescent probes; triazine-based spectroscopic labeling probes; PET (photoinduced electron transfer) based spectroscopic switches for tyrosinase and extreme pH. In this talk, special focus will be on our recent research results about (1) the development of rhodamine B based spectroscopic probes for Hg^{2+} and pyruvic acid, and (2) site-specific spectroscopic labeling and local structure analysis of proteins [6-8].

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Development of MRI Contrast Agents for Diagnosis of Tumor and Brain Lesions	

Magnetic Resonance imaging (MRI) is one of the most frequently-used diagnostic modalities for detection of tumors and space-occupying brain lesions. In recent years, contrast-enhanced MRI techniques have been developed for better detection of tumor and brain lesions. Paramagnetic chelates or nanoparticles are often used in such techniques as the contrast agents to achieve high sensitivity and specificity.

The first thing to consider when developing an MRI contrast agent is to have a high relaxivity, which can be achieved by optimizing structural parameters of the contrast agent including the number of inner-sphere water molecule per paramagnetic center, the residence lifetime of the inner-sphere water molecules and rotational correlation time of the whole molecule/nanoparticle. If the contrast agent is to be used for in vivo diagnosis, it should have optimal blood residence time and good biocompatibility. This is often achieved by connecting the paramagnetic chelate/nanoparticle to polymers such as polyethylene glycol (PEG) of certain molecular weight. The next thing to consider when designing contrast agents for diagnosis of tumor and brain lesions is the specificity of the interaction between the contrast agent and tumor cells and the ability of contrast agent to penetrate blood-brain-barrier. Many recent advances have been made in this area of research.


In this talk, I shall give an introduction to recent progresses in design, synthesis and application of paramagnetic MRI contrast agents for diagnosis of tumor and brain lesion.

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Engineering Interfacial Nanomaterials toward Capture of Cancer Cell	

Circulating tumor cells (CTCs) have become an emerging “biomarker” for monitoring cancer metastasis and prognosis.¹ Although there are existing technologies available for isolating/counting CTCs, the most common of which using immunomagnetic beads, they are limited by their low capture efficiencies and low specificities. By introducing a three-dimensional (3D) nanostructured substrate – specifically, a silicon-nanowire (SiNW) array coated with anti-EpCAM – we can capture CTCs with much higher efficiency and specificity.² Unlike conventional methods of isolating CTCs that depend on adjusting collision frequency and contact duration, nanoscaled local topographic interactions between CTCs and substrate lead to increased binding of CTCs, which significantly enhance capture efficiency. This study has been paving a doable way for nanostructured substrates toward cancer detection.

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How to breath: Analysis of Surface Topology and Chemical Composition of Nanostructured Artificial Lung Surfactant Films	

The lung surfactant is a complex mixture of lipids and proteins that covers the air/water interface of type II pneumocytes in the lung. These surface active substances are necessary to lower the surface tension to a value close to zero, a basic necessity for normal breathing.

The chemical analysis of lung lavage material shows that pulmonary surfactant mainly consists of 85-90% lipids, where not only saturated dipalmitoylphosphatidylcholine (DPPC) and unsaturated phosphatidylcholines are predominant, but also phosphatidylglycerols, phosphatidylethanolamines, phosphatidylinositols, sphingolipids, fatty acids, and cholesterol are important components. The hydrophobic proteins SP-B and SP-C. play a role in interfacial monolayer formation, formation of multilayer under compression, and reformation of the surface film on expansion. However the overall chemical composition does not allow conclusion on the molecular mechanisms of the control of the surface tension. Spatially resolved analytical techniques have thus to be applied to understand the domain formation within the surfactant monolayer based on physical as well as chemical analysis.

We have demonstrated by videoenhanced fluorescence spectroscopy, scanning force microscopy, electron microscopy, and SNOM that 3-dimensional protrusions are formed in a lipid/SP-C film under compression. This chemical phase separation allows adapting the surface tension to the change in surface area under compression and expansion on a very low level. Laterally resolved


TOF-SIMS allowed analysing the chemical composition of this surfactant film after transfer onto a solid support. Quantification of different fragments originating from the lipids or the peptide showed that the SP-C is enriched within the fluid domains forming the reservoir despite a possible electrostatic interaction with the negatively charged phosphatidylglycerols. Ca^{2+} -ions are considered to act as a switch to drive this phase separation.

This monolayer is the first target for inhaled nanoparticles. Here we will report, that nanoparticles insert into the fluid phase of the surfactant monolayer may be displacing surfactant proteins. High resolution AFM topology imaging shows that the nanoparticles cluster in the protrusions. Phase imaging, force modulation microscopy and force mapping allows to determine the mechanical elastic properties of the nanoparticles inserted in the surfactant material. With these techniques we are able to show that the nanoparticles are covered by a soft shell of lipids and possibly proteins.

A model will be presented that explains the inhalation/exhalation process during breathing by the reversible formation of nanoscaled 3-dimensional protrusions guaranteeing the constancy of the surface tension by a controlled squeeze out of the material.

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Non-cadmium Quantum Dots Emitters Covering Visible to NIR	

High quality doped and undoped InP nanocrystals were synthesized at relatively low temperature by introduction of the amine into the reaction system. Experimental results indicated that the size of the as-prepared InP nanocrystals was tunable in the range of 1 to 8 nm for the first time. Accordingly, the first exciton absorption peak of the InP nanocrystals shifted from 390 to 720 nm and their photoluminescence (PL) covered the region from visible to NIR. To further improve the emission properties of InP nanocrystals, Cu-doped InP nanocrystals (Cu:InP d-dots) emitters were successfully synthesized by epitaxial growth of ZnSe diffusion barrier for the dopants. By varying the size of the InP host nanocrystals, the emission of the Cu dopant in the Cu:InP/ZnSe core/shell nanocrystals was tunable in the region of red and near infrared (NIR) window (630~1100 nm). After being coated by ZnS shells on surface of the doped and undoped InP nanocrystals, the resulting core/shell nanocrystals showed improved stability and PL quantum yield (~40%) compared to the pure InP nanocrystals. At an extent work, multinary nanocrystals such as CuInS₂ and ZnCuInS₃ with a broad emission windows covering visible to NIR were successfully obtained. Importantly, these particles show high quatumn yield (over 60%), and do not contain heavy metal elements in it. Overall, the ideal emitters based on the non cadimium nanocrystals have a broad emission window covering visible to NIR window, which are ideal bio-labeling materials due to their low toxicity.

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In vitro/in vivo Imaging of Pt ^{II} -based Anticancer Drugs	

Cisplatin is one of the most successful clinical anticancer drugs, and several analogues have also been applied in clinical therapy. In addition, developing novel Pt^{II}-based anticancer complexes is attracting much more attention to overcome the high toxicity, intrinsic/acquired resistance and limited anticancer spectrum of clinical Pt^{II}-based drugs. The molecular fluorescence imaging as the noninvasive technique should be helpful to understand the mechanism of action and side effects of both the current Pt^{II}-based clinical drugs and novel Pt^{II} complexes. This methods displays the advantage over other X-ray based intracellular Pt^{II}-detection methods in visualizing the cellular Pt^{II} uptake, distribution and transportation in real time or in live cells, and tethering fluorophore to platinum complexes to clarify the intracellular distribution of Pt^{II} complexes is of great significance for exploring new anticancer Pt^{II}-based complexes. To clarify the anticancer behavior of the monofunctional Pt^{II} complexes reported by us, a new fluorescent Pt^{II} complex, [PtLCl]Cl, constructed by bridging a 7-nitro-2,1,3-benzoxadiazole (NBD) fluorophore with the chelated Pt^{II} centers of one positive charge and one leaving group. With the fluorescence imaging, the biological fluorescent distribution of monofunctional Pt^{II} complex was visualized in breast carcinoma MCF-7 cells and zebrafish larva.

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Water Soluble NaLnF₄ Nanoparticle for Highly Multiplexed Bioassays	

The lanthanide nanoparticles (Ln NPs) antibody complexes are designed for quantitative cellular immunoassays of biomarkers and transcripts of low copy number. The analysis of low copy number biomarkers on a cell-by-cell basis is of fundamental interest in cell biology and provides an important new diagnostic for cell based diseases such as cancer. Bioassays with NP-antibody conjugates take advantage of the multiplexing capabilities of mass cytometry, a new technique based upon the union of atomic mass spectrometry and cytometry. Current methods are able to detect biomarker expression at the level of ~2,000 per cell using antibody tags carrying ~200 ions of a lanthanide isotope.¹ To achieve the high sensitivity needed to detect biomarkers at the level of <100 copies per cell, the nanoparticles must contain at least 5,000 atoms of a single metal isotope. A series of NaLnF₄ (Ln: La, Pr, Nd, Sm, Eu, Gd, Tb and Ho) nanocrystals with a narrow size distribution have been prepared by controlling the amount of oleic acid (OA) in the high temperature synthesis, and the particle diameters are larger than 12 nm. Therefore, the NaLnF₄ nanoparticle is comprised at least 10,000 lanthanide atoms, which will yield a 1-2 order of magnitude increase in assay signal. For bioassay applications, the hydrophobic nanoparticles (NPs) need to be modified with ligands that will make the NPs colloiddally stable not only in water, but in buffer as well as in serum. In addition, the surface ligands have to have functional groups to attach antibodies. We prepared the PEG polymer ligands with orthogonal end groups. We attempted to put phosphate on one end and functionality (biotin, maleimide) on the other end to enable reaction with the antibody. The PEG-phosphate coated NPs could easily be dispersed in Di-water and buffer to form transparent stable solutions. Further more, sodium 2-mercapto-5-benzimidazolesulfonate can be attached effectively to the functional NPs by reaction of the –SH group of the dye with the 2-methylmaleimido group on the end of the PEG, indicating the suitability of lanthanide nanoparticle for antibody labeling.

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Receptor-mediated Delivery of Magnetic Nanoparticles across the Blood-Brain Barrier	


The blood-brain barrier (BBB) is a physical and physiologic barrier that regulates the passage of molecules from the systemic circulation to the brain parenchyma.¹ It consists of brain microvascular endothelial cells (BMVECs) connected by tight junctions and supporting pericytes and astrocytes endfeet. Actually, only unionized, lipophilic, and low molecular weight molecules can diffuse freely through the endothelial membrane and may passively cross the BBB. Polar molecules and small ions are totally excluded.² While the BBB constitutes a natural defense mechanism that safeguards the brain against the invasion of various circulating toxins and infected cells, it also offers one of the most exclusive biological barriers limiting the diagnostic agents and therapeutics.^{3,4}

A brain delivery probe with the ability to cross blood-brain barrier (BBB) based on the magnetic nanoparticles serving as the MRI contrast agent and a nanocarrier was constructed. In this probe, poly(ethylene glycol) coated Fe₃O₄ nanoparticles^{5,6} were severed as nanocarrier and MRI contrast agent, and the Fe₃O₄ nanoparticles were conjugated with lactoferrin as a receptor-mediated transcytosis vector. The BBB transmigration efficacy of this probe was evaluated on both in vivo and in vitro models. For in vitro experiment, an in vitro BBB model was constructed and the probes were incubated with the BBB model. For in vivo experiment, a series of in vivo experiments were performed by using SD rat as animal model to detect the BBB passing through effect of the probes by MRI techniques. The results of both in vitro and in vivo experiments revealed that the Fe₃O₄-Lf probe showed the ability of passing though the BBB and the mechanism of the BBB passing effect was supposed to be the receptor-mediated pass way.

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Potential Value of Snail in Early Detection of Colorectal Cancer Metastasis	

Background: Epithelial-mesenchymal transition (EMT) is a vital biomarker of biological behavior in malignant tumors. A lot of studies revealed that EMT was the key step in tumor invasion and metastasis. In Colorectal Cancer, EMT is involved in the procession of local invasion and distant metastasis. Multiple signal pathways and sophisticated molecular mechanisms are involved in triggering the EMT, and transcription factor Snail plays a key role in these complicated networks, the bioactivity change of Snail could be a potential marker of tumor metastasis.

Methods: The change of cellular morphology was observed after the Colorectal Cancer cell line HCT-116 was treated with TNF α for different time. Luciferase reporter assay was applied to detect the transcriptional activity of Snail. Western blot was used to detect the expression of Snail protein and RNA interference technology was used to confirm the potential effect of Snail in the procession of EMT.

Results: EMT model was successfully constructed, and the EMT-like morphology change was found obviously after HCT-116 cells were treated with TNF α for 8-12h, the protein expression and transcription activity of Snail enhanced significantly in early stage and peaked at 8-12h. After interfering Snail by siRNA, TNF α -induced EMT-like phenotype in cellular morphology was abolished.

Conclusions: Snail plays a key role in the procession of EMT, the change of Snail activity could be take as a potential marker for prediction and diagnosis in early stage of Colorectal Cancer Metastasis.

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Modulation on the Cellular Uptake of Quantum Dots	

Quantum dots (QDs) are semiconductor nanocrystals with unique optical properties such as high quantum yield, high molar extinction coefficients, narrow emission spectra, size-depending emission and high photostability. Quantum dots have emerged as an attractive alternative to organic fluorophores for fluorescent labeling and optical imaging. However, quantum dots are often cell-impermeable and require transporters to facilitate crossing over the cell membranes. Modulation on the cellular uptake of quantum dots is therefore of importance for fluorescence imaging of living cells.¹ Except for physical methods such as microinjection, electroporation and lipofectamine-mediated transfection, surface coating of quantum dots with cationic peptides or polymers has also been demonstrated to effective method to facilitate the cellular uptake of quantum dots. Our previous work has demonstrated that short peptides with a certain number of Arginine (Arg) or Lysine (Lys) residues could mediate the cellular uptake of quantum dots when they were linked to the quantum dots' surface.² It is also possible to modulate the cellular uptake of quantum dots conveniently through the surface modification using short peptides with structurally modified amino acid residues. Here we present a photo-modulation method on the cellular uptake of quantum dots by using caged lysine³ in the short peptides modified on the quantum dots' surface.

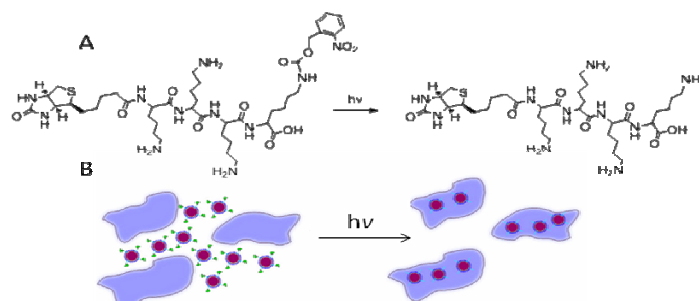



Figure 1. (A) Structure of photo-labile caged Lysine; (B) Illustration on cellular uptake of quantum dots modified by short peptides containing caged Lysine before and after light irradiation.

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Aptamers: An Emerging Class of Molecules for Diagnostics and Therapeutics	

Aptamers, evolved from combinatorial nucleic acid libraries by SELEX (systematic evolution of ligands by exponential enrichment), have attracted intense research efforts in recent years because of their potential biomedical and analytical applications^[1-2]. The first aptamer drug, Macugen, was approved by FDA in 2004, several other aptamer drugs (such as ARC1779, AS1411 and RB006) are on clinic trials. Many aptamer-based probe/sensors have been developed for the detection of various targets because of their high affinity, specificity and great flexibility in design [2,3].

Recently using cell-based aptamer selection strategy, groups of aptamers specific to different tumor cell lines have been generated^[4-6]. By labeling with fluorescence dyes or immobilizing on nanoparticles, these aptamers have been applied for tumor cell collection, detection, profiling and even tumor imaging^[5-8]. By covalently linking antitumor drugs to aptamers, targeted drug delivery to tumor cells has been realized^[9]. As affinity ligands, aptamers have been used to elucidate membrane protein specific tumor cells^[10].

Although most of the diagnostically and therapeutically relevant aptamers have so far only been tested in cultured cells and animal models, the characteristics of aptamers have make them promising tools for diagnosis and therapy. The aptamers selection and their use in diagnostic and therapeutic applications is still an expanding area. Several companies (such as Achemix and SomaLogic in USA and Noxxon AG in Germany) have been set up to capitalize the potential of aptamers as therapeutic or diagnostic agents. Presumably, in the near future further aptamers may be available for commercial use in diagnosis and therapy.

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Aggregation-Induced Emission: Promising Fluorescent Probes for Bioimaging	


In bioassays, there are only trace amounts of biomolecules. For common organic dyes, emissions from dilute solutions are often weak, leading to low sensitivity. The sensitivity cannot be enhanced by using high fluorophore concentration because of concentration-quenching effect¹. Because of the intrinsic hydrophobicity, the small fluorophore molecules may accumulate on the surfaces of the biomacromolecules and cluster in the hydrophobic pockets. Even in dilute solutions, concentration-quenching can still be involved. In addition, the small numbers of the dye molecules in dilute solutions can be quickly photobleached when a harsh laser beam is used as the excitation light source. The application of quantum dots (QDs) in bioassays is an alternative strategy to surmount the photobleaching problem, but QDs have its own problems².

Tang and colleagues discovered a family of new luminogens, which were non-luminescent in the solution state but emissive in the aggregated state, and this novel phenomenon was coined by the term of “aggregation-induced emission” (AIE)³. The novel AIE effect is exactly opposite to the notorious concentration-quenching effect. This property allows us to use high-concentration dye solutions or even use nanoparticles of dyes for bioassays and enables the development of “turn on” or “light up” sensors. The nanoaggregate-based AIE sensors are in some sense the organic counterparts of inorganic QD-based sensors. Thus may find versatile applications in bioassays. Herein, we present some typical AIE-based fluorescent molecules and their primary applications in bioimaging^{4,5}.

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New Functional Studies of Conjugated Polymers: Anticancer Activity and Apoptosis Imaging	

Conjugated polymers (CPs) have delocalized electronic structures which exhibit unique electronic and optical properties. Recently, extensive research has focused on multifunctional anticancer medicines for simultaneous cancer imaging, diagnosis and therapy, providing a new strategy in cancer treatment.

Recent advances in biological applications of conjugated polymers have focused on highly sensitive diagnostics. The therapeutics of conjugated polymers, however, remains a challenging task. Here we explore for the first time that cationic polythiophene (PMNT) is used as a multifunctional agent for simultaneous cancer therapeutic and apoptosis imaging applications. The anticancer mechanism study showed that the PMNT can uptake inside renal cell carcinoma (A498) cancer cells in a diffusion manner and induce their apoptosis. The increased activation of caspase-3 has been shown to be time- and dose-dependent on PMNT, which indicates a signalingtransduction pathway of PMNT induced-apoptosis in A498 cells. Beyond conventional endpoint analysis of apoptosis using multiplex dyes, the PMNT can image the cells and clearly distinguish the living and apoptotic cancer cells. Strikingly, the PMNT could quickly induce cellapoptosis within several minutes under irradiation. The PMNT integrates photosensitivity, anticancer activity and apoptosis imaging, which opens the door for new functional studies of conjugated polymers in disease therapeutics.


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Two-photon Ratiometric Fluorescent Sensor for Zinc Ions in Living Cells	

Recently, two-photon microscopy (TPM) is regarded as a promising technique of fluorescence microscopy for bio-imaging studies due to its NIR excitation, less excitation and emission spectra overlap, lower sample autofluorescence and self-absorption, less phototoxicity, and increased penetration depth. In this paper, we described a two-photon ratiometric fluorescent sensor for Zn^{2+} , AD2, based on β -acetonaphthone with DPA as receptor. Under physiological condition (10 mM HEPES, 0.1 M NaCl, pH = 7.4), AD2 can selectively detect Zn^{2+} with nM sensitivity under physiological conditions. When coordinated to Zn^{2+} to the carbonyl oxygen, AD2 exhibits the red-shifts of excitation/emission wavelengths and a significant increase of the TPA cross section. This observation gives ample credence to the argument that the direct interaction between acceptor site of fluorophore and Zn^{2+} in solution leads to the charge-transfer-based fluorescence modulation. Further confocal experiments demonstrate that cell-permeable AD2 can image Zn^{2+} in living cells through a ratiometric approach by utilizing two-photon excitation.

Key words: fluorescent sensor, zinc ion, ratiometric measurement, two-photon

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Silica Capsulated Nanoparticles for Bio-imaging and Bio-separation	

Silica encapsulated nanoparticles, such as dye doped silica or silica coated magnetic nanoparticles were a kind of new generation materials for bio-imaging and bio-separation. Here, negative or positive charged dye molecules were introduced into stöber system to make dye doped silica nanoparticles. Optical performance of the dye in the silica particles was improved and multi-color particles could be built by incorporated several dyes into the particles. Magnetic silica composite nanoparticles were prepared by introduce magnetic nanoparticle aggregates into the stöber system. The size and the magnetization of the composite nanoparticles can be adjusted by changing the size of the magnetite aggregates and/or the thickness of silica coating layer.

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Surface Biofunctionalized Multicore/Shell CdTe@SiO₂ Composite Particles for Immunofluorescence Assay	

Molecular probes based on fluorescent quantum dots (QDs) are attracting increasing attention owing to their remarkable optical properties governed by the quantum confinement effect.^{1,2} However, due to the dynamic nature of the QD surface capped by organic ligands, the strong surface defect-dependent fluorescence, as well as the release of toxic metal ions upon photooxidation, the use of “bare” QDs still faces inherent disadvantages in bioapplications. Encapsulation of QDs by silica has been considered as an effective approach for overcoming these drawbacks.³⁻⁴ Herein, strongly fluorescent multicore/shell structured CdTe@SiO₂ composite particles of ~ 50 nm were synthesized via the reverse microemulsion method by using CdTe quantum dots costabilized by thioglycolic acid and thioglycerol. Towards immunofluorescence assay, both poly(ethylene glycol) (PEG) and carboxyl residues were simultaneously grafted on the surface of the silanol-terminated CdTe@SiO₂ composite particles upon further reactions with silane reagents bearing PEG segment and carboxyl group, respectively, in order to suppress the nonspecific interactions of the silica particles with proteins and meanwhile introduce reactive moieties to the fluorescent particles. Via the surface carboxyl residue, various antibodies were covalently conjugated to the fluorescent particles and the resultant fluorescent probes were used in detecting cancer cells through both direct fluorescent antibody and indirect fluorescent antibody assays, respectively.

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Quantum Dot-Antisense Oligonucleotide Conjugates for Multifunctional Gene Transfection, mRNA Regulation, and Tracking of Biological Processes	

Due to the excellent optical properties, quantum dot (QDs) are greatly desirable for multiplex immunoassays, cellular fluorescence imaging, and in vivo fluorescence imaging.¹ They have also been found to be potentially useful in visually tracking biomolecules inside living cells to elucidate some biological processes at the cellular level. For example, QDs have even been used in recent gene studies.² In addition to gene transfection, QD-based gene vectors can successfully be developed for monitoring the cellular uptake of foreign genes. The cellular uptake is undoubtedly one of the most important steps for gene transfection. However, the following endosomal escape, cytoplasmic mobility, and nuclear entry of foreign genes are also very important for in vitro gene transfection with respect to nonviral gene transfection systems. Therefore, to visually track and identify the intracellular localization of a foreign gene would be greatly helpful for revealing the intracellular target sites of the transfected genes, elucidating the biological actions and processes exerted or caused by the transfected genes, and thereby probing the mechanisms of the transfected genes at the cellular level

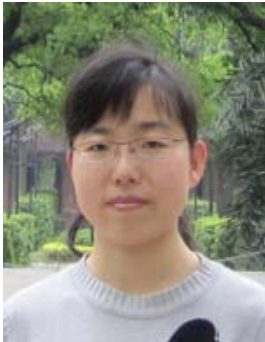
Through covalently conjugating anti-survivin antisense oligonucleotide (ASON) to thioglycolic acid-capped CdTe QDs via amide bond, we develop a fluorescent system for gene transfection and visually tracking the intracellular behavior of transfected genes. Systematic investigations reveal that negatively charged oligonucleotides covalently conjugated to CdTe QDs can effectively induce the cellular uptake of the still negative CdTe-oligonucleotide conjugates through the macropinocytosis pathway. Further experimental results demonstrate that CdTe-ASON can specifically induce the down-regulation of the survivin mRNA and ultimately induce the apoptosis of HeLa cells. Benefiting from the fluorescence of CdTe QDs, the visualization of the specific localization of the CdTe-ASON is consequently achieved. Systematic results suggest that the perinuclear region is the location where the antisense regulation process occurs. In addition, our researches also reveal that the surface modification of oligonucleotide can effectively suppress the

cytotoxicity of the CdTe QDs, which may expand the applications of QDs in cell biology investigations after further improvement.

In summary, our investigations demonstrate that CdTe QDs can not only be used as gene vectors but also offer the possibility of visually tracking the intracellular localization of a given oligonucleotide, thereby providing the possibilities to correlate the gene functions with their specific intracellular localization.

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Gelification- an Effect Measure for Achieving Differently Sized Biocompatible Fe₃O₄ Nanocrystals through a Single Preparation Recipe	

Due to the quantum confinement effect and the nanometer size effect, inorganic nanocrystals exhibit unique particle size-dependent optical, electronic, magnetic properties in comparison with their corresponding bulk materials.¹ Although great successes have been achieved over the past decades in the size control of various types of inorganic nanocrystals based on different synthetic principles,² developing new synthetic methods and further understanding the mechanisms for delicate control of the particle size remain hot subjects for wet-chemical synthesis of inorganic nanocrystals, especially for magnetic iron oxide nanocrystals due to their bright future in nanomedicine. Following on from our previous investigations on water-soluble and biocompatible Fe₃O₄ nanocrystals prepared by pyrolyzing Fe(acac)₃ in various types of high boiling point solvents,⁴⁻⁷ herein we report our recent investigations on the gelification-associated size regulation effects for biocompatible Fe₃O₄ nanocrystals produced by pyrolyzing Fe(acac)₃ in the presence of α,ω -Dicarboxyl-terminated polyethylene glycol (HOOC-PEG-COOH) and oleylamine in diphenyl oxide. It was observed from this reaction system that different sized PEG-coated Fe₃O₄ nanocrystal can simply be obtained through a single recipe of reactants by controlling the gelification degree of the reaction system. The gel formation mechanism was investigated and the gelification-associated size regulation effect was discussed in combination with theoretical simulation results.

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Lateral Flow Immunochromatographic Assay for Sensitive Pesticide Detection by Using Fe₃O₄ Nanoparticle Aggregates as Color Reagents	

Lateral flow immunoassay (LFIA) has been widely applied in food safety monitoring and clinical diagnosis¹⁻³ due to its inherent advantages, such as fast, convenient, and on-site detecting. A highly sensitive and specific magnetic LFIA method is established via the use of Fe₃O₄ particle aggregates instead of individual nanoparticles commonly adopted in conventional LFIA in our group. Magnetic Fe₃O₄ particle aggregates were prepared by crosslinking Fe₃O₄ nanoparticles bearing surface carbonyl groups with poly-L-lysine. Upon further coupling with anti-paraoxon methyl polyclonal antibody, the resultant particle aggregate-based probes were used in lateral flow immunochromatographic assay (LFIA) of pesticide residue of paraoxon methyl. The results were compared with that achieved by using the mother Fe₃O₄ nanoparticles. Owing to the significant amplification effect, the Fe₃O₄ particle aggregates offer greatly improved visual detection limit in detecting paraoxon methyl, in addition to excellent detection specificity. More quantitative analysis through relative optical density demonstrates that the detection limit is decreased by more than 40 folds, reaching 1.7 ng/mL. The current investigations thereby pave a novel strategy for developing ultra-sensitive LFIA through the amplification effect endowed by the controlled particle aggregation.

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Investigation of the Binding Stability between PEG and Fe₃O₄ Magnetic Nanoparticles	

Dicarboxyl polyethylene glycol (PEG) modified Fe₃O₄ nanoparticles synthesized via one-pot reaction have been demonstrated a bright future in the field of nanomedicine due to their simple preparation and good biocompatibility in physiological systems¹⁻⁴. However, it is still unclear on the coordination situation of dicarboxyl PEG since both carboxyl and ether groups are involved in the dicarboxyl PEG molecule. Furthermore, the binding force between iron and carboxyl is weaker than that between iron and other anchor groups used for modification of Fe₃O₄ nanoparticles such as dopamine and dimercaptosuccinic acid. More investigations are needed to enrich the knowledge of the binding stability between dicarboxyl PEG and Fe₃O₄ nanoparticles. In the present study, Fe₃O₄ nanoparticles without any surface modification were synthesized by coprecipitation method, and three different kinds of functional groups (carboxyl, hydroxyl, methoxyl) terminated PEG 2000 were decorated to the naked nanoparticles via adsorption. The obtained three kinds of Fe₃O₄@PEG nanoparticles were dialyzed in deoxygenated water for more than one month. FTIR were applied to monitor the surface modification in the dialysis process, and the surface coordination amount of PEG was characterized by TGA.

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Preparation of Magnetic Polystyrene Beads with Different Coatings and Their Potential Applications in Biological Field	

Magnetic beads have become a powerful tool used in biological and biomedical fields, such as the purification and separation of proteins, nucleic acids, cells and bacteria. Due to its narrow bead size distribution, satisfying colloidal stability and strong magnetic response, the monodispersed beads have the advantage of providing for a very uniform reproducibility of magnetic separation¹⁻⁴. In the present study, Monodispersed Magnetic polystyrene beads incorporated with Fe₃O₄ nanoparticles are prepared using well-prepared polystyrene beads. The resultant magnetic beads present well-defined composite structures, excellent colloidal stabilities, and strong magnetic response. In order to meet various biological applications, the coating technique of magnetic polystyrene beads coating with silica and proteins is investigated. The potential applications of the monodispersed magnetic beads in DNA extraction and HIV antibody detection are demonstrated. After being coated with silica, the monodispersed magnetic beads can extract up to 80.5% DNA from 2.6 ug crude DNA. When being coated with HIV antigen, the preliminary experiment showed the method using magnetic beads to detect HIV antibody in HIV carrier's blood was comparable to the traditional ELISA test.

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Synthesis of PVP-Coated Magnetite Nanocrystals in a Continuous Flow Tubular Microreactor	

Scaled-up production of monodisperse colloidal nanocrystals has become an important research subject in recent years. In this regard, continuous flow reactors are generally favored over batch reactors. This work describes the synthesis of PVP-coated Magnetite Nanocrystals in a continuous flow tubular microreactor using ferric triacetylacetonate ($\text{Fe}(\text{acac})_3$) as precursor in N-Vinyl-2-pyrrolidone (NVP). Our previous investigation showed that PVP coating on the Fe_3O_4 nanocrystals can be prepared by in-situ polymerization of NVP in the one-pot reaction, and the obtained product demonstrated superdispersity both in organic solvents and aqueous solutions. Based on this theoretical foundation, we prepared Fe_3O_4 nanocrystals which presented superdispersibility in 0.01 M phosphate-buffered saline buffer. The resultant product can also have a narrow size distribution by adjusting its residence time in tubular microreactor. Importantly, the preparation of PVP-Coated Magnetite Nanocrystals can be operated continuously under the optimum conditions.

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