CdSe quantum dot synthesis, characterisation and toxicity

Vejleder: Peter Fojan and Leonid Gurevich

Nanocrystals of II-VI semiconductors have generated tremendous interest over the past decade and are often considered for different applications, ranging from light-emitting diodes to biological sensors. This interest arises from the possibility of tuning the optical properties of semiconductor nanocrystals by simply varying their size, owing to the effect of quantum confinement. Among various kinds of materials, colloidal CdSe quantum dots are undoubtedly the most widely studied because of their tunable emission in the visible range and the advances in their preparation.

In this project you will synthesize various CdSe nanoparticles with different sizes and CdSe ratios. You will study their luminescence, absorption characteristics and shapes with Absorption spectroscopy, Fluorescence spectroscopy, fluorescence microscopy and SEM. CdSe nanoparticles can not only form differently sized Quantum Dots, but also very interesting elongated structures (nanowires). The properties of these nanowires is not yet investigated. One important factor, where very little is known today about, is their influence on cells. You will investigate the toxicity of such nanoparticles on bacterial cell cultures.

Quantum dot of different sizes, inferring different colour.

CdSe structures investigated by SEM.
1. Engineering a biosensor for detection of quantum dots

Supervisor: Evamaria Petersen and Peter Fojan

The toxic heavy metals cadmium and lead are naturally occurring elements in the environment. Due to their unique chemical and physical properties like resistance to corrosion, excellent electrical conductivity and low melting point, these metals have several widespread applications in a number of industries.

Cadmium for instance has been used in Ni-Cd batteries, paints, metal plating, stabilizers, alloys, and electronic compounds such as cadmium telluride (CdTe). Excellent properties such as bright coloring (quantum dots), resistance to detergents and other corrosive chemicals, water-insolubility, and heat stability makes cadmium pigments essential in the manufacture of plastics, ceramics, colors and enamels.

However, harmful effects are associated with these metal ions. These metals are prevalent in the environment and are toxic even at low levels. Chronic exposure to lead in humans can lead to anemia, neurotoxicity, and renal damage, and can be fatal in some cases. The most common effects of cadmium exposure are kidney disease, lung damage, fragile bones, and abdominal pain. One feature of the toxicity associated with these metals is their tendency to accumulate in the body over an extended period of time that eventually can lead to long-term effects in humans. Extensive industrial demand and other environmental sources make these pollutants a serious health concern. Therefore, it is important to monitor the presence of these metal ions in the environment and prevent the excessive exposure of various life forms to these metals.

Monitoring of most metal ions present in environmental samples is carried out using conventional analytical techniques, such as inductively coupled plasma-atomic emission/mass spectroscopy (ICP/AES, ICP/MS), or electrochemically by anodic stripping voltammetry (ASV).
However, expensive instrumentation and extensive sample pretreatment are needed in some cases. In addition, the inability to provide information on the bioavailability of the metal ions and the toxicity associated with them makes these methods less attractive for monitoring environmental samples.

Our sensor in this project is based on genetically engineered bacteria which will give a fluorescent signal upon exposure to Cadmium/quantum dots. In order to achieve that we will use an E. coli promoter (YodA promoter) that can be induced by the presence of Cadmium. As a reporter gene we will use enhanced GFP (green fluorescence protein) which will be under control of the Cadmium promoter. So, as soon as the E.coli is exposed to Cd ions it will start synthesizing GFP and a fluorescent signal can be observed.

Within the framework of the project you will synthesis water soluble quantum dots and engineer the regulation of gene expression which means we investigate/engineer the on/off of the promoter. We have already cloned the construct. The problem we are having right now is the leakyness of the promoter which means that even no Cd is present we do get a signal. The goal of this project will be to engineer the promoter by means of random mutagenesis and deletion mutagenesis to get a tight promoter.

If successful we will have generated a novel sensitive, easy to use and self-replicating biosensor which can detect quantum dots.

Mode of action of the biosensor upon induction with a target compound (Cd)
Result: Fluorescent *E. coli* upon induction with Cd
Mikrofluidsystemer baseret på electro-wetting

Vejleder: Kjeld Pedersen


Projektet kan indeholde følgende:

1. Fremstilling af hydrofobe belægninger
2. Beskrivelse af principperne bag electro-wetting (overfladespændinger mm)
3. Udlægning af elektroder med PVD og lithografi
4. Styring af spændinger på elektroder
Nanoporøse Al₂O₃ templates til dyrkning al nanostrukturer

Forslagsstiller: Kjeld Pedersen


Arbejdet med projektet kan indeholde 3 elementer.

Fremstilling af nanoporer
- Optimering af procesbetingelser
- Kontrolleret variation af porøstørrelser
- Frigørelse af porøst lag

Karacterisering
- AFM
- SEM
- Optisk (mikroskopi og spektroskopi)

Nanoporer som templates
- Dyrkning af nanowires (fx Au eller Ag)
- Flourescens på molekyler i porer

På den teoretiske eller forståelsesmæssige side kan man fx arbejde med selvorganiserende strukturer, optiske egenskaber af periodiske strukturer, flourescens fra molekyler i små kaviteter mm.

Litteratur:

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Photosensitized generation of singlet oxygen and the singlet oxygen induced depolymerization of biomolecules.

Vejleder: Esben Skovsen

In nature, molecular oxygen is everywhere. It plays an important role both in the maintenance of life and in the mechanisms in which life is destroyed and materials destroyed. The lowest excited state of molecular oxygen, commonly known as singlet oxygen, is an important intermediate in many chemical and biological processes. In particular, it has a unique reactivity that can result, for example, in polymer degradation or the death of biological cells.

Singlet oxygen can be produced in many ways, but the most common way is through a photosensitized reaction, where a so-called photo-sensitizer molecule is excited upon irradiation and subsequently quenched by molecular oxygen to create singlet oxygen. This is for example used in photodynamic therapy of cancer where singlet oxygen, created by irradiation of a photosensitizer, is used to trigger apoptosis in malignant cancer cells.

In this project we will use a porphyrin (TMPyP) as a photosensitizer to create singlet oxygen. We will measure the lifetime of singlet oxygen in a few relevant solvents by monitoring the very weak phosphorescence of singlet oxygen as a function of time. After getting more acquainted with singlet oxygen, we will proceed to study the effect of singlet oxygen on the depolymerization of biopolymers like e.g. starch.

This kind of light induced depolymerization can, in principle, be used to do lithography with biomolecules on a surface with a resolution better than ~500 nm. If time allows we will try to use photoinduced depolymerization to write simple patterns on surfaces covered with a thin biopolymer coating, but for practical reasons we will be limited to resolutions on the order of ~20 µm.
Measuring the rotational diffusion of biomolecules using time-resolved fluorescence anisotropy.

Vejleder: Esben Skovsen

In this project we will use time-resolved fluorescence anisotropy measurements to study the rotational diffusion of biomolecules. How fast large biomolecules rotate when in solution will depend on their size and shape. Therefore, one can get information about the biomolecules by studying how fast they rotate.

One way to measure how the molecules rotate is to use time resolved fluorescence anisotropy, where a short linearly polarized light pulse is used to excite a solution of fluorescent molecules. Because the excitation light is polarized, it will preferentially excite those fluorophores that have their dipole transition moment aligned with the excitation field. When the excited molecules decay by emitting fluorescence, the emitted light will be polarized at a fixed angle with regard to the molecular axes. Right after excitation, the photoselected fluorophores will still have their transition moment aligned parallel to the polarization of the excitation field. However, after some time the molecules will have rotated due to diffusion and, as a consequence, the polarization of the emitted fluorescence will have changed too. By simultaneously measuring the amount of fluorescence emitted with a polarization parallel and perpendicular to the excitation light one can calculate the so-called anisotropy of the fluorescence. If this is done as a function of time after excitation, one can then get information about the time it takes the molecules to be completely randomized in orientation due to diffusion.

In this project we will use time-resolved fluorescence anisotropy measurements to study the rotational diffusion of selected biomolecules under different conditions (temperature, solvent etc.).
Nitrogen laser

Dye laser

SH G

detecto

Crossed Polarizers

detecto

Nitrogen laser
5th semester Nanobio: Sensing the effect of Reactive Oxygen Species on biological nanostructures

Supervisor: Teresa Neves Petersen, Ane Kold

State of-the-art

For over 20 years oxidative damage has been implicated in several disease states, including cardiovascular disease, cancer, stroke, neurodegenerative diseases such as Alzheimer's disease, cataracts, age-related macular degeneration, chronic inflammatory diseases, and the actual aging process itself (free radical damage). Free radicals attack many cellular targets including membranes, proteins and nucleic acids, carbohydrates, lipids and cause structural damage to the DNA. These structural changes manifest as point mutations and chromosomal alterations in cancer-related genes. Partly because cancer incidence is strongly correlated with age, many scientists also attribute normal aging to the accumulation of unrepaired mutagenic DNA lesions, and oxidative stress is implicated in what has been called the "free radical theory of aging." The initial reaction triggered by radical species generates a second radical, which in turn can react with other macromolecules to continue the chain reaction. Among the more susceptible targets are polyunsaturated fatty acids. Similarly, modification of individual nucleotide bases, single-strand breaks and cross-linking are the typical effects of reactive oxygen species on nucleic acids. Species formed due to oxygen reduction, O²⁻, OH radicals and H₂O₂, together with singlet oxygen ¹O₂ and unstable intermediates in the peroxidation of lipids, are referred to as Reactive Oxygen Species (ROS).

Antioxidants, on the other hand, are chemical compounds that have been shown to prevent, stop, or reduce oxidative damage. Certain antioxidant supplements can prevent much oxidative damage to DNA (like vitamin C and E) and thus reduce the ability of the oxidants to induce cancer (including skin cancer). Due to the above mentioned damaging effects of Reactive Oxygen Species (ROS) significant effort in finding molecules that can scavenge those highly reactive radical species.

Proposed Research Topics

Generation of ROS species

- photochemical generation of singlet oxygen using photosensitizers

- chemical generation of hydroxyl radicals

Detection of ROS species

Fluorescence Spectroscopy
Fluorescence spectroscopy is one of the most sensitive techniques and in combination with the fluorophore BODIPY581/591 (oxidation sensitive dye) makes it possible to detect ROS species.

**Fig. 1.** Oxidation of the double bonds participating in the conjugated system of Bodipy581/591 results in a shift in fluorescence from red to green.

Upon oxidation, the fluorescence excitation and emission of this probe is shifted from red to green. The ratio of green (oxidized) to total (green + red) fluorescence eliminates variations caused by heterogeneous probe uptake and distribution. This property is unique among oxidation-sensitive probes and facilitates the visualization of ROS activities on a sub-cellular level.

**Steady State Dynamic Light Scattering**

This is a technique that will allow you to monitor changes in the molecular weight of molecules, like polymers, upon measuring the intensity of light scattered by those molecules. ROS species are known to induce depolymerisation of such polymers.

**Steady State Dynamic Light Scattering and fluorescence spectroscopy data will be correlated in order to study the effect of ROS species in biomolecules**

**Preventing damage by ROS species**

The role of different antioxidants in preventing damage by ROS species will be investigated and compared.
Tracking metabolic processes using fluorescence probes

There are approximately 9 million photons per every atom in our universe! Light is essential for life as we know it and life is only possible because light interacts with matter. The study of the interaction between biomolecules and light is at the heart of a deeper understanding of biomolecular function and this study goes far beyond photosynthesis! Whatever the energy of the photons, they will collide with the molecules, and in some instances be able to transfer energy to or from the molecule they collided with. If energy transfer to the molecule does take place, the amount of transferred energy determines what happens next.

**Project Target**

This project will focus on acquiring enough knowledge on why molecules emit light and how we can use this fantastic property in nanotechnology applications. Light emission allows you to monitor the migration of nanoparticles in cells, monitor the interaction between biological nanostructures, like proteins, follow reaction mechanisms, observe calcium gradients in a cell, and detect minute concentration of toxins ... and much more.

You will learn about which type of fluorescence molecules you can use to detect some of the above mentioned phenomena. You will learn to understand at the molecular level, why such molecules can emit light and why they are such good probes. Besides this, you will experiment with some of these molecular probes in the lab, so that you get hands-on-experiments and data analyses.

Using fluorescence microscopy will also see how hydrophobic and hydrophilic probes distribute themselves at a lipid/water interface. Afterwards, a lipophilic enzyme will be added to the film and the redistribution of the fluorescence probes will be recorded.
Title. Surface Plasmon Resonance and Microcantilever based sensors: comparative study of their response to formation of monolayers.

Introduction

Many of the biosensors currently available on the marketplace are based on the detection of mass change on a properly functionalized surface. Currently, the most sensitive systems are based on Surface Plasmon Resonance phenomena. The technique enables rapid, label-free monitoring of biomolecular reactions in real-time, and requires only a small amount of sample. SPR sensor is sensitive to changes in the refractive index of a thin layer adjacent to the metal layer and therefore is a technique of choice for measuring minute mass change upon antigen binding to an antibody coated gold layer. Another very promising mass-sensitive technique that emerged recently is based on microcantilevers similar to ones used in AFMs. Upon binding with molecules of interest those cantilevers can exhibit static bending as well as change in their resonance behaviour (e.g. resonance frequency and quality factor). Therefore optimization of binding and binding layer properties is of paramount importance for reliable and understandable SPR measurements. Within this project you will compare response of SPR-based (Reichert SR7000DC) and cantilever-based (Cantion) systems to formation further build-up of well-defined self-assembled monolayers.

Objective:-
To study, model and optimize monolayer absorption on a gold surface as well as crosslinking to the absorbed monolayer using state-of-art Reichert SPR and Cantion detectors.

Experimental plan:-

1. Measuring of in situ absorption and monolayer formation on gold surfaces using SPR. You will adsorb thiol-functionalized molecules of different length and with different terminal groups (alkane, acids, amines etc.) as well as mixed monolayers, monitor layer formation and model the SPR response.
2. At the next step you will cross link molecules of interest (e.g. antibody) to the obtained layer, measure and model the layer capacity vs. composition and structure.
3. Perform similar measurements on Cantion system, compare and explain the results.
5th semester project

Combing of Triplex and Quadruplex DNA molecular wires

Supervisors: Leonid Gurevich and Eva Petersen

**Background:**
Besides “classical” Watson-Crick double helix DNA can form a large variety of other structures. Two interesting examples here are G-C-G triplexes and 4G quadruplexes formed via Hoogsteen pairing of two G-bases. As was recently found, those molecules can be of particular interest for future molecular electronics. Generally, molecules to be used for electronic applications need to express three main features:

- **Structuring** – the possibility to tailor their structural properties (composition, length, etc.) “on demand”;
- **Recognition** – the ability to attach them to specific sites or to other target molecules;
- **Electrical functionality** – suitable conductivity and control of their electrical characteristics.

In this respect usual DNA possess all this features except electrical functionality. Early work in this field has yielded seemingly controversial results for *native-DNA* showing electrical behaviors from insulating through semiconducting to conducting. One point of view is that though theoretically DNA can be conducting, any eventual bending misaligns orbital stacking in the molecules rendering it semiconducting. DNA triplexes and quadruplexes are significantly more rigid and as tentative measurements on those new DNA structures show that they possess number of unusual physical properties e.g. enhanced polarizability and enhanced fluorescence even without any doping. We are part of an EU project centered around research on those DNA derivatives with a final goal to create functional nanodevices (sensors, transistors etc.). This project will be a part of those efforts.
**Project objective:**
- developing techniques for controlled deposition of DNA nanowires (triplex DNA and G4-DNA) on planar and patterned surfaces;
- comparative AFM study of DNA-nanowires and ds-DNA.

**Project methodology**

The project will concentrate on the use of so-called DNA combing technique. If we move a substrate where DNA can stick to through a liquid-air interface the DNA molecules will be aligned and stretched on the surface. The extent of the alignment and stretching depends on the surface properties as well as on the deposition buffer.

You will perform combing of DNA nanowires and ds-DNA on silicon surface, either planar or containing nanoelectrodes, with various types of functionalizations (initially silanes with various head groups) and using various buffers. Than you will study the resulting DNA structure (height, persistent length, degree of stretching, etc.) using AFM.
Suggested methods and research highlights: Within the project you will:
- stretch native DNA, triplex and G4 in a straight microfluidic channel
- verify the results using fluorescent microscopy and AFM. Use this data to optimize stretching procedure (buffer, slide coating and flow parameters) for each DNA derivative
- Perform EFM (electrostatic force microscopy, e.g. [2]) on stretched and “as deposited” DNA-derivatives
- Stretch across the electrodes [see e.g. [3]) and characterize electrical properties of DNA-nanowire.

References:
5th semester project

**Protein Immobilization on Metal and Polymeric Nanoparticles**

**Supervisor: Leonid Gurevich and Peter Fojan**

Placing proteins on nanoparticles (organic or inorganic) is important for improving surface area and performance of microreactors, drug delivery and retention, enzyme recycling etc.. In addition metal particles supporting localized plasmon resonance can act as an optical biosensor, as localized Plasmon peak position is sensitive the substance absorbed on the surface and to mean spacing between particles. On the other hand degradable organic nanoparticles can serve as an excellent means of timed drug release and local delivery.

The main focus of this project is developing effective immobilization techniques of lysozyme and glucose oxidase on gold nanoparticles and on polystyrene nanoparticles of similar size with carboxyl groups crafted on the surface.

Within the project you will:

- Prepare gold nanoparticles, characterize their size distribution using DLS, spectrophotometry and AFM imaging.
- Develop effective immobilization technique on self-made metal particles and commercial polystyrene particles and verify the results using fluorescent microscopy and AFM
- Check activity of the immobilized enzyme and compare with the activity of a free enzyme.

Figure 1. Left to right: Schematics showing protein mounted on a nanoparticle via carboxyl functionalized chains; Protein mounted on metal nanoparticles exhibit 2 peaks in absorption spectrum, related to nanoparticles plasmon band and absorption by protein; AFM image showing metal nanoparticlesw with mounted proteins.
Nano 5 projekt:

**Dyrkning, kontaktering og modellering af ZnO nanowires.**

*Projektforslag af Christian Fisker*

Et af de hotteste emner indenfor nanoteknologi er nanowires og nanotubes, da de har mange spændende potentielle anvendelser indenfor elektronikken og optikken. Zinkoxid har et stort bulk båndgap på 3.4eV hvilket gør at overfladetilstande og ydre påvirkninger bliver afgørende for de elektriske egenskaber for nanowires ved lave spændinger. Dette skyldes at overfladetilstandenes energier ligger i bulk båndgabet og derfor exciteres ved lavere energier end bulktilstandene.

Forskningsresultater peger på flere potentielle anvendelser af ZnO nanowires i industrien. Båndgabet gør dem til glimrende grønne lysdioder, og den velordnede struktur gør det muligt at koncentrere lysudsendelsen omkring endefladerne af wiren således at man kan skabe meget lokale felter. ZnO wires er også blevet anvendt med succes til solceller, hvor de med deres langstrakte geometri effektivt transporterer de exciterede ladninger.

**Der er mange ting at kaste sig over:**

- **Dyrkning af ZnO nanostrukturer:** Dyrkningen foregår i en rørovn, og ved at variere forsøgsparametrene og substratet fremkommer forskellige strukturer, som nanowires, nanobælter, nanoringe, tetrapods og mange andre. Der kan også eksperimenteres med at dyrke ordnede arrays af wires.

- **Kontaktering af wires:** For at måle wirernes elektriske egenskaber skal der laves nogle små elektriske kredsløb med kontakter. Dette gøres vha. UV litografi og metal pådampning, og wirernes og kontakternes struktur kan undersøges i lys- og elektronmikroskop. Her er det interessant om man kan dyrke eller manipulere wirerne så de elektriske baner kan skrives direkte ovenpå samlekt, eller om wirerne skal placeres efter.

- **Ledningsevne undersøgelse:** Når det er muligt at trække en strøm igennem wirerne kan deres ledningsevne undersøges, og denne kan forsøges påvirket af f.eks. en laser (såkaldt fotoledning), eller ved at variere temperaturen.

- **Optiske spektre:** En anden måde at undersøge den elektroniske struktur af nanowirerne kunne være at måle deres absorptionsspektrum, for derved at bestemme båndgabet.

- **Numeriske modeller:** Forskellige kvantemekaniske modeller for ZnO nanowires kan opstilles, f.eks. kan en wire betrægtes som en 2D hexagonal kvantebrønd, eller vi kan lave tightbinding hvor krystalstrukturen tages i betragtning.

**Litteratur:**