Mendel University in Brno

Department of Chemistry and Biochemistry

Nanoparticles in bioanalytical applications

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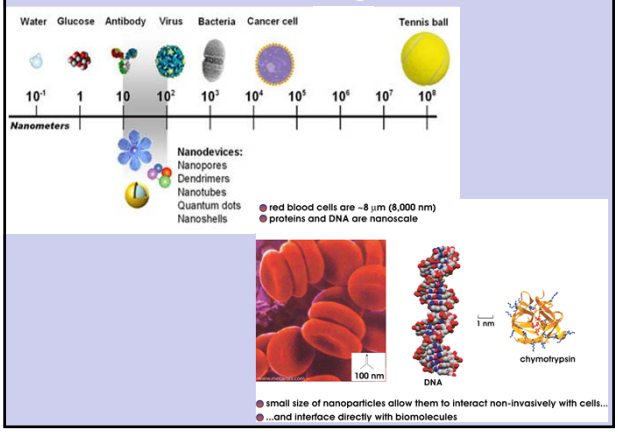
The size of Things



Definition of Nanotechnology

The name “nanomaterials” is a general term that covers an extremely large group of materials. A generally accepted definition is that a nanomaterial is “any material that has an average particle size of **between 1 and 100 nanometres at least in one dimension**.”

The European Commission defines a nanomaterial as “a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for **50%** or more of the particles in the number size distribution, one or more external dimensions is in the size range **1 nm–100 nm**”

✕ albumin molecule is approximately 7 nm in diameter

Richard Feynman’s famous presentation “**There’s Plenty of Room at the Bottom**” was in the 1959 at the American Physical Society.

Here he asked:

•Why can’t we manipulate materials atom by atom?

• Why can’t we control the synthesis of individual molecules?

•Why can’t we write all of human knowledge on the head of a pin?

•Why can’t we build machines to accomplish these things?

The challenge involved the possibility of scaling down letters small enough so as to be able to fit the **entire *Encyclopedia Britannica*** on the head of a pin, by writing the information from a book page on a surface 1/25,000 smaller in linear scale

What is nanotechnology, and why do we care?

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- nanotechnology is the study of matter from 1-100 nm

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- for chemists: the interface between molecules and material

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- for physicists: where quantum ends and bulk begins

What is nanotechnology, and why do we care?

- **nanotechnology is the study of matter from 1-100 nm**
- **for chemists: the interface between molecules and material**
- **for physicists: where quantum ends and bulk begins**
- **for you: faster computers, better communication, and new approaches to medicine**

Interdisciplinary area :

Biology, Physics, Chemistry, Material science, Electronics, Chemical Engineering, Information technology

Why Now?

- New tools for atomic-scale characterization
- New capabilities for single atom/molecule manipulation
- Computational access to large systems of atoms and long time scales
- Convergence of scientific-disciplines at the nanoscale

What's the BIG deal about something so SMALL?

Materials behave differently at this size scale.

It's not just about miniaturization.

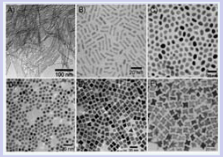
At this scale---it's all about INTERFACES

Size Matters!

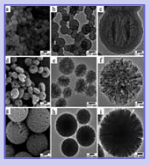
Color depends on particle size
Quantum dots 3.2 nm in diameter have blue emission
Quantum dots 5 nm in diameter have red emission

Particle types

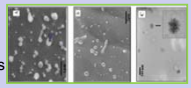
Metal particles
- Noble metals
- Iron oxide
- Semiconductors



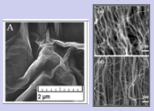
Polymer particles
- Silica
- Chitosan
- Polystyrene
- Dendrimers



Bioparticles
- Liposomes

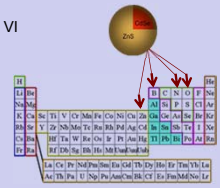


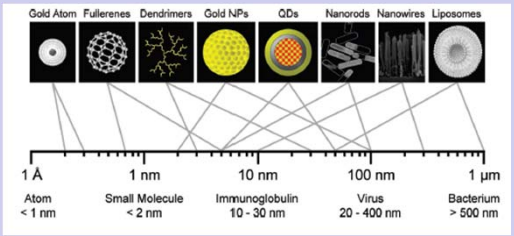
Carbon particles
- Carbon nanotubes
- Graphene
- Fullerenes



Quantum dots

- Semiconductor nanocrystals synthetized from II and VI or III and V elements of PSE
- 1970 developed first low dimensional structures quantum well (QW)
- 1980 – Ekimov, Efros – first description of quantum dots



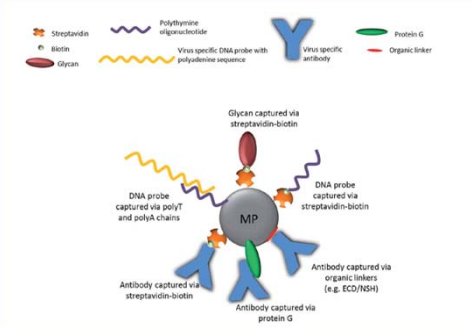


Nanoparticles for sample pretreatment

Sample preparation

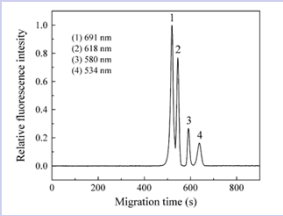
- Isolation form complex biological matrixes
  - Large surface
  - Simple functionalization
  - Magnetic properties

- Magnetic nanoparticles (Iron oxides), Carbon nanoparticles (nanotubes, graphene), gold nanoparticles



CE characterization of nanoparticles

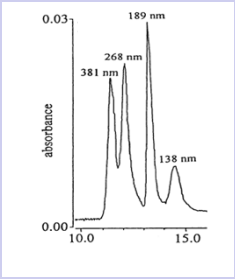
Separation of Quantum dots



Electropherograms of a mixture of four QDs. Sample were water-soluble CdSe/ZnS core–shell QDs with emission wavelength at 534, 580, 618 and 691 nm, and concentrations of QDs in the mixture were all 2.038×10−6 M. 4% PEG was used as sieving medium and applied voltage was 14 kV

Li. Analytica Chimica Acta 647 (2009) 219–225

Separation of latex nanoparticles

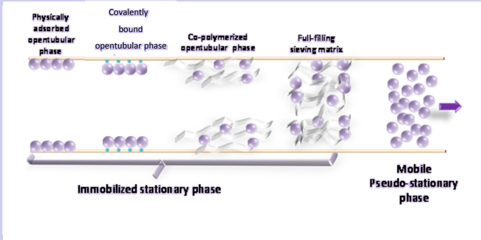


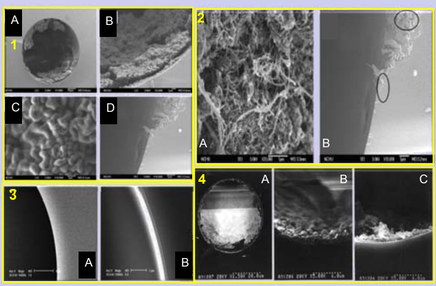
separation of sulphated polystyrene latex particles. BGE: 89 mmol/L Tris,

89 mmol/L boric acid, 2 mmol/L Na_2EDTA, pH 8.3

Radko, S. P., Stastna, M., Chrambach, A. Electrophoresis 2000, 21, 3559–3562

Separation





Scanning electron micrographs of various nanomaterials immobilized on the capillary wall. **1)** Scanning electron micrographs images of prepared CNTs-polymer composites. (A) Mono-(2-(methacryloyloxy)ethyl) succinate **carbon nanotubes** (MES-CNTs) coated on capillary wall; (B) five-fold magnification of (A); (C) the MES-CNTs formed on aluminum foil; (D) butyl methacrylate carbon nanotubes (BMA-CNTs) coated on capillary wall. **2)** SEM images of (A) the BMA-**CNTs** composite, and (B) as a coating on a capillary wall **3)** Scanning electron micrographs of (A) bare fused-silica capillary column, and (B) capillary column modified with octadecylamine-capped **AuNPs**. **4)** Scanning electron micrographs of **TiO_2 NPs**-coated column. (A) Cross section of the column prepared by two-cycle coating procedures. (B) Edge of the column prepared by two-cycle coating procedures. (C) Edge of the column prepared by a single coating procedure.

Separation of 100 bp DNA step ladder using (a) 0.10% PEO only and (b) 0.02% **yttrium oxide nanoparticles** and 0.10% PEO, peak assignment; 1 = 100 bp. 2 = 200 bp. 3 = 300 bp. 4 = 400 bp. 5 = 500 bp. 6 = 600 bp. 7 = 700 bp. 8 = 800 bp. 9 = 900 bp. 10 = 1000 bp. 11 = 1500 bp.

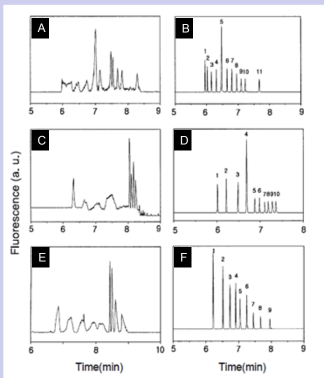
Separation of 500 bp DNA ladder using (c) 0.02% PEO only and (d) 0.02% **yttrium oxide nanoparticles** and 0.02% PEO,

Separation of 1 kbp DNA ladder using (e) 0.02% PEO only and (f) 0.02% **YNP** and 0.02% PEO,

peak assignment; 1 = 1000 bp. 2 = 2000 bp. 3 = 3000 bp. 4 = 4000 bp. 5 = 5000 bp. 6 = 6000 bp. 7 = 7000 bp. 8 = 8000 bp. 9 = 9000 bp.

Electrokinetic injection at 4 kV for 3s; separation at 5.4 kV, fused-silica capillary; 360 μm o.d., 75 μm i.d.,

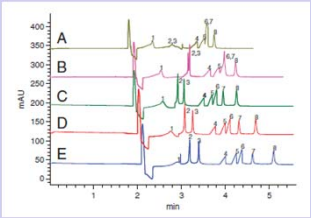
30 cm total length, and 22 cm effective length.



Kwon, H.; Kim, Y. *Bull. Korean Chem. Soc.* **2009**, *30*, 297.

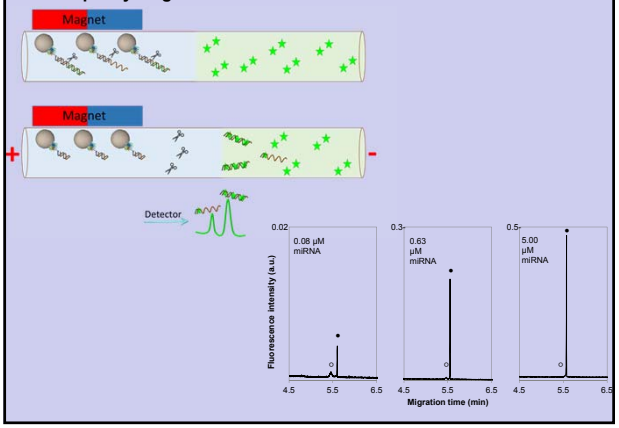
Influence of SC-SWNTs concentrations on the separation of eight analytes using MEEKC: (A) without SC-SWNTs, (B) 1.5 mg/L SC-SWNTs, (C) 3.0 mg/L SC-SWNTs, (D) 4.5 mg/L SC-SWNTs, and (E) 6.0 mg/L SC-SWNTs.

Microemulsion buffers: 0.5% (57 mM) w/v ethyl acetate, 0.6% (30 mM) w/v lauric acid, 4.0% (666 mM) w/v propanol, 50 mM Tris solution of pH 9.0 and 0–6.0 mg/L SC-SWNTs additives. Other operating conditions: analyte concentration, 0.07 mg/mL; capillary length, 40 cm total (31.5 cm effective length) 50 μm id; temperature, 30°C; voltage, 23 kV; injection, 50 mbar, 3 s; detection, 200 nm; analytes: 1. epicatechin, 2. epigallocatechin gallate, 3. epicatechin gallate, 4. Caffeic acid, 5. Gallic acid, 6. Protocatechuic acid, 7. Quercetin, and 8. Kaempferol



Cao, J.; Dun, W. L.; Qu, H. B. *Electrophoresis* **2011**, *32*, 408.

In-capillary magnetic isolation



Bioanalýza - separace

27

In-capillary magnetická izolace

Detection

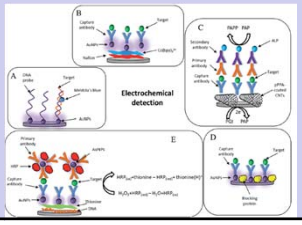
Analyte Detection

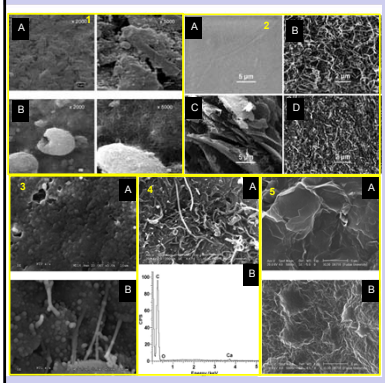
Nanomaterials in electrochemical detection

- **Nanoparticle modified electrodes**
- **Nanoelectrodes**

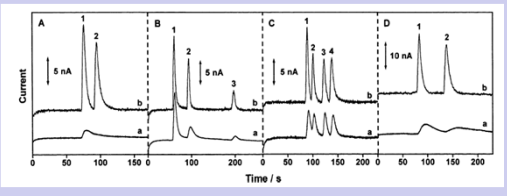
High surface → high adsorption capacity → increased sensitivity

Carbon nanotubes, Au and Pt nanowires





Scanning electron micrographs of various nanomaterial-modified electrode surfaces used for electrochemical detection in CE. **1)** SEM micrographs of bare SPE electrode (A), and CNTs dispersed on the SPE electrode surface (B). **2)** SEM images of the surfaces of A) pure PMMA sheet, B) pure **CNTs**, and the cross sections of C) graphite/PMMA and D) CNT/PMMA composites. **3)** SEM pictures of carbon nanotube paste electrode prepared with short (A) and long (B) **carbon nanotubes**. **4)** A) SEM image, and B) energy dispersive spectroscopy results for the CNT-alginate composite on a carbon disk electrode. **5)** SEM images of pristine **graphene** (A), and graphene/poly(urea-formaldehyde) (B) composite.



Electropherograms for hydrazines (A), dopamine, catechol, and ascorbic acid (B), phenols (C), and purines (D) at the bare (a) and **CNT-modified** (b) screen-printed carbon electrodes.

Sample A: 100 μM hydrazine (1) and 200 μM dimethylhydrazine (2).

Sample B: 100 μM dopamine (1), 100 μM catechol (2), and 100 μM ascorbic acid (3).

Sample C: 100 μM phenol (1), 100 μM 2-chlorophenol (2), 200 μM 2,4-dichlorophenol (3), and 200 μM 2,3-dichlorophenol (4).

Sample D: 200 μM guanine (1) and 200 μM xanthine (2).

Conditions: run buffer, phosphate buffer (20 mM, pH 7.5) (A); 20 mM MES (pH 6.5) (B); 10 mM borate/20 mM phosphate buffer (pH 8.0) (C); 5 mM borate/10 mM phosphate buffer (pH 8.0) (D). Separation voltage, +1000 (A) + 1500 (B–D) V; injection voltage, +1000 (A) +1500 (B, C), +2000 (D) V; detection potential, +0.6 (A), +0.7 (B), +0.9 (C), and +0.8 V (D) (vs Ag/AgCl wire).

Wang, J.; Chen, G.; Chatrathi, M. P.; Musameh, M. *Anal. Chem.* **2004**, *76*, 298.

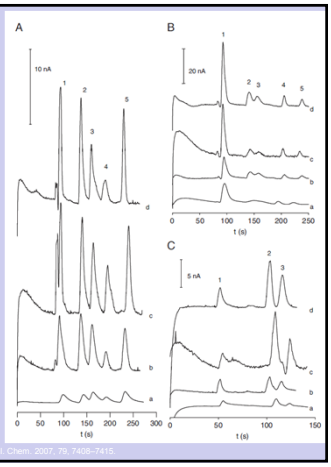
Representative examples of analytical performance of CNT-film detectors in **microchip electrophoresis**

(a) Bare SPE
(b) SPE–SWCNT,
(c) SPE–MWCNT-A
(d) SPE–MWCNT-B

(A) antioxidant standard peaks: (1) arbutin, (2) phloridzin, (3) catechin, (4) rutin, (5) ascorbic acid

(B) flavor standard peaks: (1) vanillic alcohol, (2) ethyl maltol, (3) maltol, (4) ethyl vanillin, (5) vanillin

(C) water-soluble vitamin standard peaks: (1) pyridoxine, (2) vitamin C, (3) folic acid.

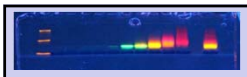


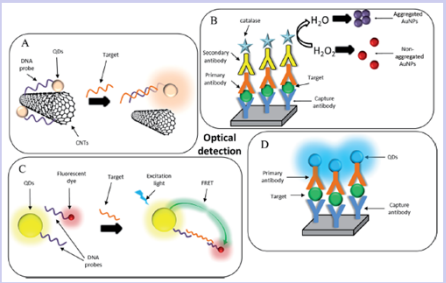
Davies, A. E.; Avila, M.; Pinilla, M.; Gonzalez, M. C.; Escarpa, A. *Anal. Chem.* **2007**, *79*, 7408–7415.

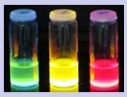
Analyte Detection

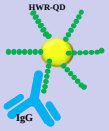
Nanomaterials in optical detection
- Nanoparticles as labels for fluorescent labeling
- Nanoparticles for colorimetric detection
  - (i.e. pregnancy test)

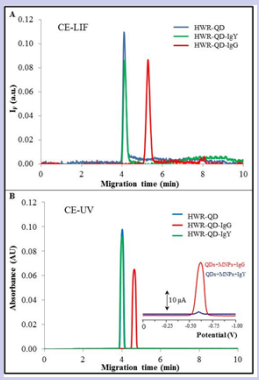
Simple surface modification → applicable for different analytes
- Quantum dots (semiconductor, carbon)
- Gold nanoparticles











L. Janu, M. Stanisavljevic, S. Krizkova, P. Sobrova, M. Vaculovicova, R. Kizek, V. Adam, Electrophoretic study of peptide-mediated quantum dot-human immunoglobulin bioconjugation, Electrophoresis 34 (2013) 2725-2732.

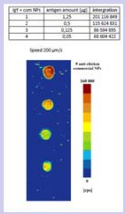
Analyte Detection

Nanomaterials in elemental detection
- Nanoparticles as labels for labeling

Simple surface modification → applicable for different analytes
- Quantum dots (semiconductor, carbon)
- Gold nanoparticles

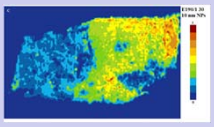
Laser ablation ICP MS

Dot blot

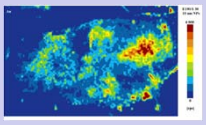


Tissue section

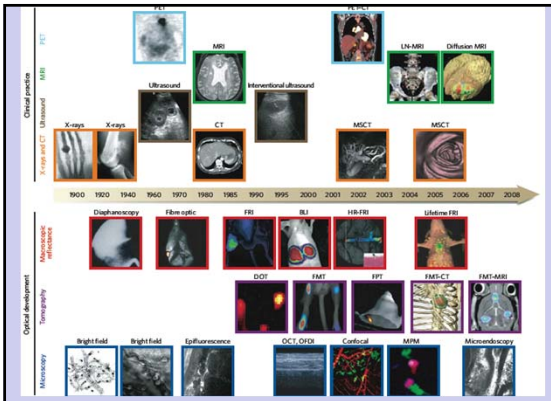
Carbon



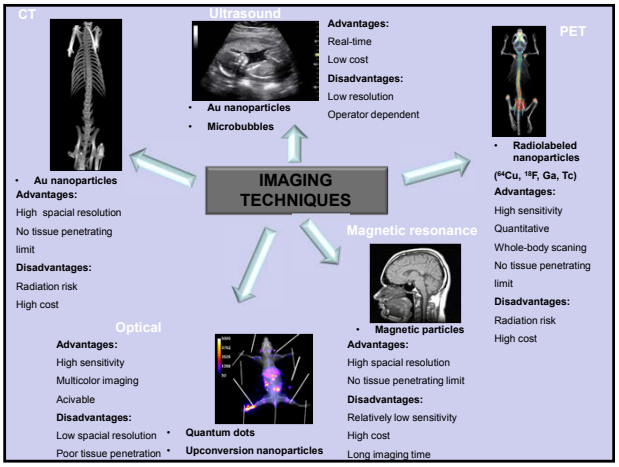
Gold



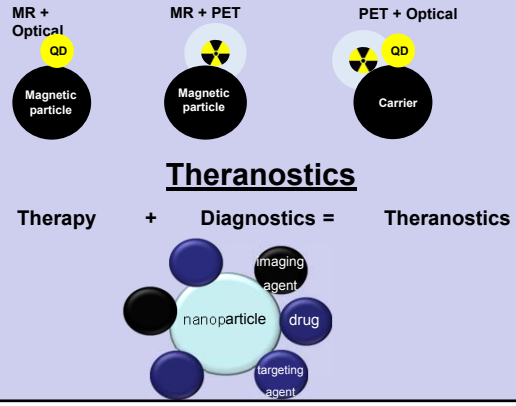
Imaging



BLI - bioluminescence imaging; **CT** - computed tomography; **DOT** - diffuse optical tomography; **FMT** - fluorescence-mediated tomography; **FPT** - fluorescence protein tomography; **FRI** - fluorescence reflectance imaging; **LN-MRI** - lymphotropic nanoparticle-enhanced MRI; **MPM** - multiphoton microscopy; **MRI** - magnetic resonance imaging; **MSCT** - multislice CT; **OCT** - optical coherence tomography; **OFDI** - optical frequency-domain imaging; **PET** - positron-emission tomography

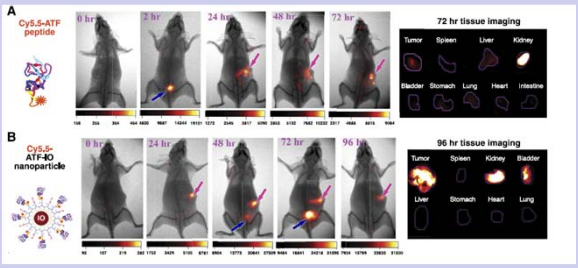


Multimodal imaging



Theranostics

Therapy + Diagnostics = Theranostics



Iron oxide (IO) particles functionalized with amino-terminal fragment (ATF)

peptides labeled with near infrared (NIR) dye (Cy5.5)

Passive targeting

enhance permeability and retention (EPR) effect

"Leaky vessels" – solid tumor, myocardial infarction…

Wide inter-endothelial junctions, fenestrae, transendothelial channels, discontinuous basement membrane

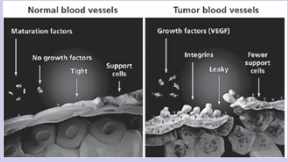
Pore size (tumor):

200 nm-1.2 μm

380-780 nm a typical value

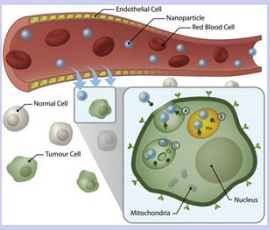
Pore size (normal vessels):

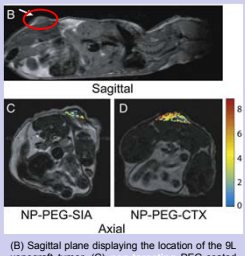
6-7 nm



Peter Carmeliet. Oncology 2005;69(suppl 3):4–10

Active targeting





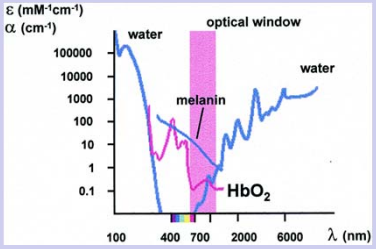
(B) Sagittal plane displaying the location of the 9L xenograft tumor. (C) non-targeting PEG-coated iron oxide nanoparticles and (D) CTX-targeted PEG-coated iron oxide nanoparticles 3 h post nanoparticle injection.

- **Antibody-antigen**
- **Ligand-receptor**
- **Lectin-carbohyrdrate**
- **Aptamers**

Future prospective

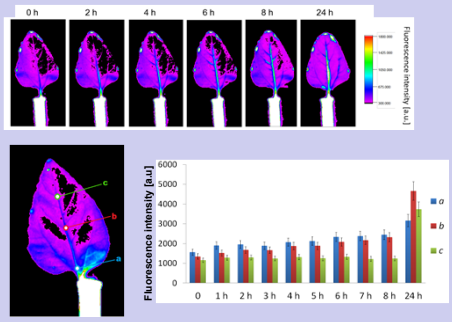
- To meet the requirements for nanotheranostic agents, nanoparticles must be developed with high stability in **extreme conditions** (high salt concentrations and wide pH and temperature ranges) and retain minimum active interaction with serum proteins, which would allow the nanoparticles to conjugate alternative or additional biomolecules without substantially alternating its colloidal stability.
- **The biocompatibility and toxicity** of theranostic nanoparticles need to be thoroughly evaluated as addition of each component material would potentially alter the pharmacokinetic profiles of the nanoparticle.
- To further identify new molecular **targets that are fully correlated with diseases** and discover new targeting ligands with high specificity, small molecular footprint, and good stability.
- Future improvement will also need to focus on development of **innovative strategies to allow efficient tissue penetration of nanoparticles** and offer controlled delivery of therapeutics to a particular type of tissue or subcellular compartments

Fluorescence imaging



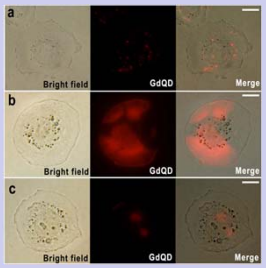
http://research.stowers-institute.org/wiw/external/Technology/NLO/index.htm

Fluorescent imaging of plants



CdTe-PVP QDs (λ_{ex} 480 nm, λ_{em} 535 nm). Fluorescence intensity was analyzed in 3 leaf regions (*a*-*c*) of the main venation as illustrated in the picture (left). Graph (right) shows the mean fluorescence intensity in these regions.

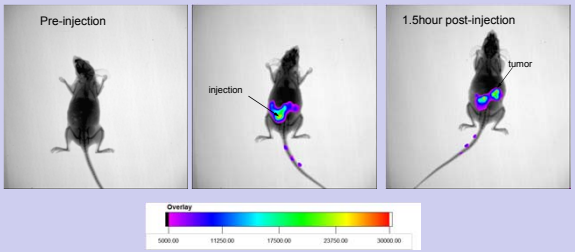
Fluorescent imaging of cells



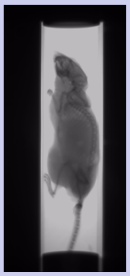
(**a**) Micrograph of PC3 cells that had been cultured in Ham's F12 medium for 5 days and stained with GdQDs. The red fluorescent spots indicate the presence of damage on the plasma membrane caused by the old medium, in which most of the nutrients had already been consumed by the cells. Micrographs of PC3 cells treated with carboplatin (**b**) or cisplatin (**c**) and subsequently stained with GdQDs. Different amounts of red fluorescence indicate different effects of the chemotherapeutic drugs on the plasma membrane.

Fluorescent imaging of animals

QDs injected into mouse with breast tumor



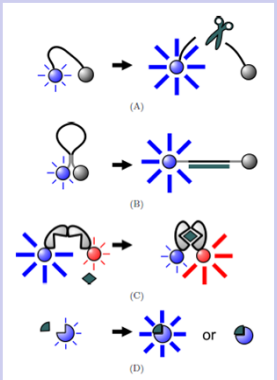
Fluorescent imaging of animals



Cleavable probes

- Enzymes
- Nucleic acids
- Reactive Oxygen Species
- pH
- Ions
- External stimuli (ultrasound, temperature, light, etc.)

➢ **Lower background signal**
➢ **Higher sensitivity**



Summary

- Nanomaterials are great ☺

Analytical tools for characterization of nanomaterials → Nanomaterials as tools for improvement of analytical methods

Thank you for your attention

http://ucb.af.mendelu.cz/ustav/laboratore/27216-laborator-bioanalyzy-a-zobrazovani

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