Functional nanoparticles for biological applications

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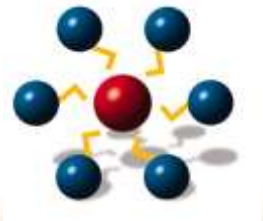
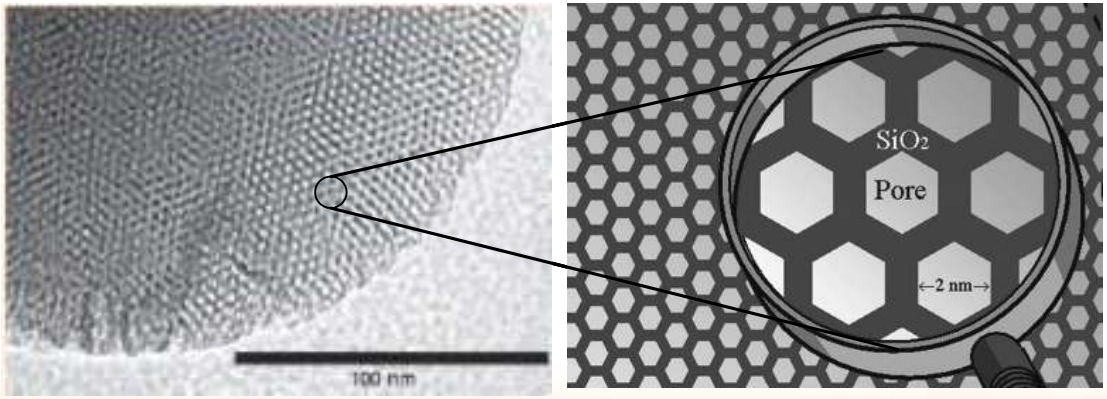


Mesoporous silica (SiO_2) nanoparticles

- Extremely high surface areas and pore volumes enable these matrixes to ***host a large amount of cargo*** (drugs, proteins...)
- The regular pore structure provides a ***homogeneous distribution*** of guest molecules (e.g. drugs), followed by a ***sustained release***
- Pores can be tuned in the molecular size range → ideal for hosting drug molecules
- The **pore walls** can be surface functionalized to provide anchoring points for the cargo molecules and enhance drug immobilization
- The outer **particle surface** can be functionalized independently to ***regulate the release*** (e.g. molecular gate properties), ***tune the surface charge*** and provide ***suspension stability*** and/or attach functional moieties via standard ***bioconjugation reactions***



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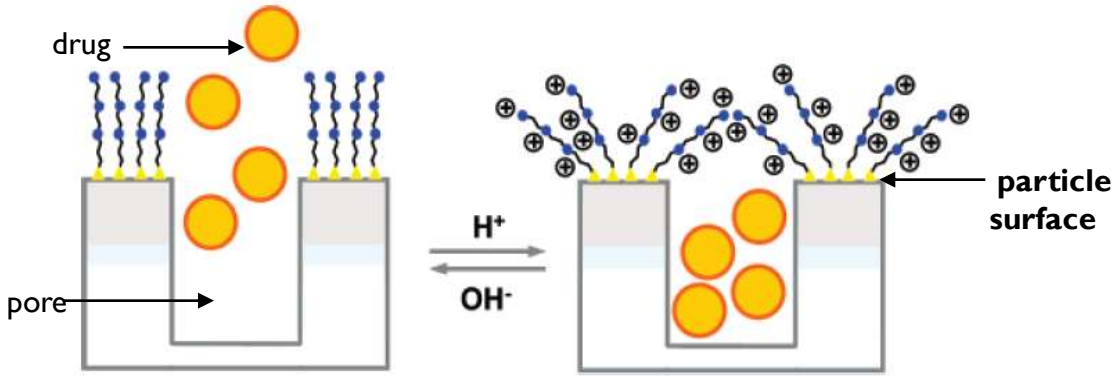
Temporal control: Therapeutic delivery

- Cargo release from the NPs can occur while the NP is still intact or through its decomposition
- Integrating and retaining the cargo components within NPs by ***matching chemical or physical characersitics*** of the cargo with those of the carrier system → ***carrier design + surface functionalization***
- If the surface function comprises inherent responsiveness, the carrier system can be made stimuli-responsive *(triggered release)*

  e.g. PNiPAM thermoresponsive polymer or pH-regulated ionic gate (see figure)



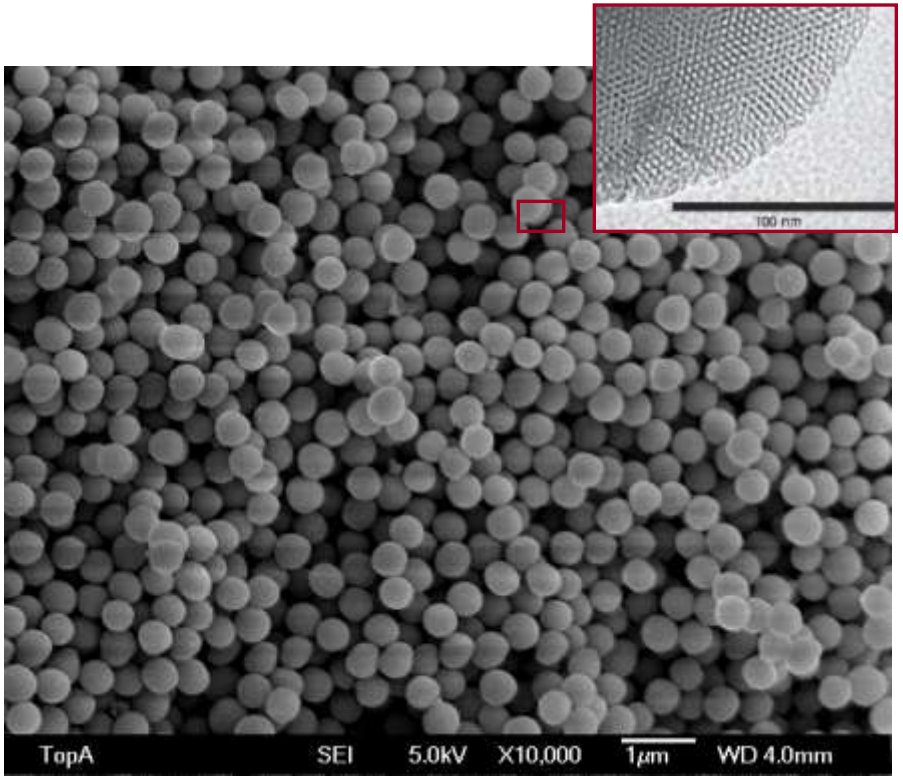
Casasús et al. *J. Am. Chem. Soc.* 130, **2008**, 1903.

Spatial control: Size and surface charge

SiO_2-NP size control:

- The carrier *physicochemical properties*, i.e. **size** and **surface charge**, are the two main determining factors that affect a nanoparticle's fate in the body
- ***Stöber process*** (non-porous & mesoporous NPs)

  50 nm – 2 µm
- ***Mesoporous silica***: Colloidal approach, growth quenchers etc.



In nanobiomedicine, nanoparticles have even been defined as "solid, colloidal particles consisting of macromolecular substances that vary in size from 10 nm to 1000 nm"

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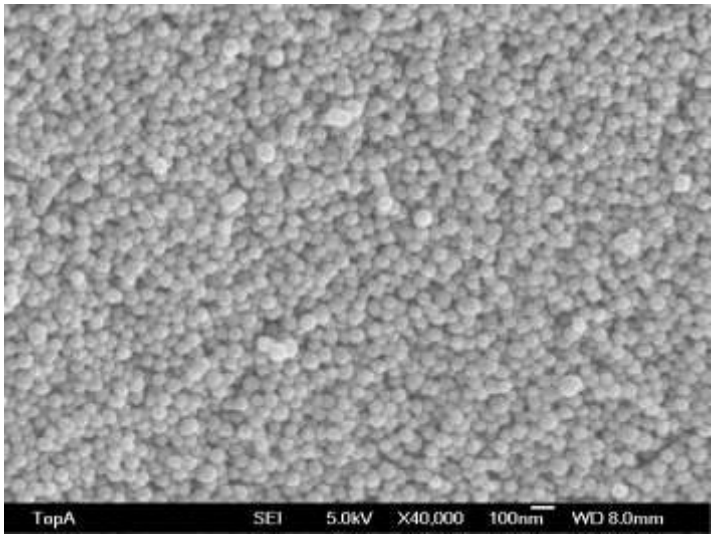
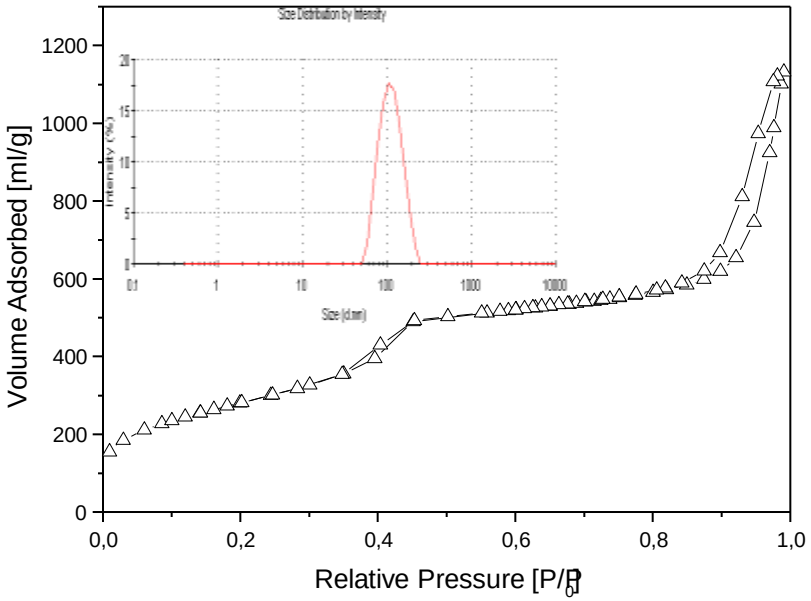
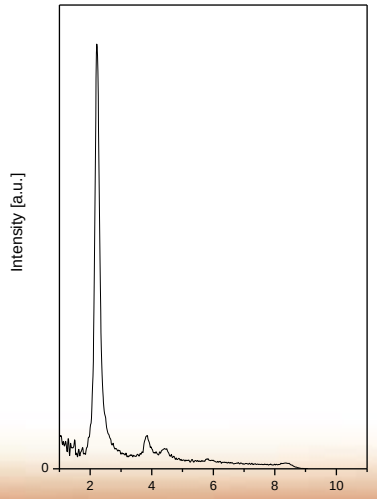
Mesoporous SiO_2 nanoparticle synthesis

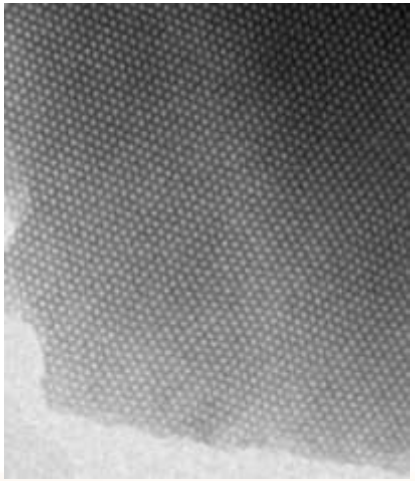




- Synthesis carried out under alkaline conditions using C_{16}TAB as the structure directing agent and glycol as a particle growth quencher

- Particles fully dispersable in water





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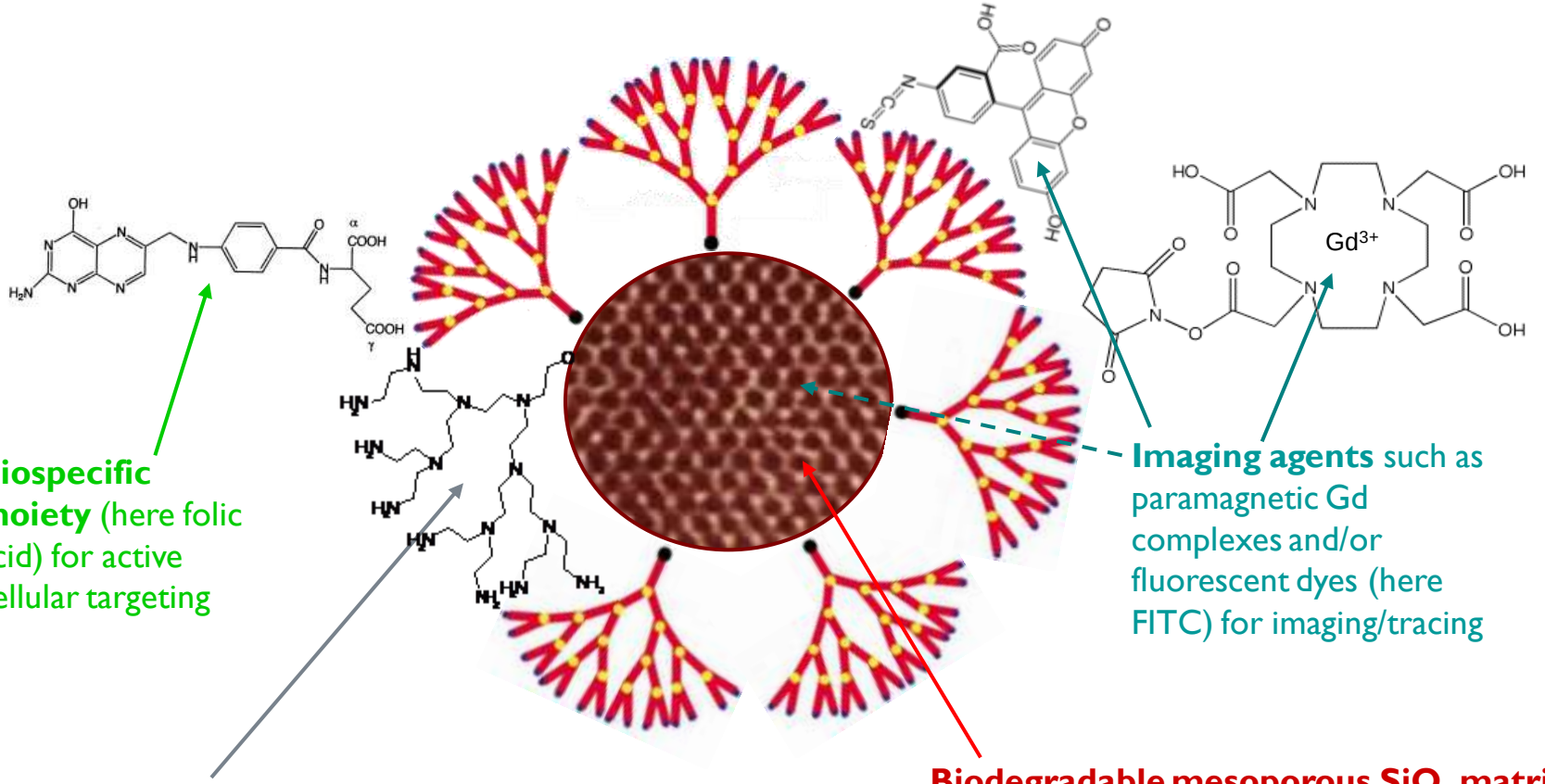
Our design: Multifunctional NP system



Biospecific moiety (here folic acid) for active cellular targeting

Imaging agents such as paramagnetic Gd complexes and/or fluorescent dyes (here FITC) for imaging/tracing

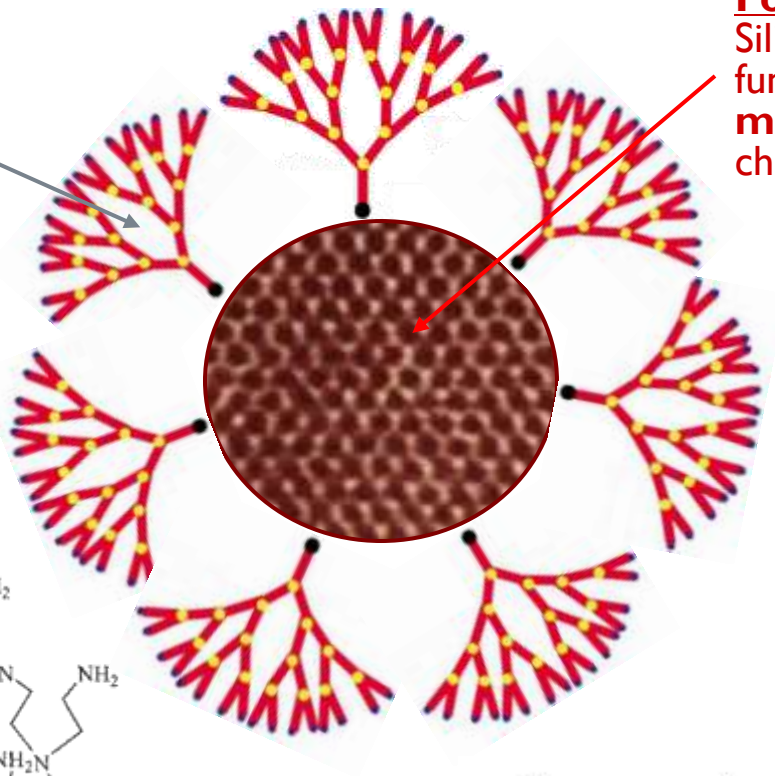
PEI-layer for a) providing reactive "hooks" for attachment, b) particle suspension stabilization, c) surface charge tuning → promoting RES evasion d) promoting endosomal escape, and possibly also: e) molecular gate properties

Biodegradable mesoporous SiO_2 matrix

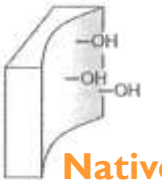
for loading of large amounts of **drugs** for therapeutic delivery

Our design: Additional levels of flexibility

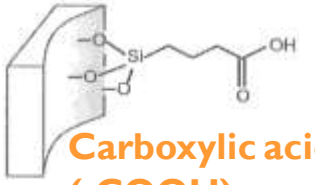
Outer surface engineering: Particle surface can be tuned to yield a highly negative or positive charge at physiological pH



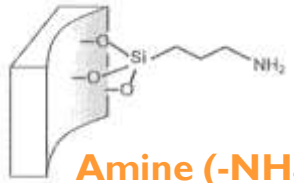
Pore surface engineering: Silica pore walls can be functionalized *(silanized)* to **match cargo molecules** of choice:



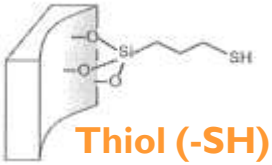
Native silanols



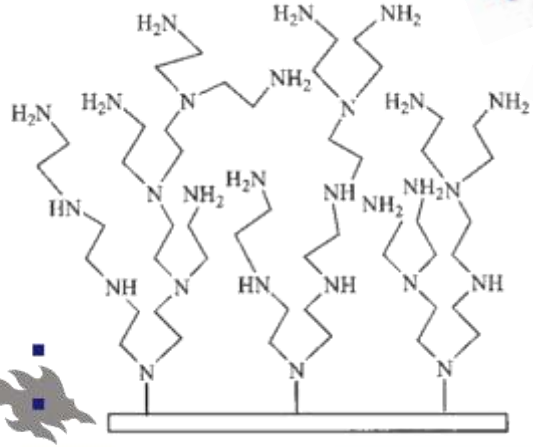
Carboxylic acid (-COOH)



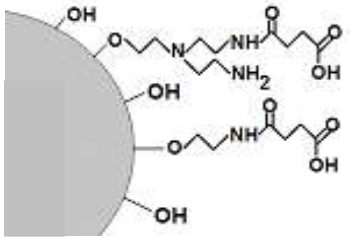
Amine ($-NH_2$)



Thiol (-SH)



succinylation



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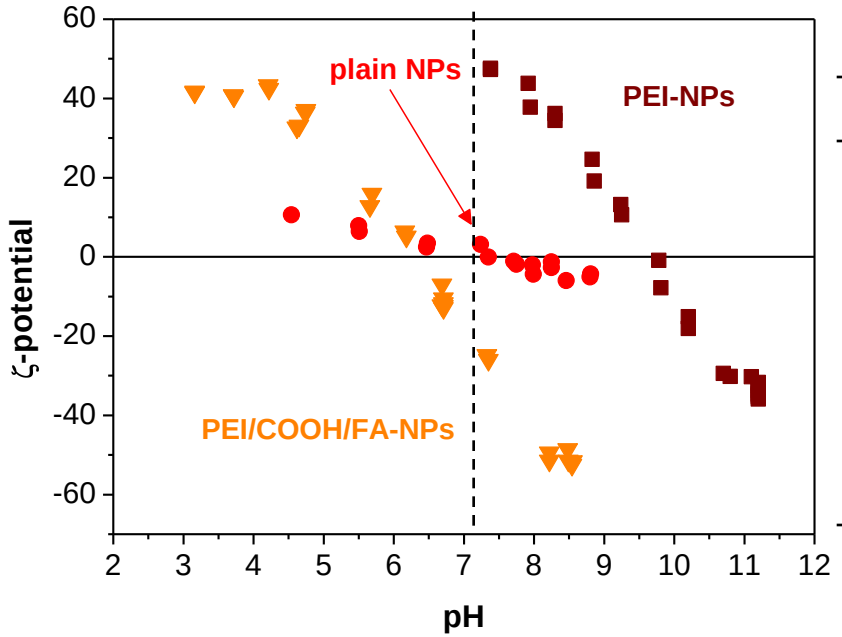
Surface charge modulation



IEP-values of functionalized NPs.

Particle type	IEP
plain NPs	7
PEI-NPs	9.6
FITC/PEI-NPs	9.5
FITC/PEI/FA-NPs	8.7
FITC/PEI/COOH-NPs	5.65
FITC/PEI/COOH/FA-NPs	6.35

- NPs were surface functionalized via surface hyperbranching polymerization of PEI + succinylation to yield opposite surface charge at physiological pH (7.4)
- Surface polymerized NPs show considerable enhancement in electrostatic suspension stability as compared to plain NPs (*in situ* functionalized with an aminosilane)

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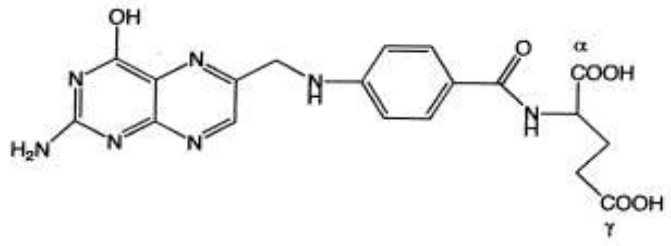
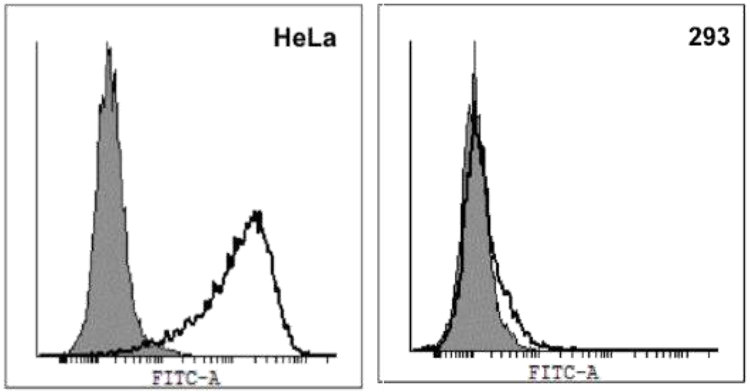
Cellular targeting via the folate receptor

- Folic acid (vitamin B9) as targeting ligand
- The ***cell surface receptor for folate is inaccessible from the circulation to healthy cells*** owing to its location on the apical membrane of polarized epithelia, but it is ***overexpressed on the surface of various human cancers***, including ovary, brain, kidney, breast, and lung malignancies



Molecular structure of folic acid (FA).



Cell surface expression of the folate receptor (FR) in cancer (HeLa) and normal (293) cells.

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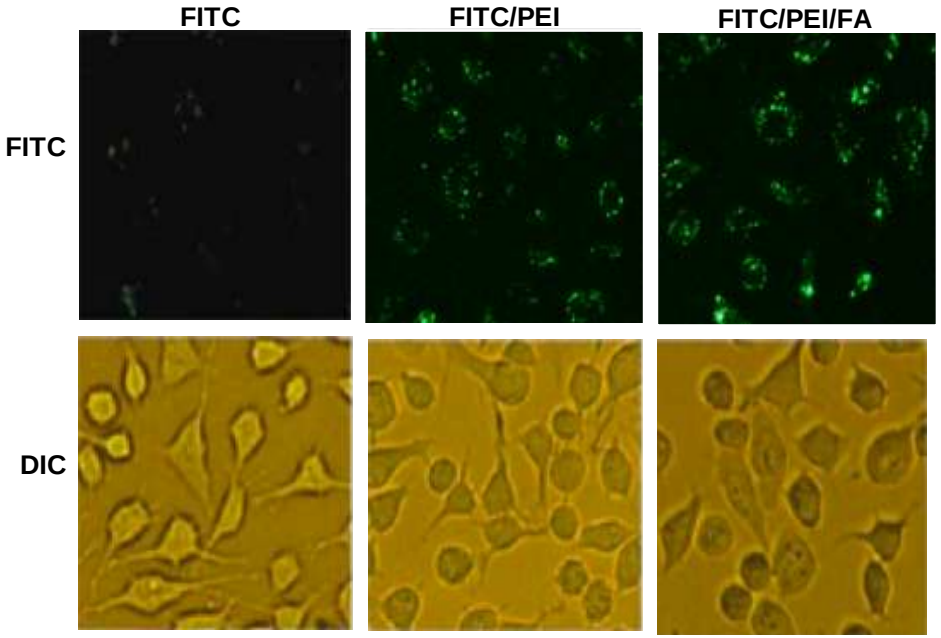
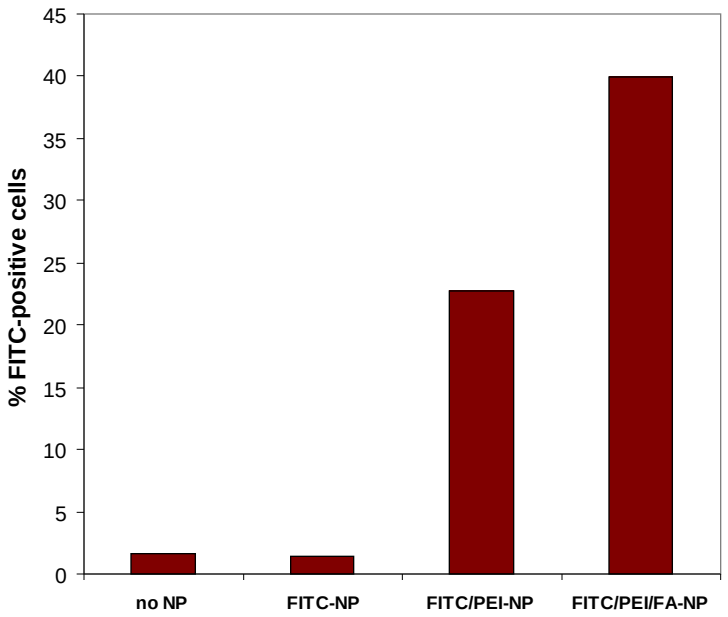
Internalization of mesoporous SiO_2-NPs by human cervical cancer cells (HeLa)





Internalization of fluorescent (FITC – green fluorescence) mesoporous silica nanoparticles in HeLa cells as a function of surface modification

The number of HeLa cells that have internalized the nanoparticles as detected by flow cytometry after 2h incubation + quenching of extracellular fluorescence.

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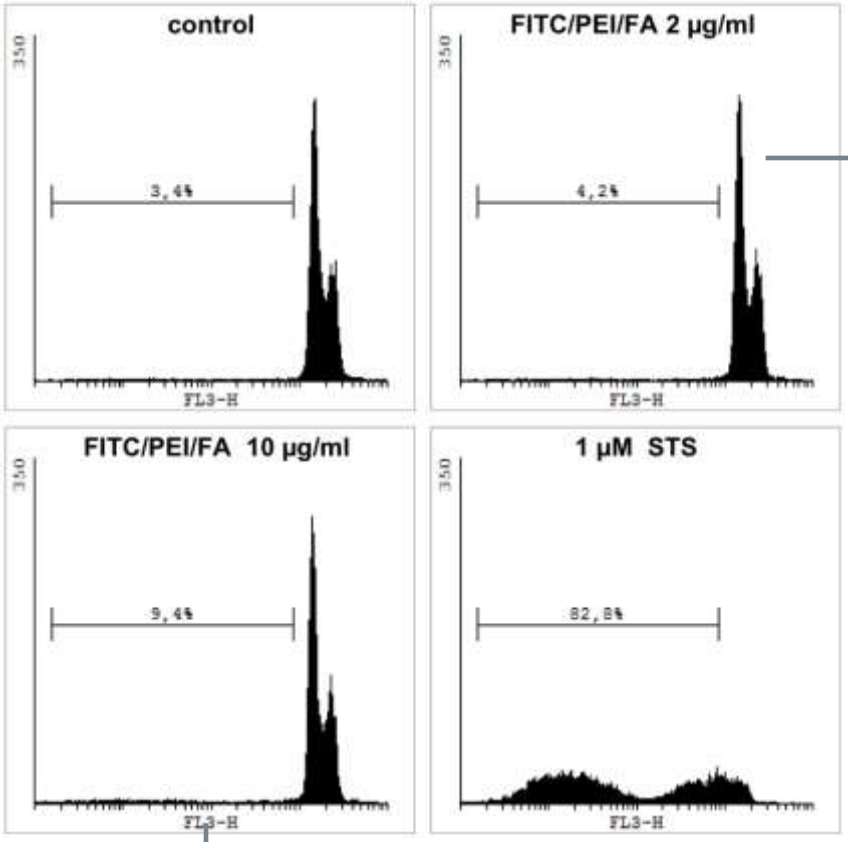
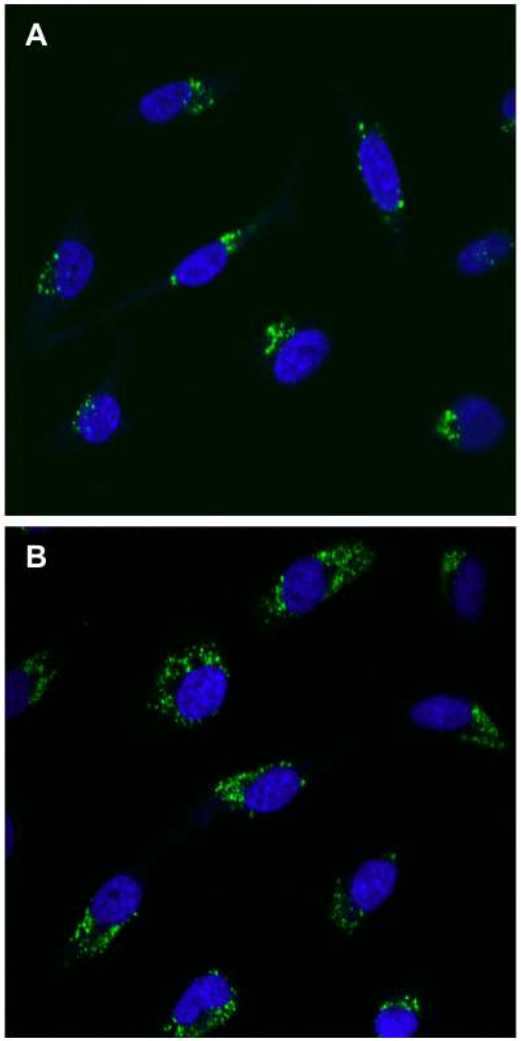
J.M. Rosenholm et al. ACS Nano 2009

Cytotoxicity assays

- Surface modification may change toxicity
- The vehicle is not cytotoxic





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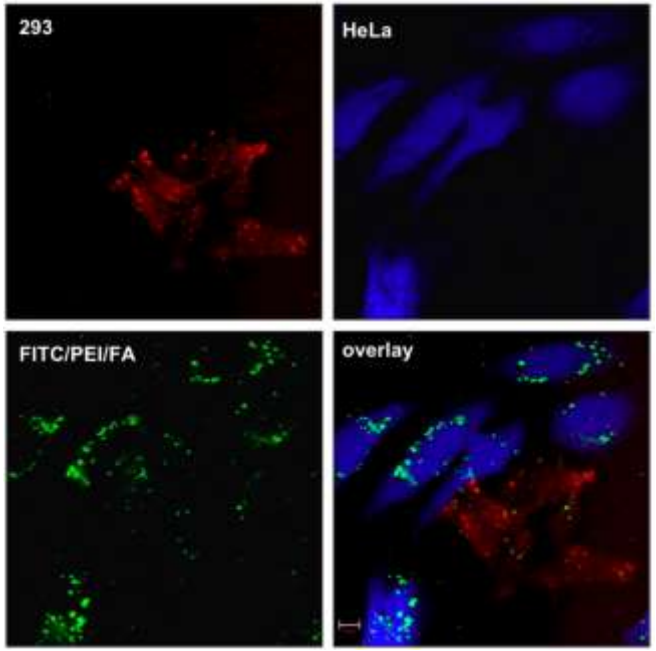
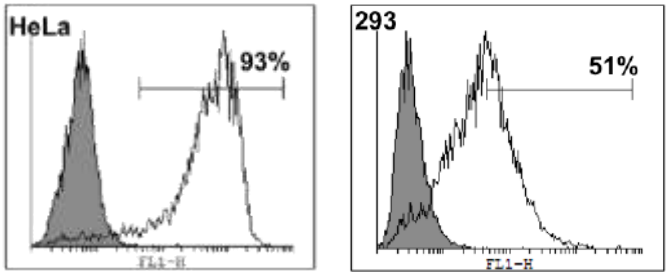
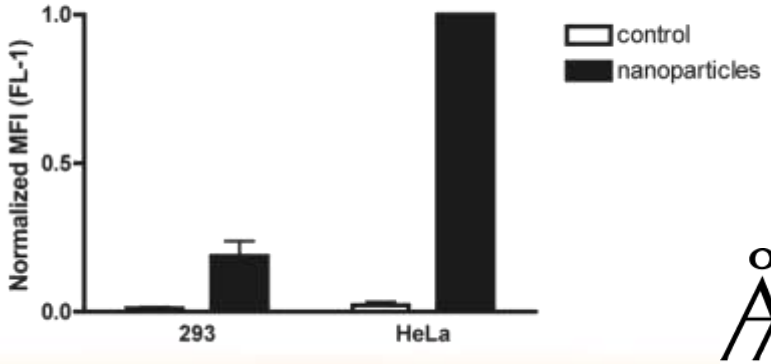
Cellular targeting to cancer cells

- Biospecifically tagged (FA) NPs can be specifically targeted to cancer cells
- Co-culture conditions of cells expressing different levels of the FA-receptor (embryonic kidney epithelial 293 vs. HeLa)
- In total, an ***order of magnitude*** more NPs are internalized by the cancer cells than the normal cells!







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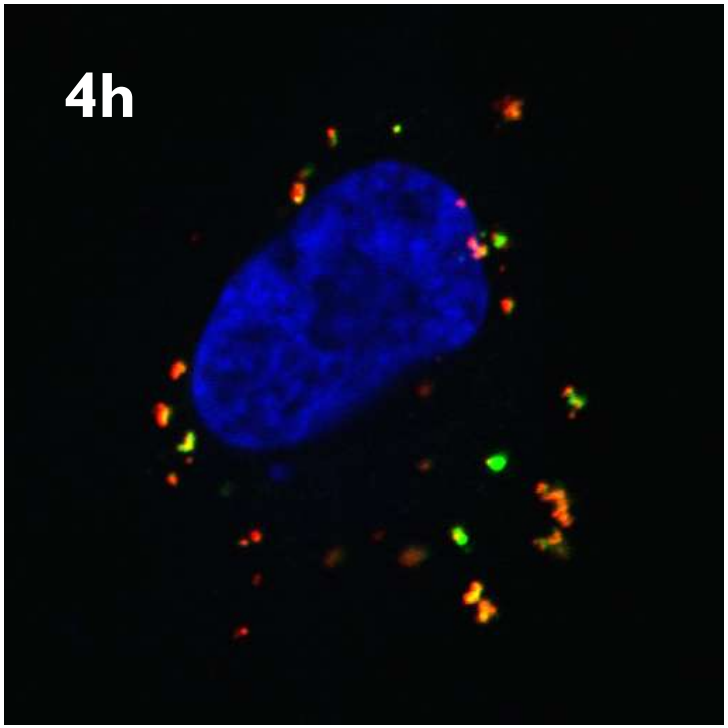
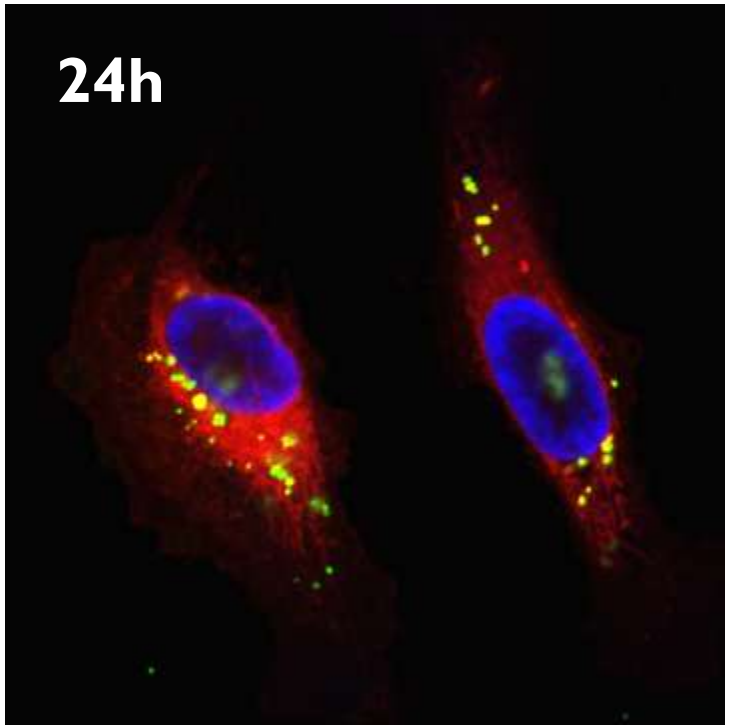
Intracellular delivery of hydrophobic agents





- The green fluorescent silica particles and the red fluorescent model drug cargo (DiI, 1 wt%) are co-localized in endosomes in the HeLa cells (nucleus stained blue) at shorter times, but the DiI is released into the cytoplasm at longer times, while the particles seem to remain compartmentalized (24 h)

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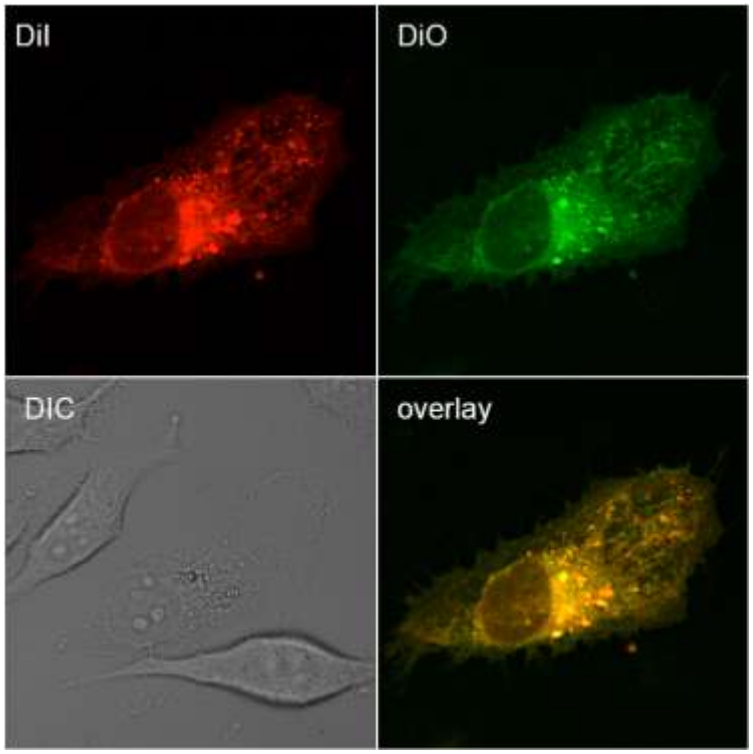
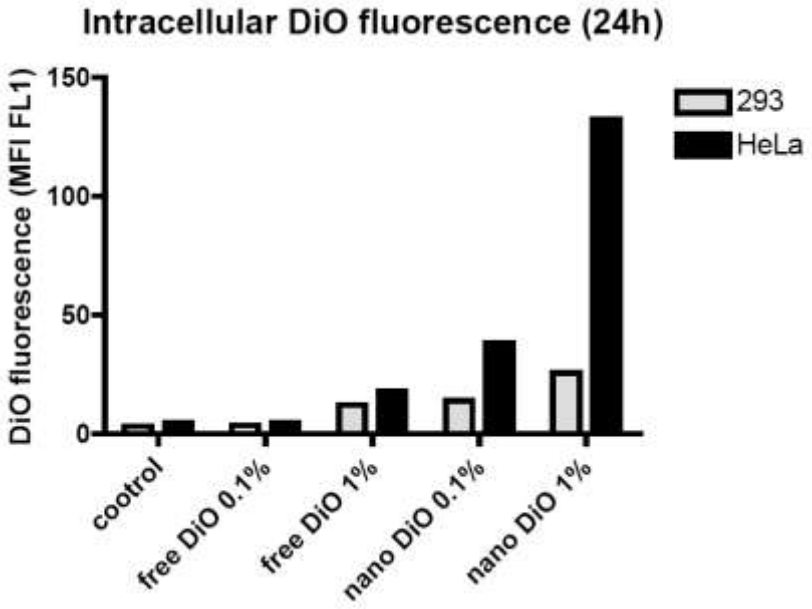
The delivery can also be targeted





- The intracellular delivery of hydrophobic fluorophores can easily be quantified using flow cytometry, indicating the NP-mediated delivery is selective to the HeLa cells and considerably more efficient than free agent.
- Co-delivery of two or more agents is also possible

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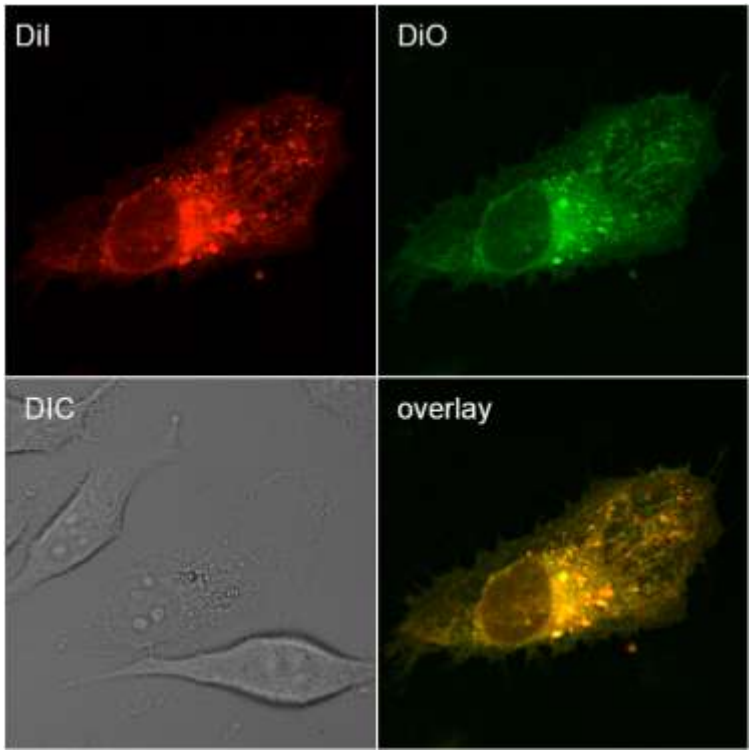

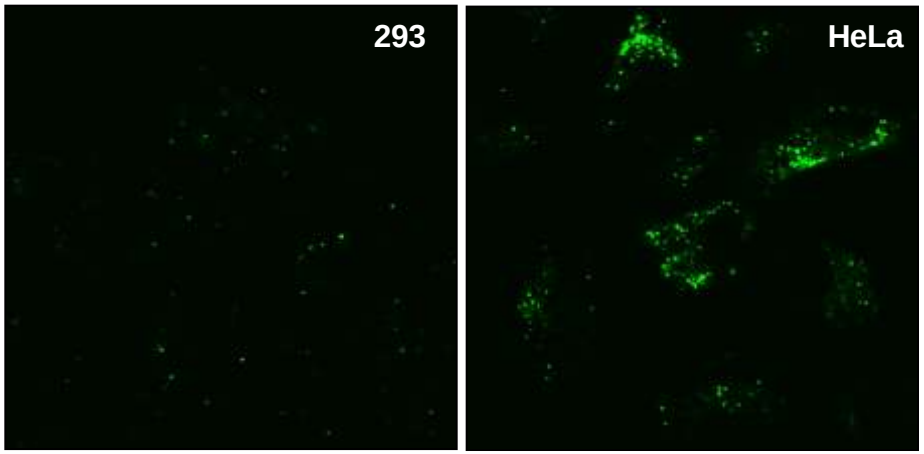

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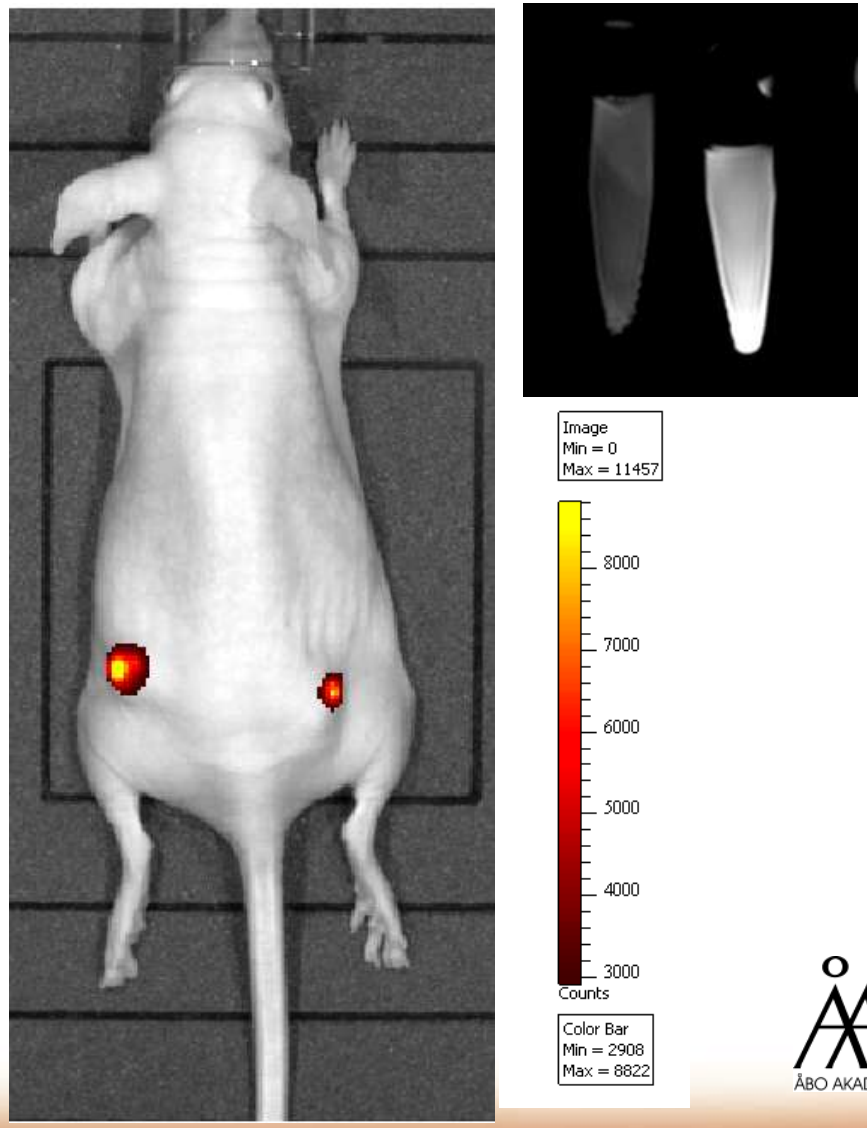
Imaging & Diagnostic Tool

- Principles used to target delivery of drugs may also be applied to target imaging and diagnostic agents to enhance detection sensitivity in medical imaging
- Silica particles encapsulating organic fluorophores are photostable → *fluorescent imaging*
- Silica particles conjugated to a Gd-complex or with a magnetite core → *MRI contrast agents*
- Adding targeting moieties provides a diagnostic tool for instance for early tumor diagnosis
- Also cell tracking (no targeting needed) e.g. stem cells



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Summary

- A highly flexible platform for the synthesis of functionalized silica NPs for biomedical applications has been developed
- Successful targeting of silica NPs to cancer cells has been demonstrated under *in vitro* conditions (*in vivo* on-going)
- The biospecifically tagged particles are taken up by receptor-mediated endocytosis, followed by compartmentalization where the payload can be released into the cytoplasm
- Selective (targeted) NP-mediated delivery of hydrophobic agents have been demontrated and successfully quantified
- Anticancer agent-conjugated NPs can be targeted to tumor originating cells for specific apoptosis (cell death) induction as compared to normal epithelial cells, while both cell types are equally sensitive to the free drug !
- Preliminary *in vivo* results indicate an accumulation of the biospecifically tagged nanoparticles in the tumor – as followed by fluorescence imaging (targeted NPs) but also MRI (non-targeted NPs)
- **Please also see poster "Targeted Intracellular Delivery of Hydrophobic Agents using Mesoporous Silica Nanoparticles as Drug Carrier Systems"**

Acknowledgments

- Dept Phys Chem: Mika Lindén, Alain Duchanoy, Lotta Bergman...
- Dept Biology (ÅAU) & Turku Centre for Biotechnology: Cecilia Sahlgren, Emilia Peuhu, Annika Meinander, Veronika Mamaeva...
- Funding sources: Biomaterials and Tissue Engineering Graduate School, EU project NanoEar; Academy of Finland; Tor, Joe and Pentti Borg Memory Foundation
- The audience for listening !!!

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