ORMOBEAD® - New Generation of Multifunctional Particle Systems for Diagnostics and Therapy

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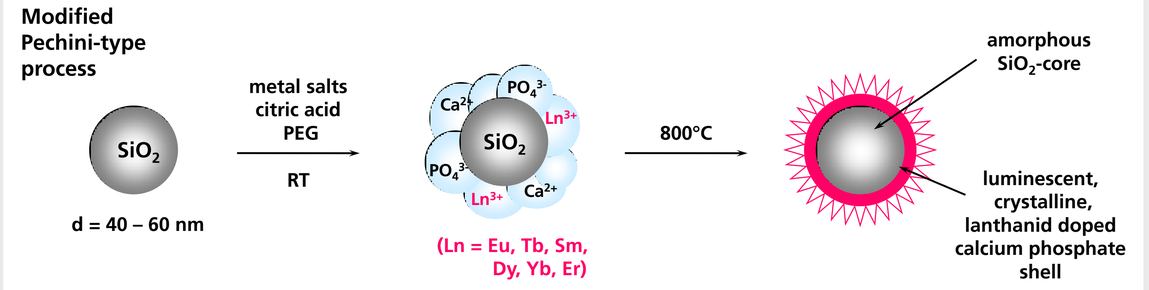
The multifunctional particle concept *ORMOBEAD*® currently in focus of our research activities is able to bind biomarkers or encapsulate active substances or drugs and will open up new pathways to individualized diagnoses and therapies. The great potential of nanoparticles (NPs) as a diagnostic tool was recently demonstrated by the development of *ORMOBEAD*® *vivo* NPs.
Here, we present our recent activities for medical diagnostics concerning the fabrication and surface functionalization of luminescent inorganic NPs on the basis of calcium phosphate (CP). Structure, size, and composition of these NPs can easily be controlled to tailor their properties. We have further demonstrated a subsequent surface modification of the resulting NPs with various functionalities for a later attachment of biomolecules to enable their use as luminescent markers in biological or medical diagnostics. The characterization of CP-based NPs is done by conventional methods. The newly developed NPs were tested for their biocompatibility, e.g. by in vitro cell culture based assays.

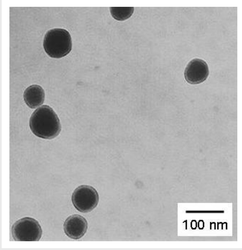
Synthesis



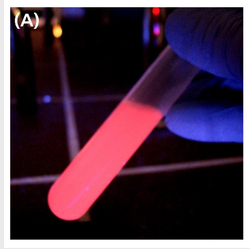
An elegant synthesis strategy offers new possibilities to produce nanoparticles with adjustable properties:

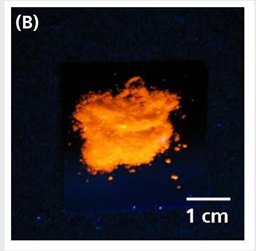
- »Module« principle
- Control of particle size and distribution as well as aggregation grade
- Variation of the core size, shell thickness, composition, and crystal structure
- Adjustment of optical properties
- High particle stability in various environments

Characterization

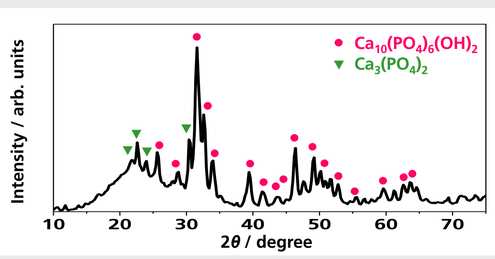


TEM micrograph of SiO_2/CP:Eu^{3+}-core/shell NPs

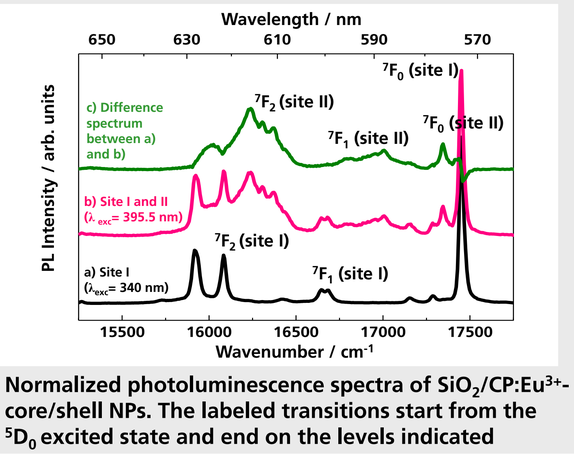




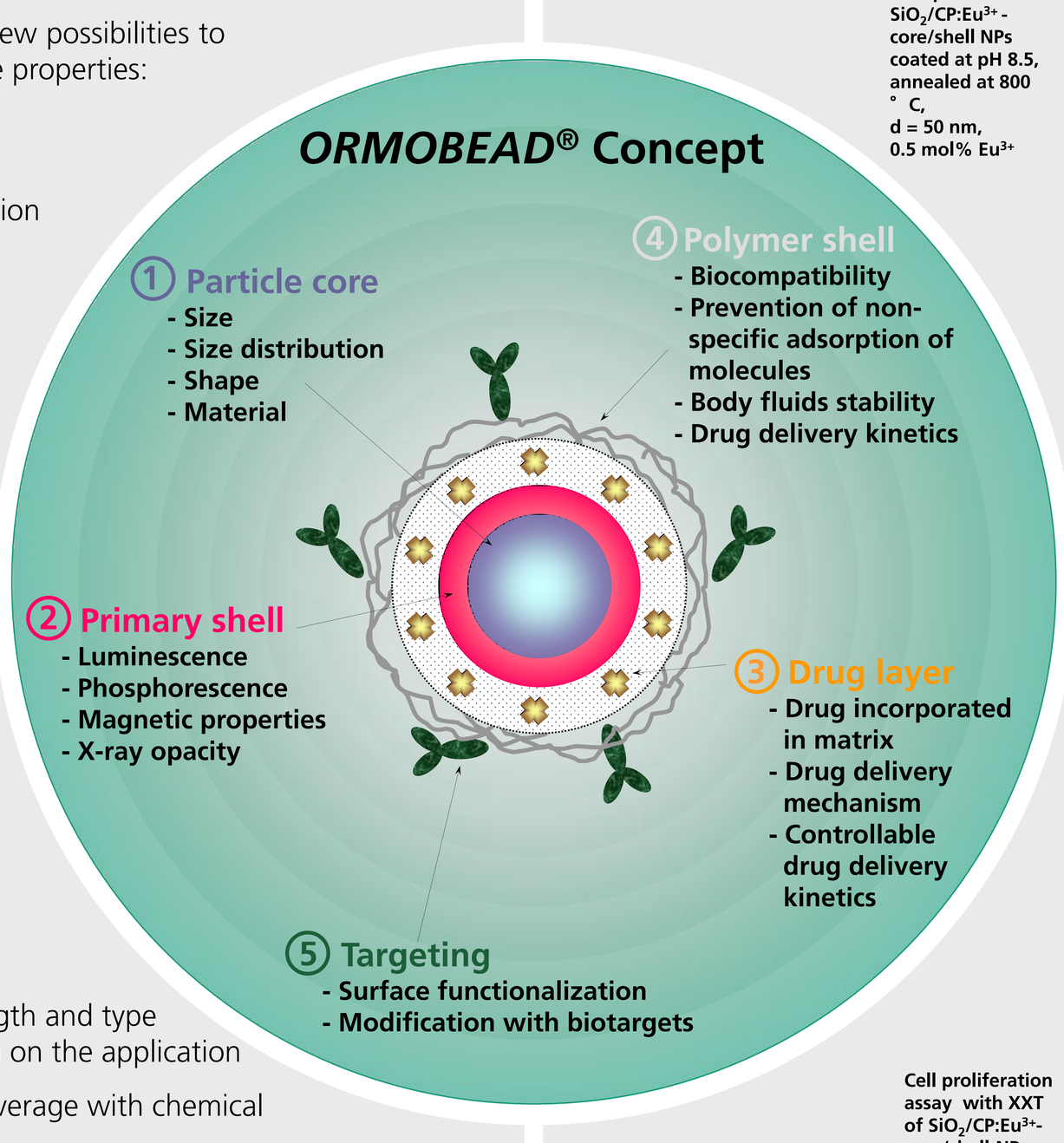
Suspension of luminescent SiO_2/CP:Eu^{3+}-core/shell NPs (A) and NP-powder (B) under excitation with UV-lamp (λ_{ex} = 254 nm)



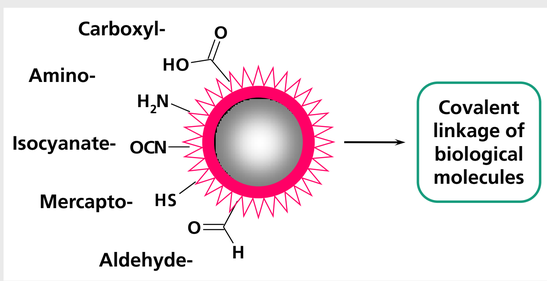
XRD pattern for SiO_2/CP:Eu^{3+}-core/shell NPs coated at pH 8.5, annealed at 800 °C, d = 50 nm, 0.5 mol% Eu^{3+}



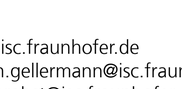
Normalized photoluminescence spectra of SiO_2/CP:Eu^{3+}-core/shell NPs. The labeled transitions start from the $^5D_0$ excited state and end on the levels indicated



Surface Modification



- Subsequent introduction of reactive functionalities to the surface of NPs
- Systematic adjustment of spacer length and type of chemical functionality, depending on the application
- Qualitative analysis of NP surface coverage with chemical functionalities

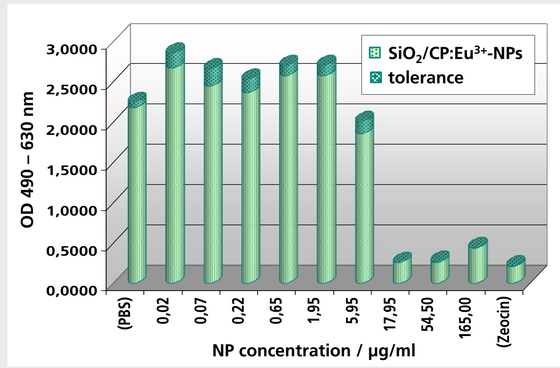
Reagent	Formula	Reactive function
Polyethylenimine (PEI)		Amine
N-[3-(Trimethoxysilyl)-propyl] diethylene-triamine (TRIAMO)		Amine
6-Aminohexanoic acid (AHA)		Carboxyl
Adipic acid (AA)		Carboxyl

Overview of structure and functionalities of different chemicals used for the modification of the NP surface

ζ-potential of non-modified and functionalized SiO_2/CP:Tb^{3+}-core/shell-NPs as a function of pH

Biocompatibility

- In vitro cell culture based XXT (2,3- bis(2-methoxy-4-nitro-5-sulfo-phenyl)-5-[(phenylamino)carbonyl]-2H-tetrazolium hydroxide) viability assay



Cell proliferation assay with XXT of SiO_2/CP:Eu^{3+}-core/shell-NPs

Experimental conditions:
Cell culture: murine cell line L929
Incubation: 72 h, 37 °C (10% FCS, 5% CO_2, 100% humidity)
Negative control: PBS
Positive control: Zeocin (cleaves and intercalates into DNA)

- Only viable cells convert the XXT compound metabolically into a water soluble formazan product (optical detection).
- The EC_{50} (half maximal effective concentration) of SiO_2/CP:Eu^{3+}-core/shell-NPs is 6.58 µg/ml. The corresponding EC_{50} value of quantum dots is up to 1000 times lower*.

*S. T. Stern, B. S. Zolnik, C. B. McLeland, J. Clogston, J. Zheng, S.E. McNei, *Toxicol. Sci.* 106, **2008**,140–152

Conclusion

- Wet-chemical synthesis of luminescent NPs on the basis of calcium phosphate
- Adjustment of the NP structure, size, composition, and optical properties
- Successful surface modification and analysis of NP cytotoxicity

Outlook

- Doping to match optical tissue window (650 – 1200 nm)
- Biofunctionalization of NPs

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