Multifunctional Nanoparticles for Medical Imaging

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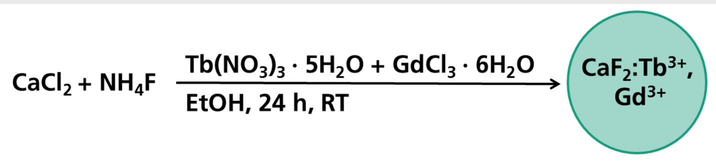
We present our recent activities in the research field of medical diagnostics on the development of multifunctional biocompatible nanoparticles (NPs). Our work is focused on the synthesis and characterization of **calcium fluoride** and **iron oxide** based NPs.

The **CaF_2-based** NPs are produced by wet-chemical synthesis [1] and doped with multiple lanthanide ions, e.g. Gd^{3+} and Tb^{3+}, leading to paramagnetism and fluorescence, making them suitable for T_1-weighted magnetic resonance imaging (MRI) and fluorescence microscopy [2]. The characterization is done by conventional methods such as transmission electron microscopy (TEM), X-ray diffraction analysis (XRD) and photoluminescence (PL) spectroscopy. The capability of these NPs to be used as positive contrast agents for MRI was investigated. In addition, the cytotoxicity of the NPs was tested by a cell viability assay with respect to a later in vivo application.

The **Fe_3O_4-based** NPs are synthesized by methods such as co-precipitation [3], thermal decomposition [4] or sonochemistry [5] and are optionally coated with silica [6]. The latter is envisaged to improve the biocompatibility. The characterization of these NPs is also done by TEM and XRD. Additionally the magnetization curves were measured with a vibrating sample magnetometer (VSM).

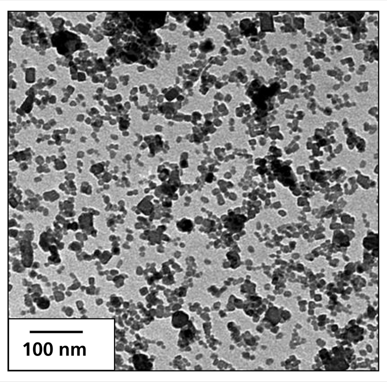
Calcium fluoride

Synthesis and Structural Properties

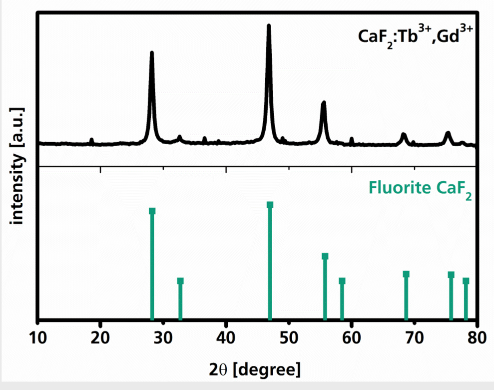


Synthesis of multifunctional NPs on the basis of CaF_2

- Adjustment of NP properties by doping with different lanthanide ions in various concentrations:
  - Gd^{3+} leading to paramagnetism
  - Tb^{3+} or Yb^{3+} and Er^{3+}, respectively, inducing fluorescence into the NPs



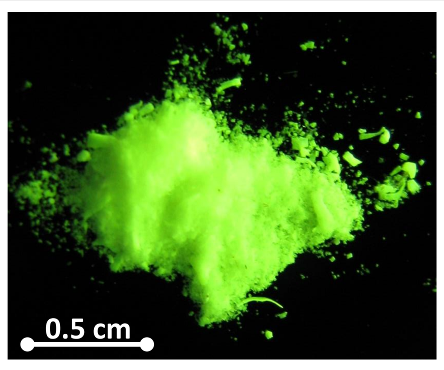
TEM micrograph of CaF_2:Tb^{3+},Gd^{3+}-NPs



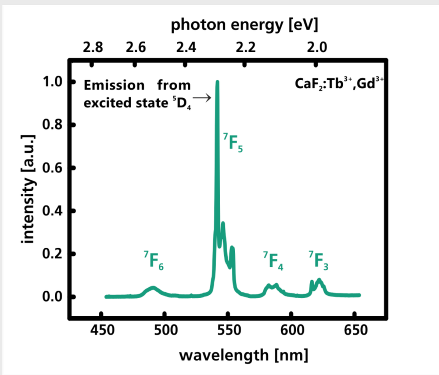
XRD pattern for crystalline CaF_2:Tb^{3+},Gd^{3+}-NPs (d = 10 nm, Tb^{3+} and Gd^{3+}: 1 mol%)

Optical Properties

- Doping with Gd^{3+} (seven unpaired electrons) leads to paramagnetic CaF_2.



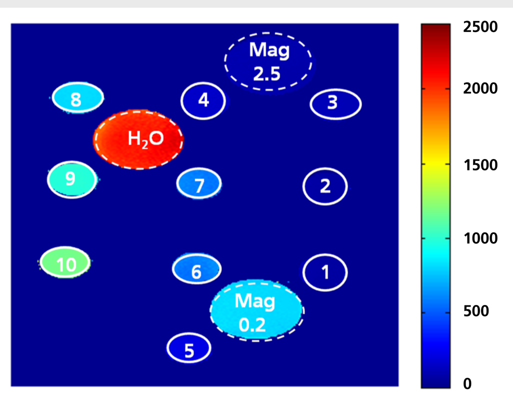
NP-powder of CaF_2:Tb^{3+},Gd^{3+}-NPs under excitation with UV-lamp (λ_{ex} = 254 nm)



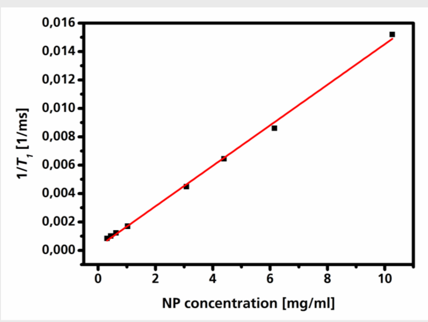
Normalized PL spectrum of CaF_2:Tb^{3+},Gd^{3+}-NPs
The transitions start from the $^5D_4$ excited state of Tb^{3+} and end on the levels indicated (λ_{ex} = 254 nm).

MRI Studies

- Positive contrast agents, such as Gd^{3+} ions induce a shortening of the longitudinal relaxation time T_1 leading to a local signal increase in the MR images.
- The efficiency of the contrast agent is constituted by the relaxivity r_1.



T_1-map from 0 to 2500 ms. (1)-(10): CaF_2:Tb^{3+},Gd^{3+}-NP-samples.
References:
Mag2.5 = Magnevist c = 2.5 mmol/l;
Mag0.2 = Magnevist c = 0.2 mmol/l and water.
Relaxivity r_1 of CaF_2:Tb^{3+},Gd^{3+}-NPs (1 mol% of Gd^{3+}): 0.5 ml/mg·s
Relaxivity r_1 of Magnevist: 4.89 ml/mg·s



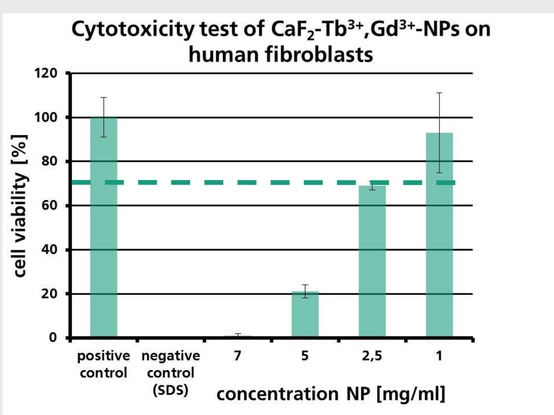
Inverse relaxation time $1/T_1$ of CaF_2:Tb^{3+},Gd^{3+}-NP plotted against the concentration

Biocompatibility

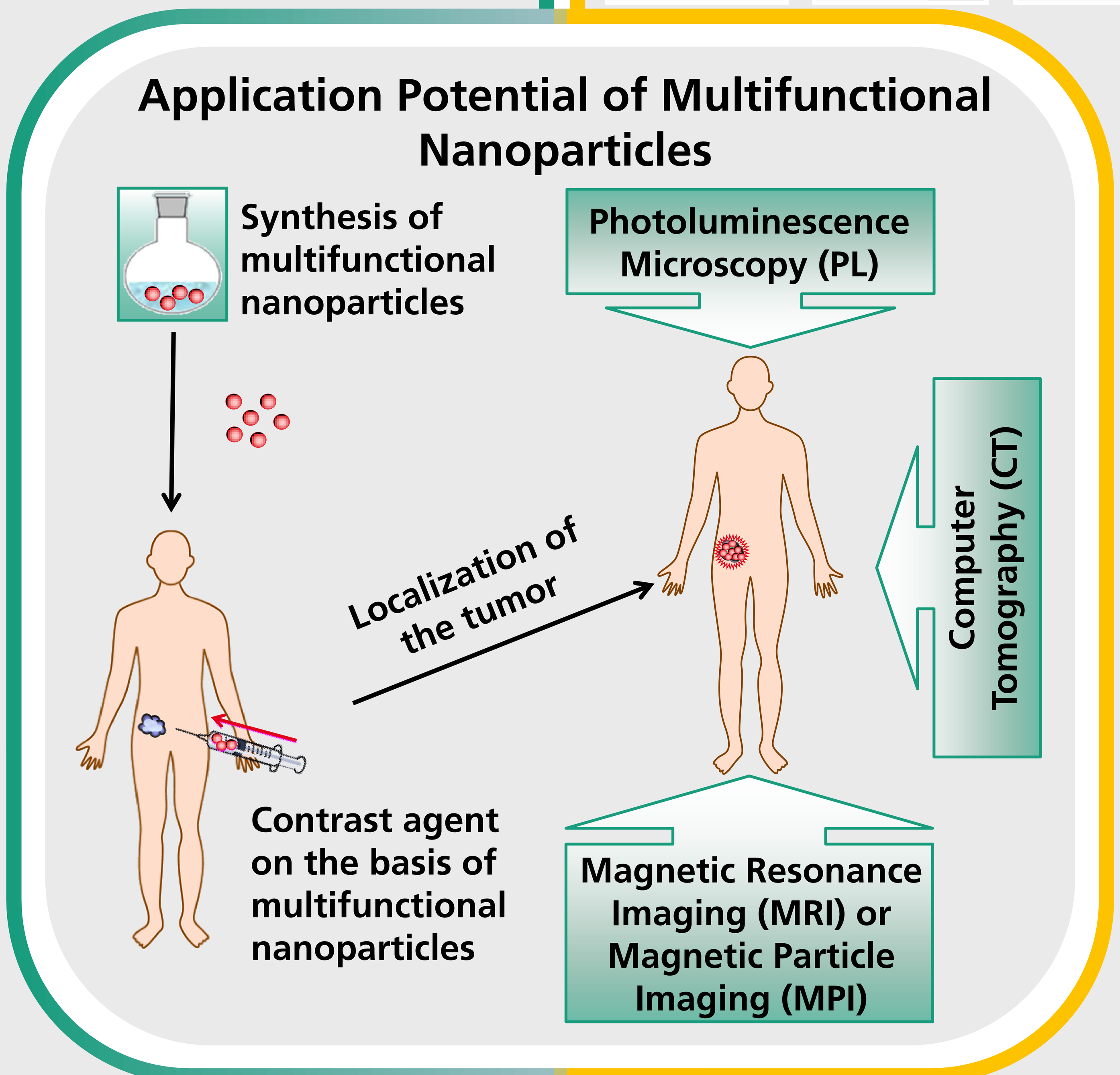
- CellTiter-Glo® Luminescent Cell Viability Assay [7] of CaF_2:Tb^{3+},Gd^{3+}-NPs on human fibroblasts
- Particle samples with the cellular activity over 70% are biocompatible.

Experimental conditions:

cell culture:	human fibroblasts
incubation:	24 h, 37 °C
negative control:	Sodium dodecyl sulfate (SDS)
positive control:	human fibroblasts in serum-supplemented medium

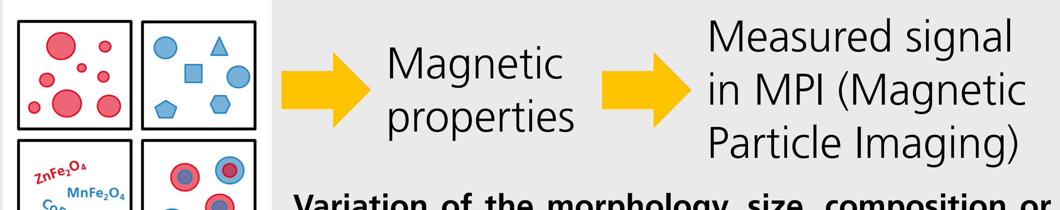


Application Potential of Multifunctional Nanoparticles



Iron oxide

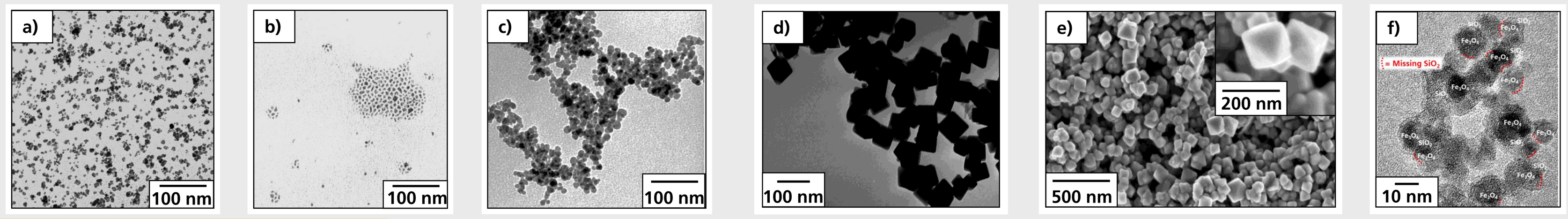
General idea



Variation of the morphology, size, composition or structure of the NPs influences the MPI signal

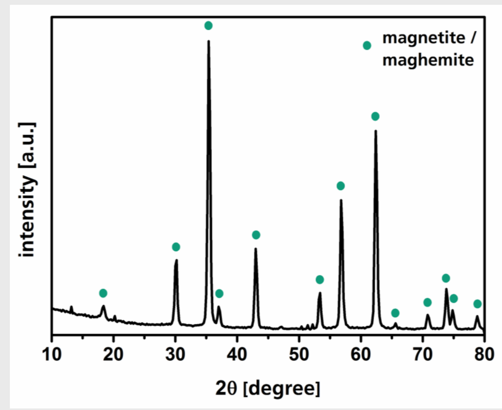
Magnetic properties of Fe_3O_4-based NPs can be adjusted by variation of their:
- size
- composition
- morphology or
- architecture.

Synthesis and Structural Properties



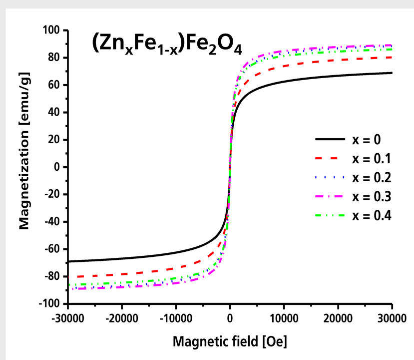
TEM micrographs of Fe_3O_4-NPs after a) co-precipitation, b) thermal decomposition and c) co-precipitation and doping with Zn^{2+}. d) TEM image of octahedral NPs obtained after 2d ageing time. e) SEM image of the same sample. The octahedral shape is emphasized in the inset in image e). f) Patchy silica-coated Fe_3O_4-NPs.

Exemplary X-ray diffractogram of Fe_3O_4-NPs. The peaks, indicating the crystallinity of the particles, can either be assigned to magnetite (Fe_3O_4) or maghemite (γ-Fe_2O_3).



Variation of the Composition

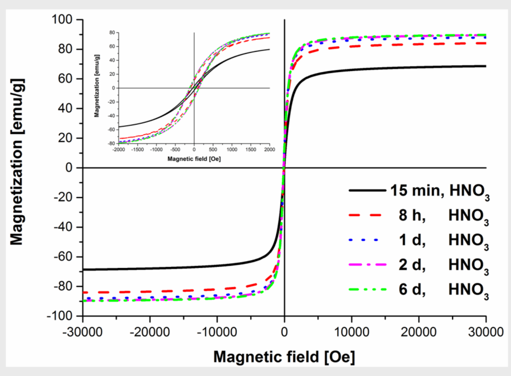
- Doping with Zn^{2+} (TEM c) leads to increased saturation magnetizations. The values vary for different doping concentrations.



Magnetization curves of Fe_3O_4-NPs (doped with x = 0, 0.1, 0.2, 0.3 and 0.4 parts of zinc) after co-precipitation. All samples possess a hysteretic magnetic behavior.

Variation of the Morphology

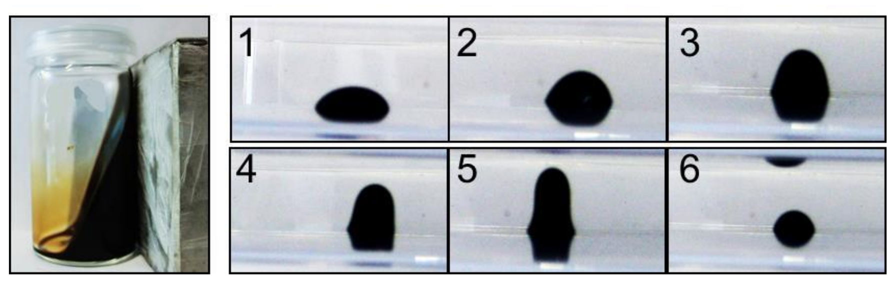
- Combination of ultrasound and nitric acid treatment yields octahedral shape (TEM d,e).



Magnetization curves of Fe_3O_4-NPs after ageing times of 15 min, 8 h, 1 d, 2 d and 6 d. All samples possess a hysteretic magnetic behavior; saturation magnetizations increase with increasing ageing times.

Variation of the Architecture

- A core-shell structure of the NPs (TEM f) can make them biocompatible. Coating with silica results in a true ferrofluid, which is not only stable over a wide range of pH but also in physiological solutions.



Fe_3O_4-NPs coated with silica. Left image: in a vial next to a magnet; right image series: for a single droplet. For the image series 1–6, a magnet was approached from the top.

Summary and Outlook

- Wet-chemical synthesis of multifunctional CaF_2- and Fe_3O_4-based NPs
- Characterization of structural, optical and magnetic properties
- The CaF_2-NPs are biocompatible and suitable for T_1-weighted MRI
- The Fe_3O_4-NPs can be used for MRI and MPI
- Attachment of biomolecules such as antibodies
- Investigation of the X-ray-opacity.

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