Target Nanoparticles for Cancer Therapy : Structure Investigation by SAXS, SANS, ASAXS, DLS and Electron Microscopy

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Therapy of cancer and specific imaging can be extended by **indirect radiation therapy IRT** using **heavy metal targets** with synchrotron X-ray radiation, as shown in figure 1, or neutrons (Gd).

In our concept the ratio of healing to radiation damage effects is improved with **magnetic target nanoparticles** which are based on two principles (figures 1):

– **concentration** of about 1000,000 target atoms in nanoparticles
– **local enrichment** of the nanoparticles by magnetic forces at the tumor site

We use two kinds of **magnetic target nanoparticles** shown in fig.2: i) **magnetic target liposomes**, which bear the **water soluble target** in the entrapped lumen, and ii) **double shell poly-Ferrofluids**, containing the target bound in a surface layer. Our target nanoparticles are biocompatible. The heavy metal is applied as stable metal-DTPA complex, e.g. Lutetium-DTPA, Gd-DTPA (no metabolism; Gd-DTPA is usual in MRI (2g)).

The magnetic target nanoparticles and their components were characterized by various methods, due to the wide size distribution of the components (5 nm – 1 µm) and variing chemical composition and scattering:

- **size distribution** and **structure** of poly-Ferrofluids and magnetite cores were obtained by **DLS, SAXS** (ID1)
- **structure** and **magnetic superstructure** were characterized by **electron microscopy** and **magnetic DLS**
- **magnetic moments** of poly-Ferrofluids and γ-Fe_2O_3 cores were investigated with a **magnetic balance**
- **structure dynamics** of magnetic shell liposomes (fig.2c) and liposomes was studied by **SANS** at ILL-D22
- **magnetic liposomes with metal targets** were analyzed by **ASAXS** at the **L-edge** of absorption at ESRF-ID1
- **medical applications and imaging** were done at the **K-edge** of absoption (50-80 keV) at ESRF-ID17

Indirect radiation therapy with magnetic target nano-particles

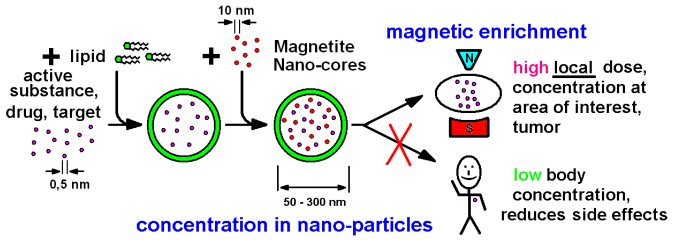


Fig.1: Nanotherapy improves the effect of molecular active substances (drug, target) twice: ~1,000,000 molecules are concentrated in nanoparticles, which are enriched at the tumor locally.

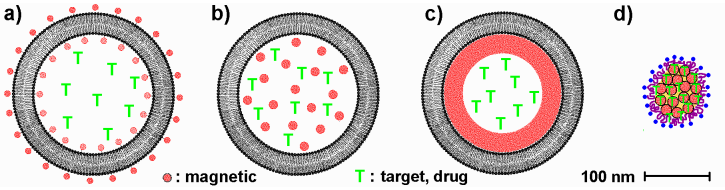
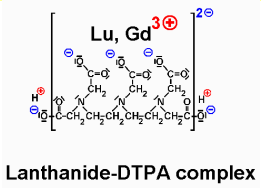


Fig.2: Magnetic and target entities (T) for nanotherapy can be introduced in magnetic liposomes (a-c: metal-lipd, entrapped core, double-shell liposomes), or in double-shell poly-Ferrofluids (d).



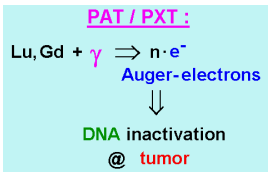


Fig.3,4: Lanthanide-DTPA complexes are biocompatible targets for radiation therapy and imaging with synchrotron X-rays, neutrons, photobiology or MRI. Photon Activation Therapy PAT generates free electrons upon K-edge X-ray photon absorption.

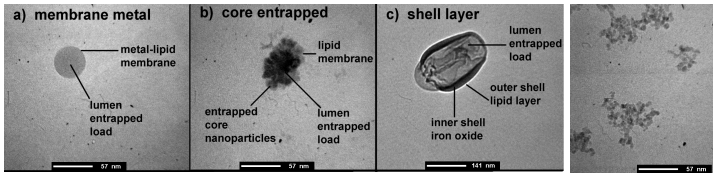
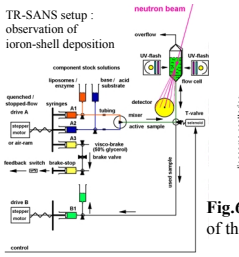
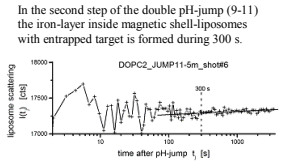


Fig.5: Non-stained **electron micrographs** of magnetic nanoparticles (metal images) : magnetic liposomes (a-c: metal-lipd, entrapped core, double-shell liposomes), disk-shaped poly-Ferrofluids (d).

TR-SANS time-resolved neutron scattering of magnetic liposomes





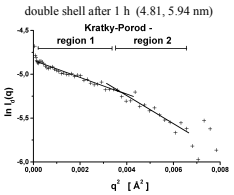


Fig.6-8: TR-SANS of magnetic shell-liposomes (fig.2c, 5c) during precipitation of the γ-Fe_2O_3 -layer (ILL-D22): A double pH-jump yields stable nanoparticles

SAXS – ASAXS - XAS of magnetic target liposomes & poly- Ferrofluids

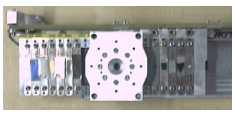


Fig.9: Thin layer cell for ASAXS of fluids at ESRF-ID1 (adjustable flat layer of 0.05 – 10 mm path)

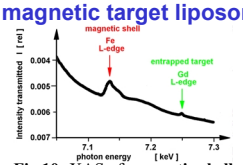


Fig.10: XAS of **magnetic shell liposomes** with entrapped **Gd-target** (fig2c, 5c) during ASAXS at ESRF-ID1 (MD118)

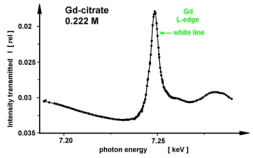
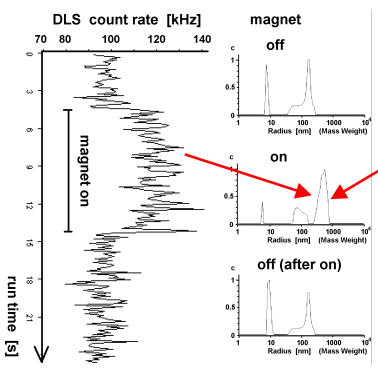


Fig.11: XAS of **Gd-target** during ASAXS of liposomes (ESRF-ID1)

Magnetic superstructure during therapy conditions : M-DLS & M-EM



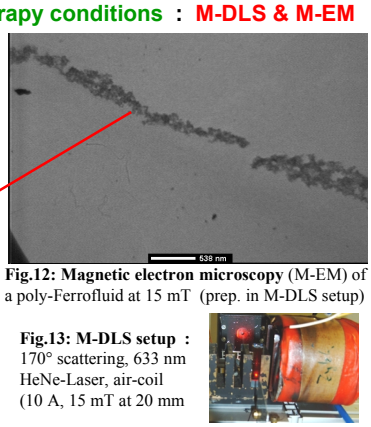


Fig.12: Magnetic electron microscopy (M-EM) of a poly-Ferrofluid at 15 mT (prep. in M-DLS setup)

Fig.13: M-DLS setup : 170° scattering, 633 nm HeNe-Laser, air-coil (10 A, 15 mT at 20 mm

Fig.14: Magnetic dynamic light scattering M-DLS of a shell poly-Ferrofluid at 15 mT indicates the reversible formation of a µm-sized magnetic superstructure, which is the structure persent during therapy.

The **structure of target nanoparticles** have for the **medical application** to fulfill **critical demands:**

1) The **structure** has to be smaller than 500 nm, because of embolic risks (blocking blood capillaries);
2) The particle **shape** has to be free of cell demaging edges;
3) The particle **entrapped target volume & concentration** has to be sufficient for radiation therapy;

Metallo-liposomes containing a **metal lipid** (DTPA-DMPE + Eu, Tb, Gd) shown in fig2a, 5a, as studied by EM and ASAXS at ESRF-ID1 (LS1554, 1718, 1843), were homogenous spheres (45 nm) but contained only 0.4 mM target metal bound outside (liposome post sonication lanthanide-loading).

The **double layer magnetic liposmes** containing an inner magnetite/ maghemite and an outer lipid layer [1] were investigated by time resolved neutron scattering TR-SANS at ILL-D22 during preparation (γ-Fe_2O_3-deposition), as shown in fig.6-8. Optimized liposomes of 250 nm size were target loaded [3] with Boron-diol esters for **neutron capture therapy** (B-NCT), and Gd-DTPA (Gd-NCT).

Poly-Ferrofluids for chemo- and radiation-therapy of cancer (fig.2d, 5d) and Magnetite cores for entrapping in magnetic liposomnes (fig.2c, 5c) were studied by unstained and magnetic electron microscopy, dynamic light scattering (M-DLS), SANS (ILL-D22) and (A)SAXS (ESRF-ID1: MD118). The commercial shell poly-Ferrofluids obtained by limited etching were **disk-shaped** (h=15 nm; d=50-100 nm) and consisted of dense iron oxide spheres embeded in disks of lower density. Material from double synthesis contained 3% **Hafnium** in the outer shell (ASAXS + XAS; analysis by L-edge shift).

Reversible formation of a **magnetic superstructure** from poly-Ferrofluids was observed at conditions equivalent to medical treatment by **magnetic DLS** and **magnetic EM**;. The dynamics has to be studied by time resolved methods, because the effect is important for the medial application, blood interaction.

Magnetic target liposomes containing **magnetite cores** (fig.2c, 5c) where analylized at the absorption L-edge in structure and target distribution by ASAXS at ESRF-ID1 (MD118) after loading with **water soluble heavy metal targets** (**Gd**-DTPA, **Tm**-DTPA, **Lu**-DTPA, **Hf**-DTPA, cis-**Platinun**). Prior to the ASAXS experiments the excess target outside of the liposomes was freshly removed by gel filtration chromatogrphy at the beamline (Sephadex G25m). Thus the L-edge (white line) XAS analysis yielded the entrapped target content, the ASAXS the target localization; both required for medical application (at K-edge). With magnetic Gd-liposomes the iron and gadolinium edges were observed in parallel. Target reference solutions (0.7-2 M) had to be diluted because of the very low transmission at the L-edge („black“; white lines). With the Lanthanide-DTPA's in citrate, pH7.5 no interference with liposome structure up to 0.7 M was observed. By energy (transmission) **Lutetium-DTPA** was optimal.

ASAXS and time-resolved scattering are required for medically shure target nanoparticles

References :

1. T. Nawroth, M. Rusp and R.P. May, Physica **B 350**, e635-638 (2004) "Magnetic liposomes and entrapping : time resolved neutron scattering TR-SANS and electron microscopy"
2. T. Nawroth, Ch. Meesters, H. Decker, M. Rusp, G. Le Duc, St. Corde, A. Bravin, H. Requardt, T. Brochard; ESRF experiment report **MD163-1** (2005) „Imaging of imobilised magnetic target.nanoparticles: liposomes and ferrofluids entrapping target for magneto-photodynamic therapy of cancer M-PXT“
3. T. Nawroth, R. Gebhardt, M. Rusp, I. Grillo, R.P. May; ILL experiment reports **8-03-413** & **9-10-661** (2004) „Time resolved neutron scattering of magnetic liposomes entrapping target“
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This report is sufficiently critical and actual, but limited on Boron therapy (B-NCT).

Acknowledgements

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