Magnetic Liposomes entrapping Target – Hollow Magnetic Nanoparticles for Bio-Medical Applications : Imaging, Neutron- and Photodynamic X-ray Therapy of Cancer

Thomas Nawroth[1a], Monika Rusp[1b], Günter Goerigk[2], Roand May[3], Peter Boescke[4]

www.mpsd.de

1) TU München , Physics Department , E17 & E22 , James-Franck-Str. , D- 85747 Garching , Germany
2) DESY-HASYLAB, Beamline B1 (Jusifa), and Forschungszentrum FZ, IFF, D-52425 Jülich, Germany
3) ILL Institut Laue Langevin, Beamline D22 , Avenue des Martyrs, F-38042 Grenoble Cedex , France
4) ESRF Synchrotron, Beamline ID01 , BP220 , Rue Jules Horowitz, F-38043 Grenoble,Cedex , France

Membrane Protein Structure Dynamics (MPSD) project

SRMS4 : Grenoble 23.-25.8.2004 Synchrotron Radiation in Material Sciences, BP07

DN2004 : Dresden 1.-4.9.2004 Deutsche Neutronenstreutagung, H06

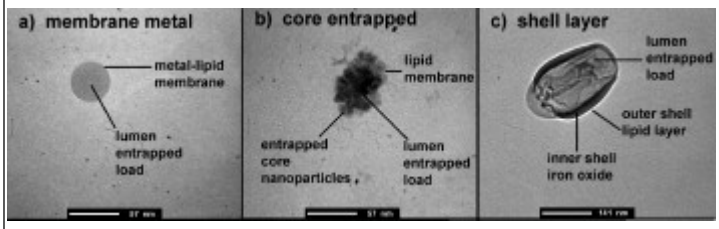
Liposomes – hollow Bio-Nanoparticles

Liposomes are **biocompatible hollow Nano-particles** covered by a lipid bilayer. They can be used as carriers for **material entrapped inside** the lumen and at the surface in cell-biological and medical applications [1].

Liposome applications can be improved, if the liposomes can be detected or manipulated by **magnetic forces** [2]. In this case the liposomes can either be dragged or deposited in a body region of interest, e.g. a tissue or **tumor**.

Magnetic liposomes entrapping target for **imaging (MRI)** and radiation therapy with **Neutron capture NCT** or **photodynamic X-ray therapy PXT** [3] of **cancer** were prepared by three methods yielding: **a) metal membrane** liposomes, **b) metal core** vesicles, and **c) metal shell** liposomes. During preparation **targets** as Boron-compounds and X-ray absorbers were **entrapped inside the liposomes**. This enables the application in cancer therapy by **local radiation therapy**, as well as **imaging diagnostics** or **rheological experiments** with magnetic tweezers. The formation of the liposomes and the internal metal structure was observed by ASAXS[6], time resolved neutron scattering TR-SANS[4,5], dynamic light scattering DLS and electron microscopy EM[9] using a stopped-flow mixing device. The internal volume was used for entrapping of **water-soluble target** material, which produces **secondary radiation of short range** upon irradiation, or **drug targeting** applications. The magnetic shell liposomes revealed a **size** of 50-400 nm, as required for applications in vivo (< capillary diameter).

Electron microscopy of Metallo-liposomes : Magnetic structure

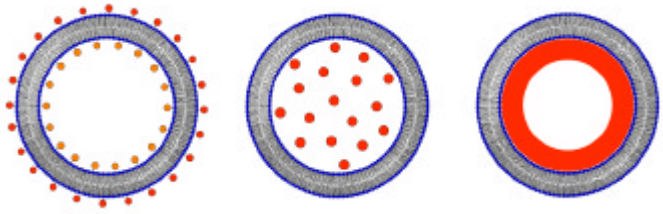


Non-stained electron micrographs of the three types of metallo-liposomes: a) membrane metal liposomes, b) core entrapped vesicles, and c) shell layer metal liposomes. The EM images depict the buried metal directly.

The membrane metal liposomes (a) contained 5% Europium-loaded chelate-head lipid [6,7] DTP-DMPE in DMPC, as in ESRF-experiments LS1554, 1843 (ASAXS at ID01). The entrapped core vesicles (b) contained 7 nm sub-nanoparticles (chromatographically purified ?-Fe_2O_3). The shell layer liposomes(c) had a double layer of 4.8 nm lipid and 5.9 nm Fe_2O_3 according to neutron scattering at ILL-D22 [5]. The original magnifications were: 52,000 (b); 52,000 (b); and 21,000 (c).

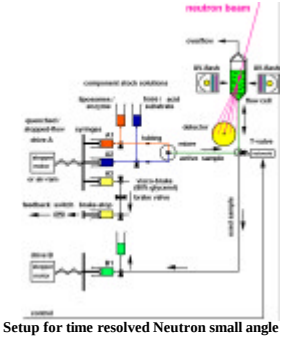
The lumen entrapped load was 1 M KCl / KJ; 0.1 – 0.25 M unique DPTA-Chelates [6] of Eu, Sm, Ho, Gd, Fe; 250 to 25 mM of the Boron acid diol-esters BGB, BBG, BBT [8] ; cis-Platin (1-10 mM); or dyes (10 mM BTB, BCG, Pyranin).

Structure principles of Metallo–liposomes : Magnetic liposomes

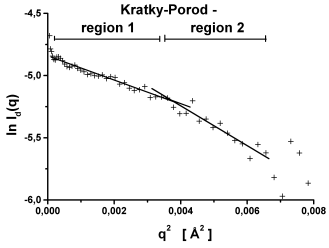


Metallo-liposomes can bear the **metal** supplying **magnetic properties** as well as **specific radiation interaction**, in three structures: **a) metal-lipid liposomes**, e.g. Me-DTPA-DMPE or Me -DTPA -StearylAmide, bearing the metal inside and outside (different metals possible); as used for ASAXS at ESRF-ID1 and DESY [1], **b) liposomes entrapping metal-oxide nanoparticles** $(Me_xO_y)_n$ or **metal-chelate** (DTPA-**Gd**, **-Sm**, **-Fe**, **-Ho**, **-Dy**, or **cis-Pt**, and **c) metal-oxide shell liposomes** bearing a double wall structure : lipid (outside) and metal-oxide (inside). For **biomedical applications** the metal **Me** is **Iron** or **Gadolinium**: Fe-chelate, or Gd-chelate (DTPA-lipid [6]), or Fe–oxide; e.g. ?-Fe_2O_3

Time resolved Neutron scattering TR-SANS : Structure generation



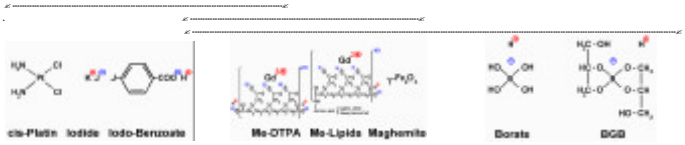
Setup for time resolved Neutron small angle scattering TR-SANS at ILL-D22. The crude liposomes (from fast GPC) with entrapped iron chelate and Boronate are subjected to a pH-jump by fast mixing with a stopped-flow device [5b]. The structure film is collected with logarithmic time scale (5.3 % time increase / frame).



Neutron scattering of crude magnetic liposomes from 10 g/l SbPC (purified Soy bean Phospholipids, mainly DiLinoleylPhosphatidylCholine) after a pH-jump at proton permeation equilibrium (30 min) as Kratky-Porod plot. The straight lines indicate layer thickness of d_1 = 4.81 ? 0.08 nm and d_2 = 5.94 ? 0.25 nm.

Target entrapping in Metallo–liposomes and radiation therapy

PXT Photodynamic X-ray Therapy | **MRI** Magnetic Resonance Imaging | **NCT** Neutron Capture Therapy



Metallo-liposomes can carry soluble or membrane-bound targets for photodynamic X-ray therapy PXT, magnetic imaging MRI, or neutron capture therapy NCT of cancer.

Most targets are bound by entrapping water-soluble compounds, metal-oxide sub-nanoparticles $(Me_xO_y)_n$, metal-chelate (DTPA-Gd, -Sm, -Fe, -Ho, -Dy, Iodine or cis-Pt in the lumen. The targets can be combined by co-entrapping during liposome preparation.

NCT : ^{10}B + n ⇙ ^{7}Li + alpha (short range secondary radiation) ⇙ DNA-inactivation @ tumor

PXT : Pt, I, Gd + ? ⇙ *n* e⁻ Auger electrons (short range secondary radiation) ⇙ DNA-inactivation @ tumor

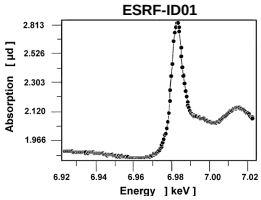
Local radiation therapy reduces the non-specific body load and radiation damages by two priniciples:

1) the target is concentrated at the tumor, e.g. with magnetic liposomes (hollow nanoparticles); and

2) the target produces tumor-killing secondary radiation of short range (< 30 µm) upon irradiation with neutrons (NCT) or specific X-ray absorption energy (PXT at absorption edge). For Platinum the energy is 75keV, which avoids body transmission problems (neutrons, iodine).

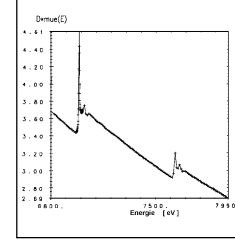
EXAFS / ASAXS

ESRF-ID01



EXAFS spectrum of 50 mM EuDTPA in H_2O from unique pH-shift preparation [7] for entrapping in magnetic liposomes obtained during **ASAXS** experiments at ESRF-ID01 (undulator source, 5 min./scan, experiment LS1554, Helium-cooled sample environment [ESRF lett.33/10, 10 www.mpsd.de]

DESY-HASYLAB-B1



EXAFS spectrum of 50 mM EuDTPA from pH-shift preparation [7] for entrapping obtained at DESY-HASYLAB-B1 (bending magnet, 4h / scan, 8 mm flat cell with adjustable pathlength).

Conclusions

- The liposome structure and the burried metal was observed by TR-SANS, ASAXS, DLS and EM.

- The metal - oxide was located by three concepts:

a) at metal-membranes,
b) as entrapped metal core nanoparticles, and
c) as double metal shell at the inner surface of the lipid layer.

-Target entrapping was examinated with Boronates, metal chelates, oxide and ions for NCT, PXT and MRI.

- The magnetic liposomes revealed a size of 50-400 nm, as required for appli-cations in vivo [5]. For 0.25M target the entrapping rate was 10^9/ liposome of 250 nm size.

References

[1] C. Oja, P. Tardi, M.P. Redelmeier, P.R. Cullis, *Biochim. Biophys. Acta* **2000**, 1468, 31. [2] Y.R. Chemia, H.L. Grossman, Y. Poon, R. McDermott, R. Stevens, M.D. Alper, J. Clarke, *PNAS* **2000**, 97, 14268. [3] M.-C. Biston, A. Joubert, J.F. Adam, H. Elleaume, S. Bohic, A.-M. Charvet, F. Esteve, N. Foray, J. Bolasso, Cancer Res. **(2004) 64**, 2317. [4] T. Nawroth; et al., *PhysicaB* **1989** 156, 489, [5] T. Nawroth, M. Rusp , R.P. May, **2004** *Physica B350/1-3S, E635*, [6] T. Nawroth, R. Gebhardt, K. Zwicker, G. Goerigk, DESY-HASYLAB Annual report **2000, part1 (online)**.
[7] T. Nawroth, Fresenius Z. Anal. Chemie **1992** 343, 174.
[8] ILL reports 8-03-413, 9-10-661, **2004**.
[9] T. Nawroth, M. Rusp, G. Goerigk, R.P. May, P.Boesecke, **2004** submitted.