Target Nanoparticles for Therapy – SANS and DLS of Drug Carrier Liposomes and Polymer Nanoparticles

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Abstract. Target Nano-Pharmaceutics shall improve therapy and diagnosis of severe diseases, e.g. cancer, by individual targeting of drug-loaded nano-pharmaceuticals towards cancer cells, and drug uptake receptors in other diseases. Specific ligands, proteins or cofactors, which are recognized by the diseased cells or cells of food and drug uptake, are bound to the nanoparticle surface, and thus capable of directing the drug carriers. The strategy has two branches: a) for parenteral cancer medicine a ligand set (2-5 different, surface-linked) are selected according to the biopsy analysis of the patient tissue e.g. from tumor.; b) in the oral drug delivery part the drug transport is enforced by excipients/ detergents in combination with targeting materials for cellular receptors resulting in an induced drug uptake. Both targeting nanomaterials are characterized by a combination of SANS + DLS and SAXS or ASAXS in a feedback process during development by synthesis, nanoparticle assembly and formulation.

1. Synopsis and Concept

Heavy metal loaded target Nanoparticles [1-3] shall improve therapy and diagnosis of severe diseases, e.g. cancer, by individual targeting [4,5] of drug-loaded nano-pharmaceuticals towards cancer cells. Specific protein or cofactor ligands, which are recognized by the diseased cells (cancer) or the drug uptake system, are bound to the nanoparticle surface, and thus capable of directing the drug carriers. In the concept a ligand set is coupled by a fast assembly technique (click link) in the very last step of the formulation. In the later clinical cancer therapy application the ligands set (2-5 different) will be selected according to the differential biopsy analysis of the patient tissue (tumor, healthy). The targeting nanomaterials are characterized and optimized by a combination of SANS+DLS [7] and SAXS, ASAXS, and radiotherapy tests with cells in a feedback process during development.

2. Materials and Methods

All materials were at least of analytical grade and obtained from Sigma-Aldrich Schnelldorf and Boehringer Ingelheim. The synthesis was done at the pharmaceutical institute of the Gutenberg University and Nanovel Ltd. & Co KG, Langenlonsheim (www.nanovel.de). The liposomes were prepared by the

film method with subsequent vortexing (1 min, 2400 rpm) yielding multilamellar vesicles MLV (160g/l), followed by addition of the drug stock solution and 11-fold extrusion through 100 nm polycarbonate membranes (Millipore). The DLS experiments were done as described earlier [7] at 633 nm (5 mW HeNe laser, Uniphase) with a 5000/EPP correlator, SPIC-II single photon detector (ALV, Langen) and projecting DLS optics devive with 170° backscattering ProSpecD501 (Nanovel). The neutron scattering experiments were done at the ILL at the D11 instrument, and the KWS-2 instrument at the FRM-II reactor, MLZ Garching, with neutrons of 0.6 nm wavelength (9% $\Delta\lambda/\lambda$).

3. Results and Discussion

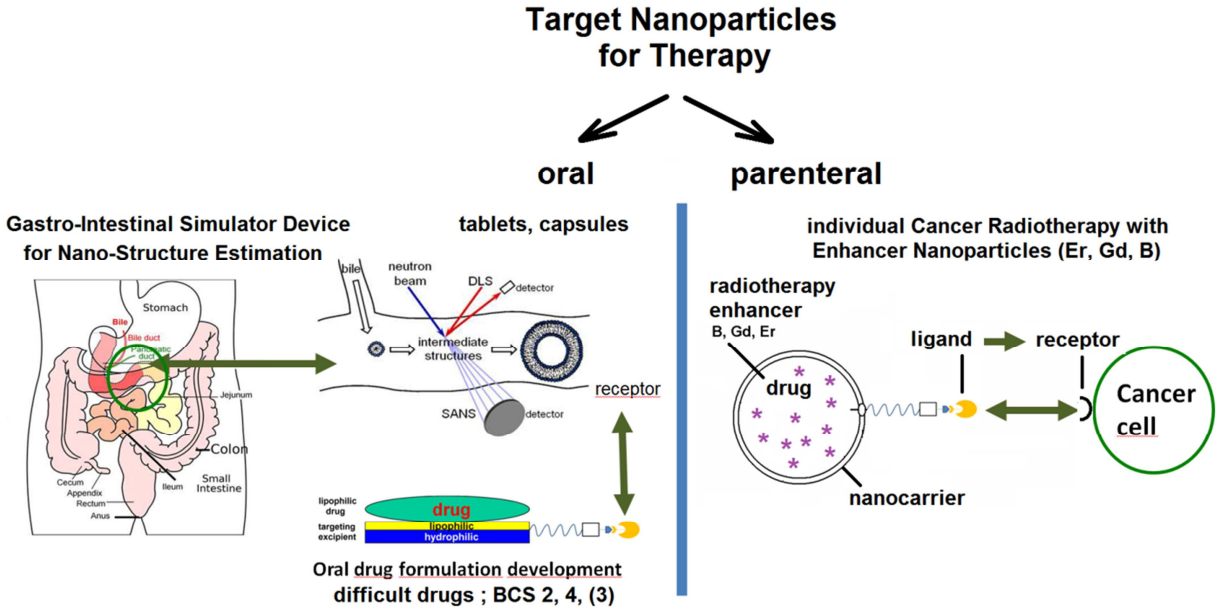

Fig.1: Target Nanoparticles can be applied by oral or parenteral ways. The ligands direct the drug carriers to specific cell receptors. The nanoparticle-ligand structure and dynamics, and the intermediate nano-assemblies with proteins and bile are studied by a combination of SANS and DLS.

We synthesize and combine targeting excipients for oral drug delivery and modifiers of metal drug loaded nanoparticles for radiotherapy, which bear a specific receptor/carrier ligand domain at the end of a linker domain. The other end domain is hydrophobic, i.e. capable of drug binding (drug co-drug complex for oral application), or embedding into the surface of hydrophobic drug carrier nanoparticles for parenteral supply. In both cases the result is a drug carrier directed to specific receptor, as depicted in fig.1. All components are biocompatible and bio-degradable, which limits the selection of chemical structures, but prepares pharmaceutical licensing and medical application. The nanoparticles are biodegradable polymer [3], e.g. PLGA; ferrofluids [6], micelles [5,11,12], lipidic particles [7] or liposomes [8-10]. For oral application the ligand coupling is covalent, while the linkers for parenteral nano-pharmaceuticals are bound by cleavable S-S-bonds as terminal step of the preparation of a person- and case-specific medicine.

The structure of the modified nanoparticles is analyzed by neutron small angle scattering SANS in combination with dynamic light scattering DLS [8,11,12], SAXS and metal specific X-ray scattering ASAXS, while the therapy effect of the drug is investigated with kinetic cell cultures as tumor models [1]. The attached protein shell resembles the protein corona formed by nanoparticles in the blood [13], but it consists of specific receptor targeting proteins, which are bound covalently. The particles in pharmaceutical samples depict a size scale of 1 nm to 100 µm in parallel. The nano-solutions are highly concentrated (up to 30 %, crowded conditions). Thus a combination of SANS, (A)SAXS and DLS is required [8], the latter as back scattering to avoid multiple scattering.

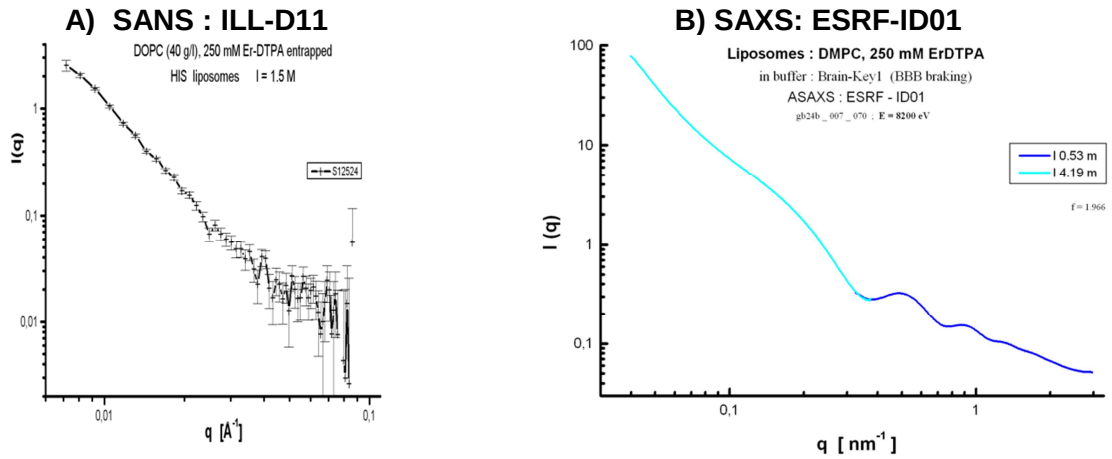

Fig.2: Structure analysis of lanthanide entrapping liposomes (0.25M ErDTPA with (A) neutron small angle scattering and (B) synchrotron X-ray small angle scattering SAXS.

Fig.2 depicts the structure analysis of liposome nanoparticles for radiotherapy with entrapped lanthanides [1-3] by SANS (ILL-D11) and SAXS (ESRF-ID01). The neutron small angle scattering gave radius of gyration (R_g = 21.3 ± 0.12 nm) and membrane span (4 nm) of the metal nanoparticles in highly concentrated D_2O buffer (1.5 M ionic strength). The (A)SAXS investigation revealed a metal-membrane interaction, which is indicated by the oscillation of the scattering curve in Fig.2B. The radiotherapy tests with cells as kinetic tumor model [1] depicted an increased photon induced cell inactivation, when the appropriate drug release was observed ($t_{1/2}$ = 1 h @ c > 0.1 M entrapped drug).

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