Experimental report

16/02/2018

Proposal: 9-12-363 **Council:** 4/2014

Title: Domain Structure and Dynamics of Target Nanoparticles for cancer therapy - Polymer and Liposomes with surface bound protein (TR-SANS)

Research area: Biology

This proposal is a new proposal

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Samples: Cholesterol
DMPC, dimiristoyl-phosphatidylcholine
Cholesterol-PEG-Poly-glycerol (PG stealth-lipid)
PLGA (Poly-Lactate-Glycolate)
bovine serum albumin (BSA), thiolated
DOPC, dioleyl-phosphatidyl-choline (lecithin)
Cholesteryl-Hemisuccinyl-Hexamethylenediamido-Glycyl-Glycyl-Cysteamide
Cysteamido-DTPA
DMPC-d54, dimiristoyl-phosphatidylcholine deuterated

Instrument	Requested days	Allocated days	From	To
D33	2	0		
D22	1	0		
D11	3	2	02/12/2014	04/12/2014

Abstract:

Targeted Nanoparticles as drug carriers for cancer therapy are equipped for cell recognition by an artificial protein-ligand shell. These will be Lanthanide loaded polymer (PLGA, patent: Gutenberg-university), and liposomes (fast development system).
Here PLGA and liposomes with entrapped radio-therapy (RT) enhancers (B, Gd, Er) are surface linked to serum albumin BSA through a cleavable peptide-SH-linker. BSA is recognized by cells (clinical studies). The S-bridge binding principle is general applicable.
The proteo-NPs shall be investigated in the structure and dynamics by SANS in combination with DLS in a size range of 1nm - 50ìm & time resolved TR-SANS : double-beam technique.
The following structure aspects shall to be addressed:
1) How stable are nanoparticles if: a) a high concentration of target is incorporated?;
b) when and how the ligand-protein is inserted (reconstitution in membranes, PLGA) ?
c) upon beam-absorption in the Nanoparticles: TR-SANS + neutron absorption (B, Gd)?
2) How is the NP-ligand-protein structurally organized, especially in ligand exposition?
- D-contrast variation (solvent and lipid) in SANS
3) Stabilization of the NPs by detergent, sugar

Experiment N° **9-12-363** **on experiments @ ILL - D11**

Domain structure and dynamic of target nanoparticles for cancer therapy – Polymer and Liposomes with Surface bound Protein (TR-SANS)

Experiments: Thomas Nawroth, Lidija Krebs, (Inst. Pharmacy and Biochemistry Mainz); **Lc:** Ralf Schweins (ILL)

Target nanoparticles [1] bear a surface modification for cell specific direction of the drug carriers towards special cells, e.g. in a tumor. In case of radiotherapy drugs [2], the terminus "target nanoparticles" has an intended double meaning: biological cell-targeting (cancer), and physical beam-targeting by special isotopes, elements for neutron therapy NCT (Boron, Gadolinium, Lithium) or for photon therapy PT (Erbium, Gadolinium, Platinum, Bismuth).

Target nanoparticles, manly liposomes, for cancer therapy were constructed by mixtures of a neutral matrix material (98%), novel amphiphilic target-anchors, capable of ligand protein binding by formation of a di-thio-bond (click-link technology), and a dummy drug load, without neutron activation problems. The nanoparticles were investigated by SANS with the solvent and specimen D-contrast method, and by time resolved SANS (TR-SANS) upon binding of SH-bearing bovine serum albumin (BSA) to contrast-matched liposomes.

For this experiment, the liposomes were constructed from the deuterated lipid DMPC-d_{54} (deuterated fatty acid) in order to yield a high contrast of the bound (H)-protein, and novel cholesterol-based amphiphilic anchor-linkers. These were synthetized [3] and introduced by up to 2% into the lipid film, from which the liposomes (SUV) were prepared and investigated at various D_2O-content by SANS, and dynamic light scattering DLS. After activation by di-thiopyridine BSA as target SH-protein was added. The protein binding was observed by time resolved neutron scattering (TR-SANS) to D-matched liposomes (in 86% D_2O), i.e. using the scattering technique which we introduced earlier for the investigation of membrane proteins in situ (in D-matched liposomes) [4]. By this ligand-loading strategy, a later medical use shall lead to patient-specific medicine by surface modification of preformed drug-liposomes (stock) with a specific ligand protein, according to a tumor receptor analysis, as the very last step in therapy preparation, about 1-3 hours before patient treatment.

Fig.1 depicts two successful anchor-linkers: Cholesterol-succinyl-Spacer-linker-Thiol with a length of 22 Å (Chol-L22) and 40 Å (Chol-L40), for incorporation into the nanoparticle surface, and SH-protein loading after activation of the linker. As shown in fig.2 a short peptide part (GG) leads to an early ligand protein attachment by additional H-bonds prior to the formation of the SS-bridge (dual link bond). Figures 1,2 show the dimensions for anchor-linker and the BSA (67000) as ligand protein for cell receptor targeting [5]. The proteo-liposomes form a protein-corona [6] by artificial membrane proteins. The anchor-linkers were investigated in DMPC-d_{54} liposomes with and without protein loading. As control empty liposomes (without the anchor-linker entity) were investigated upon BSA addition. All samples were investigated after SANS by DLS in the same cuvettes as shown earlier [7].

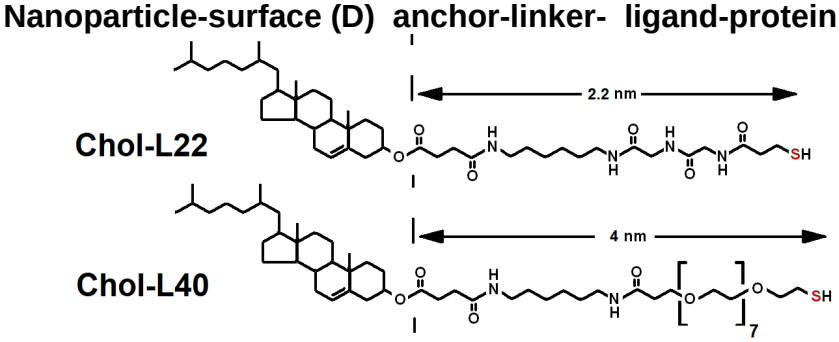


Fig.1.: Modular target Nanoparticles for radiotherapy of cancer, PLGA-polymer and liposomes, bear protein ligands for cell recognition. Structure and dynamics of the protein shell shall be investigated by D-contrast variation (SANS+DLS), and time resolved (TR-) SANS.

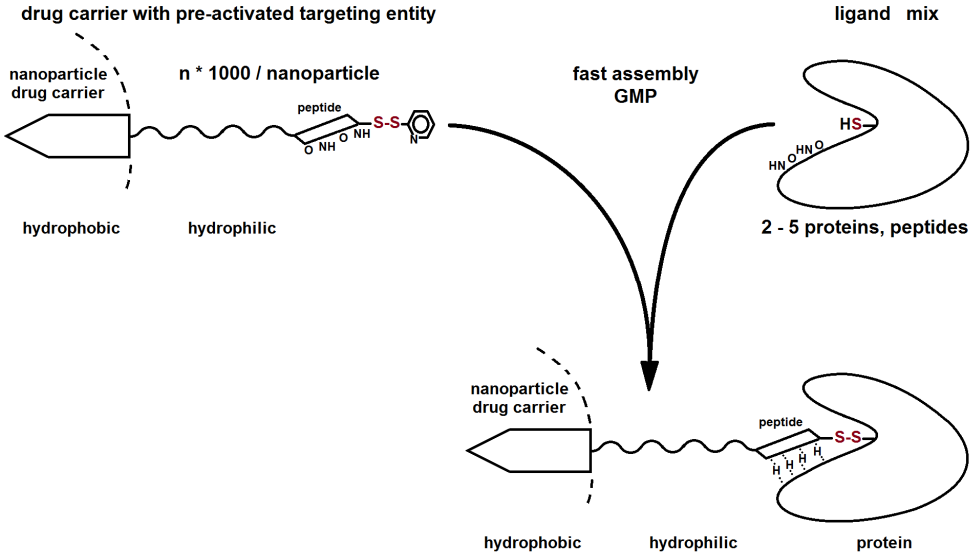


Fig.3: The ligand-protein is bound to the activated anchor-linker by two domains sequentially, according to our dual link concept: A fast attachment to a hydrophilic / peptide entity (Gly-Gly-… and/or PEG…) by hydrogen bonds is followed by the formation of a stable covalent S-S-bridge.

By the limited beamtime the experiments were focused on the liposome part (aims 2, 3 of the proposal), with PLGA polymer-NP initial experiments on contrast matching were done.
Fig.3a depicts the neutron scattering of empty liposomes (SUV from DMPC-d_{54}), 3b) the liposomes with anchor (DMPC-d_{54}, 2% anchor Chol-L22) both in high contrast buffer (97 % D_2O), while fig.3c depicts the anchor liposomes at lipid matching (86% D_2O buffer, 2% glucose, pH7.2). The SANS of liposomes without protein in D_2O (fig.3a,b) depicts a broad side maximum at ~0.15 A^{-1}, resulting from the non-deuterated headgroups of DPMC-d_{54}; the structure with and without the anchor-linker is equivalent. At lipid near-matching (fig.3c, 83.8%D) this is the dominant signal, the particle-SANS, q<0.1 A^{-1} is mostly diminished.

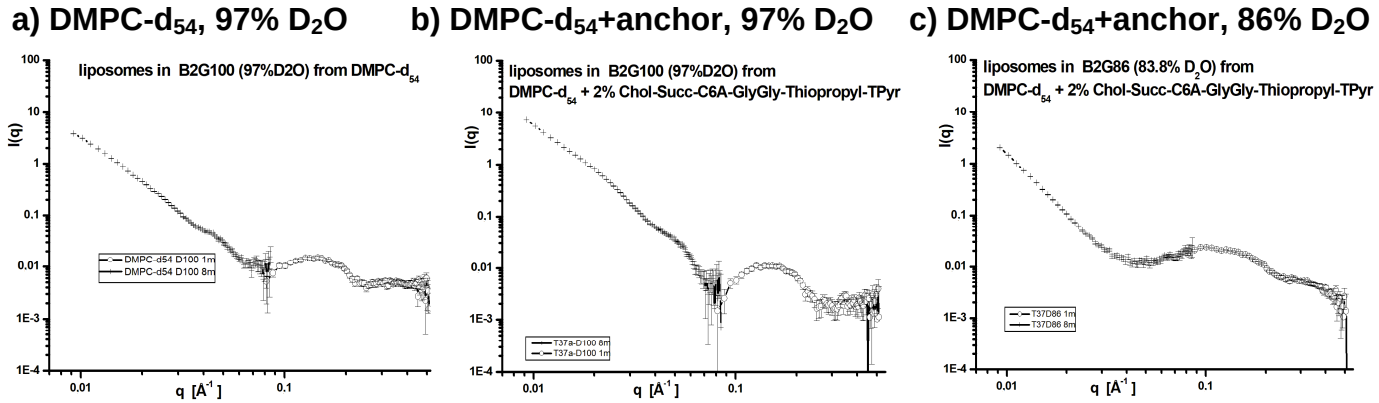


Fig.3: SANS of liposomes (SUV) from DPMC-d54 a) without addition, b) with 2% activated cholesteryl-anchor-linker Chol-L22 in D_2O-buffer, 2% glucose (97% D); and c) with anchor-linker at lipid matching conditions (86% D-buffer, 83.8% D); all without protein addition.

For the observation of the ligand protein loading of the pre-activated drug liposomes by time resolved SANS (TR-SANS), including a dual detector distance change, we have developed the DD-TR-SANS strategy depicted in fig.4. By this an expensive stopped-flow device is obsolete, if an initial SANS-delay of ~ 1 minute is acceptable, and if a time resolution of 1 minute is sufficient: The sample is prepared by fast manual mixing inside the SANS-cuvette (2 experimentalists); after the closing interlock system the pre-set SANS-script is started. This includes a series of SANS time frames with logarithmic middle-frame time, according to fig.3a. After frame 9 (24 min), the detector distance is changed without beam, in our case between 8 and 34 m, which takes as long as the “blind” frame 10” (8 min). The next SANS-frame 11, and optional further, are observed. Then the sequence is repeated, starting with the alternate distance, than all. By this shot-shot differences in SANS can be avoided, i.e. frame 9 and 11 of one shot give a complete SANS-set, if the sample dynamics is slow enough.

a) TR-SANS time frames

1: (mix-shot; interlock, start SANS)
2 : 1min, start auto-framing script
3: 1 min
4: 1 min
5: 2min
6: 2 min
7: 4 min
8: 4 min
9: 8 min
10: detector distance change (8min)
11: 16 min
optional: further frames

b) distance-time schedule

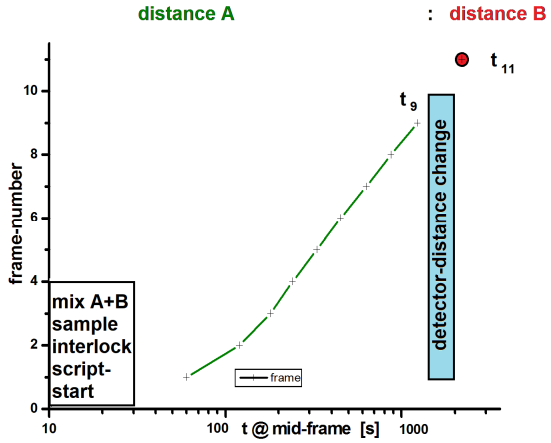
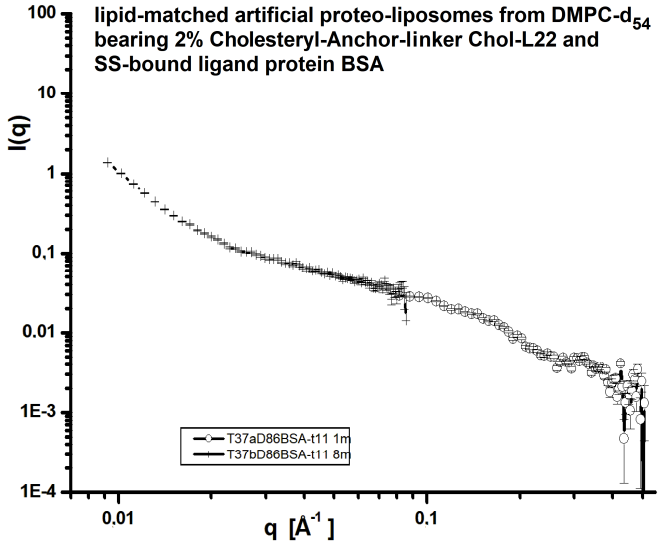


Fig.4: TR-SANS strategy with fast manual mixing and start (1 min delay), logarithmic time.

The SANS of anchor-linker liposomes according to fig.1 and covalent ligand-protein loading with SH-BSA after 36.5 min protein coupling time (t_{11}), all in lipid-matching D_2O-buffer B2G86, is depicted in fig.5a. The difference plot to protein-free reference liposomes in fig.5b yielded the SANS of the protein *in situ*, i.e. while bound to anchor-linker by an SS-bridge: The bound protein (artificial membrane protein) *in situ* appeared slightly smaller (–5%) as the free BSA reference (without anchor-linker, $R_g = 3.487 \pm 0.037$ nm) in 86% D_2O-glucose-buffer. Thus the bound protein appears to be monomer on the membranes. In further projects the expensive D-lipid shall be exchanged by cheaper DOPC, and polymer-excipient (PLGA).

a) DMPC-d_{54}+anchor+BSA for 36 min., 86% D_2O



b) Difference-SANS yields protein *in-situ*

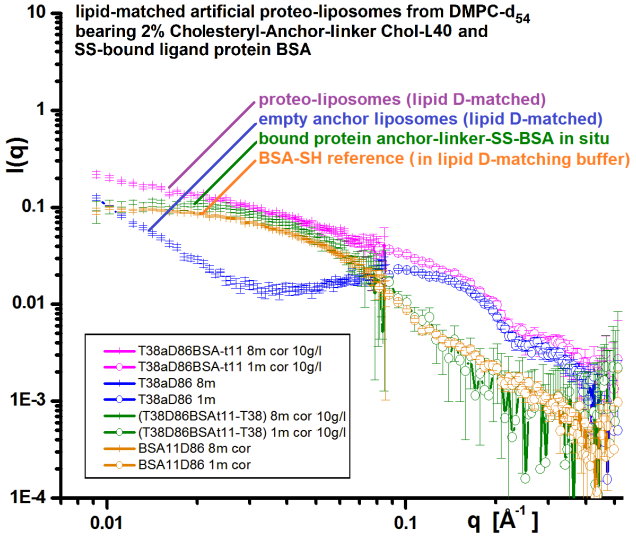


Fig.5: a) SANS of D-matched proteo-liposomes from DPMC-d54 with 2% activated cholesteryl-anchor-linkers in 86% D_2O-buffer B2G (2% glucose) + BSA; b) difference-SANS with protein-free reference yields the in-situ SANS of the bound protein achnchor-link-SS-BSA.

References:

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