Experimental report

03/03/2018

Proposal: 9-13-593 **Council:** 10/2014

Title: Therapeutic nanoparticles with receptor targeting by polymer-ligand constructs for individual cancer therapy

Research area: Other...

This proposal is a new proposal

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Samples: DMPC-d54
PLGA (Poly-Lactate-Glycolate)
Boron-Phenylalanine (BPA)
PLA (Poly-Lactate)
Cholesteryl.succinyl-Hexamethylenediamido-GlycylGlycyl-ThioPrionamide
CHAPS (detergent)
Erbium-DTPA (Radiotherapy agent, no N-activation)
GdDTPA*Tris2

Instrument	Requested days	Allocated days	From	To
D11	3	2	22/06/2015	24/06/2015
D33	2	0		
D22	1	0		

Abstract:

Nanoparticles for individual cancer therapy are modified by NP-core penetrating polymer modules for cell receptor targeting. In contrast to surface binding cholesterol based constructs, these are based to short polyester chains (PLA), which integrate into the nanoparticle core. The therapeutical nanoparticles will be Lanthanide loaded polymer (PLGA, patent: Gutenberg-university), and to a minor extent liposomes (fast development system). The radio-therapeutic entites are non-toxic compounds of elements depicting a high cross section for xray/gamma and neutron beams, Gadolinium, Erbium and Boron, which produce free radicals upon external irradiation. The individual targeting (person and case specific) is obtained by fast binding of receptor ligands (protein: Albumin, thiolated Transferrin; and small ligands as hemin, folate) by disulfide bridges.
The structure shall be estimated by deuterium contrast SANS, the stability and development in cell culture media by time resolved SANS in combination with dynamic light scattering DLS. The cell targeting with the ligand excipients (0.5 5% of the polymer) shall be compared with a detergent stabilization of the nanoparticles.

Experiment N° **9-13-593** **on experiments @ ILL - D11**

Therapeutic nanoparticles with receptor targeting by polymer-ligand constructs for individual cancer therapy

Experiments: Lidija Krebs, Thomas Nawroth, Christian Siewert (Pharma Inst. Uni Mainz); Raphael Johnson (Uni Mainz and Kwame Nkrumah University of Science, Kumasi, Ghana)

Lc: Ralf Schweins (ILL)

In the targeted-Nanotherapy project, a cooperation of the Gutenberg-University and the University-Medicine (clinics) Mainz, a novel method of cancer therapy with case-specific target nanoparticles is developed, radiotherapy by enhancer neutron capture and photon radiation therapy NCT & PT. The therapeutic nanoparticles work by two principles: a) the nanoparticles are guided towards the cancer cells by surface modifications by recognition and uptake receptor ligands, presented after binding to a linker structure; b) the therapy beam is absorbed by entrapped material of high cross sections, Gadolinium+Boron, plus Erbium.
Target nanoparticles [1], e.g. for radiotherapy [2] bear a surface modification for cell specific direction of the drug carriers towards special cells, e.g. in a tumor. The nanoparticles, manly foam-like double emulsion PLGA-polymer particles (w/o/w) [2], plus some liposomes, for cancer therapy were constructed by mixtures of a neutral matrix material (98%) and novel amphiphilic target-anchors, capable of ligand protein binding by formation of a di-thio-bond (click-link technology). The nanoparticles were investigated by SANS with the solvent D-contrast method, and by time resolved SANS (TR-SANS) upon binding of SH-bearing ligand-proteins to contrast-matched nanoparticles. For this experiment, the PLGA-nano-particles were constructed from the bio-degradable polymer Resomer 502H (Evonik, Boehriger), which is licensed for human application. Proteins, which act as cell address ligand in nano-therapy, were attached to these by two methods: a) hydrophobic attachment (Ovalbumin-PLGA), and b) after covalent S-S-bridging to 2% activated SH-anchor polymer according to fig.1. The novel material was synthetized [3] from oligomeric Amino-PLA of two different chain length (A-PLA2500, A-PLA4000) and various linker chains including a short peptide (S## in fig.1; ## = synthesis number code [3]). Structure and dynamics of the nanoparticles, pure and upon SH-protein shell formation, were investigated by D-contrast variation (SANS+DLS), and time resolved (TR-) SANS. A part of the proteins were equipped with additional SH-groups by iminothiolan (Trout's reagent), which can convert any protein to a SH-protein by lysine modification. The ligand proteins were:

- OvA: Ovalbumin (amphiphilc and native SH-group)
- BSA: Bovine serum albumin (amphiphilc and 1 native SH-group)
- BSA-tSH artificially thiolated Bovine serum albumin (3-5 SH-groups @ Lysine)
- Tf: native Transferrin (no SH-group, control)
- Tf-SH: artificially thiolated Transferrin (3-5 SH-groups @ Lysine)

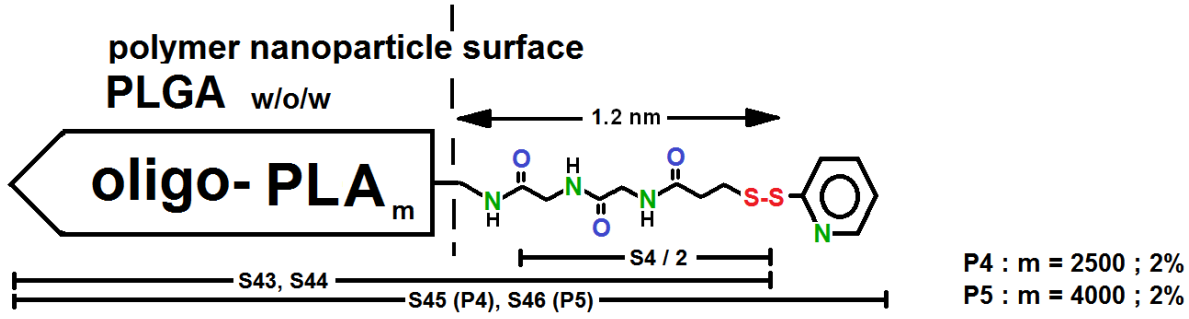

Fig.1.: Modular target Nanoparticles for radiotherapy of cancer, PLGA-polymer and liposomes, bear protein ligands for cell recognition after coupling to activated anchor, in this case based on Amino-PLA.

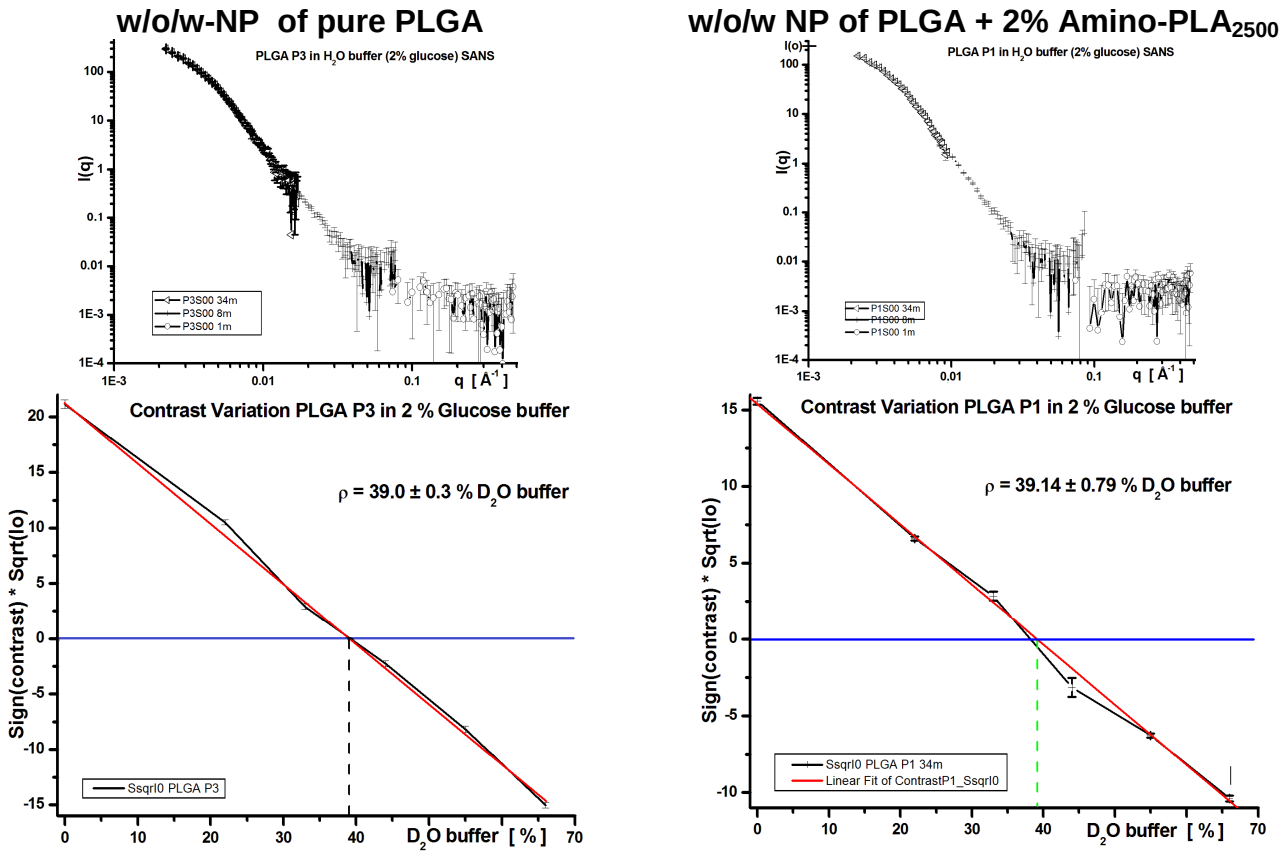

Fig.2.: SANS (upper, in H_2O buffer B2G, 2% glucose, 10 mM HEPES) and D-contrast variation of PLGA double emulsion nanoparticles without and with 2% target-ligand anchor-root Amino-PLA2500.

The SANS investigation of foam-like PLGA double emulsion nanoparticles (w/o/w) with and without Amino-PLA anchor-root (2%) in Glucose-buffer is shown in fig.2. As depicted in SANS (upper) and D-contrast variation (lower), the A-PLA anchor doesn't change the structure; the particles appear homogenous in SANS and DLS (not shown). The scattering length density (SLD) of these foam-NP differ from that of massive PLGA-nanoparticles by a precipitation method [8]. The SLD of the w/o/w-nanoparticles is 39.0 ± 0.3 % D_2O, i.e. rather similar to that of proteins (43%). Thus the w/o/w-PLGA polyester nanoparticles can be described as O-analogon of a protein, which would be poly-Glycine-Alanine (M= 15000).

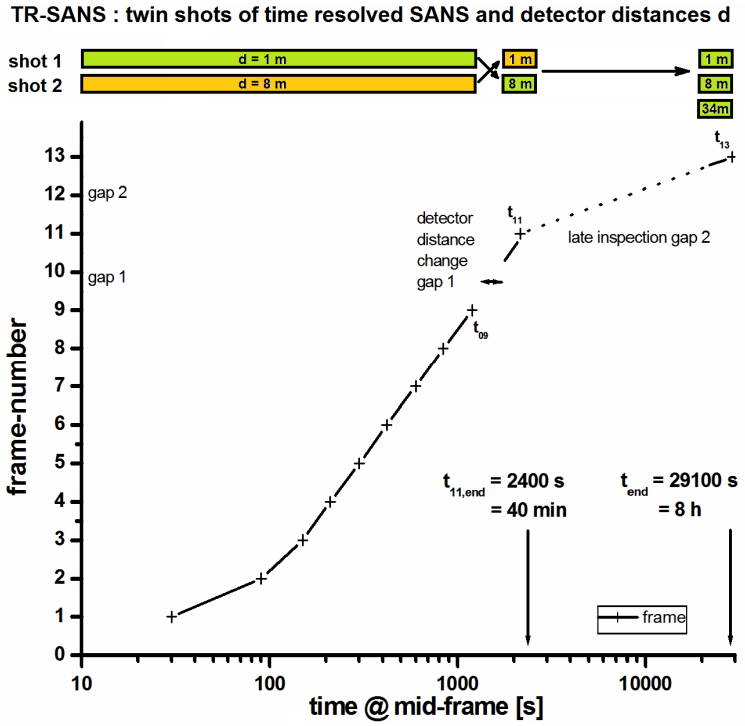

Fig.3.: Improved scheme for time-resolved neutron scattering TR-SANS of protein binding to contrast matched nanoparticles with twin shots and observation at 3 detector distances.
In each of the twin SANS-shots the detector distance is changed after time frame t09 the first time (change between 1m and 8m distance during "blind-frame" t10). A late time point "t13" is observed, e.g. after 8h (in sample equilibrium) at 1m, 8m and 34 m detector distance. The whole twin sequence is then repeated, at least for a short observation time (t01-t09). The twin strategy avoids shot-to-shot variations.

The investigation of the SH-protein binding to the w/o/w-PLGA particles with A-PLA anchor and additional linkers (fig.1), and by hydrophobic attachment was done by time resolved SANS upon SH-protein coupling to the contrast matched nanoparticles (in 38.5% D_2O-buffer). By the small excess contrast of the protein in PLGA-matching buffer (fig.2), the time resolved observation was limited to BSA, BSA-tSH, Tf and Tf-SH. Further samples were investigated in a two time point scheme (SANS before and 1 d after protein binding).
The result of the TR-SANS investigation of covalent coupling of BSA forming an artificial protein corona on D-matched PLGA w/o/w double emulsion nanoparticles bearing 2% of the Amido- PLA construct (fig.2) is shown in fig.4. The protein corona [6] formed in 30min. and only slightly increased the particle size. Results were presented at the BMBF-conference [9].

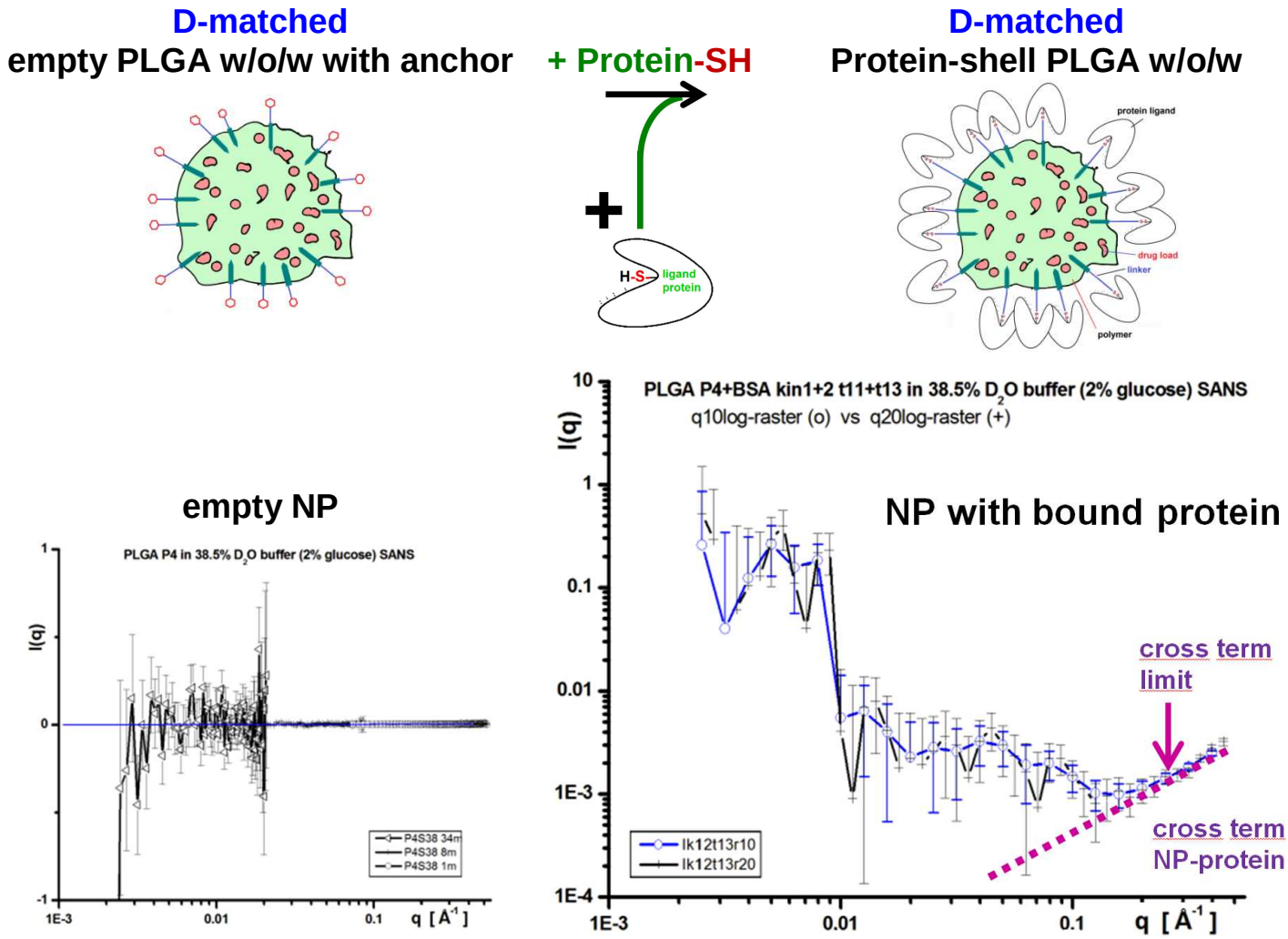

Fig.4.: Artificial protein corona (BSA) of PLGA-w/o/w-NP with 2% Amido-PLA-anchor detected by TR-SANS.

References:

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