Experimental report

01/03/2021

Proposal: 9-13-857 **Council:** 4/2019

Title: Probing the interaction between lipidic drug nanoparticles and low density lipoproteins with SANS

Research area: Soft condensed matter

This proposal is a continuation of 9-13-696

Main proposer: Frederic GOBEAUX

Experimental team: Fabienne TESTARD
Patrick GUENOUN
Frederic GOBEAUX

Local contacts: Anne MARTEL

Samples: Low Density Lipoproteins
Squalene-adenosine nanoparticles

Instrument	Requested days	Allocated days	From	To
D22	0	2	04/02/2020	05/02/2020
			09/09/2020	10/09/2020
D33	2	0		

Abstract:

If we want to use nanoparticles (NPs) in biomedical applications, it is of utmost importance to understand their interactions with complex biological media. We have thus started to investigate these interactions using squalene adenosine NPs, which have shown a positive effect in neuroprotection of spinal cord in rodents, and model biological media by combining cryo-TEM, small angle neutron scattering and various spectroscopies. We have already demonstrated that BSA could partially disassemble SqAd NPs by forming SqAd-BSA complexes. In the proposed scheme, the NPs act as ~100 nm reservoirs in the bloodstream and the BSA act as transporter of the SqAd monomeric bioconjugate. Other transporters of lipids in the plasma are lipoproteins, which are large (20-30 nm) hydrosoluble protein-lipid complexes. Previous studies have demonstrated that they were indeed potent transporters of SqAd bioconjugates. The main aim of the proposed experiment is thus to decipher the effect of the low density lipoproteins (LDL) on the size and structure of the SqAd NPs and conversely the effect of the SqAd on the LDL complexes by analyzing several mixtures of both components in D2O.

Experimental Report / Experiment 9-13-857 / D22

Probing the interaction between lipidic drug nanoparticles and low-density lipoproteins with SANS

02/02/2020 & 09/09/2020

Frédéric GOBEAUX, Fabienne TESTARD, Patrick GUENOUN, Anne MARTEL

Deciphering the fate of nanodrugs in complex biological media is a prerequisite before they can be used in human body. Previous experiments carried out at the ILL (experiment 9-13-696 reported in ref[1]) have demonstrated that squalene-adenosine (SQAd) nanoparticles suspension have a relative colloidal stability in fetal bovine serum (FBS) and in the presence of bovine serum albumin (BSA). Furthermore, it was demonstrated that BSA could partially disassemble SQAd nanoparticles by forming SQAd-BSA complexes. Low-density lipoproteins (LDL) are large (20-30 nm) hydrosoluble protein-lipid complexes and are another important component of plasma involved in the transport of cholesterol and other lipophilic molecules. As such, several studies have already pointed their role in the uptake of squalene-based nanodrugs[2–6] and probably other lipid-based nanodrugs. However, the specifics of the supposed interaction are still unknown. The aim of the present experiment was to clarify this point.

Before assessing these interactions, we wanted to get more details of the inner structure of the nanoparticles formed by the SQAd bioconjugate. We have thus performed a contrast variation experiment (Figure 1). The minimum of contrast for the SQAd nanoparticles was found to occur around 30% D_2O. Although this variation of contrast does not make any specific feature appear or disappear, which denotes a homogeneous organization inside the nanoassemblies, this information could be of valuable use for future studies of adsorbed proteins around the NPs.

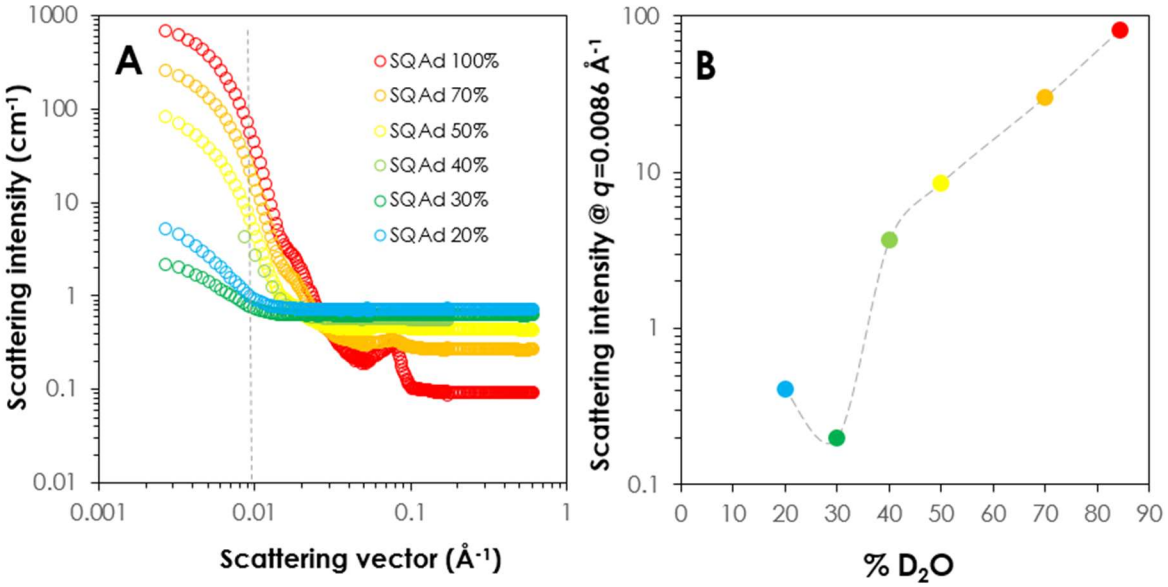

Figure 1 : Contrast variation experiment (SI) ***A)*** *SANS patterns of a 4 mg/ml suspension of SQAd nanoassemblies in different D_2O/H_2O mixtures (respectively 100%, 70%, 50%, 40%, 30% and 20% D_2O).* ***B)*** *Plot of the scattering intensity at q = 0.0086 $Å^{-1}$ (measured on the background-subtracted patterns at the position indicated by a dashed grey line in panel A) as a function of the D_2O content of the solvent. The dashed grey line is a guide to the eye.*

We have then analysed the scattering patterns of LDL and different mixtures of SQAd nanoparticles and LDL. First, we noticed that the mixtures were stable over time (admittedly, the LDL saline buffer was removed beforehand by dialysis against D_2O). As expected, the scattering pattern of both components in the mixtures can be easily differentiated because of the large difference in size. Moreover, two series of mixtures were prepared: one with a constant concentration of nanoparticles with different excesses of LDL and one with a constant concentration of LDL and different excesses of nanoparticles. However, in both series the scattering signals obtained can be reproduced by linear combinations of the signals of the individual components using the concentration ratio as coefficients. Overall, these experiments suggest that both components coexist in the mixtures without any structural modification or interaction. We hypothesize that the uptake of the SQAd bioconjugates is conditional on the preliminary disassembly of the nanoparticles (like BSA, e.g.) or the presence of third party molecules to catalyse the lipid transfer like phospholipid transfer proteins.

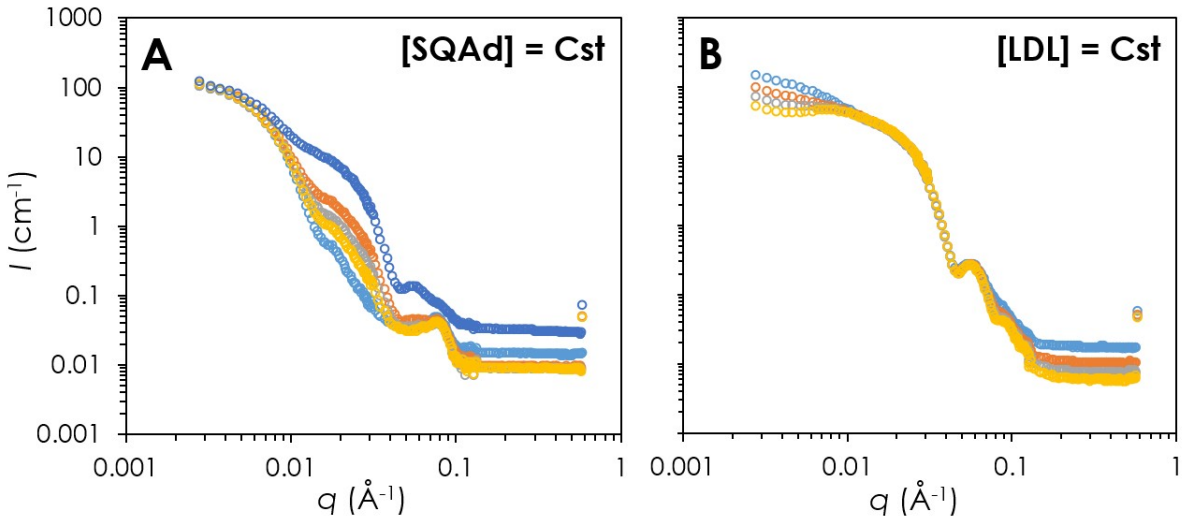

Figure 2 : ***A)*** *SANS patterns of mixtures of SQAd nano-assemblies and LDL with [SQAd] = 0.5 mg/ml and [SQAd]/[LDL] = 5, 10 and 20.* ***B)*** *SANS patterns of mixtures of SQAd nano-assemblies and LDL with [LDL] = 1 mg/ml and [LDL]/[SQAd] = 2, 5 and 10.*

The experiments were performed without any problem.

1. Gobeaux, F. *et al.* Albumin-driven disassembly of lipidic nanoparticles: the specific case of the squalene-adenosine nanodrug. *Nanoscale* **12**, 2793–2809 (2020).
2. Gaudin, A. *et al.* Transport Mechanisms of Squalenoyl-Adenosine Nanoparticles Across the Blood–Brain Barrier. *Chem. Mater.* **27**, 3636–3647 (2015).
3. Sobot, D. *et al.* Conjugation of squalene to gemcitabine as unique approach exploiting endogenous lipoproteins for drug delivery. *Nature Communications* **8**, 1–9 (2017).
4. Sobot, D. *et al.* Circulating Lipoproteins: A Trojan Horse Guiding Squalenoylated Drugs to LDL-Accumulating Cancer Cells. *Molecular Therapy* **25**, 1596–1605 (2017).
5. Yesylevskyy, S. O., Ramseyer, C., Savenko, M., Mura, S. & Couvreur, P. Low-Density Lipoproteins and Human Serum Albumin as Carriers of Squalenoylated Drugs: Insights from Molecular Simulations. *Mol. Pharmaceutics* **15**, 585–591 (2018).
6. Rouquette, M. *et al.* Squalene-adenosine nanoparticles: ligands of adenosine receptors or adenosine prodrug? *Journal of Pharmacology and Experimental Therapeutics* jpet.118.254961 (2019) doi:10.1124/jpet.118.254961.
7. Stuhrmann, H. B. *et al.* Neutron scattering study of human serum low density lipoprotein. *Proc Natl Acad Sci U S A* **72**, 2270–2273 (1975).
8. Meyer, D. F., Nealis, A. S., Bruckdorfer, K. R. & Perkins, S. J. Characterization of the structure of polydisperse human low-density lipoprotein by neutron scattering. *Biochemical Journal* **310**, 407–415 (1995).