Experimental report

06/03/2019

Proposal: 9-13-781 **Council:** 4/2018

Title: Sq-siRNA nanoparticles for Charcot-Marie-Tooth disease: formulation and colloidal stability in serum albumin solutions

Research area: Soft condensed matter

This proposal is a continuation of 9-13-696

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Frederic GOBEAUX

Local contacts: Isabelle GRILLO

Samples: BSA
Squalene-SiRNA
Serum bovine albumine

Instrument	Requested days	Allocated days	From	To
D33	1	1	30/06/2018	01/07/2018

Abstract:

In the framework of the Nanoprotection Labex program, the squalenoyled concept for the fragile macromolecules (siRNA) is used to preserve their efficacies and propose a new treatment of the Charcot-Marie-Tooth disease type 1A (CMT-1A). In this proposal, we aim to fully characterize the nanoparticles suspensions of Sq-siRNA after their formation and their evolution when incubated in different biological media. This is a mandatory step to control the formulation and to obtain first insight on the mechanism of the siRNA's pharmocinetics after injection. In addition, this new investigation will determine if our first results on Squalene adenosine nanoparticles suspension incubated in BSA solutions could be extrapolated to a macromolecular conjugate of a squalenoyl compound.

Experimental Report / Experiment 9-13-781 / D33

Sq-SiRNA nanoparticles for Charcot-Marie-Tooth disease: formulation and colloidal stability in serum albumin solutions

29/06/2018-01/06/2018

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10.5291/ILL-DATA.9-13-781

The aim of this SANS experiment was to characterize new nanoparticles for drug delivery and provide a preliminary assessment of their fate in a model biological medium.

Nanometer size carrier materials indeed appear as a very promising tool for drug delivery[1] combining vectorization to a chosen target with protection of the drug. This approach was chosen in the Nanoprotection Labex program[2] (held by Liliane Massade) to develop new possible treatments for the Charcot-Marie-Tooth disease type 1A (CMT-1A), a devastating pathology currently deprived of efficient treatment. This neuropathy is due to altered peripheral nerve myelin, resulting in demyelination and causing a severe and invalidating disease[3]. With the "squalenoyl" concept,[4] nanoparticles (NPs) are formed by nanoprecipitation with a conjugate (SQ-siRNA) made of the natural and biocompatible squalene moiety linked to the fragile siRNA in order to target the overexpression of a protein responsible for the disease[5]. The advantage is a high loading siRNA capacity in the lipid NPs with its preservation from degradation. This concept has been successfully used with adenosine to provide neuroprotection after stroke and spinal injury.[6]

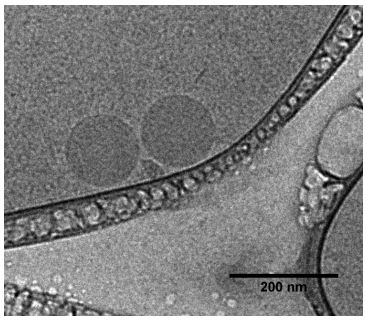

Figure 1 : Cryo EM imaging of Sq-SiRNA nanoparticles prepared by nanoprecipitation. (Image: Marie Caillaud)

Recently, a new synthetic pathway has led to high yield of the conjugate (SQ-siRNA) which can spontaneously self-assemble into NPs in aqueous solution[7] (Figure 1).

Results

We first have collected SANS diagrams of two types of SQ-siRNA nanoparticles nanoprecipitated in D_2O at 0.6 mg/ml concentrations (Figure 2). As expected, and in agreement with experiments carried out with nanoparticles prepared with other squalene-bioconjugates (Squalene-Deoxycytidine, Squalene-Adenosine etc…), we can fit the diagram with a lognormal distribution of sphere with a rather high polydispersity. The average radii are about 50 nm. Additionally, we did not observe any change in the SANS diagrams upon 1:1 dilution in D_2O.

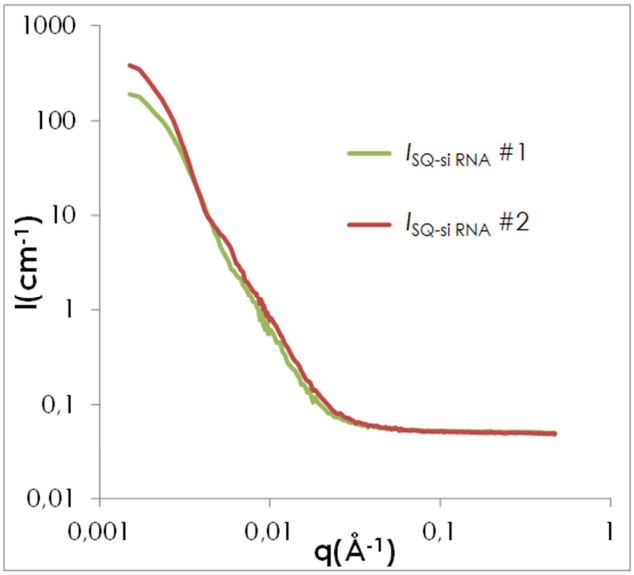

Figure 2: SANS diagrams of two types of SQ-SiRNA nanoparticles suspensions in D_2O (0.6 mg/ml)

Then, we have mixed the 0.6 mg/ml nanoparticles solutions with 13 mg/ml solutions of bovine serum albumin (BSA) in D_2O. The SANS patterns (Figure 3) of the mixture appears to result from the linear combination of the SANS patterns of each individual components. The nanoparticles are thus colloidally stable. However, the parameters suggest that the volume fraction of nanoparticles decreases of in presence of the protein. This has been observed before with SQAd nanoparticles.[8-9] We have interpreted this as an unraveling of the nanoparticles as BSA extracts the bioconjugate to form a complex.[9]

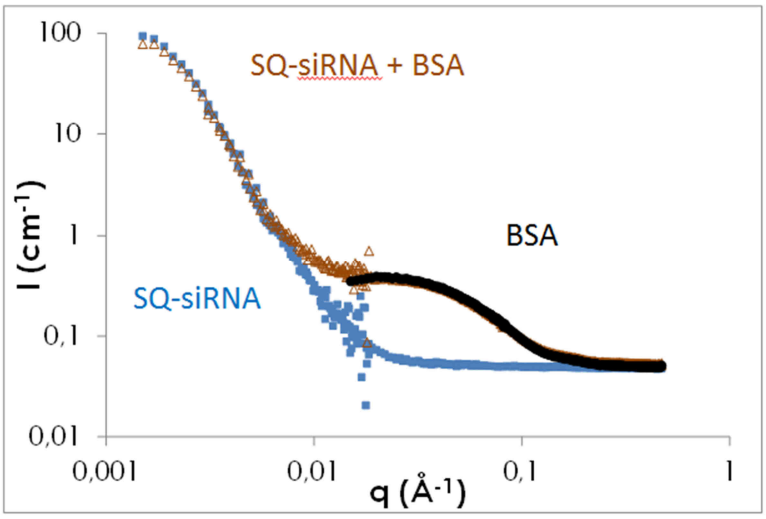

Figure 3 : SQ-siRNA nanoparticles (0.3 mg/ml) without (blue) and with BSA (red). 6 mg/mL BSA (black)

Finally, we have mixed the 0.6 mg/ml nanoparticles solutions with fetal bovine serum (FBS) of equal volume. The FBS had been previously dialyzed overnight against a phosphate buffer saline (PBS) prepared in heavy water to replace H_2O by D_2O. Here again no sign of aggregation is observed and the SANS pattern result from a

linear combinations of SANS patterns of nanoparticles and FBS (Figure 4). This can be attributed to the higher concentration in BSA (~20 mg/ml) and also to the presence of lipoproteins which are transporters of lipids. The unravelling of the nanoparticles is thus stronger than with only 6 mg/ml BSA.

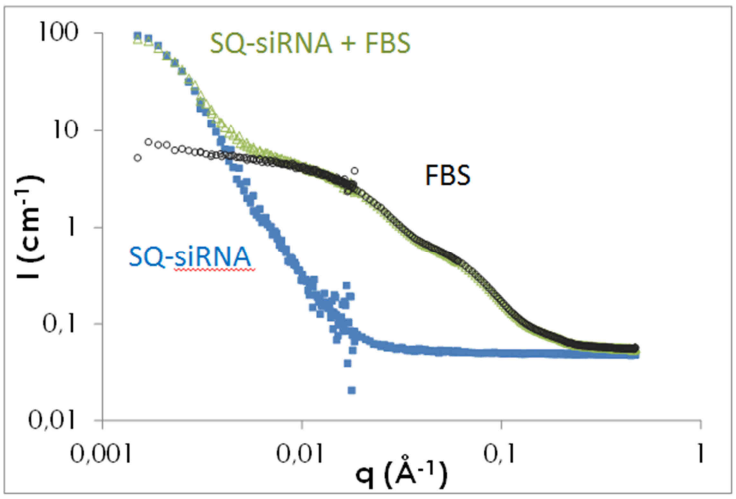

Figure 4 : SQ-siRNA nanoparticles without (blue) and with FBS (green). FBS alone (black).

Overall, these results show that SQ-SiRNA nanoparticles are colloidally stable in a complex biological medium, but their volumic fraction decreases. Along with complementary fluorescence quenching experiments also suggest that BSA is able to form complexes with SQ-SiRNA molecules, we can conclude that these nanoparticles behave as the other types of squalenoyl drugs. These results will be further analyzed in combination to parallel studies of the activity of the SQ-siRNA *in vivo* in a mouse disease model of CMT-1A.

In addition to the main experiment, we have also shown that Squalene-Adenosine nanoparticles could be recovered with same size distribution after freeze-drying. This result has important implication for the formulation and conservation of the nanodrug and has already been reported in a publication.[10]

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[1] Kunjachan S. et al , *Chem. Rev.* (2015), 115, 10907.

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[8] Testard F. et al. Experimental report 10.5291/ILL-DATA.9-13-696

[9] Gobeaux F. et al. *to be submitted*

[10] Rouquette M. et al. *Journal of Drug Targeting* 2019 DOI: 10.1080/1061186X.2019.1566340