Experimental report

17/02/2025

Proposal: 9-13-1085 **Council:** 4/2023

Title: Probing the interaction of miRNA-loaded fluorinated dendrimer with model membranes: cellular and endosomal

Research area: Chemistry

This proposal is a new proposal

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Samples: LIPIDS
fluorinated dendrimer
miRNA

Instrument	Requested days	Allocated days	From	To
D17	2	0		
FIGARO	2	2	11/09/2023	13/09/2023

Abstract:

Gene alteration is responsible for several complex illnesses. Gene therapy, working on altering, modifying, or silencing defective or mutated genes, has emerged as a promising therapeutic strategy. Dendritic-based vectors are a good candidate for gene delivery due to their well-defined chemical structure, easy tunability of density of terminal groups, and easy surface modification. The addition of fluorinated chains enhances serum stability of dendriplexes (complexes between dendritic vectors and genes), facilitates endosomal escape, enhances cellular uptake and reduces cytotoxicity. We combined Bis-MPA-based dendrimers with fluorination to improve their efficacy as gene vectors. We selected branched fluorinated moiety bearing three perfluoro-t-butoxyl groups as hydrophobic part and synthetized a fluorinated Janus-type dendrimer (FJD2N). The presence of four primary ammonium groups allows complexation with nucleic acids and has the potential to enhance endosomal escape.
We aim to understand the mechanism of interaction of miRNA-FJD2N with cellular and endosomal model membranes for the development of more efficient gene delivery systems.

Report on Experiment 9-13-1085
11-13 September 2023

In this experiment, we aimed to understand the mechanism of interaction of a novel fluorinated Janus-type dendrimer (FJD_2N) with cellular and endosomal model membranes for the development of more efficient gene delivery systems. To investigate the mechanism behind the increased cellular uptake and endosomal escape of fluorinated drug delivery systems, we investigated the interaction of FJD_2N in presence and absence of siRNA with cellular model membranes (i.e. DOPC) at pH 7.4 (HEPES 10 mM NaCl 150 mM).
We studied the interaction of FJD_2N in presence of siRNA with endosomal model membranes (i.e. DOPC:DOPE:DOPS) at physiological pH 7.4 and tried to investigate the same interaction at mildly acidic pH 5.5, mimicking the early endosomal compartment. The FJD_2N-siRNA complex showed instability in acidic conditions with the buffer chosen (citrate 10mM NaCl 150 mM) and the dataset collected in these conditions was not usable.
We experienced unexpected problems with the solvent exchange system, which resulted in exchange of solvent for longer time than expected and the need to check NR signal before continuing with acquisitions; this has impacted our sample plan and had to skip one of the planned samples.
We collected data on the following 4 samples:

1. DOPC pH 7.4 interaction with FJD_2N (Fig. 1)
2. DOPC pH 7.4 interaction with FJD_2N-siRNA (Fig. 2)
3. DOPC:DOPE:DOPS pH 7.4 interaction with interaction with FJD_2N-siRNA (Fig. 3)
4. DOPC:DOPE:DOPS pH 5.5 interaction with interaction with FJD_2N-siRNA – <u>unstable</u>

As expected, FJD_2N was visible even in hydrogenated SLBs. We used two model membrane compositions and characterised them in four isotopic contrasts: i) cellular model SLBs prepared with DOPC and ii) endosomal model SLBs prepared with DOPC, DOPE and DOPS (59:23:18 mol %).[1]
For the DOPC SLB, the interaction with FJD_2N results in dendron accumulation in the inner leaflet, while the outer leaflet maintains a high coverage (Fig.1). The same SLB interacting with the siRNA- FJD_2N dendriplex results in removal of lipids combined with dendriplex accumulation in the inner leaflet. (Fig.2)
For the DOPC:DOPC:DOPC SLB, the interaction of siRNA- FJD_2N dendriplex results in insertion of the dendriplexes in the inner leaflet while the outer leaflet does not show major lipid removal (Fig.3).
We have collected data on the missing samples at both pH 7.4 and 5.5 on OFFSPEC (ISIS RAL) and plan to optimize the data analysis and submit a manuscript in the next few months.

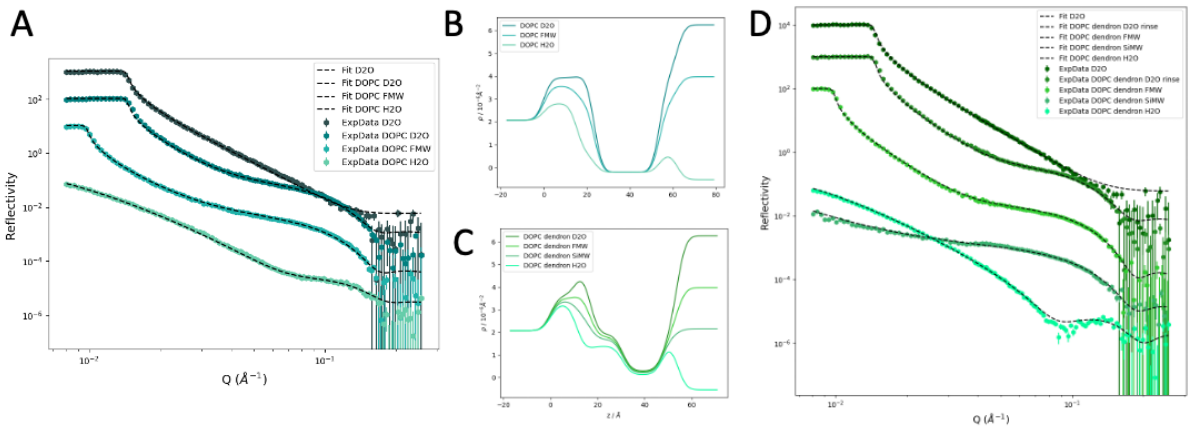

Figure 1. Reflectivity profiles of DOPC SLB measured before (A) and after interaction with 0.56 mM dendron (D). The darkest shade refers to the block without SLB in D2O, while the remaining curves go from 100 to 0 % D-buffer. SLD profiles for SLB prior (B) and after interaction with dendron (C). Dendron seems to bind to the SLB and accumulate in the leaflet toward the substrate.

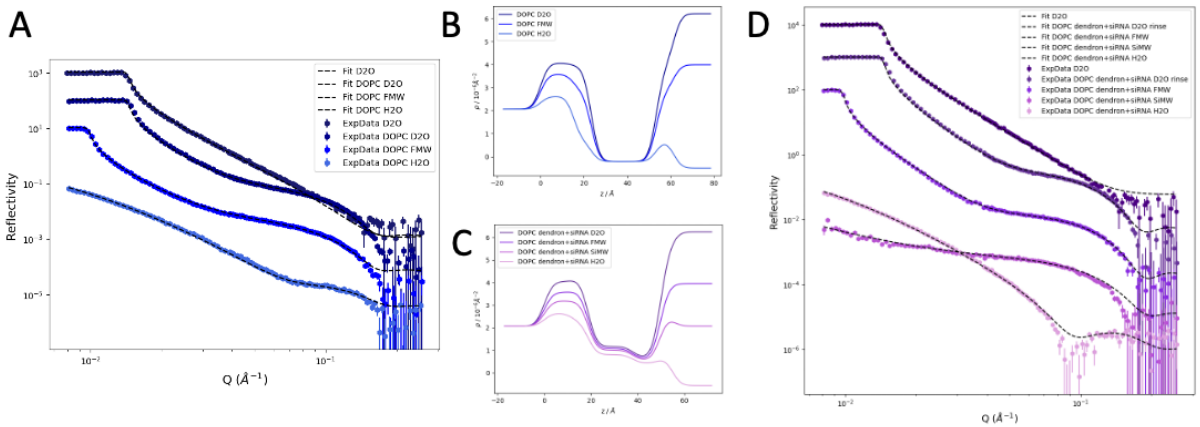

Figure 2. Reflectivity profiles of DOPC SLB measured before (A) and after interaction with 0.56 mM dendron-siRNA N:P 30 (D). The darkest shade refers to the block without SLB in D2O, while the remaining curves go from 100 to 0 % D-buffer. SLD profiles for SLB prior (B) and after interaction with dendron-siRNA (C). Dendriplexes seems to bind to the SLB and accumulate in the tail layer especially toward the substrate.

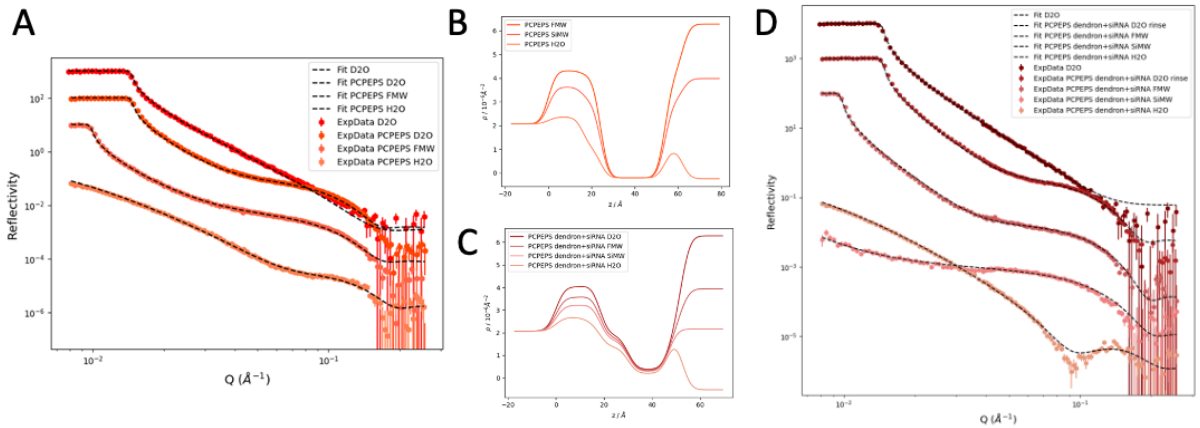

Figure 3. Reflectivity profiles of DOPC:DOPE:DOPS SLB measured before (A) and after interaction with 0.56 mM dendron-siRNA N:P 30 (D). The darkest shade refers to the block without SLB in D2O, while the remaining curves go from 100 to 0 % D-buffer. SLD profiles for SLB prior (B) and after interaction with dendron-siRNA (C). Dendriplexes seems to bind to the SLB and accumulate in the tail layer toward the substrate, while the outer leaflet of the SLB seems to maintain a high lipid coverage.