

# **Brain-targeted delivery of pre-miR-29b using lactoferrin-stearic acid-modified–Chitosan/Polyethylenimine polyplexes**

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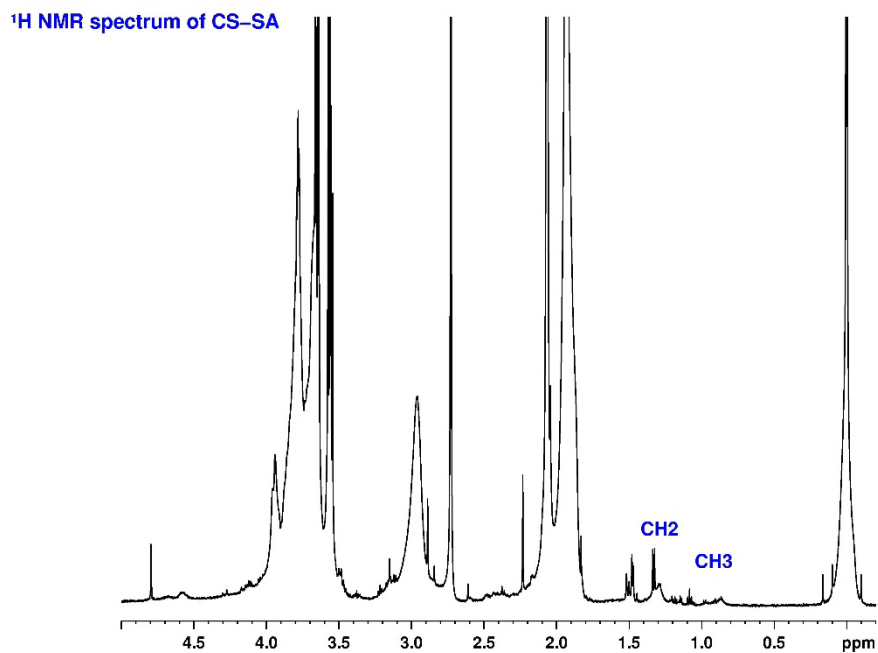
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**Figure S1** - <sup>1</sup>H NMR spectrum of stearic acid-chitosan (CS-SA) conjugate.

The spectrum of the CS-SA shows 3 broad peaks, which correspond to the resonance of the monosaccharide residue protons, namely a signal at  $\delta$  1.5 ppm of the carboxyl group (-COCH<sub>3</sub>), the methylene group (-CH-NH-) next to hydroxyl group at 2.88 ppm and the internal CH/CH<sub>2</sub> groups in the chitosan ring at 3.61–4.0 ppm.

<sup>1</sup>H NMR spectrum of PEI-SA

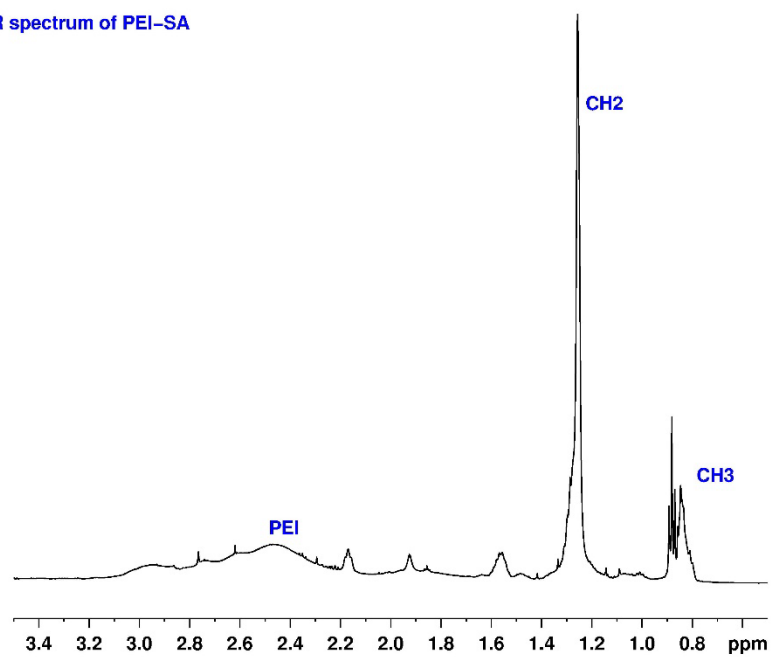


Figure S2 - <sup>1</sup>H NMR spectrum of stearic-polyethyleneimine (PEI-SA conjugate).

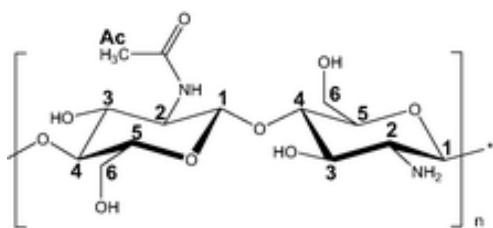
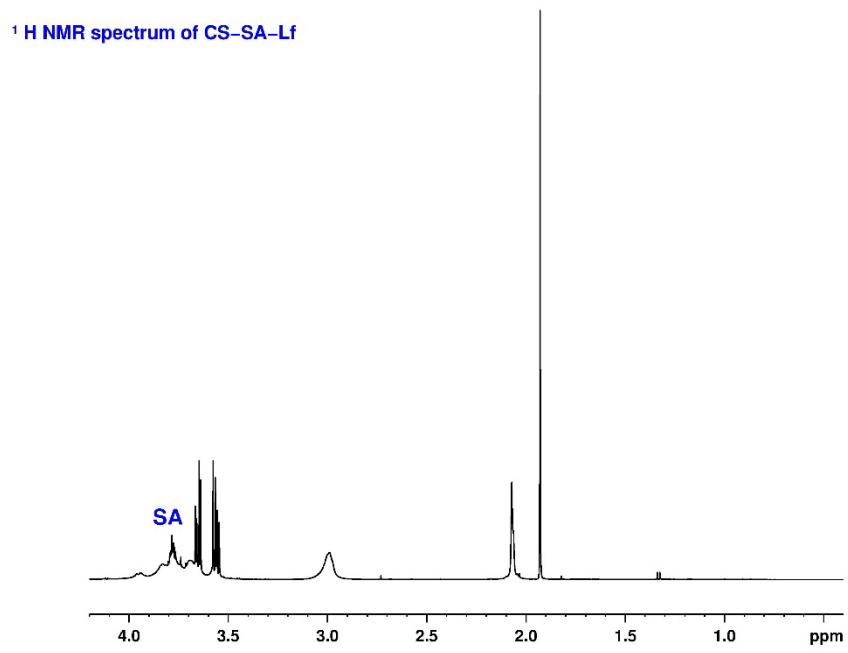
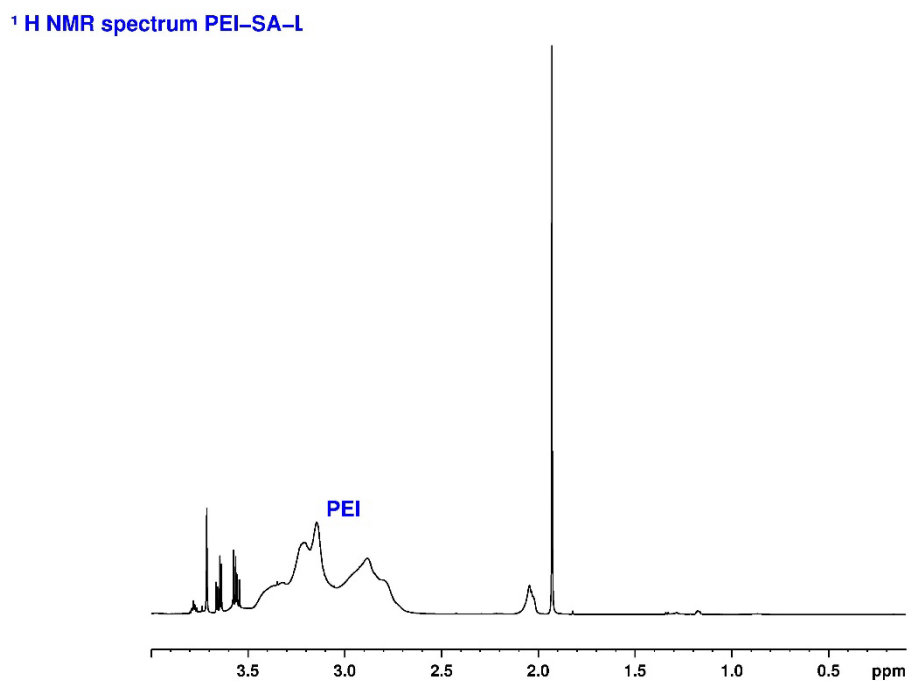


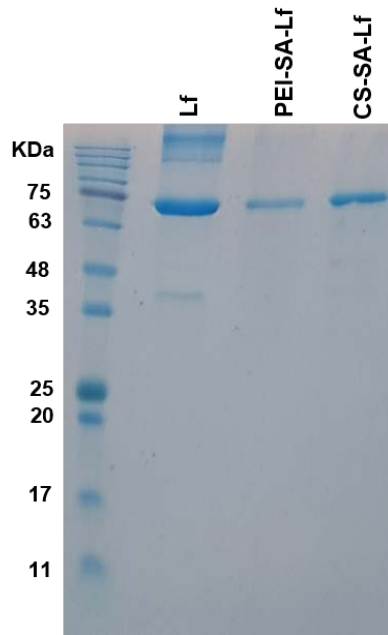
Figure S3 - Chemical structure of chitosan (CS).



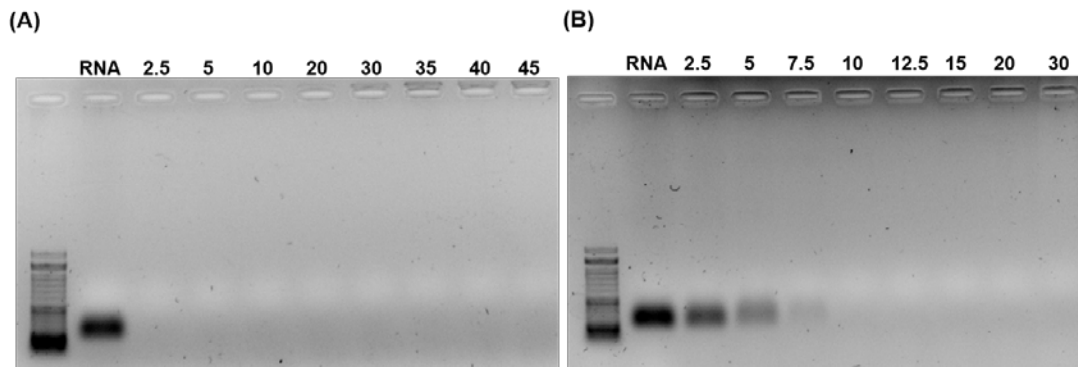
**Figure S4** - <sup>1</sup>H NMR spectrum of chitosan-stearic acid (CS-SA) conjugate coupling to lactoferrin (Lf) in D<sub>2</sub>O.



**Figure S5** - <sup>1</sup>H NMR spectrum of polyethyleneimine-stearic acid (PEI-SA) conjugate coupling to lactoferrin (Lf) in D<sub>2</sub>O.



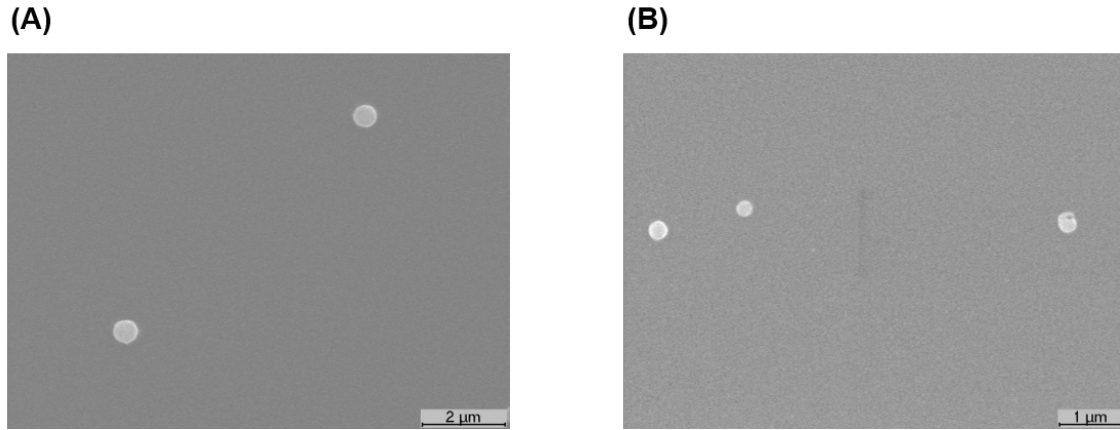
**Figure S6** - SDS-PAGE of the PEI-SA-Lf and CS-SA-Lf conjugates.



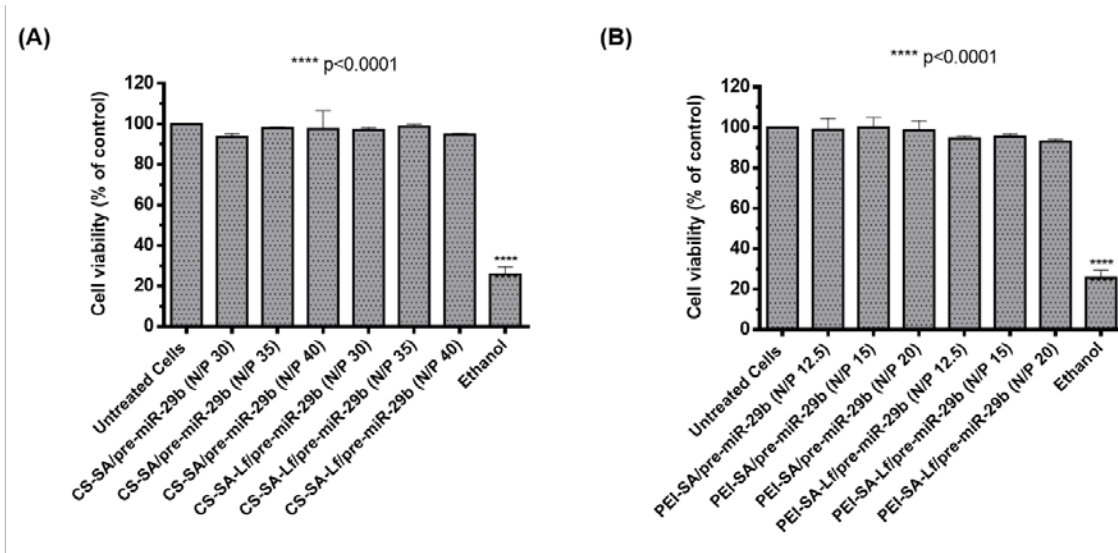
**Figure S7** - Agarose gel electrophoresis of polyplexes at various N/P ratios: **(A)** CS-SA/RNA and **(B)** PEI-SA/RNA. The first lane of the gel corresponds to the molecular weight marker and the second lane corresponds to free pre-miR-29b. The numbers in each lane indicate the N/P ratios. Each experiment was performed three times.

According to the electrophoresis analysis shown in Figure S7, it was possible to observe that the RNA complexation by CS-SA conjugates starts at very low ratios, namely  $N/P=2.5$ , suggesting that RNA is fully complexed. In turn, in the case of the PEI-SA/RNA polyplexes, it was observed that the lanes corresponding to the N/P ratios of 2.5, 5 and 7.5 contain free RNA. However, above the N/P ratio of 7.5,

the band corresponding to free RNA was not observed, indicating that the RNA was efficiently conjugated with the PEI-SA.



**Figure S8** – SEM images for CS/PEI modified with stearic acid and lactoferrin: (A) CS-SA-Lf/RNA and (B) PEI-SA-Lf/RNA.



**Figure S9** – Relative cell viability of N2A695 cells treated with different pre-miR-29b-loaded polyplexes at various N/P ratios for 72 hours: (A) CS-based polyplexes and (B) PEI-based polyplexes.

### CS-SA/pre-miR-29b

### CS-SA-Lf/pre-miR-29b

0 h

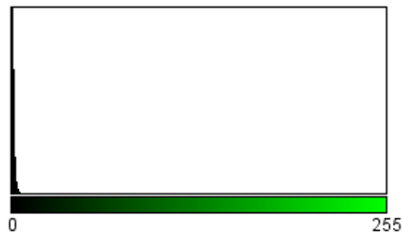


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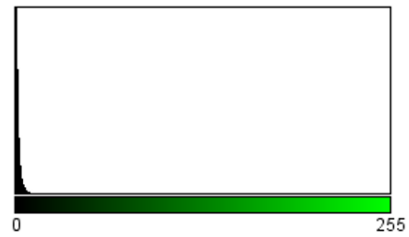


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0.5 h

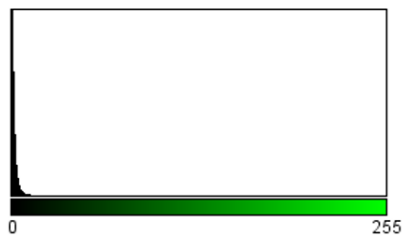


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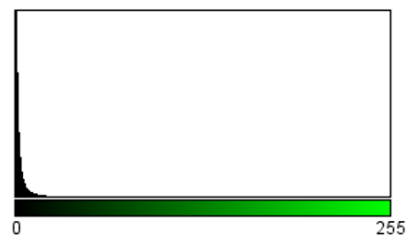


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1 h

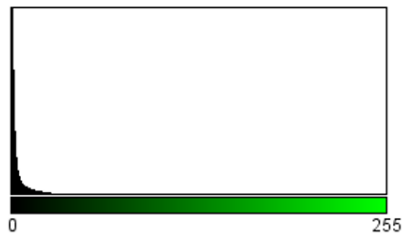


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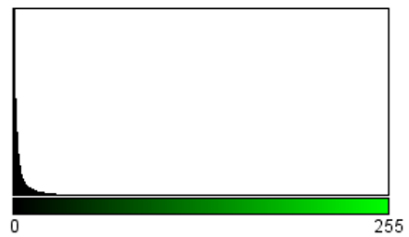


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1.5 h

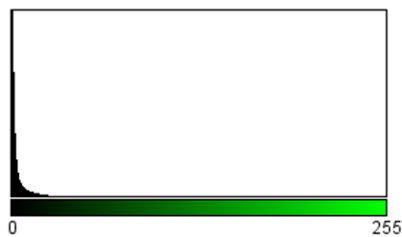


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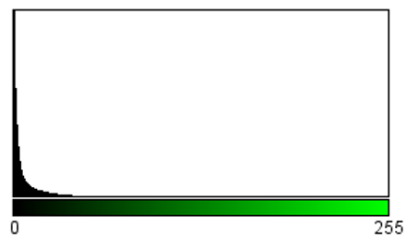


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StdDev: 8.988    Mode: 0 (20101956)

2 h



Count: 62914560    Min: 0  
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StdDev: 5.240    Mode: 0 (34149685)



Count: 62914560    Min: 0  
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StdDev: 10.874    Mode: 0 (16558960)

**Figure S10** – Histogram of FITC-fluorescence intensity obtained by confocal microscopy on N2a695 cells.