

SUPPORTING INFORMATION

Nucleic Acid-Based Supramolecular Structures: Vesicular Spherical Nucleic Acids from a Non- Phospholipid Nucleolipid

Erik Dimitrov,¹ Natalia Toncheva-Moncheva,^{1} Pavel Bakardzhiev,¹ Aleksander Forys,² Jordan Doumanov,³ Kirilka Mladenova,³ Svetla Petrova,³ Barbara Trzebicka,² Stanislav Rangelov^{1*}*

¹ Institute of Polymers, Bulgarian Academy of Sciences, Akad. G. Bonchev St. 103A, 1113 Sofia, Bulgaria

² Centre of Polymer and Carbon Materials, Polish Academy of Sciences, M. Curie-Skłodowskiej 34, Zabrze, Poland

³ Department of Biochemistry, Faculty of Biology, Sofia University *St. Kliment Ohridski*, Dragan Tsankov Blvd. 8, 1164 Sofia, Bulgaria

* Corresponding authors: rangelov@polymer.bas.bg, ntoncheva@polymer.bas.bg

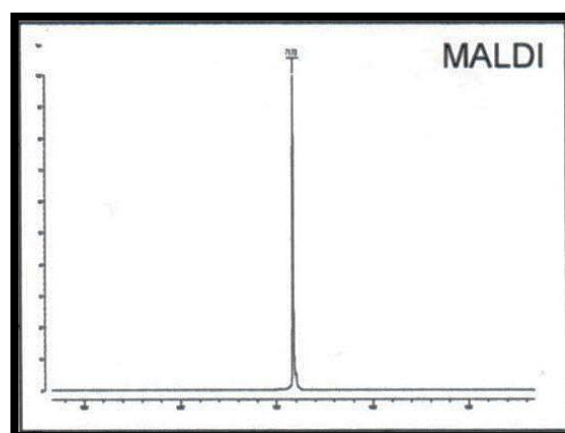
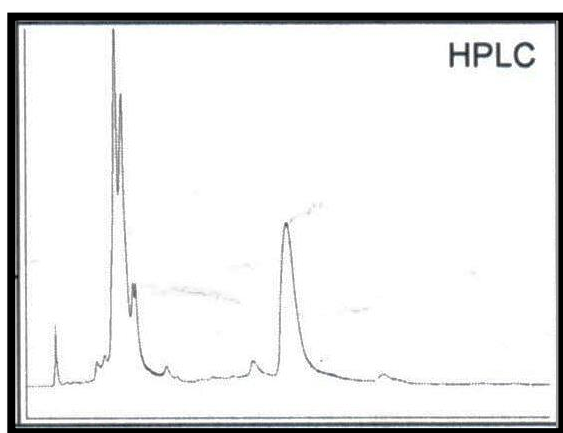
I. Composition and characterization of DBCO-oligonucleotide

Dibenzocyclooctyne-functionalized oligonucleotide (DBCO-oligonucleotide) was purchased from Biomers.net GmbH. The sequence and composition as well as characterization data, according to the producer, are presented in Table SI1 and Figure SI1.

Table SI1. Sequence and composition of DBCO-oligonucleotide.

Code	Oligonucleotide composition* Sequence (5'→3')	M _w (g.mol ⁻¹)
DBCO-oligonucleotide	DBCO-(EG) ₄ -5ta ata cga ctc act ata ggg	7169

* DBCO – dibenzocyclooctyne, EG – ethylene glycol, 5 – spacer 18, phosphodiester followed by 6 ethylene oxide units, C6 = (CH₂)₆ groups.



5'-5ta ata cga ctc act ata ggg -3'
Length* 21
GC Content 38,00%
A:7,0 C:4,0 G:4,0 T/U:5,0
Ext. Coeff.: 230600

MW Calc.	7169 g/mol
MW Found	7169 g/mol
Purific.	HPLC preparative**
5' Mod.:	DBCO-PEG4
3' Mod	None
5: Spacer 18	

*The DBCO-oligonucleotide has a typical length of 21 bases, 20 nucleotides and 1 internal modification (Spacer18) which is marked in the sequence with the number 5.

** The preparative HPLC was used for quality control, separation and purification of the synthesized end functionalized oligonucleotide. The isolated fraction of the main product was further analyzed by MALDI mass spectrometry. The presence of a single peak on the spectrum together with the expected molecular weight data confirmed the composition of the DBCO end functionalized oligonucleotide.

Figure SI1. HPLC chromatogram (left), MALDI spectrum (right), and other characterization data of DBCO-oligonucleotide.

II. Synthesis of 1,3-dihexadecyloxy-propane-2-ol (DHP).

DHP was synthesized following a procedure described elsewhere.^{1,2} Details for the synthetic procedure are given below. 13.068 g (0.054 mol) of 1-hexadecanol were placed in a two-necked round bottom flask flushed with nitrogen. 16.092 g (0.054 mol) of hexadecyl glycidyl ether and 0.0675 mL of SnCl₄ were added under stirring. The stirring was maintained at 110-120°C for 24 h, then another portion of 0.0675 mL of SnCl₄ was added and the mixture was stirred for other 70 h. The quantitative conversion of hexadecyl glycidyl ether is evidenced by the lack of resonances of the epoxide protons at 2.6–3.2 ppm in the ¹H NMR spectrum of DHP. The product was recrystallized twice from hexane. Yield 16.709 g, 57.3%. The synthetic pathway and a ¹H NMR spectrum of DHP are presented in Figure SI2 and Figure SI3, respectively.

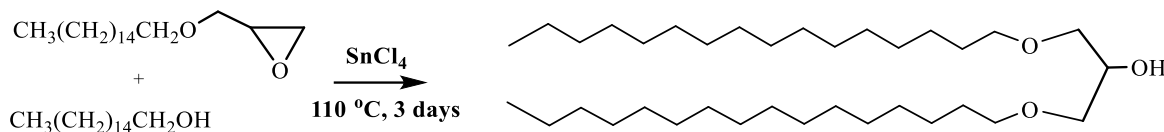


Figure SI2. Schematic presentation of the synthesis of 1,3-dihexadecyloxy-propane-2-ol.

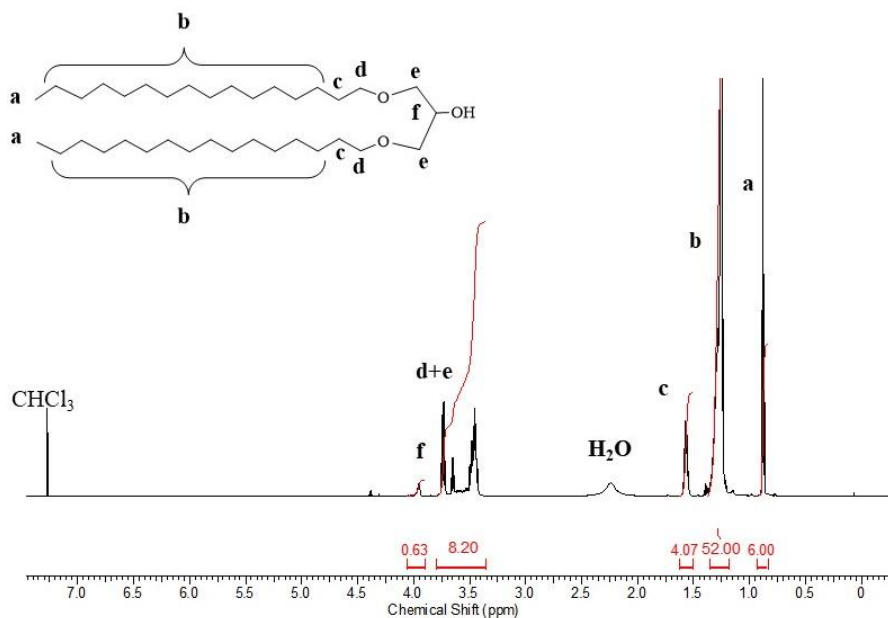


Figure SI3. ¹H-NMR spectrum of 1,3-dihexadecyloxy-propane-2-ol in CDCl₃.

III. Functionalization of DHP

To obtain the desired nucleolipid from cyclooctyne-functionalized oligonucleotide by an azide-alkyne *click* reaction, the secondary hydroxyl group of DHP must be converted to azide. A realistic synthetic approach involved mesylation of DHP and then conversion of the mesylate group to azide. The expected synthetic pathway is presented in Figure SI4, whereas the procedures are described in the following. DHP (Mw = 540 g.mol⁻¹) (1.5121 g, 2.8 mmol, 1 eq.) was dissolved in dry CH₂Cl₂ (80 mL) in a 200 mL round-bottom flask under an inert atmosphere. Then, DMAP (0.17 g, 1.4 mmol, 0.5 eq.) and TEA (2.83 g, 28 mmol, 10 eq.) were added to the reaction mixture. After stirring for 5 min at 0 °C, methanesulfonyl chloride (3.20 g, 28 mmol, 10 eq.) dissolved in 20 mL of dry CH₂Cl₂ were added and the reaction mixture was stirred overnight at 25 °C (Figure SI4a). The resulting product was added to water and formed a turbid dispersion. The product was extracted several times with CH₂Cl₂. The extracts were collected, dried using anhydrous Na₂SO₄, and filtered. The mesylated DHP was recovered after evaporation of the solvent and dried for 24 h in a vacuum oven at 25 °C. Yield 1.43 g (95%), degree of conversion 99%. The successful transformation of the end hydroxyl group into a mesylate group was proved by ¹H NMR (Figure SI5).

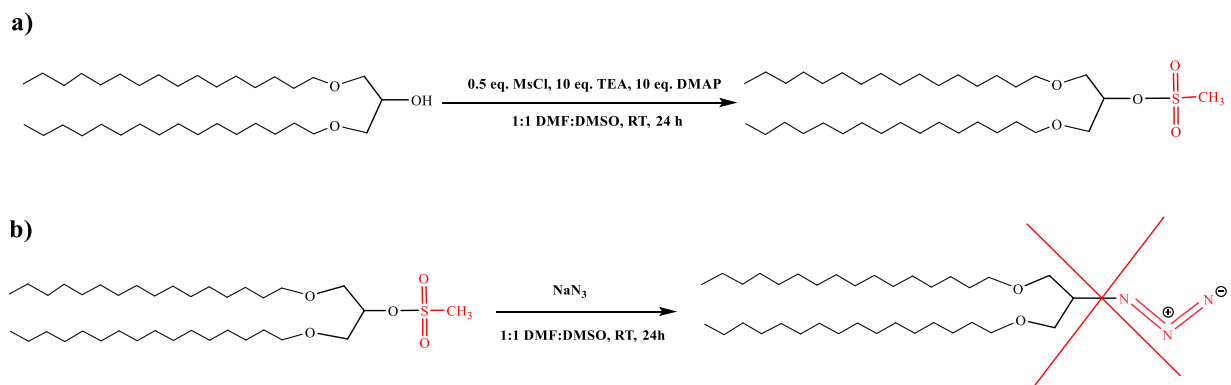


Figure SI4. Functionalization of 1,3-di(hexadecyloxy)-propane-2-ol.

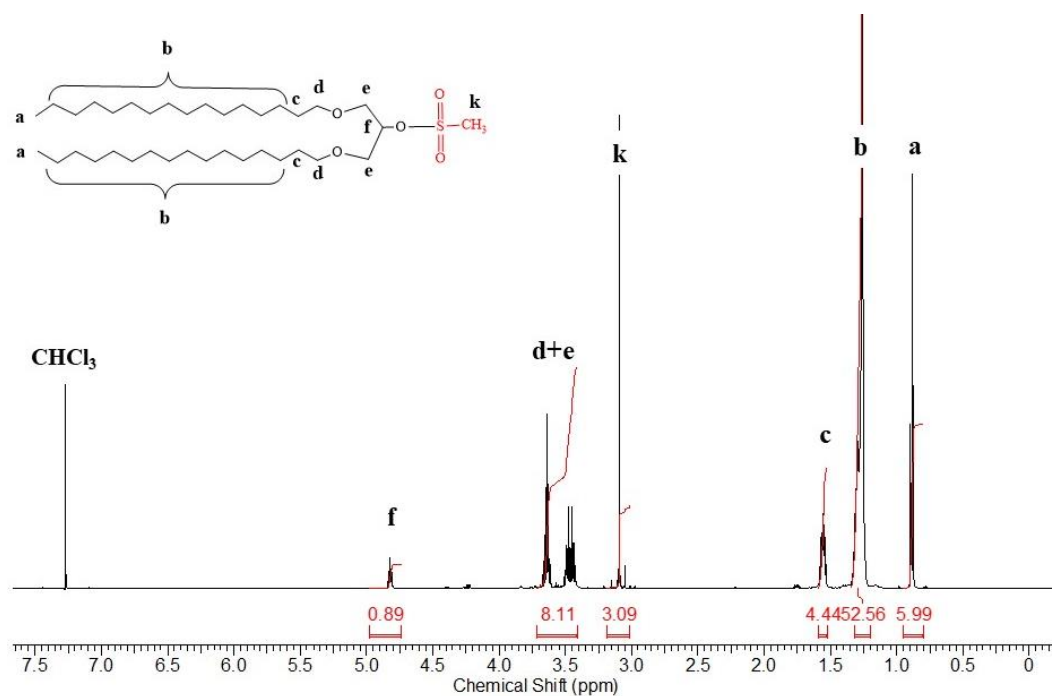


Figure SI5. ¹H NMR spectrum of mesylated DHP in CDCl₃.

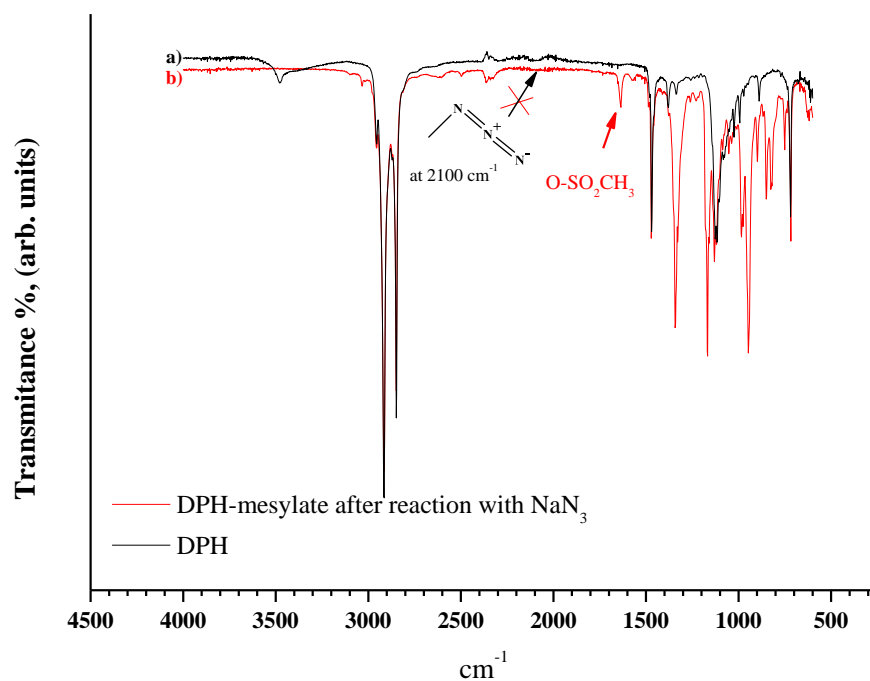
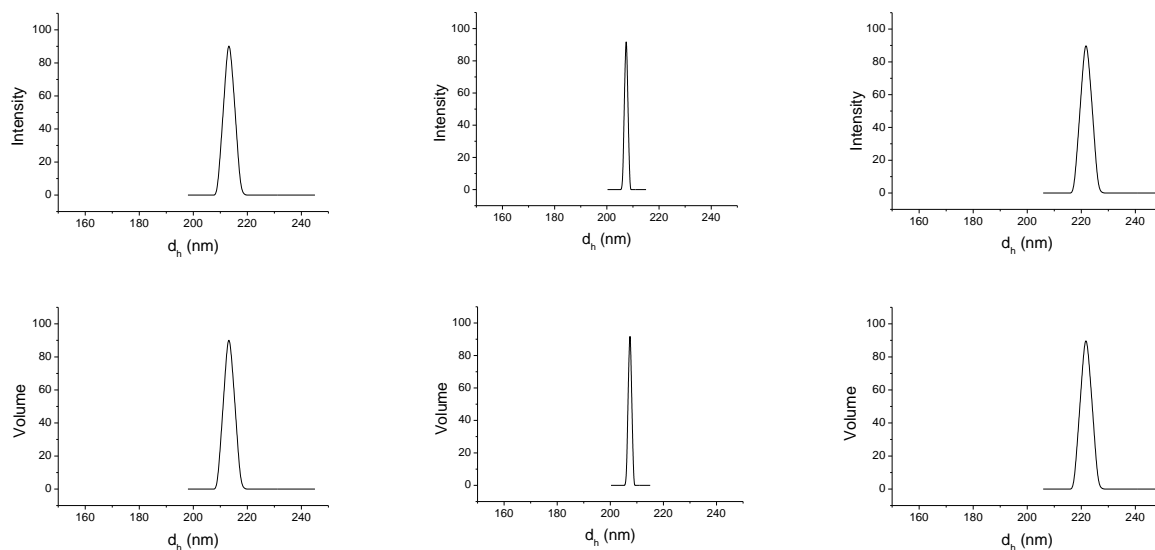


Figure SI6. FTIR spectra of DHP (a) and the product after azidation (b).

The mesylated DHP ($M_n=618 \text{ g.mol}^{-1}$) (1.4 g, 2.26 mmol, 1 eq.) was dissolved in a mixture 1:1 of dry DMF:DMSO (20 mL) under an inert atmosphere in a 50 mL round-bottom flask and NaN_3 (4.4174 g, 68 mmol, 30 eq.) was added. The reaction was carried out at 25 °C overnight (Figure SI4b). The purified product was characterized by FTIR analysis (Figure SI6). As seen from Figure SI6, the characteristic band at 2100 cm^{-1} for azide stretching vibration is not seen in the spectrum. The spectrum is dominated by the characteristic bands of the mesylate group, implying that the reaction was not successful. The efforts to obtain well-defined N_3 -functionalized DHP by prolonging reaction time and/or varying reaction temperature did not bring any success.

IV. Additional and supporting DLS data for vesicular SNAs, prepared by self-assembly of NucL2 in aqueous solution

DLS measurements were performed at nine angles θ in the range 50 – 130° and concentrations in the interval $0.0398 - 0.0636 \text{ mg.mL}^{-1}$ at 25 °C. Selected particle size distributions weighted by intensity, by volume, and by number are displayed in Figure SI7. The practically identical values of the hydrodynamic diameter, d_h , determined by intensity-, volume-, and number-weighting evidence the narrow and monomodal size distributions.



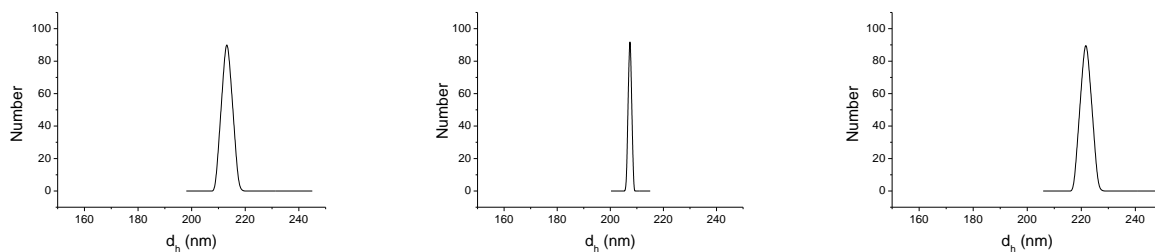
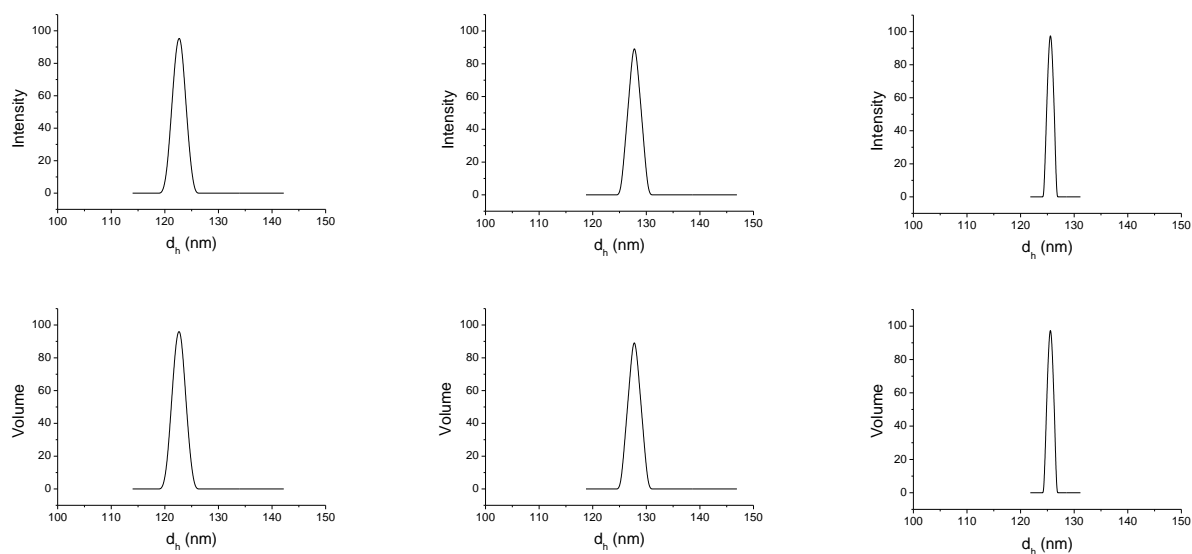


Figure SI7. Particle size distributions by intensity (top row), volume (middle row), and number (bottom row) for vesicular SNAs, prepared by self-assembly of NucL2 in aqueous solution, measured at different concentrations and angles: $c = 0.0398 \text{ mg.mL}^{-1}$, $\theta = 80^\circ$ (left column); $c = 0.053 \text{ mg.mL}^{-1}$, $\theta = 70^\circ$ (middle column); $c = 0.0636 \text{ mg.mL}^{-1}$, $\theta = 60^\circ$ (right column).

V. Additional and supporting data for vesicular SNAs, prepared by co-assembly of NucL2 with DPPC and Chol

DLS measurements were performed at nine angles θ in the range $50 - 130^\circ$ and total lipid concentration of 2 mM at 25°C . Selected particle size distributions weighted by intensity, by volume, and by number are displayed in Figure SI8. The practically identical values of the hydrodynamic diameter, d_h , determined by intensity-, volume-, and number-weighting evidence the narrow and monomodal size distributions.



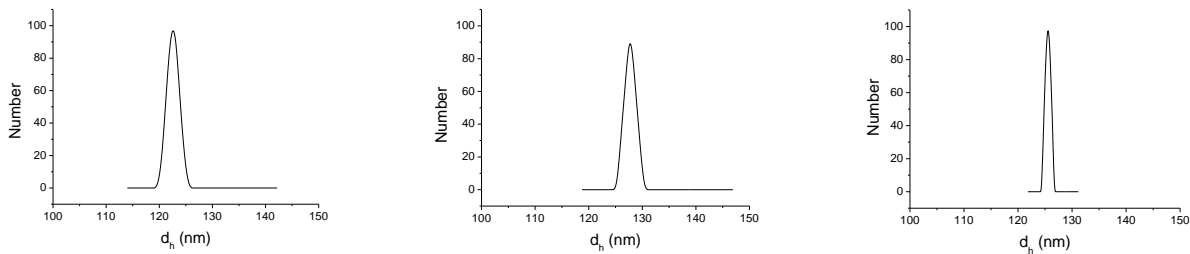


Figure SI8. Particle size distributions by intensity (top row), volume (middle row), and number (bottom row) for vesicular SNAs, prepared by co-assembly of NucL2 with DPPC and Chol in aqueous solution, measured at different angles: $\theta = 60^\circ$ (left column), 70° (middle column), and 110° (right column).

The diffusion coefficient, D , at a single concentration (2 mM) was determined from the angular dependence of the relaxation rate (inversely proportional to relaxation time), measured at different scattering angles (Figure SI9). D was used for calculation of the apparent hydrodynamic radius, R_h .

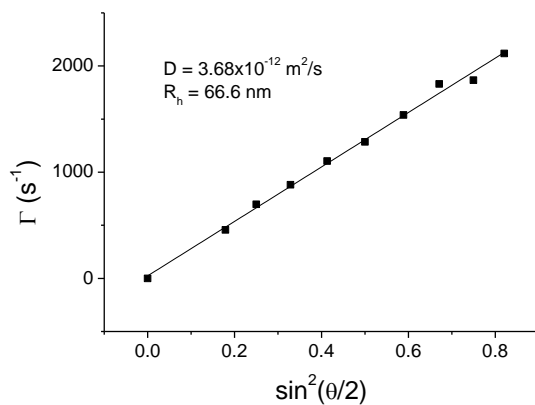


Figure SI9. Angular dependence of the relaxation rate (Γ) of DPPC/Chol/NucL2 vesicular SNAs at $c = 2$ mM total lipid and 25°C for determination of apparent R_h .

Cryo-TEM images of DPPC/Chol/NucL2 vesicular SNAs are shown in Figure SI10. The size (diameter) of the vesicles ranged from 50 to 290 nm. The size distribution from cryo-TEM is presented in Figure SI11. By measuring the size of 150 objects, the average diameter of 140 nm was determined, which is in good agreement with the results from DLS. The membrane thickness, also measured over a large number of objects, was ~ 5 nm.

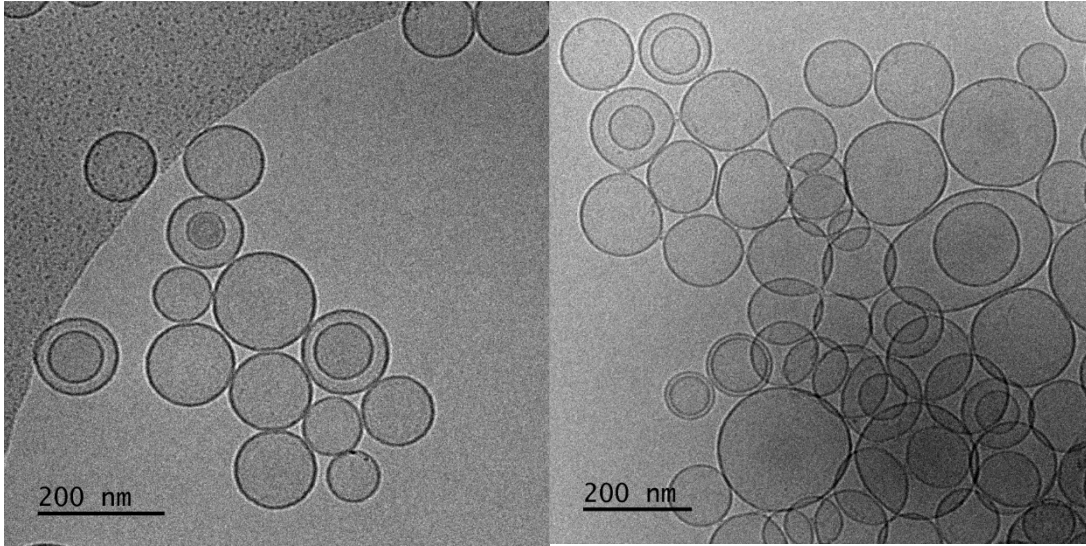


Figure SI10. Cryo-TEM images of DPPC/Chol/NucL2.

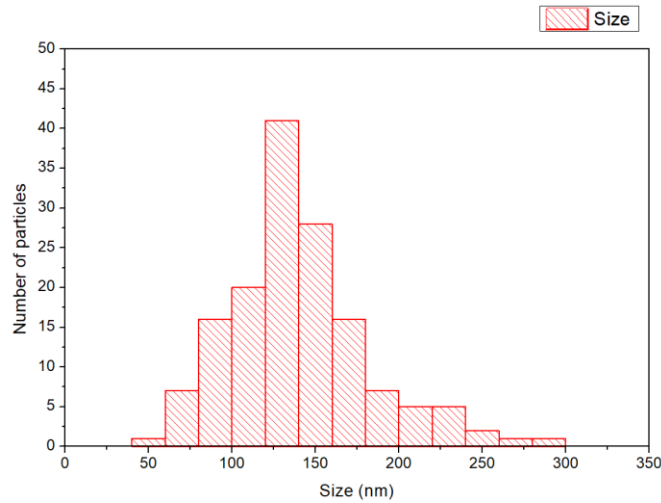


Figure SI11. Size distribution from cryo-TEM of DPPC/Chol/NucL2 vesicles at $c = 2$ mM total lipid.

VI. Calculation of the Flory radius, R_F , and determination of the critical grafting density (σ_{tr}) at the transition from mushroom to brush conformation

The Flory radius, R_F , is the end-to-end distance of a free polymer coil in a theta solvent, resulting from the balance between the expanding steric forces and the counteracting entropic forces from stretching of the coils. In its simplest form it is given by equation (1):³

$$R_F = an^{3/5} \quad (1)$$

Here a is the length of the monomer unit and n is the degree of polymerization. The hydrophilic section of the nucleolipid NucL2 is composed of two hexaethylene glycol spacers and 21 bases. If the lengths of ethylene oxide unit and nucleotide unit are taken as 0.39 nm and 0.34 nm, respectively, and the total degree of polymerization is 33 ($= 2 \times 6 + 21$), then for R_F we obtain:

$$R_F = \left(\frac{12}{33} \times 0.39 + \frac{21}{33} \times 0.34 \right) \times (12 + 21)^{\frac{3}{5}} = 2.92 \text{ nm}$$

The polymer coils start to interact laterally when the distance between the grafting points is equal to or less than the Flory radius. Thus, the grafting density at the transition from an unextended (mushroom) to brush conformation (σ_{tr}) can be determined by $\sigma_{tr} = 1/R_F^2$. Thus, for σ_{tr} of the nucleolipid NucL2 we obtain $\sigma_{tr} = 0.117 \text{ nm}^{-2}$. When $\sigma < \sigma_{tr}$ the tethered polymer coils (or oligonucleotide strands) are isolated; if $\sigma > \sigma_{tr}$ they interact laterally, overlap, and adopt a more extended (brush) conformation.

REFERENCES

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