Supporting Information

DNA-Controlled Assembly of Soft Nanoparticles

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Materials: 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC, lyophilized powder) was purchased from Avanti Polar Lipids and 1,1'-dioctadecyl-3,3,3',3'- tetramethylindocarbocyanine perchlorate (DiI-C₁₈) and 3,3'-dioctadecyloxacarbocyanine perchlorate (DiO-C₁₈) from Invitrogen/Molecular Probes. 2,2'-(1,8-Dihydroxy-3,6-disulfonaphthylene-2,7-bisazo)bisbenzene arsonic acid (arsenazo III) and all other chemicals were obtained from Sigma in the purest form available. Ultrapure Milli-Q water was used in all experiments. 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) buffer (10 mM HEPES, 110 mM Na⁺) was prepared by mixing appropriate amounts of HEPES, HEPES sodium salt and NaCl followed by correction of pH to 7.0. The chemicals for DNA synthesis were purchased from Glen Research. All solvents (HPLC grade) and reagents were purchased commercially and used without further purification. DNase I (RNase-free) was purchased from Roche Diagnostics Corporation. Muscovite mica (75 mm x 25 mm x 200 µm sheets) was from Plano GmbH, Germany. Mica sheets of 10 mm x 10 mm were preglued onto round (0.17 mm, Ø24 mm) microscope coverslips using a transparent and biocompatible silicone glue (MED-6215, Nusil Technology, Santa Barbara, CA). Immediately prior to use, the mica was cleaved with a knife leaving a thin and highly transparent mica film on the coverslip.

Measurement of transition temperatures: Transition analyses were carried out on a Perkin Elmer UV/VIS spectrometer Lambda 30 with a PTP-6 (Peltier Temperature Programmer) device by using PE TempLab 2.0 and UV Winlab 2.8. Melting temperatures (T_m , in °C) were determined as a first derivative of thermal denaturation curves, which were obtained by recording absorbance at 260 nm as a function of temperature at a rate of 0.5°C min⁻¹ for measurements with liposomes and with 1°C min⁻¹ for measurements without liposomes. The solutions were heated to 90 °C, maintained for 5 min at this temperature, and then gradually cooled before performing thermal denaturation experiments. All melting temperatures are reported with an uncertainty of ±1 °C, as determined from multiple experiments.

Experimental procedure for liposome assembly: In a typical experiment we use POPC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine) liposomes with an average diameter of 65 nm ± 15 nm and a lipid concentration of 10 mM, prepared by extrusion through 50 nm filters, a final concentration of 0.5 mM is used for all experiments. Addition of lipophilic probe DNA in HEPES buffer solution (pH 7.0, 110 mM NaCl, 10 mM HEPES, 62 nM probe strand) followed by addition of the target sequence initiates assembly. Studies at higher DNA concentrations (2 µM) resulted in particle aggregation as observed visually by a rapidly increasing turbidity and finally precipitation of the liposome aggregates with fully intact liposomes as shown by an arsenazo III dye assay (see figure 1). Experiments with lower target concentrations (target concentration < 100 nM) produce stable aggregates as observed by light scattering, but without macroscopic precipitation, even after prolonged time (24 h).

Preparation of POPC liposomes: POPC was suspended in aqueous HEPES buffer at 10 mM lipid concentration and stirred to a uniform milky solution. Liposomes were prepared by repeated extrusion (10 times) through double polycarbonate filters with a 50 nm pore size using compressed N₂ (30 bar) and a LIPEX™ Extruder from Northern Lipids. DiO-C₁₈-labeled liposomes were prepared by co-solubilization of POPC and DiO-C₁₈ in CHCl₃ followed by evaporation of CHCl₃ and hydration in HEPES buffer prior to extrusion.

Preparation of Ca²⁺ loaded liposomes: Calcium loaded liposomes for the leakage test were prepared as described above in HEPES buffer containing 6 mM CaCl₂. After extrusion, the buffer surrounding the liposomes was exchanged with a calcium-free HEPES buffer by dialysis. 3 ml Slide-A-Lyzer 10.000 MW Cut-Off dialysis cassettes (Pierce, Thermo Fisher Scientific Inc. Rockford, IL) were used. The cassettes were filled with the liposome suspension and immersed in 600 ml calcium-free buffer. The calcium-free buffer was exchanged 5 times with >3 hours intervals.

Leakage test using arsenazo III: Liposomes and arsenazo III (3 mM) were combined and absorbance was measured from 550 to 750 nm with expected maxima at 600 and 660 nm for the Ca²⁺-arsenazo III dye complex.

Figure 1: Arsenazo III assay^[1] demonstrating the structural integrity of Ca²⁺-loaded liposomes after addition of the probe-DNA and the subsequent liposome aggregation after addition of the target strand seen as a precipitate (B). Membrane lysis induced by Triton X-100 led to Ca²⁺-release and a color change of the arsenazo III dye evident by an absorbance peak at 660 nm and 600 nm (C).

The experiments were carried out using 5 mM solutions (50 μ l of a 100 mM liposome stock solution added to 946 μ l HEPES buffer, 10 mM HEPES, 110 mM Na⁺, pH 7.0) of 65 nm POPC liposomes with encapsulated Ca²⁺ (6 mM in the entrapped volume). A 3 mM solution of arsenazo III (4 μ l, 0.012 mM) was added to a total volume of 1 ml.

Continuous recording of ultraviolet spectra from the supernatant solution even after prolonged time (24 h) showed no leakage of Ca^{2+} -ions from the entrapped volume in the liposomes. Addition of 10 μL 5 % Triton X-100 solution in HEPES buffer (10 mM HEPES, 110 mM Na^+ , pH 7.0) caused 100% leakage with a visible colour change from light red to blue, further addition of Triton X-100 did not increase Ca^{2+} release (fig. 1).

Thermal denaturation data of DNA-conjugate duplexes with modification Y (single palmitoyl substituent):

Table 1. Thermal denaturation data of DNA-conjugates modified with Y.^[a,b]

Nr.	Duplex	T_m [°C]	ΔT_m [°C]	T_m [°C]	ΔT_m [°C]
	17mer complementary strand region			with liposomes ^c	without liposomes ^d
1	5'-TTT ^Y TGTGGAAGAAGTTGGTG ^Y TTT 3'-ACACCTTCTTCAACCAC	nd	-	57.0	4.0
2	3'-TTT ^Y ACACCTTCTTCAACCAC ^Y TTT 5'-TGTGGAAGAAGTTGGTG	nd	-	58.5	5.5
3	3'-TTT ^Y ACACCTTCTTCAACCAC ^Y TTT 5'-TTT ^Y TGTGGAAGAAGTTGGTG ^Y TTT	nd	-	64.0	11.0
4	3'-ACACCTTCTTCAACCAC 5'-TGTGGAAGAAGTTGGTG	nd	-	53.0	-
<p>^[a] ^Y denotes the pamitoyl substituted building block, ^[b] Conditions: T_m values/°C (ΔT_m = change in T_m value calculated relative to the DNA:DNA reference duplex) measured as the maximum of the first derivative of the melting curve (A_{260} vs. temperature) recorded in HEPES buffer (10 mM HEPES, 110 mM NaCl, pH 7.0). ^[c] 62 nM concentrations of the two complementary strands in presence of liposomes ^[d] 1 μM concentrations of the two complementary strands in absence of liposomes. Exp. error: $\pm 1^\circ\text{C}$. nd denotes “not detected”.</p>					

Preparation of supported membranes: Supported membranes were prepared by hydration of spincoated lipid films as described previously.^[2] The procedure consists of the following steps: To prepare a dry spincoated POPC film on mica a stock solution of 10 mM POPC containing 0.7 % DiO- C_{18} in hexane/methanol (97:3 volume ratio) was used. A droplet (30 μL) of this lipid stock solution was then applied to freshly cleaved mica with a size of 10 mm x 10 mm and immediately thereafter spun on a Chemat Technology, KW-4A spin-coater at 3000 rpm for 40 s. The sample was placed under vacuum for 10-15 hours to ensure complete evaporation of solvents. The dry spin coated film was subsequently hydrated by immersing the sample in HEPES buffer followed by heating in an oven at 55°C for 1 hour. The sample surface was flushed with HEPES buffer at 55°C which left a single and uniform membrane on the mica surface. After this, the buffer was replaced 10 times to remove membrane fragments in solution.

Experimental conditions for DNA controlled tethering of liposomes to a supported POPC membrane: In a typical experiment for DNA controlled tethering of liposomes to a surface, a supported POPC membrane containing

fluorescent dye (DiO-C18, 0.5%) was first prepared on a mica surface by spincoating according to our recent protocol.^[2,3]

Experimental conditions for DNA digestions using DNase I: POPC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine) liposomes with an average diameter of $65 \text{ nm} \pm 15 \text{ nm}$ and a lipid concentration of 10 mM, prepared by extrusion through 50 nm filters, a final concentration of 0.5 mM is used for all experiments. Addition of lipophilic probe DNA in Tris buffer solution (pH 7.4, 50 mM Tris, 50 mM NaCl, 20 mM MgCl_2 , 62 nM DNA probe and target strand) followed by addition of the target sequence initiates assembly, after recording a conventional thermal denaturation curve from 20 to 80°C and cooling to r.t. 2 μl DNase I is added followed by another thermal denaturation experiment.

Fig. 2 DNase I digestion experiment. UV monitored thermal denaturation experiment (black curve) followed by enzymatic digestion of the dsDNA links between individual liposomes (red curve).

Fluorescence Microscopy: Epi-fluorescence microscopy of supported membranes coupled to liposomes was performed with the sample placed with the lipid side facing up in a custom made microscope chamber (2 ml volume) and placed on a Nikon TE2000 inverted microscope using an oil immersion objective (Plan Apo, 60X NA=1.4, Nikon). Fluorescence excitation was done using a Xenon lamp with monochromator (Polychrome V, Till Photonics, Gräfelfing, Germany) as well as G-2A (DiI) and B-2A (DiO) filtercubes from Nikon. Images were recorded with a high sensitivity CCD camera (Sensicam em, 1004 x 1002 pixels, PCO-imaging, Kelheim, Germany) and operated with Camware software (PCO). The experiment was performed in a total volume of 1 ml HEPES buffer (10 mM HEPES, 110 mM Na^+ , pH 7.0) with a concentration of 100 nM for each strand (DNA 3'-ACACCTTCTTCAACCAC : 5'-TTT~~X~~TGTGGAAGAAGTTGGTG~~X~~TTT). Successful membrane formation was confirmed by independent fluorescence imaging. Thereafter liposomes labeled with a second fluorophor (DiI-C18, 0.5%) and probe strand were

added, but without liposomes adhering to the supported membrane. Finally, the target strand was added leading to duplex formation and attachment of liposomes to the surface within a time of 1-2 hours. However an almost complete attachment (immobilization on the supported membrane) was observed already after 15 min. Thermal denaturation of liposome aggregates could thereby be visualized directly by fluorescence. Heating to above the denaturation temperature of the duplex ($T_m = 46^\circ\text{C}$), led to complete release of the attached liposomes into solution and re-aggregation upon cooling below the T_m .

Thermal denaturation data of DNA-conjugate duplexes with modification X:

Table 1. Thermal denaturation data of DNA-conjugates modified with X.^[a,b]

Nr.	Duplex	T_m [$^\circ\text{C}$]	ΔT_m [$^\circ\text{C}$]	T_m [$^\circ\text{C}$]	ΔT_m [$^\circ\text{C}$]
target	5'-TTT X TGTGGAAGAAGT TT GG TG X TTT	with liposomes ^c		without liposomes ^d	
1	3'-ACACCTTCTTCAACCAC	42.5	-	47.0	-
2	3'-ACACCTTCTTCA CCC AC	36.0	7.5	39.0	8.0
3	3'-ACACCTTCTTCA G CCAC	39.0	3.5	43.0	4.0
4	3'-ACACCTTCTTCA T CCAC	37.5	5.0	41.5	5.5

^[a] **X** denotes the polyaza crown ether base surrogate. ^[b] Conditions: T_m values/ $^\circ\text{C}$ (ΔT_m = change in T_m value calculated relative to the fully complementary reference duplex) measured as the maximum of the first derivative of the melting curve (A_{260} vs. temperature) recorded in medium salt buffer (10 mM HEPES, 110 mM NaCl, pH 7.0). ^[c] 62 nM concentrations of the two complementary strands in presence of liposomes ^[d] 1 μM concentrations of the two complementary strands in absence of liposomes. Exp. error: $\pm 1^\circ\text{C}$. Mismatch position in blue.

Chemical structure of the building blocks X and Y:

Fig. 3 Chemical structure of the amidite building block (top structure) 3-[19-{2-[bis(4-methoxyphenyl)(phenyl)methoxy]ethoxy}-3,15-dihexadecyl-6,12-dioxo-3,9,15,21-tetraaza-bicyclo[15.3.1]heni-cosa-1(21),17,19-trien-9-yl]propyl-2-cyanoethyl-diisopropylphosphoramidite and the corresponding modification **X** in the DNA-conjugate (bottom structure).

Fig. 4 Chemical structure of the amidite building block (2S,3R,4S)-2-((bis(4-methoxyphenyl)(phenyl)-methoxy)methyl)-4-palmitamidotetrahydrofuran-3-yl-2-cyanoethyl diisopropylphosphor amidite and the corresponding modification **Y** in the DNA-conjugate (bottom structure).

Light scattering: Light scattering experiments were performed using a Brookhaven BI-200SM.

Fig. 5. Light scattering data for the formation of liposome assemblies. Liposome aggregate size distributions obtained by fitting to dynamic light scattering data reveals an increase in particle size during aggregation.

Oligonucleotide synthesis and MALDI-TOF analysis of DNA-conjugates: Standard procedures for automated DNA synthesis have been used except pyridinium hydrochloride as activator reagent and coupling times of 20 min. Incorporation of monomers into oligodeoxynucleotides (ONs) was performed on a 0.2 μ mol scale and was followed by purification of the ONs by HPLC as previously described.^[4] MALDI-TOF analysis was performed on a Voyager Elite Biospectrometry Research Station from PerSeptive Biosystems.

Table 2. Molecular weight of oligonucleotides (MALDI-TOF-MS)

Entry	sequence	mass _{calculated}	mass _{found}
1	5'-TTT X TGTGGAAGAAGTTGGTG X TTT	9012.6	9014.9
2	5'-TTT X CACCAACTTCTTCCACA X TTT	8701.6	8705.7
3	5'-TTT Y TGTGGAAGAAGTTGGTG Y TTT	8033.7	8034.4
4	5'-TTT Y CACCAACTTCTTCCACA Y TTT	7722.7	7728.3

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