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Electronic Supplementary Information (ESI) for

DNA surface coating of calixarene-based nanoparticles: a sequence-dependent binding mechanism

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EXPERIMENTAL

General

Ethidium bromide and sodium cacodylate were purchased from Sigma-Aldrich. The following oligonucleotides (30-mer) were purchased from Microsynth.

Table S1 DNA sequences used

Name	Sequence
AT	d(5'-AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA-3') d(5'-TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT-3')
GC	d(5'-CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC-3') d(5'-GGG GGG GGG GGG GGG GGG GGG GGG GGG GGG-3')
ATGC	d(5'-GTG GCT AAC TAC GCA TTC CAC GAC CAA ATG-3') d(5'-CAT TTG GTC GTG GAA TGC GTA GTT AGC CAC-3')

Double-stranded DNA preparation

The lyophilized oligonucleotides were resuspended in nanopure water (300 μM) and stored at -20°C. Double stranded oligonucleotides 30-mer were prepared by mixing the same volume of the two complementary single stranded oligonucleotide in HEPES buffer (20mM Hepes, 100mM NaCl, pH 7.1). The solutions were heated 5 min at 95°C, slowly cooled down to room temperature and the volume of the solution was adjusted to a final concentration of 54 μM. Double-stranded DNA samples used for circular dichroism experiments were prepared following the same protocol with a sodium cacodylate buffer solution (20 mM sodium cacodylate, 100 mM NaCl, pH 6.8).

SLNs preparation

The SLNs suspensions were prepared by dissolving 15 mg of **GC12** in 5 mL THF. After 1 min stirring, a volume of 50 mL of nanopure water was added and the solution was stirred one more minute. The THF was subsequently evaporated under reduced pressure at 40°C and the volume adjusted to 50 mL with nanopure water to obtain a final concentration of 300 μg/mL.

Critical Micellar Concentration (CMC)

A Krüss K100 tensiometer was used to measure the surface tension of **GC12** suspensions by the du Noüy ring detachment method, at room temperature. Solutions of **GC12**–based SLNs

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in nanopure water were prepared at different concentrations ranging from 2.0×10^{-7} to 7.0×10^{-4} M. Measurements were made after an equilibration time of 10 minutes in triplicate to ensure reproducibility.

Dynamic Light Scanning (DLS) and ζ-potential measurements

The particles size and zeta potential were measured using a Zetasizer Nano ZS system (Malvern Instruments). The **GC12** SLNs suspensions were diluted to a concentration of 6 µg/mL in nanopure water. All values were measured three times. The system was thermostated at 25°C.

Atomic Force Microscopy (AFM)

A solution **GC12** SLNs at a final concentration 100 µg/mL was deposited on freshly cleaved mica. Imaging was performed in noncontact mode in air using a NTegra Prima system (NT-MDT) equipped with diamond rectangular cantilevers (NT-MDT). The image is presented unfiltered.

Ethidium bromide displacement assay

Fluorescence measurements were carried out in a 96-well plate (flat bottom, black) at room temperature (excitation at 510 nm, emission at 595 nm) with a Tecan Infinite M200 reader. Ethidium bromide (0.5 equivalents per base pair) was incubated with DNA (1 mg/mL, 54 µM) in a buffer solution (20 mM Hepes, 100 mM NaCl, pH 7.1) during 15 min prior to titration by SLNs. Increasing volumes of the stock solution (300 µg/mL) of **GC12** SLNs in a buffer solution (20 mM Hepes, 10 mM NaCl, pH 7.1 or 20 mM Hepes, 100 mM NaCl, pH 7.1) were mixed with the premixed DNA-EtBr solution (final concentration of oligonucleotide 40 µg/mL) 30 min prior to measurement. Standard calibration curves were fitted to a linear curve using Origin software.

Circular dichroism (CD)

All CD experiments were recorded on an Applied Photophysics ®Chirascan circular dichroism spectrometer using a quartz cell with a 1cm path length. Volumes of **GC12** SLNs stock solution (300 µg/mL, 204 µM, in buffer 20 mM Sodium Cacodylate, 100 mM NaCl, pH 6.8) were added to a solution of oligonucleotide (650 µL, 2.72 µM, in the same buffer), the spectra were recorded from 200 to 350 nm at 20°C and data recorded in 0.5 nm increments with an average of 2s.

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Isothermal Titration Calorimetry (ITC)

Isothermal titration calorimetry measurements were conducted at 20°C on a MicroCal ITC_{200}.Volumes of 1.5 µL of oligonucleotide (147 µM in 20 mM Hepes, 100 mM NaCl, pH 7.1) were injected at 20°C into an isothermal sample chamber containing SLNs (400 µL, 7.37 µM in 20mM Hepes, 100 mM NaCl, pH 7.1) via a 40 µL rotary syringe (500 rpm). The interval time between each injection was 240 s. The initial delay prior to the first injection was 60s. Injections of oligonucleotide solutions at the same concentration in buffer solution were used as a blank. The experimental data were fitted to a theoretical titration curve using the software supplied by MicroCal (OriginLab).

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RESULTS

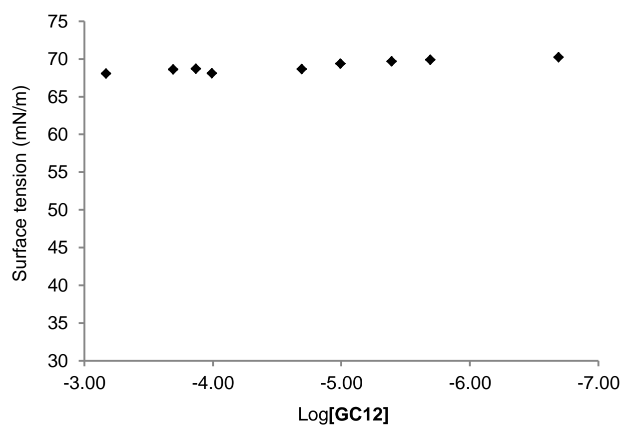


Figure S1: Graph of the surface tension versus the logarithm of the concentration of **GC12** SLNs

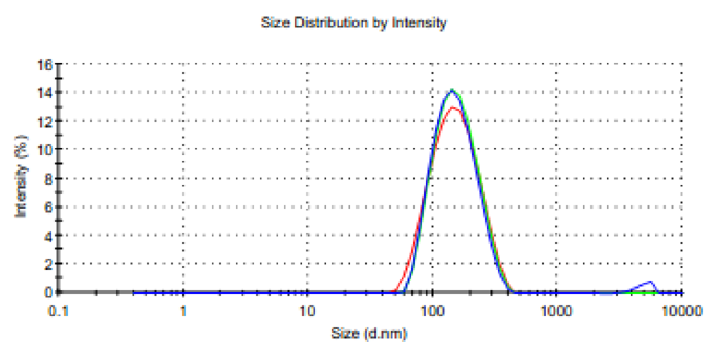


Figure S2: DLS size-distribution intensity measurements of **GC12** SLNs (6 μg/mL) in nanopure water

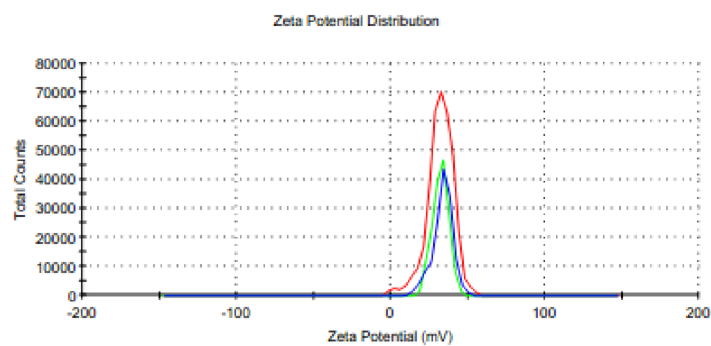
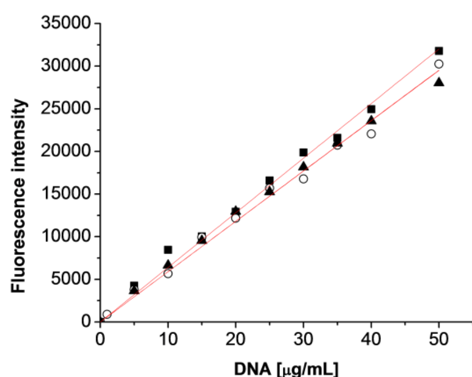
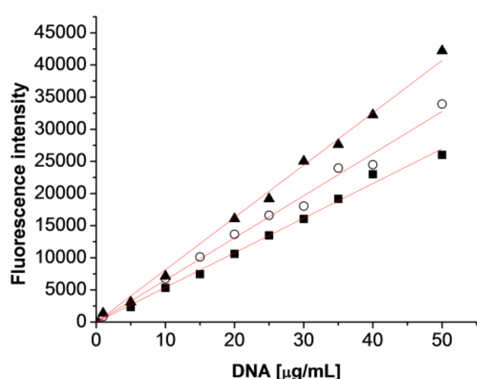


Figure S3: Zeta potential measurements of **GC12** SLNs (6 μg/mL) in nanopure water



Equation		F = A + B*[DNA]		
		Value	Standard Error	R^2
AT	A	0	0	0.99129
	B	640.47	10.48	
GC	A	0	0	0.99254
	B	590.07	9.51	
ATGC	A	0	0	0.9924
	B	590.79	9.03	

Figure S4: Standart calibration curve of **AT**-EtBr (square), **GC**-EtBr (circle) and **ATGC**-EtBr solution (triangle) fluorescence intensity as function of the oligonucleotide concentration (μg/mL) in a buffer solution (20 mM Hepes, 10 mM NaCl, pH 7.1) after 15 min of incubation with EtBr 0.5 equivalents per base pair (left); Table values of the parameters fitting the linear equation (right).



Equation		F = A + B*[DNA]		
		Value	Standard Error	R^2
AT	A	0	0	0.99482
	B	539.460	7.23	
GC	A	0	0	0.99199
	B	655.51	11.15	
ATGC	A	0	0	0.99621
	B	814.27	10.33	

Figure S5: Standart calibration curve of **AT**-EtBr (square), **GC**-EtBr (circle) and **ATGC**-EtBr solution (triangle) fluorescence intensity as function of the oligonucleotide concentration (μg/mL) in a buffer solution (20 mM Hepes, 100 mM NaCl, pH 7.1) after 15 min of incubation with EtBr 0.5 equivalents per base pair (left); Table values of the parameters fitting the linear equation (right).

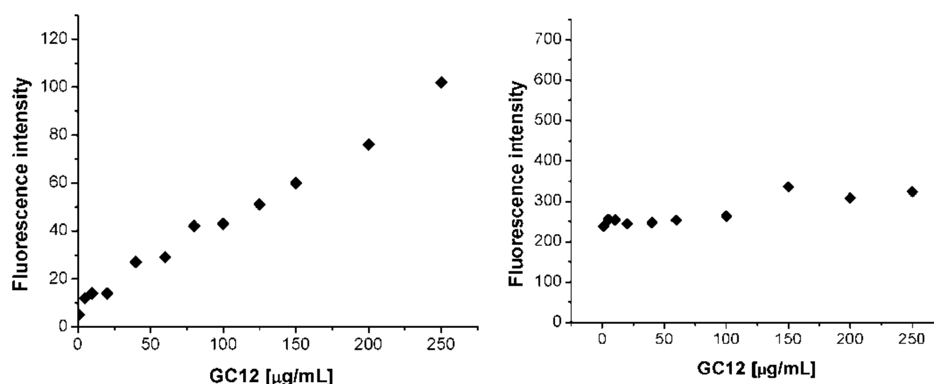


Figure S6: Fluorescence intensity of **GC12** SLNs measured in nanopure water (left) and after 15 min of incubation with EtBr in buffer solution (20 mM Hepes, 100 mM NaCl, pH 7.1) (right).

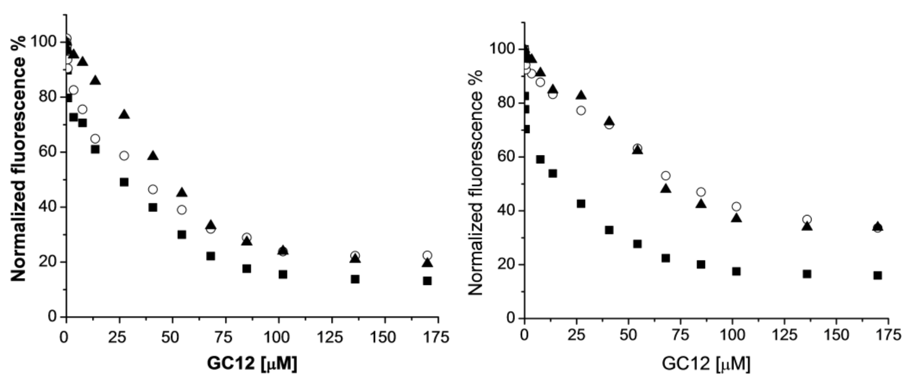


Figure S7: Titration of **AT**-EtBr (square), **GC**-EtBr (circle), **ATGC**-EtBr (triangle) complexes by **GC12** SLNs in buffer solution (20 mM Hepes, 10 mM NaCl, pH 7.1) (left), in buffer solution (20 mM Hepes, 100 mM NaCl, pH 7.1) (right)

Table S2 AC_{50} values of the SLNs with polyoligonucleotides

Polynucleotide	**GC12** [μM]	
	NaCl 100 mM[a]	NaCl 10 mM[b]
AT	14 ± 4	33 ± 6
GC	75 ± 11	50 ± 15
ATGC	108 ± 35	60 ± 12

[a] Interaction condition in a buffer solution 20 mM Hepes, NaCl 100 mM NaCl, pH 7.1. [b] Interaction condition in a buffer solution 20 mM Hepes, 10 mM NaCl, pH 7.1

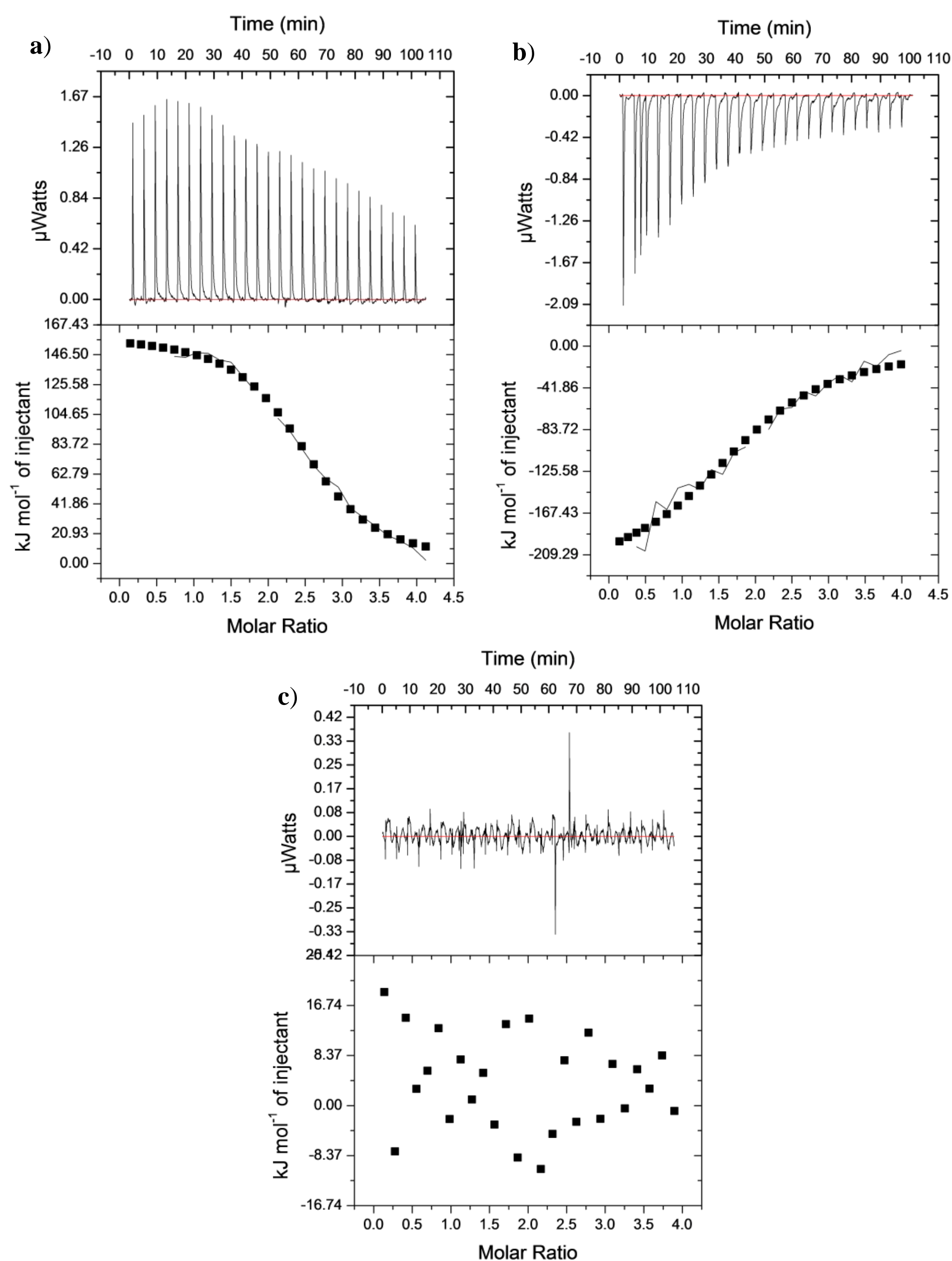


Figure S8: Isotherm titration calorimetry profiles for the binding of **GC12** SLNs at 20°C in buffer solution (20 mM Hepes, 100 mM NaCl, pH 7.1) with **a) AT**; **b) GC**; **c) ATGC**

Table 3 ITC thermodynamic profile for the binding of **GC12** SLNs with oligonucleotides

	K_A [L/mol]	K_D [mol/L]	ΔH [kJ/mol]	ΔS [J/(molK)]	ΔG [kJ/mol]
AT	1.47E+06	6.80E-07	160.5 ± 2.87	665	-34.3
GC	4.32E+05	2.31E-06	231.1 ± 19.1	-680	-31.9
ATGC	-	-	-	-	-