

Supplementary Information for

Interplay of Mechanical and Structural Properties of DNA Nanostructures Determines  
Their Electrostatic Interactions with Lipid Membranes

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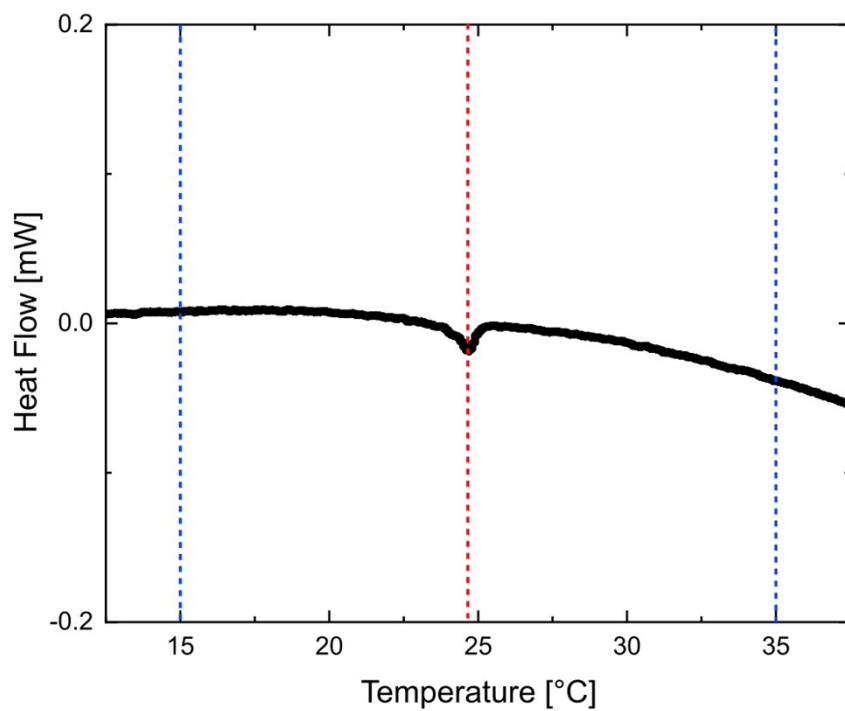


Figure S1 Differential scanning calorimetry DSC measurements of extruded DMPC vesicles (2.5 mg/ml), measured in the range of 10-40 °C, with a rate of 1 °C/min. The dashed lines represent the phase transition temperature (red) and the temperatures at which zeta potential measurements were performed, to ensure working in either liquid (35 °C) or gel (15 °C) phase. (blue).

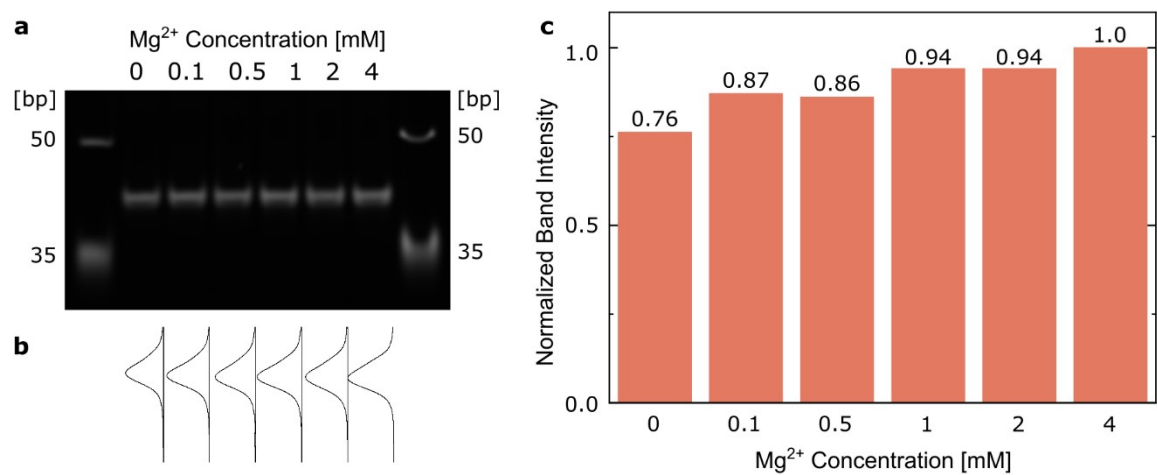


Figure S2 Polyacrylamide gel electrophoresis analysis of 40 bp-long duplex in various  $Mg^{2+}$  concentrations. (a) The gel image, also presented in Fig. 2e, alongside (b) the collected intensity profiles from the wells and (c) analyzed values plotted. The obtained, normalized intensities are added above each column.

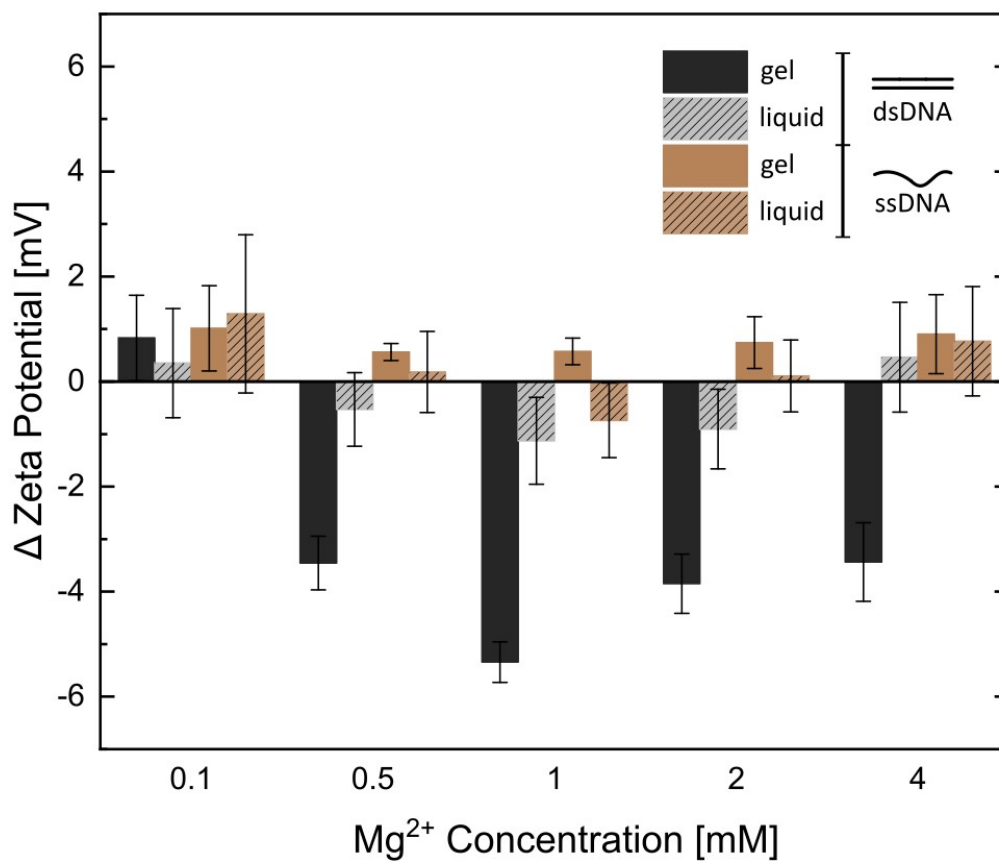


Figure S3 Differences in zeta potential of vesicles with and without DNA ( $\Delta\zeta = \zeta_{(\text{vesicles}+\text{DNA})} - \zeta_{\text{vesicles}}$ ). Values obtained when incubating ssDNA (40 nt) or dsDNA (40 bp) with DMPC in liquid or gel phase. While dsDNA with liquid-phased vesicles and ssDNA show marginal change after DNA was added to the vesicles, dsDNA shows a large drop of zeta potential above 0.5 mM  $\text{MgCl}_2$ , indicating DNA attachment to gel-phased lipid surface.

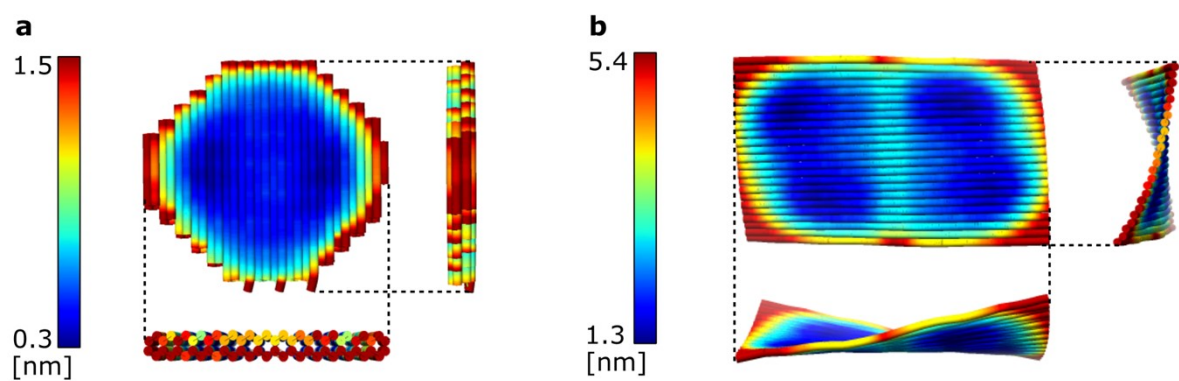


Figure S4 CanDo analysis of the two studied DNA origami structures: (a) *disk* and (b) *plate*. The colour scale represents the RMSF. Note the large difference between fluctuation scales of the two designs.

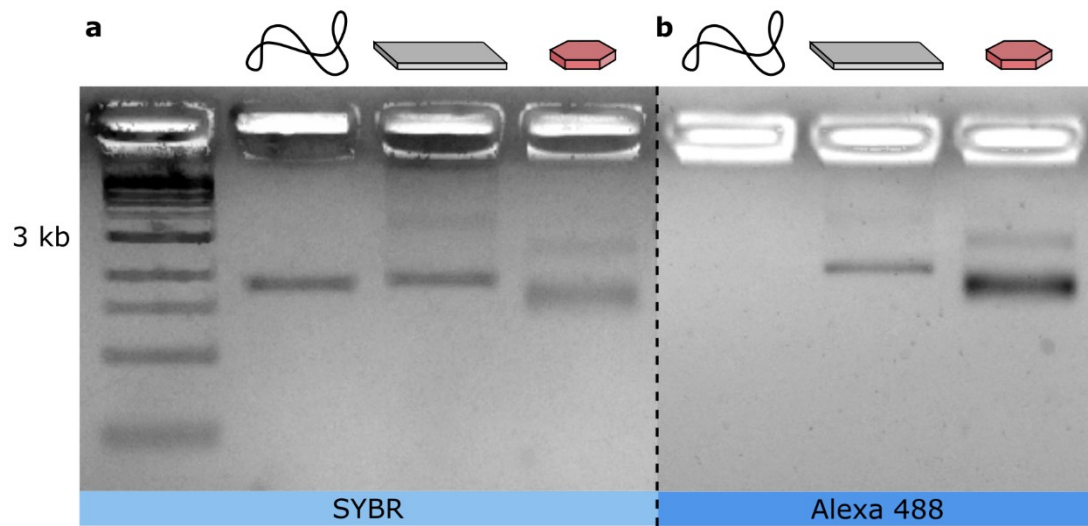


Figure S5 Agarose gel electrophoresis confirmed the folding of *disk* and *plate* origami structures (Fig 4), compared with p7560 scaffold. Panel (a) represents the SYBR signal, while (b) shows the Alexa488 modification. The same gel was imaged sequentially, starting with Alexa488 signal, and only then incubated with SYBR Safe solution, due to overlapping fluorescence spectra. Relative intensities of Alexa488 signal were measured (*plate* – 1x, *disk* – 3.8x), and used to adjust and interpret results shown in Fig. 4.

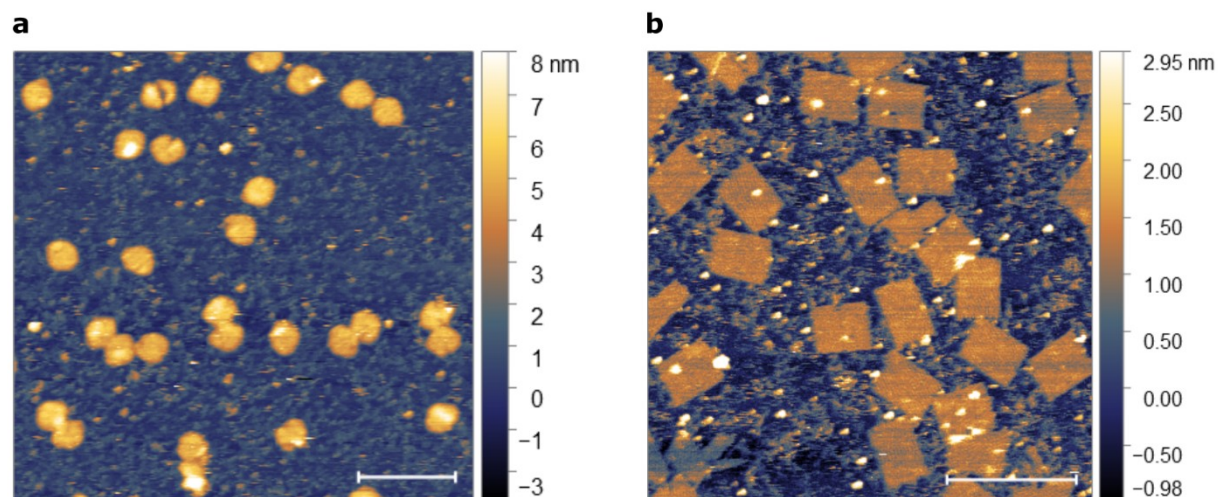


Figure S6 Atomic force microscopy images of (a) *disk* and (b) *plate* DNA origami structures. Scale bars: 200 nm.

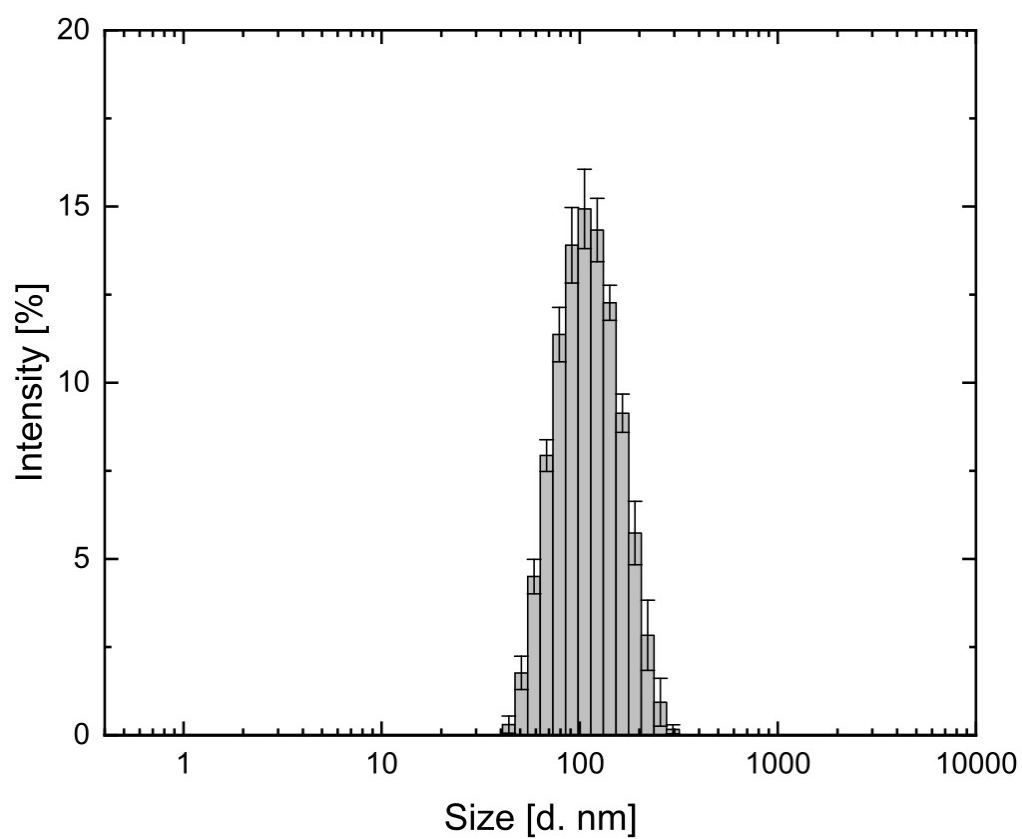


Figure S7 Dynamic light scattering (DLS) measurements of DMPC vesicles extruded through 100 nm filter. Error bars represent standard deviation from three measurements, each consisting of 12 runs.



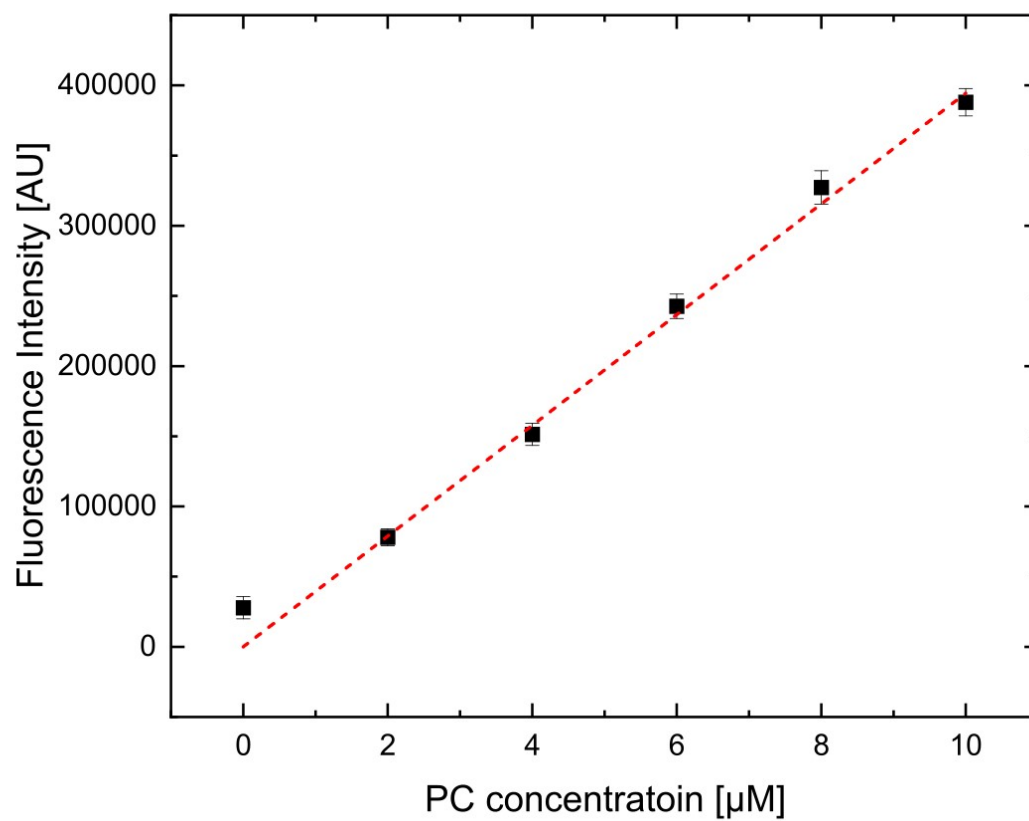


Figure S8 Calibration curve for PC concentration assay, used to compare the available lipid surface between DOPC and DPPC vesicles (Fig. 1b, Fig 4e-f). Error bars represent standard deviation from four measurements done on two separate days.

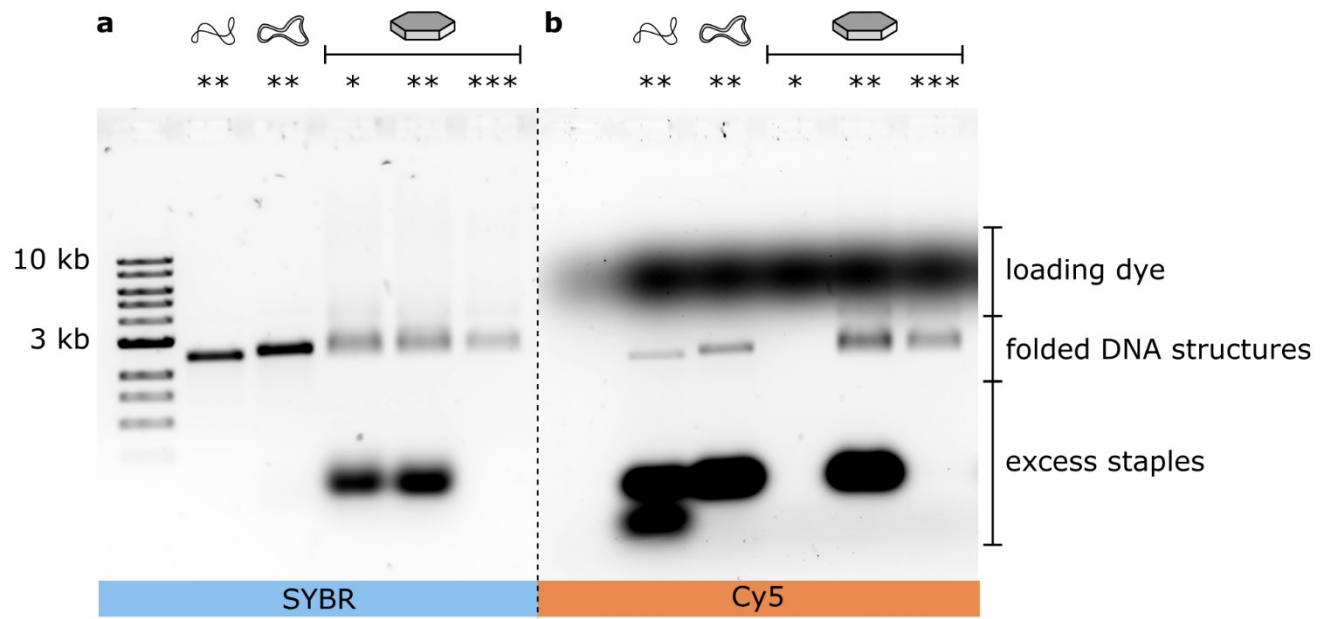


Figure S9 Agarose gel electrophoresis of Cy5-labelled long ssDNA, long dsDNA and origami *disc*. Signal was obtained from simultaneous (a) SYBR and (b) Cy5 excitation. Legend for the asterisks markings is as follows: \* before labelling, \*\* after labelling, \*\*\* after purification

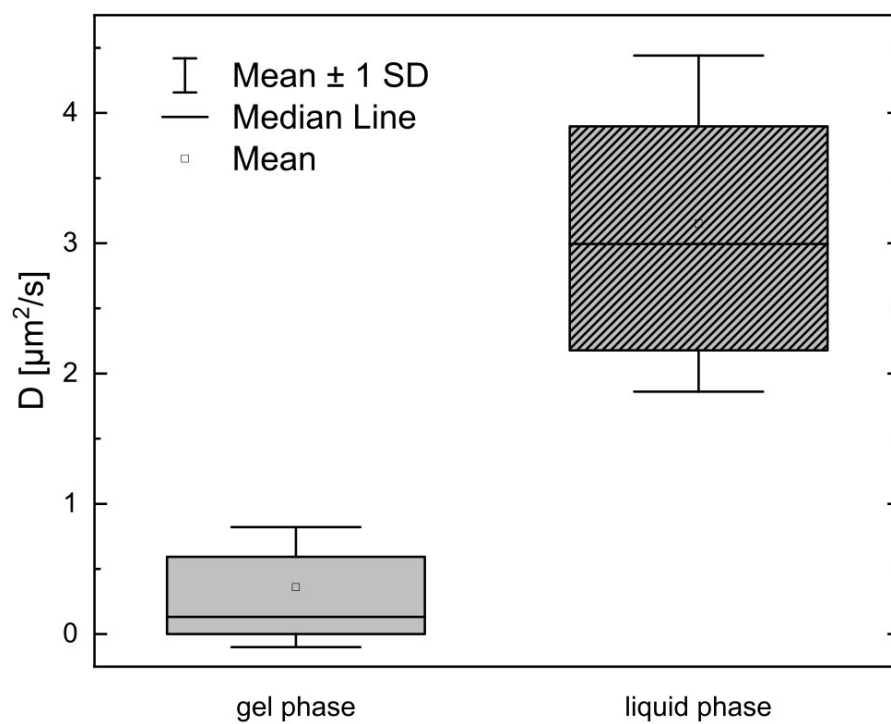


Figure S10 Box plots of diffusion coefficients ( $D$ ) obtained from FRAP experiments on vesicles in their gel (DPPC) and liquid (DOPC) phase. Collected number of vesicles:  $N_{\text{DPPC}} = 10$ ,  $N_{\text{DOPC}} = 28$ .

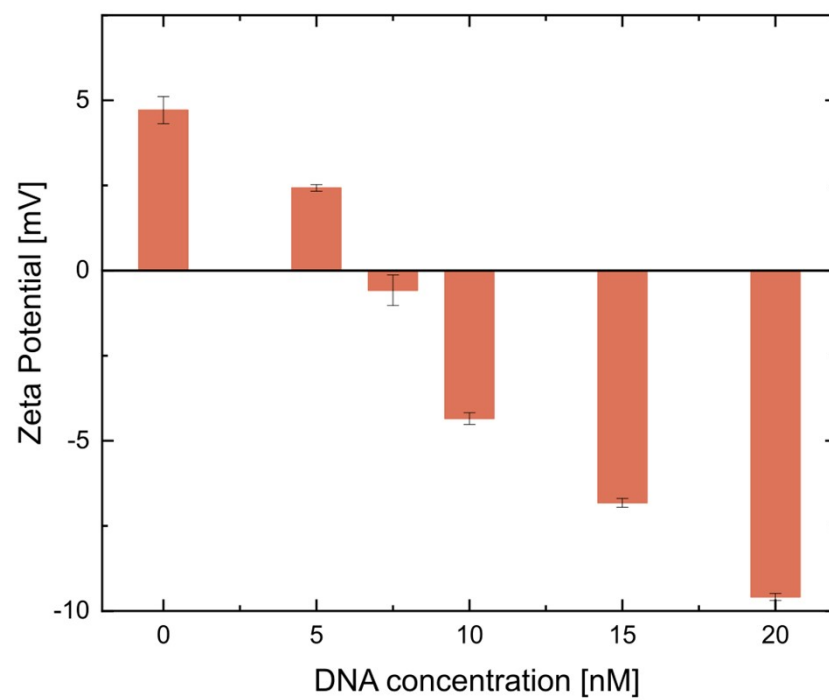


Figure S11 Zeta potential values obtained for various DNA:lipid ratios. The analyzed DNA structure was a 40 bp-long duplex in stated concentration, incubated with 12.5  $\mu\text{g/ml}$  of DMPC vesicles in gel phase.

Table S1

Sequences of oligonucleotides used to build a library of DNA duplexes presented in Fig. 3. Mod. stands for modification. Nucleotides highlighted in red are unpaired.

Structure	Strand	Sequence	Length	Mod.
=	S1	ACTGATTACATCGACATGCA	20	5'Cy5
	S2	TGTCATGTCGATGTAATCAGT	21	
==	S1	ACTGATTACATCGACATGCATAGCTAACGGTCTCAATCTA	40	5'Cy5
	S2	TAGATTGAGACCGTTAGCTATGCATGTCGATGTAATCAGT	41	
===	S1	ACTGATTACATCGACATGCATAGCTAACGGTCTCAATCTACAACATGCAGATATCGTCTT	60	5'Cy5
	S2	TAAGACGATATCTGTCATGTTGTAGATTGAGACCGTTAGCTATGCATGTCGATGTAATCAGT	61	
==	S1	ACTGATTACATCGACATGCATAGCTAACGGTCTCAATCTACAACATGCAGATATCGTCTT	60	5'Cy5
	S2A	TAAGACGATATCTGTCATGTTG	21	
	S2C	TGCATGTCGATGTAATCAGT	20	
==	S1	ACTGATTACATCGACATGCATAGCTAACGGTCTCAATCTACAACATGCAGATATCGTCTT	60	5'Cy5
	S2A	TAAGACGATATCTGTCATGTTG	21	
	S2B	TAGATTGAGACCGTTAGCTA	20	
===	S1	ACTGATTACATCGACATGCATAGCTAACGGTCTCAATCTACAACATGCAGATATCGTCTT	60	5'Cy5
	S2A	TAAGACGATATCTGTCATGTTG	21	
	S2B	TAGATTGAGACCGTTAGCTA	20	
	S2C	TGCATGTCGATGTAATCAGT	20	
===	S2-1T	AAGACGATATCTGTCATGTTGTAGATTGAGACCGTTAGCTATGTCATGTCGATGTAATCAGT	62	5'Cy5
	S1	ACTGATTACATCGACATGCA	20	
	S1B	TAGCTAACGGTCTCAATCTA	20	
	S1C	CAACATGCAGATATCGTCTT	20	
===	S2-2T	AAGACGATATCTGTCATGTTGTAGATTGAGACCGTTAGCTATGTCATGTCGATGTAATCAGT	64	5'Cy5
	S1	ACTGATTACATCGACATGCA	20	
	S1B	TAGCTAACGGTCTCAATCTA	20	
	S1C	CAACATGCAGATATCGTCTT	20	
===	S2-3T	AAGACGATATCTGTCATGTTGTAGATTGAGACCGTTAGCTATGTCATGTCGATGTAATCAGT	66	5'Cy5
	S1	ACTGATTACATCGACATGCA	20	
	S1B	TAGCTAACGGTCTCAATCTA	20	
	S1C	CAACATGCAGATATCGTCTT	20	

Table S2 Number of vesicles used to create box plot in Fig. 2c

MgCl <sub>2</sub> concentration [mM]	0.1	0.2	0.6	1.1	2.1	4.1
Number of vesicles	122	160	114	87	135	113

Table S3 Number of vesicles used to create box plot in Fig. 3e










DNA structure									
Number of vesicles	290	256	400	254	503	354	341	374	422

Table S4 Number of vesicles used to create box plot in Fig. 4e

DNA structure/lipid phase	<i>plate</i> /gel	<i>plate</i> /liquid	<i>disc</i> /gel	<i>disc</i> /liquid
Number of vesicles	63	81	75	191