Supplementary Material (ESI) for Soft Matter

Supplementary Information

Interaction of Reducible Polypeptide Gene Delivery Vectors with Supported Lipid Bilayers: Pore Formation and Structure-Function Relationships

Mahmoud Soliman[a,d], Rujikan Nasanit[b], Stephanie Allen[a], Martyn C. Davies[a], Simon S. Briggs[c], Leonard W. Seymour[c], Jon A. Preece[b] and Cameron Alexander[a]*

Table S1 – Properties of polypeptide vectors

RPC	Monomer	M_n (kDa)	Polyplex width/ (AFM)	No of Positive charges per unit at pH 7.4[a]
RPC1	CK_8C	111.48	109.14 ± 8.80	9
RPC2	CK_4H4C	118.01	91.43 ± 13.75	5.7
RPC3	$CK_2H_2K_2H_2C$	115.11	85.29 ± 8.67	5.2
RPC4	CK_2HKHKH_2C	102.96	83.57 ± 2.88	5.4
RPC5	CKHKHKHKHC	94.83	79.71 ± 13.19	5.3

a-Obtained via NMR titration

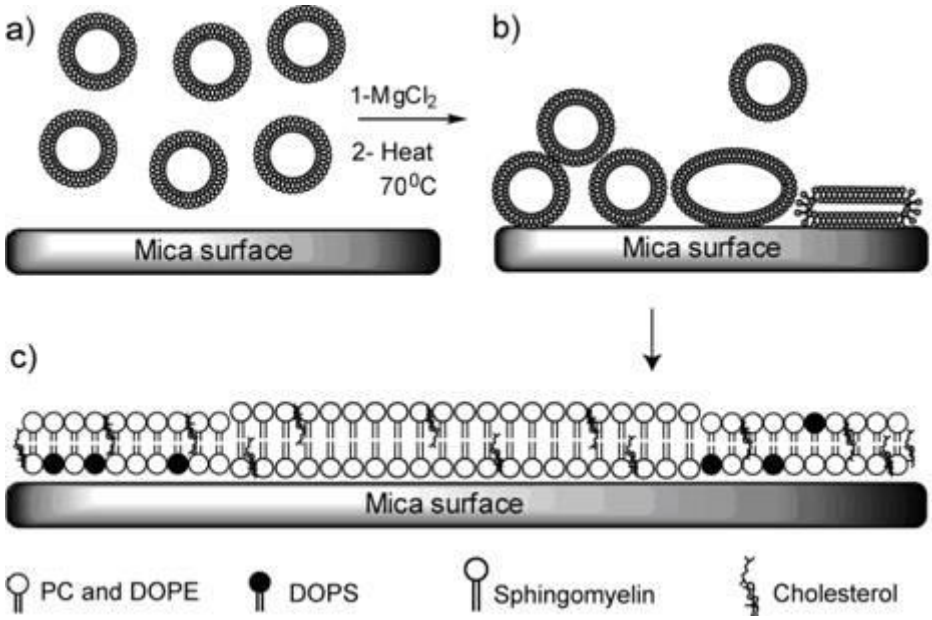

Fig. S1: Schematic of preparation of SPB. In (a) negatively charged liposomes adsorbed to mica surface in Tris buffer containing 10 mM $MgCl_2$. Heating at 70 °C leads to liposome fusing on the mica surface (b) and subsequent cooling to R.T. generates supported bilayer (c). PC = phosphatidylcholine, DOPE = dioleoyl phosphatidylethanolamine, DOPS = dioleoyl phosphaptidylserine.

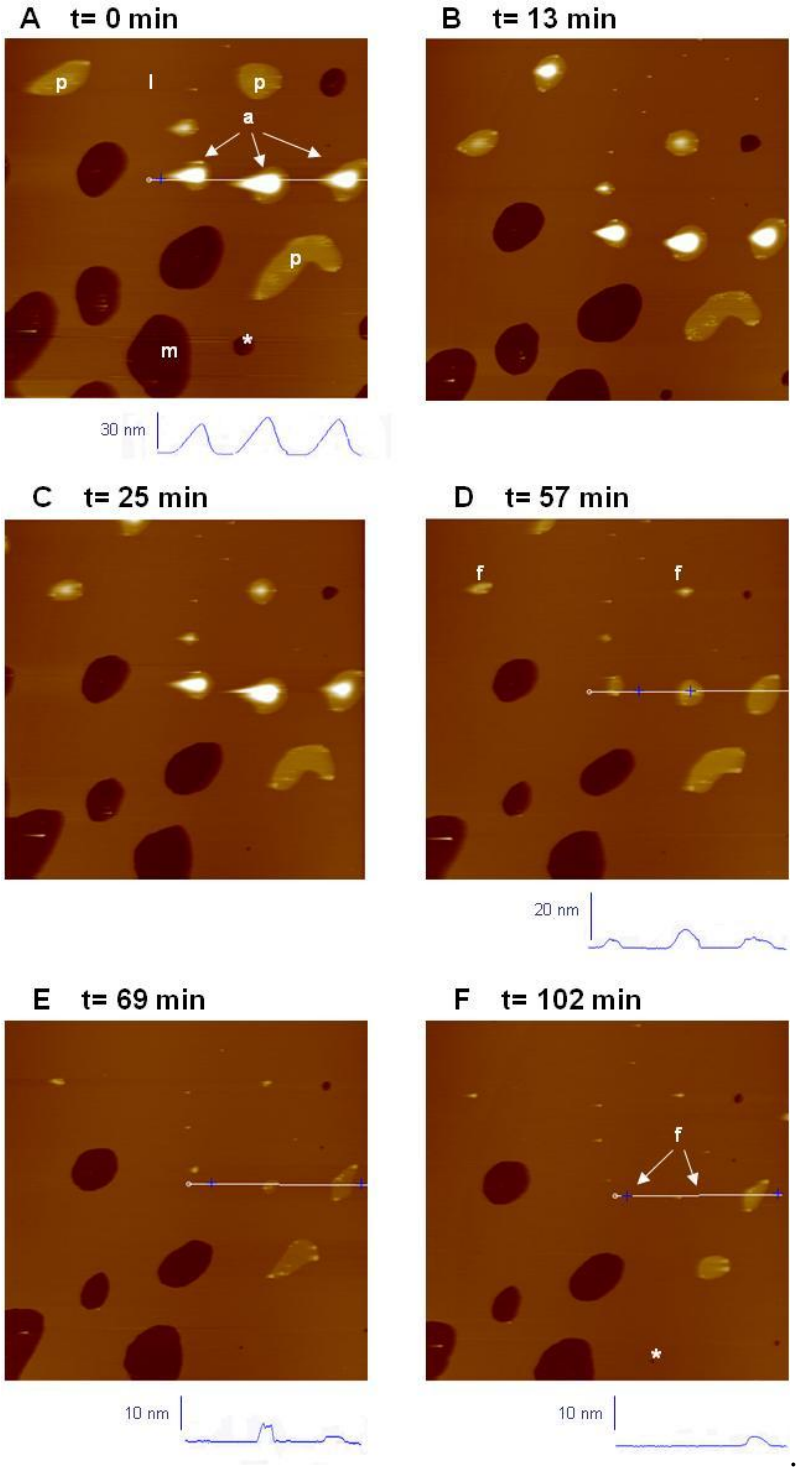

Fig. S2; Time-lapse AFM images of the same regions of the sample demonstrating liposome fusion, rupture, and bilayer spreading on mica (as also shown schematically in Fig. S1(i). The images were collected in Tris-HCl/$MgCl_2$ buffer (10mM, pH 7.4) at 60 °C. (A) The attached liposomes (a), partially fused liposomes (p), and fused lipid bilayers (l) are clearly distinguished from the bare mica surface (m). The two partially flattened liposomes (p) in the upper part of the image fuse and spread through frames A to C, until they are almost completely flattened (f), as seen in D. The three attached liposomes (a) in the middle of the image also fuse, as seen in frames A to E, until flattened (f), as seen in (F). The height of the flattened liposome is reduced from a 25 nm (A) to < 1 nm (F).The lipid material spreads to cover bilayer imperfections (*). All scan sizes are 6.9 x 6.9 µm, z-scale 10 nm

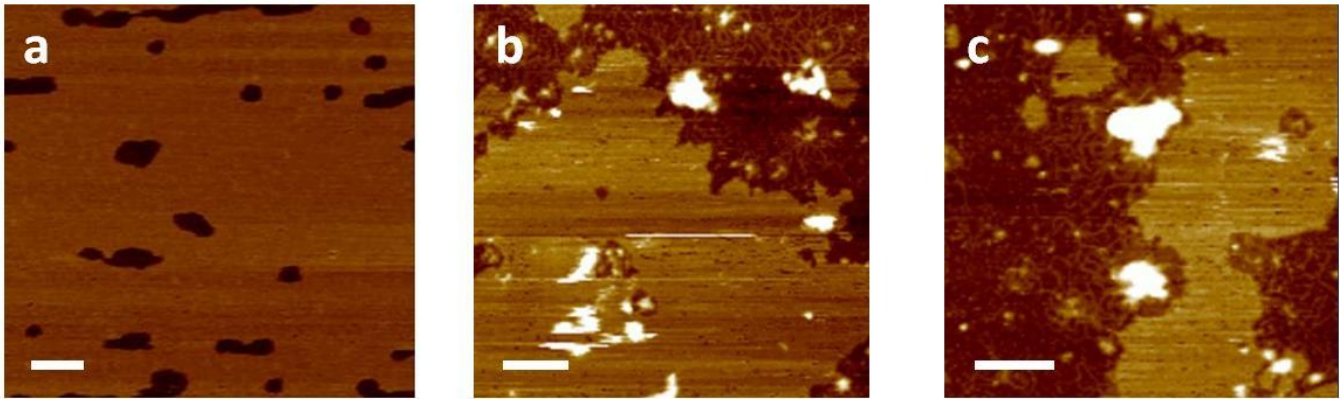

Fig. S3. AFM images of DPPC- SPB surfaces under Tris HCl buffer (10mM, pH 7.4) a) before and (b,c), 35 minutes after the addition of RPC1-DNA complexes. Note the partial disruption of SPB surface and partial dissociation of the complex when in contact with DPPC surface. Scale bar 200 nm, z-scale 15 nm.

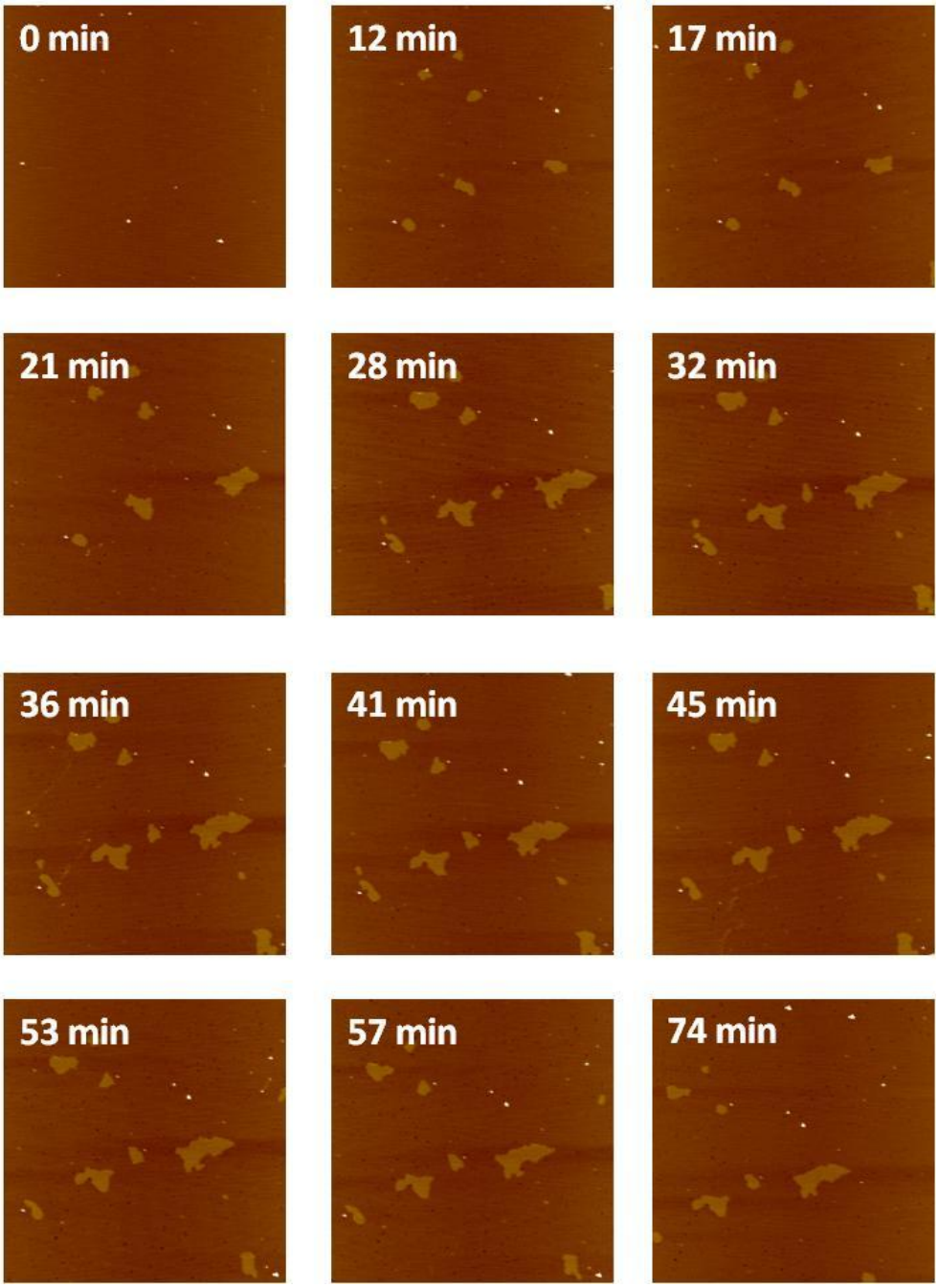

Fig. S4 AFM images of SPB imaged in Tris HCl buffer (10mM, pH 7.4) containing 10 mM Mg^{2+} showing minimal SPB defects after scanning it for up to 74 min. Only small sphingolipid-rich domains began to appear dispersed in the scan area with no formation of large coalesced domains: control experiments did not show SPM domain aggregation upon scanning the bilayer 12 times and over long intervals~ 80 min. All scan sizes are 5 x 5 µm. z-scale: 10 nm and depict the same regions of the sample.

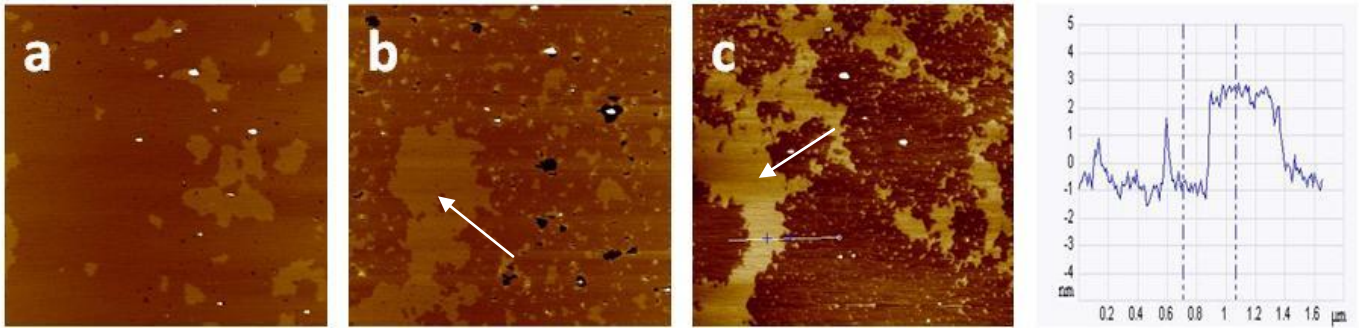

Fig. S5 – AFM images of SPB in Tris buffer (10mM pH 7.4) containing 10 mM Mg Cl_2 (a,b) 7 and 64 min. after adding 22 nM RPC1/ DNA complexes, (c) SPB after the addition of 120 nM complex, phospholipid rich domains are totally desorbed from the SPB, while SPM rich domains (arrowed) remain attached to mica surface. All scan sizes are 5 x 5 µm. z-scale: 10 nm.

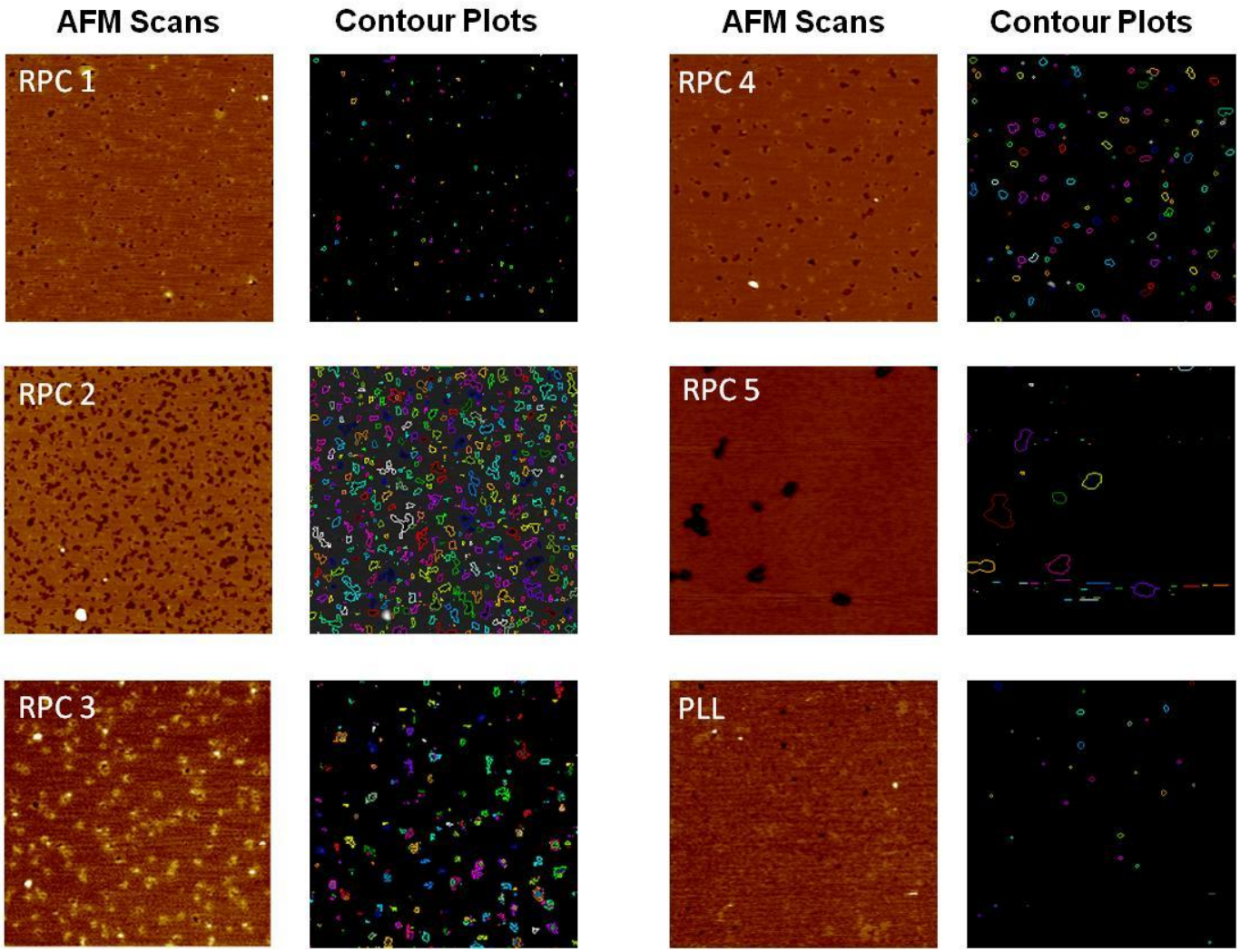

Fig. S6: Pore analysis of SPB after addition of 3.6 nM RPCs 1-5, images were pre-equalized for their local mean height profiles, image noise was reduced to 10% then pores were analyses using the thresholding method at a detection level of 0.2-0.5. All scan sizes are 2 x 2 µm. z-scale: 10 nm.

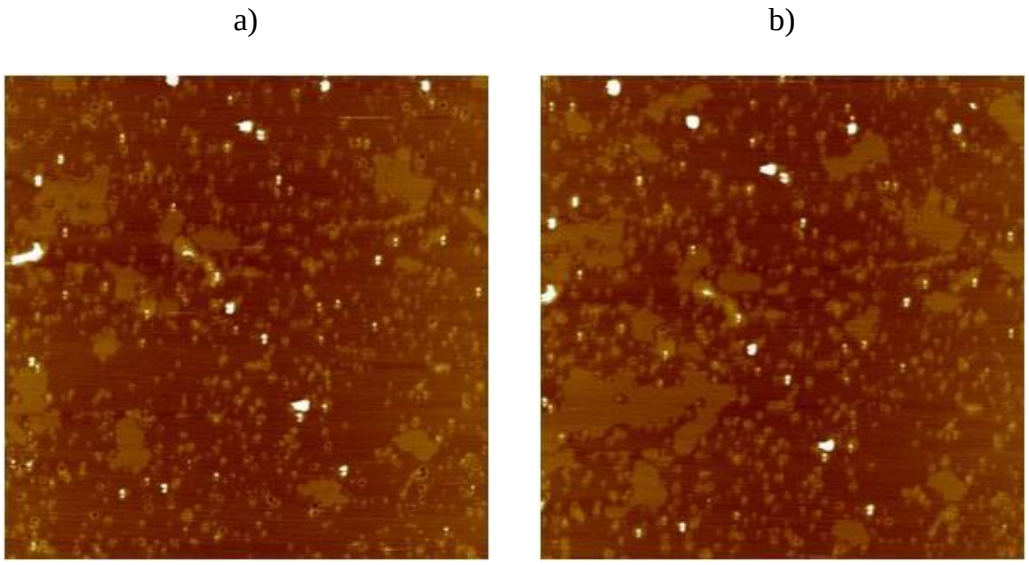

Fig. S7:AFM micrographs of RPCs 2 complexed to pEGFP-DNA at SPB surfaces imaged under Tris buffer (10mM pH 7.4) containing 10 mM Mg Cl_2 The same area of SPB surface imaged with AFM, Plates (a, b) depict RPC2-pEGFP-DNA at 42 and 57 minutes after co-incubation start. All scan sizes are 5 x 5 µm, z-scale 10 nm. Images are enlargements of those shown in Fig. 4d,e.

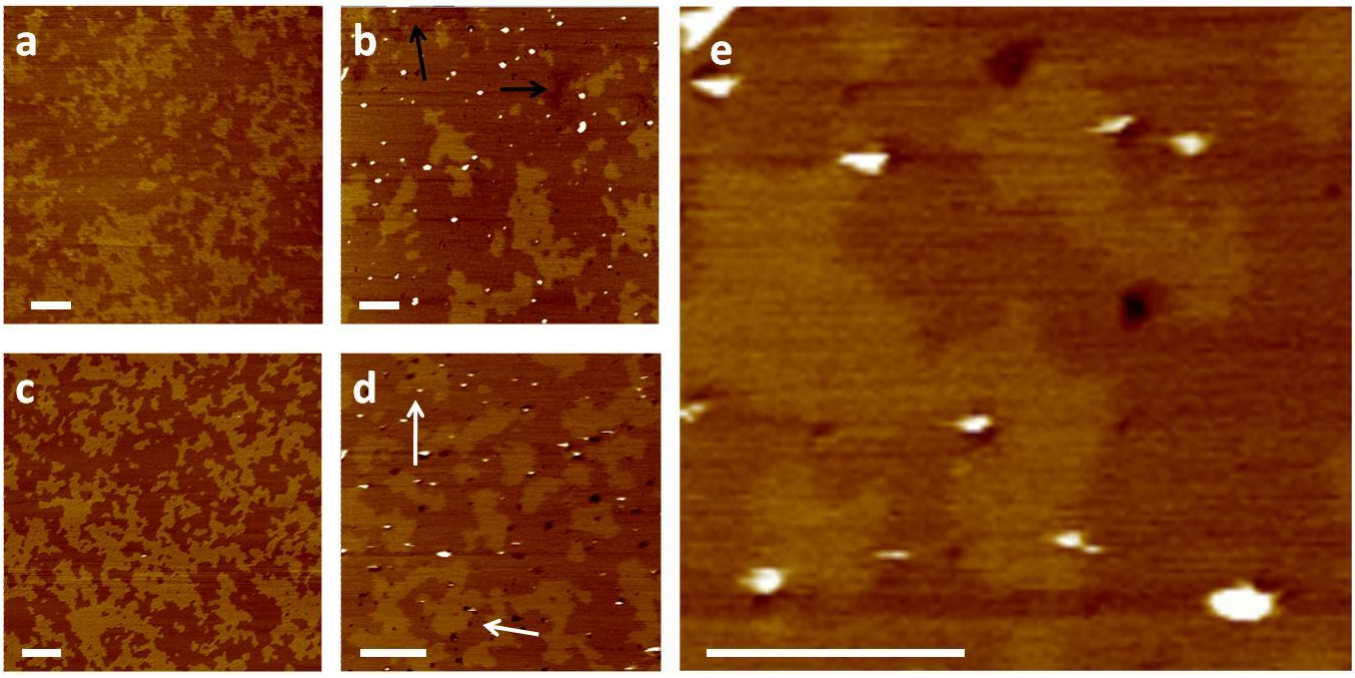

Fig. S8: AFM images of SPB on mica under Tris buffer (10mM pH 7.4) containing 10 mM Mg Cl_2 (a) before and (b) after addition of RPC1/DNA polyplexes (3.6nM, N/P=5) showing preferential adsorption and localization of polyplexes in fluid areas of the bilayer , black arrows denote membrane thinning effect of the RPC1 on fluid regions regions with minimal pore forming ability on SPM rich domains. Plates (c-d) showing AFM images of SPB without DOPS (c) before and (d) after addition of RPC1/DNA polyplexes (3.6nM, N/P=5). White arrows denotes pores in the SPM rich domains. (e) Showing RPC1/DNA polyplexes ~70-120 nm in size with some DNA threads and loops protruding from polyplex outer surface. Scale bar 500 nm, z-scale 10 nm.