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Peculiarities of adsorption of nanocarriers on planar lipid surfaces

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Appendix 1. Experimental Section

Carboxymethyl-β-cyclodextrin (CM-β-CD) with a degree of substitution of 3, according to the information of the supplier, was purchased from Sigma-Aldrich (St. Louis, USA). Asolectin, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), and 1,2-dioleoyl-3-trimethylammonium-propane chloride (DOTAP) were purchased from Avanti Polar Lipids (Alabaster, USA). Tris, NaCl, $CaCl_2$ and KOH were purchased from Reachim (Moscow, Russia). Organic solvents – ethanol, methanol, chloroform and tetrahydrofuran (THF) were obtained from Component-Reactiv (Moscow, Russia). Deionized water (DI water) with conductance 0.05 μS/cm was used for the preparation of the solutions.

Cationic liposomes were prepared by the following extrusion procedure. The weighted amounts of DOPC and DOTAP were dissolved in a methanol/ chloroform mixture so that the molar fraction of the cationic lipid [DOTAP]/([DOPC] + [DOTAP]) was 0.3. Then the organic solvent was evaporated on a Laborota 4000 vacuum rotor evaporator by Heidolph (Schwabach, Germany). The resulting thin lipid film was dispersed in 0.01 M Tris buffer with pH 7. The freshly formed suspension was subjected to extrusion using a LiposoFast extruder by Avestin (Ottawa, Ontario, Canada) with a 200 nm Nuclepore polycarbonate membrane from Avanti Polar Lipids (Ottawa, Ontario, Canada). The average size of the obtained liposomes was 180 nm. The samples were used within one day after preparation. The sizes and electrophoretic mobility (EPM) of liposomes and their complexes were controlled by Brookhaven ZetaPlus (Brookhaven, USA) equipment with software provided by the manufacturer.

Lipid monolayers were obtained using the Langmuir technique with the use of the KSV NIMA KN 1002 setup (Espoo, Finland) with the software provided by the manufacturer. Saline solution (0.9% NaCl) with pH 7.2 was used as the bulk phase. Experiments on modelling the membranes of cationic liposomes were carried out with monolayers composed of a mixture of electroneutral DOPC and cationic DOTAP lipids; the mole fraction of DOTAP was 0.3. Experiments on modelling the cell's membrane were carried out with asolectin monolayer. All experiments were conducted at room temperature.

Appendix 2. Results Section

The supramolecular complexes were obtained by electrostatic adsorption of anionic CM-β-CD onto cationic liposomes, yielding stable colloids that remained positively charged even after surface saturation. Electrophoretic mobility measurements showed a decrease from +2.5 to +1.3 (μm/s)/(V/cm) upon addition of CM-β-CD to a suspension of liposomes in physiological saline buffer, with no change in particle size (~180 nm) and no aggregation [Figure S1(*a*)]. Langmuir monolayer studies further confirmed CM-β-CD adsorption, showing a rightward shift of compression isotherms and suggesting the formation of stacked CM-β-CD ensembles on cationic lipid surfaces [Figure S1(*b*)]. These results demonstrate that CM-β-CD-coated liposomes act as multifunctional nanocarriers with high drug-loading capacity and potential biomedical utility.

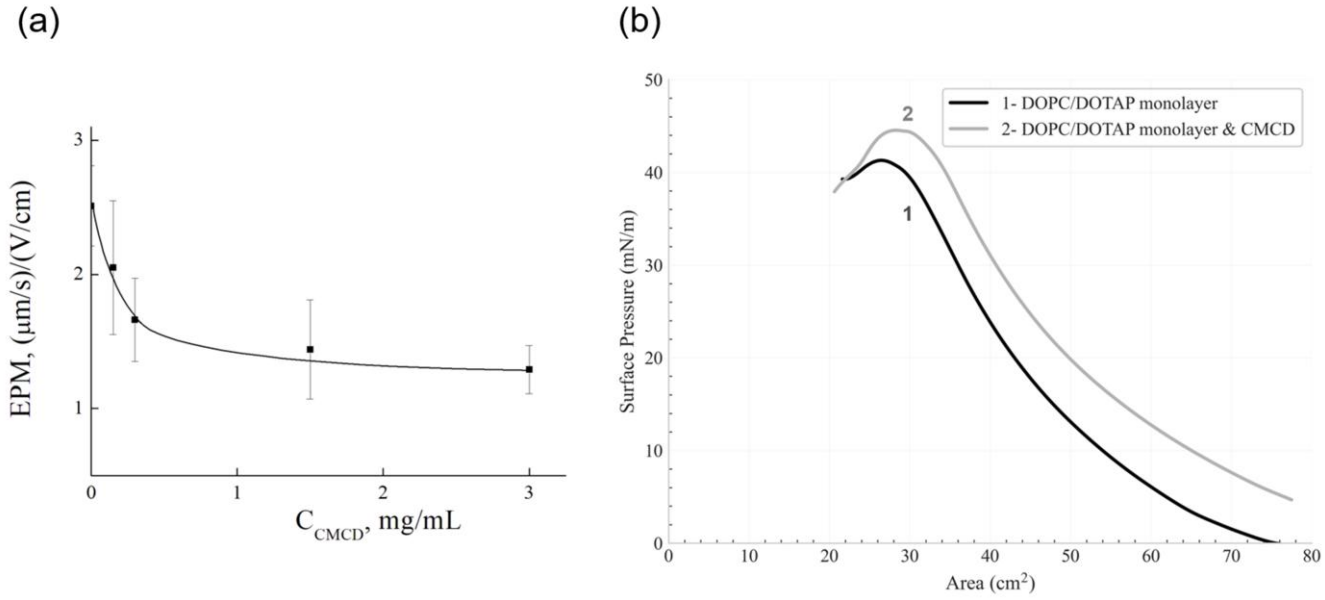

Figure S1. (*a*) Electrophoretic mobility of suspension of cationic DOTAP/DOPC liposomes upon concentration of added CM-β-CD in physiological saline buffer. (*b*) Surface pressure–area isotherms (π–A) for a DOTAP/DOPC lipid monolayer (curve 1) and for the DOTAP/DOPC lipid monolayer after addition of CM-β-CD into the subphase (curve 2).