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Supporting Information for Capacious and Programmable Multi-Liposomal Carriers

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(S1) Synthesis of spherical polycationic brushes (SPBs).

SPBs were prepared in three steps. In the first step 7.98 g of cationic surfactant, cetyltrimethylammonium bromide (CTAB, Fluka) was dissolved in 1240 ml deionized water under stirring. When 312 g of styrene was added, the reactor was degassed under vacuum and cationic thermal initiator, 2,2'-azobis(2-amidinopropane)dihydrochloride (V50, WAKO Chemicals), which was dissolved in 20 ml deionized water at the temperature of 65 °C. The reaction lasted for 120 minutes at 65 °C under 300 rpm stirring. Afterwards, 17.52 g of 2-[p-(2-hydroxy-2-methyl propiophenone)]ethylene glycolmethacrylate (HMEM) (2 mol% of the used styrene) dissolved in 20 ml acetone was added using a dosing motor under starved condition (about 0.2 ml/min) at 65 °C to obtain a well-defined thin layer of photoinitiator on the poly(styrene) core. Finally, UV/vis radiation was used to generate radicals on the surface of the particles. The polystyrene core particles modified with a thin layer of HMEM were filled in an UV-reactor (volume: 2000-3000mL, range of wavelengths: 200-600nm) and diluted to the weight concentration of 2.5 wt% with water. The total volume of particles was adjusted to about 2500 mL. Then defined amounts of monomer, 2-(acryloyloxy)ethyl) trimethylammonium chloride (30 mol% of polystyrene), were added under vigorous stirring. Photo-polymerization was performed by means of UV/Vis-radiation at 15 °C. Strong stirring ensured homogenization of particles in UVreactor. The photo-emulsion polymerization was finished within 30 minutes. After that, SPB particles were filtered over glass wool to remove possible coagulum. In this way a dense layer of cationic polyelectrolyte chains was generated on the particle surface by a grafting-from technique. The polycationic corona was analyzed as described earlier [X.Guo, et al., Macromolecules, 32 (1999) 6043]. Polycationic chains were cleaved off by addition of a strong base. The molecular weight of the chains was determined by viscosimetry (29000 g/mol)

that corresponded to their degree of polymerization equal to 140. The concentration of SPBs was given in base-mol/L.

(S2) Preparation of liposomes.

Small unilamellar anionic liposomes were prepared by the standard sonication procedure: appropriate amounts of EL, PS^{1-} and MOCH solutions in methanol were mixed in a flask, after which the solvent was evaporated under vacuum. A thin lipid film was dispersed in a TRIS buffer (pH 7.0, 10^{-2} M) for 400s with a 4700 Cole-Parmer ultrasonic homogenizer. Liposome samples were separated from titanium dust by centrifugation for 5 min at 10,000 rpm and used within one day. Liposomes with a molar fraction of anionic PS^{1-} head-groups $[PS^{1-}]/([PS^{1-}] + [EL]+[MOCH]) = 0.1$ and fraction of pH-sensitive MOCH $[MOCH]/([PS^{1-}] + [EL]+[MOCH]) = 0.3$ and 0.0 were thus obtained; liposomes with a fluorescent dye incorporated into the membrane, were prepared by the same procedure, except 1 wt.% of 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) ammonium salt was added to the lipid mixture solution before methanol evaporation.

Liposomes loaded with a NaCl solution were prepared by suspending and sonicating EL/PS^{1-}/MOCH or EL/PS^{1-} lipid film in 1M NaCl 10^{-3}M borate buffer solution. Then the liposome suspension was separated from the excess of external NaCl by dialysis against 10^{-3}M TRIS buffer. Integrity of NaCl-loaded liposomes was controlled by measuring conductivity of the liposome suspensions on conductometer Radiometer CDM- 83.

Freshly prepared liposomes were characterized by measuring hydrodynamic diameter and surface charge. For both binary and ternary liposomes the mean diameter was 40 nm with polydispersity index of 0.2-0.3. The electrophoretical mobilities for the binary and ternary liposomes at pH 7 were equal to -1.9±0.1 (μm/s)/(V/cm). At pH 5 the electrophoretical mobility of EL/PS^{1-} liposomes was -1.8±0.1 (μm/s)/(V/cm) while that of the ternary EL/PS^{1-}/MOCH liposomes was 0.8 ± 0.1 (μm/s)/(V/cm).

(S3) Determination of the number of the liposomes bound to SPBs.

First, the suspensions of fluorescent-labeled EL/PS^{1-}/MOCH liposomes with different liposome concentrations were prepared and their total fluorescence intensities were measured. Thus obtained a linear dependence of the fluorescence on liposome concentration (Figure S3, bold squares) used as a calibration curve. Then the fluorescent-labeled liposomes suspensions were mixed with a 10^{-4} M SPB suspension and 5 min after SPB/liposome complexes were separated by centrifugation at 16000 rpm for 40 min. The supernatants were analyzed by fluorescent spectroscopy and dynamic light-scattering. The dependence of fluorescence in the supernatants on initial concentration of liposomes is presented on Figure S3 (hollow circles). No fluorescence in the supernatants (in other words the complete binding of liposomes to SPB) was observed up to saturation to 0.7 mg/mL. Further increase of liposome concentration resulted in a linear growth of fluorescence in the supernatants parallel the calibration curve that showed appearance of unbound liposome in the supernatant over the saturated liposome concentration (0.7 mg/mL). Additionally, DLS measurements demonstrated only particles of liposomal size (40 nm in diameter) were found in the supernatants at liposome concentration over 0.7 mg/mL.

Using the calibration curve the liposome concentrations in the supernatants were calculated as shown in Figure 5 of the manuscript.

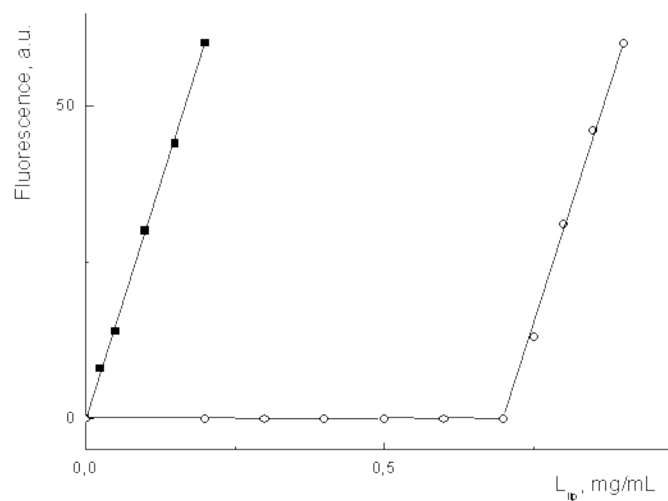

Fig.S3. The dependence of fluorescence of fluorescent-labeled EL/PS^{1-}/MOCH liposomes suspension before centrifugation (■),and supernatants of complexes of SPBs with liposomes (○); [SPB+] = 10^{-4} M; λem=595 nm, λex=565 nm