Electronic Supplementary Material (ESI) for Soft Matter

Electronic Supporting Information

Electrostatic-driven interaction of silica-supported lipid bilayer nanoplatforms and a nerve growth factor-mimicking peptide

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(1) Peptide loading efficiency determination

Peptide loading was assessed by combined centrifugation and spettrophotometric analyses with UV-visible and fluorescence spectra. In particular, the absorption/emission bands of phenylalanine (in NGF peptide), at 260/300 nm, and that of rodhamine (in lipids), at 550/590 nm, were monitored before and after centrifugation (15 min at 14,000 r.p.m) of the SUV dispersion obtained by membrane rehydration in NGF(1-14) solution in PBS.

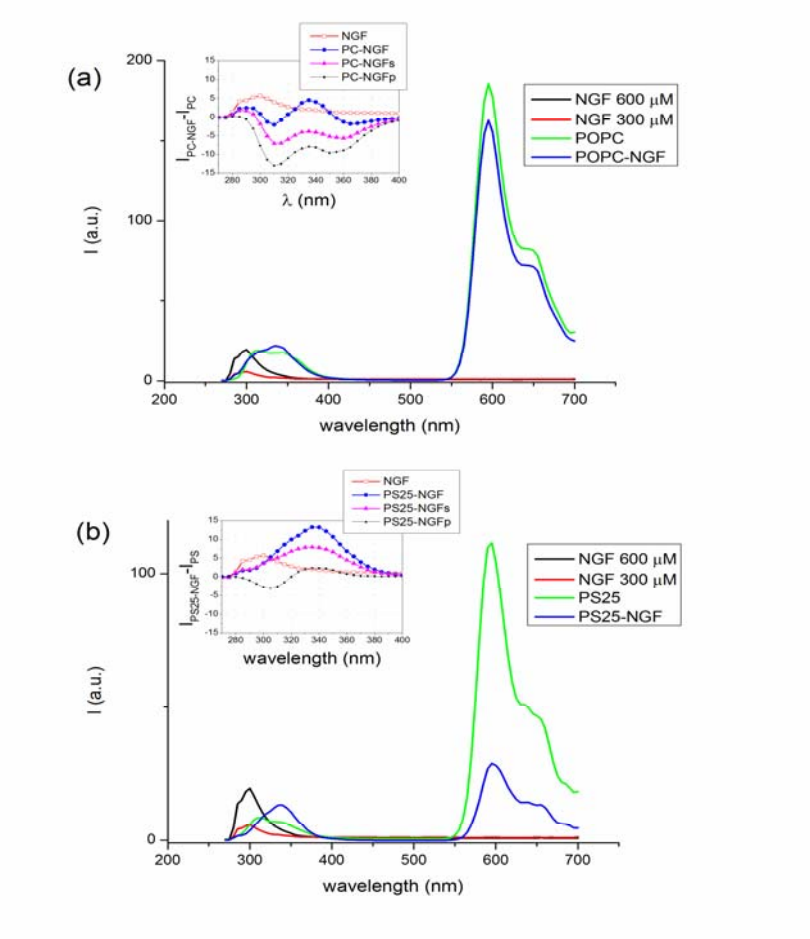

Figure S1– Emission spectra (excitation at 260 nm) of bare and NGF-associated lipid vesicles of PC (a) and PS25 (b), in comparison with spectra of free NGF(1-14) peptide at the concentrations of 300 and 600 μM in phosphate buffer saline (PBS, for PC) or 10 mM $MgCl_2$-added PBS (for PS25). In the insets the difference spectra between the NGF-associated vesicles and the corresponding bare vesicles are shown for as prepared and after centrifugation of the vesicle dispersions, both pellet ('p' subscript) and surnatant ('s' subscript); the spectrum of free NGF peptide at two concentrations (600 and 300 μM) is reported for comparison.

Peptide emission spectra (black and red curves) show a broad emission band at about 300 nm, due to the presence of two phenylalanine units per each molecule fragment. On the other hand, the bare lipid vesicles (green curve) exhibit a major emission band at about 590 nm and a smaller double picked band at about 340 nm, related to the rhodamine-labeled lipids. The spectra obtained for the SUV- NGF(1-14) system (blue curve), evidence significant changes for negative PS25 (Fig.S1b) but not for zwitterionic PC(Fig.S1a) in comparison with bare vesicles. From the ratio between the intensities of the bands related to free and lipid-associated peptide molecules the efficiencies of NGF(1-14) loading of about 76% and 96 % respectively for PC and for PS25, have been calculated. The difference spectra in the insets evidence for negative lipids the depletion of free NGF(1-14) molecules in the pellet, confirming the efficiency of association of the peptide to PS25.

(2) Kinetic curves of fluorescence recovery for FRAP experiments

The kinetics curves for fluorescence recovery are shown in Figure S2.

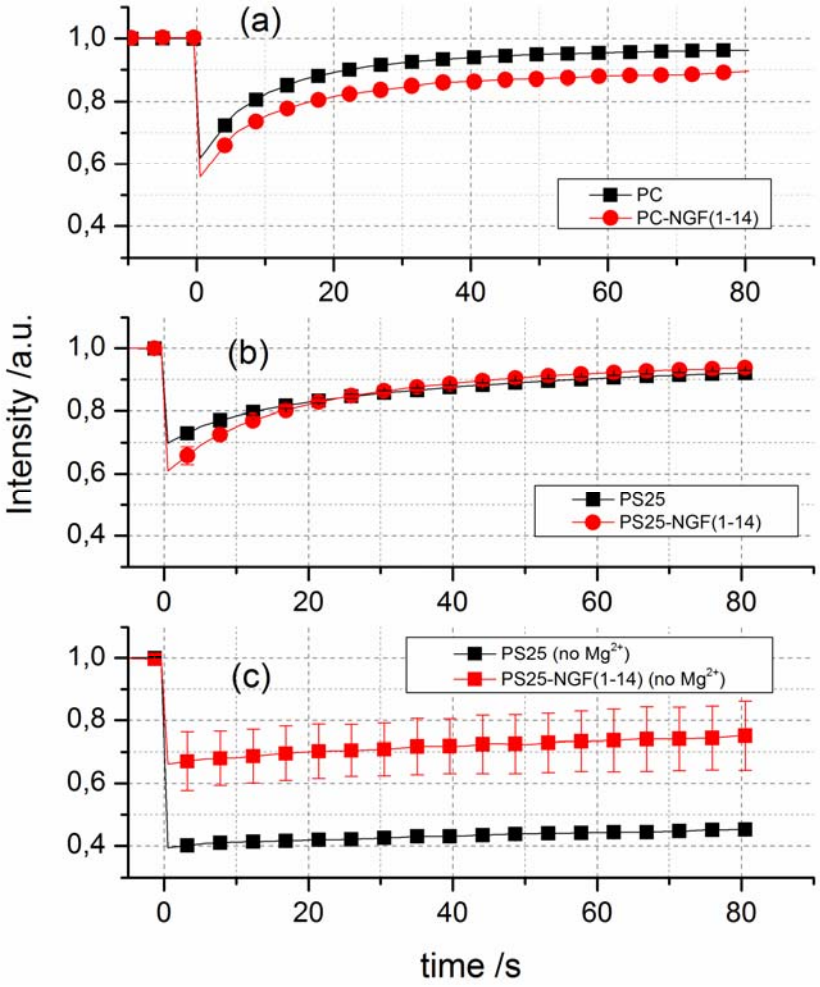

Figure S2- Fluorescence intensity normalized to the pre-bleach values for FRAP experiments on SLBs or lipid adlayers obtained by adsorption on planar silica of SUVs made of: (a) PC and PC-NGF(1-14) in PBS; (b) PS25 and PS25-NGF(1-14) with addition of 10 mM $MgCl_2$; c) PS25 and PS25-NGF(1-14) without addition of 10 mM $MgCl_2$. Mean values of ten experiments ± S.E.M.

As to the zwitterionic lipids similar curve trends are displayed for both bare and NGF-associated lipids, with a quick and almost complete recovery of fluorescence (Fig. S2a). Similarly, the fluorescence intensity curves for negatively charged PS25 and PS25-NGF(1-14)

adsorbed on silica in the presence of divalent cations (Fig. S2b) evidence an emission recovery that is a little slower than the previous case, but still representative of the formation of mobile SLBs.

As to the negatively charged lipids without the addition of divalent cations (Fig. S2c), none or very poor lateral diffusion for PS25, but a certain level of fluorescence recovery for PS25-NGF(1-14) is found. In fact, PS25-NGF(1-14) adlayers exhibit a minimum of fluorescence emission (i.e., immediately after bleaching) very similar to that found for the SLBs (~30% bleach of the initial intensity), however the further fluorescence recovery is modest compared to the SLB. Diffusion back into the spot during irradiation can explain this behavior. This is not the case for the bare PS25 adlayers, where about 60% bleach of the initial intensity is attained. Therefore PS25-NGF(1-14) adsorption on silica, without addition of divalent cations, results in a mixed adlayer where the fraction of mobile lipids, likely as SLBs patches, is significantly higher than in the corresponding bare PS25. Further measurements by using different spot sizes and irradiation times are in progress to account for this interpretation.