Supporting Information

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Lipid Head Group Charge and Fatty Acid Configuration Dictate Liposome Mobility in Neurofilament Networks

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Lipid head group charge and fatty acid configuration dictate liposome mobility in neurofilament networks

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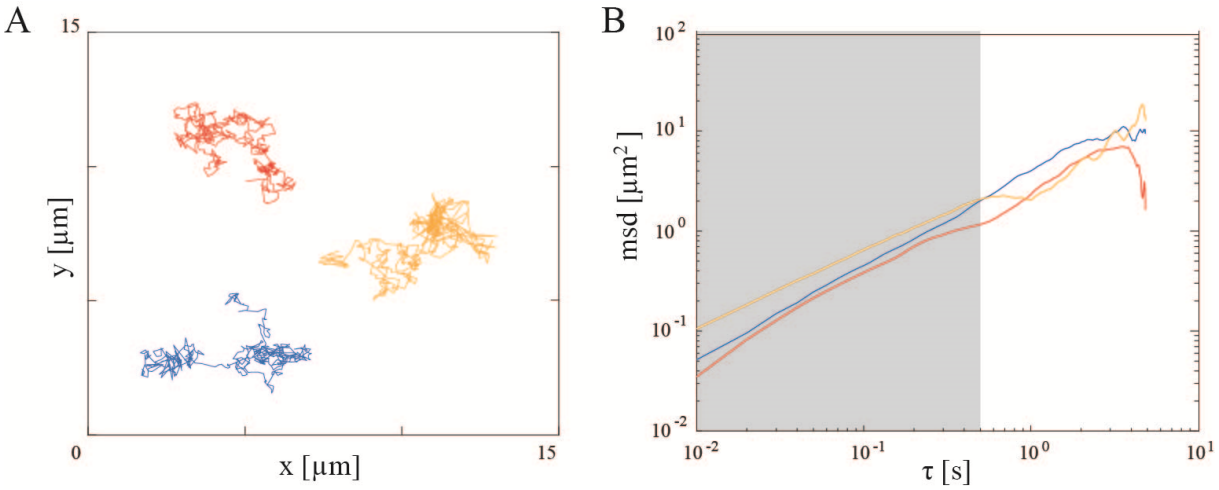

Figure S1. Data processing procedure for determining diffusion coefficient values from the Brownian motion of test particles. From the x-y-trajectories of individual particles (A) as obtained from single particle tracking, mean square displacement (msd) curves are calculated (B) as described in the methods section of the manuscript. The first 10 % of this msd data (grey shaded regime in B) is then fitted with a linear function to obtain apparent diffusion coefficient values for each particle. Three exemplary particle trajectories from the experiment performed with PEGylated polystyrene particles (full data set is shown in Figure 1a of the manuscript) are depicted. This procedure is repeated until at least 1000 particle trajectories are obtained and the corresponding diffusion coefficients are determined. The distribution of those diffusion coefficient values is then plotted in the main paper as cumulative relative frequencies.

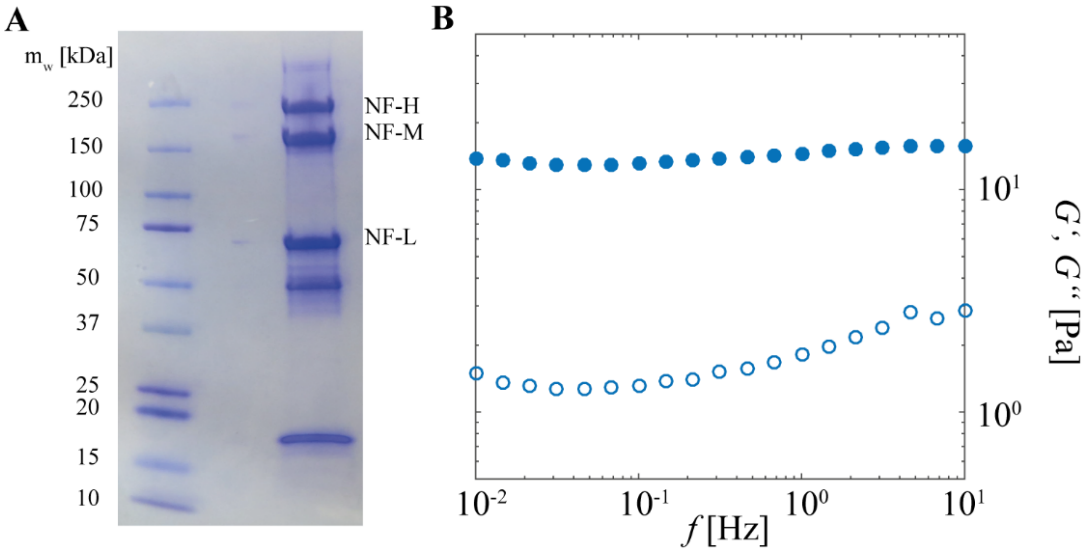

Figure S2. Characterization of the neurofilament preparation. (A) 10 µL of neurofilament sample were mixed with 10 µL Laemmli buffer (Bio-Rad) and heated to 90 °C for 5 min. The sample was then loaded onto a Mini-PROTEAN TGX precast 4 % - 20 % gradient gel (Bio-Rad) and SDS-PAGE was performed. The protein bands were visualized by a Coomassie staining. SDS-PAGE shows three bands at molecular weights where the three subunits NF-H, NF-M and NF-L are expected. The additional bands probably represent actin and tubulin. (B) Successful gel formation of those purified neurofilaments is verified by shear rheology using a stress-controlled macrorheometer (MCR 302, Anton Paar, Graz, Austria) with a 25 mm plate-plate geometry at a plate separation of 200 µm. A small torque of 0.5 µNm is applied to ensure linear response. In the viscoelastic frequency spectrum of a reconstituted neurofilaments network (1 mg/mL protein) shown, the closed circles represent the storage modulus (G') and the open circles denote the loss modulus (G''). G' clearly dominates over G'' over the whole frequency range probed which demonstrates successful gel formation of the neurofilaments. The rheometer plate was cooled to 4 °C before 150 µL of the neurofilament sample was added, and the frequency spectrum was obtained from 0.01 Hz to 10 Hz at 21 °C. A thin layer of polydimethylsiloxane oil (ABCR, Karlsruhe, Germany) was applied to the outer rim of the sample to avoid drying artefacts.

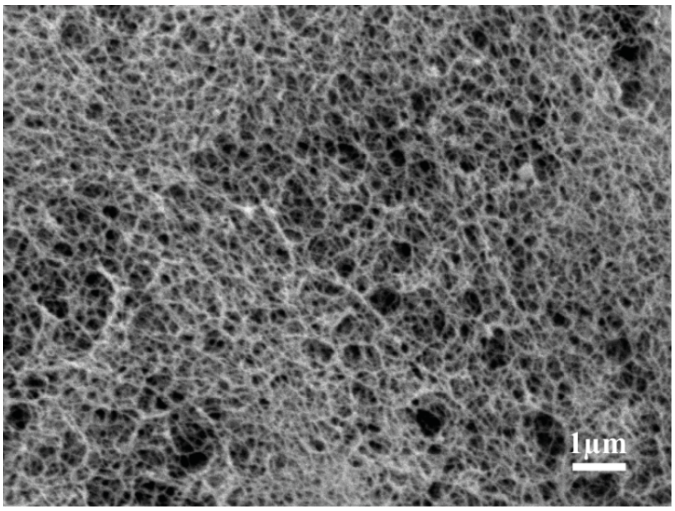

Figure S3: SEM-image of the assembled NF network. For obtaining scanning electron microscopy (SEM, JEOL-JSM-6060LV, Jeol, Germany) images, the frozen NF sample was defrosted on ice. A volume of 30 µL was pipetted onto a sample holder and incubated for 24 h at room temperature. The samples were fixed in 2.5 % glutaraldehyde (in 50 mM HEPES, pH 7.4) for one hour and washed with ddH_2O for another hour. For dehydration, the samples were incubated in an increasing ethanol series of 50 %, 70 %, 80 % and 99.8 % ethanol for 30 min, each. Then, the samples were critical point dried, sputtered with a conductive gold film (40 mA, 40 s) and imaged at 5 kV. The image indicates that the mesh size of neurofilament networks reconstituted at a protein concentration of 1 mg/mL is on the order of several hundred nanometers which agrees with literature values reported earlier.

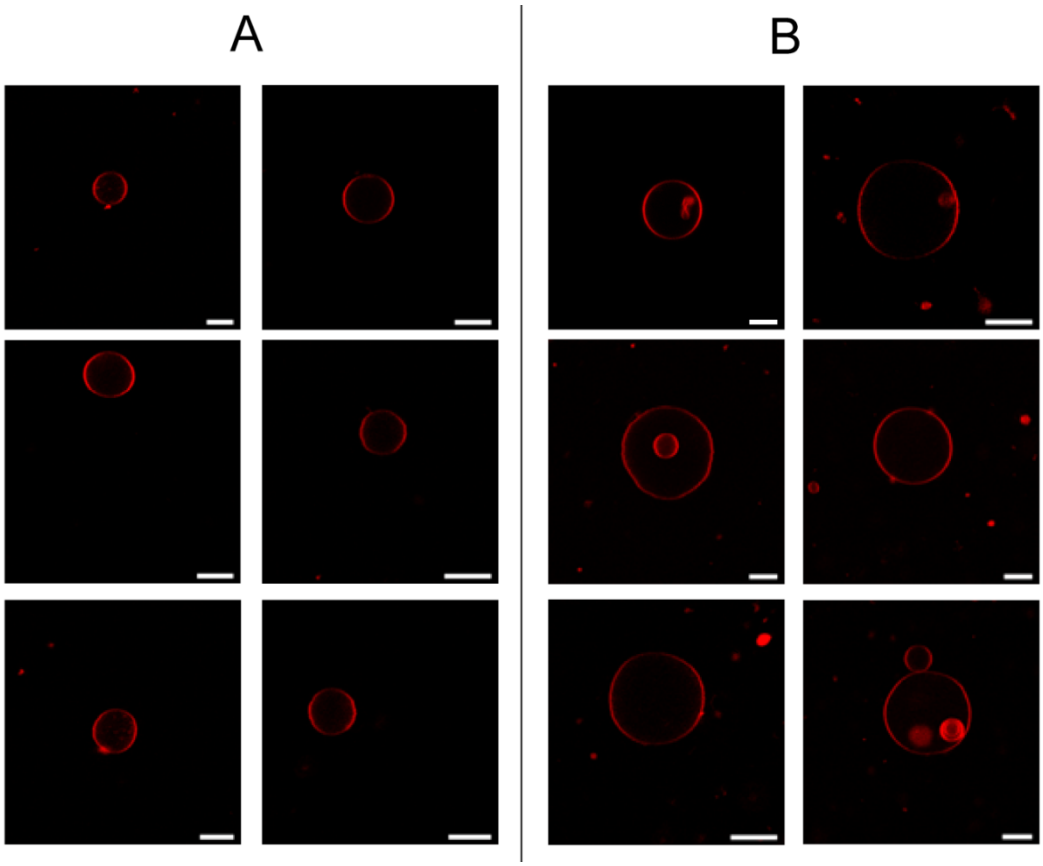

Figure S4. Visualization of Rh-DOPE distribution in GUVs in the presence of neurofilaments. When a 250 nM neurofilament solution was incubated with (A) POPC:POPG:Rh-DOPE (95:4:1) GUVs or (B) DOPC:DOPG:Rh-DOPE (95:4:1) GUVs, the distribution of Rh-DOPE fluorescence remained homogeneous. There was no sign of enrichment of Rh-DOPE in macroscopically visible patches. Scale bars represents 10 µm.