Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

Tailored Multivalent Targeting of Siglecs with Photosensitizing Liposome Nanocarriers

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Experimental Procedures

Materials

Chemicals were purchased from Sigma-Aldrich, otherwise indicated. 1,2-Dioleoyl-sn-glycero-3-phosphocholine (DOPC), and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(cap biotinyl), sodium salt (DOPE-biotin) were purchased from Avanti Polar Lipids. Ultrapure water (Milli-Q) obtained by ultrafiltration was used in all the experiments. PBS (0.01 M sodium dihydrogen phosphate and 0.15 M sodium chloride, pH 7.4) was prepared dissolving commercial PBS tablets in ultrapure water. Streptavidin was purchased from ThermoFisher and dissolved in PBS. Biotinylated human siglec-10 was purchased from Acro Biosystems and dissolved in PBS to reach a 0.1 µM solution. Amino undecaethylene glycol-biotin (PEG-biotin) was purchased from ThermoFisher.

Preparation of Small Unilamellar Vesicles (SUVs)

Batches of 1 mL of 0.5 mg/mL of DOPC SUVs in PBS pH= 7.4, with/without different molar percentages of **PcSA** (0.1, 0.2, 0.5, 1, 2 and 5%), were prepared using a stock solution of 25 mg/mL of DOPC in chloroform (with 0-2 mol% DOPE-biotin in case of SUVs for SLB formation), and a stock solution of 1.44 mg/mL of **PcSA** in PBS. After adding the required amount of DOPC in chloroform into a glass vial, a gentle nitrogen flux was used to evaporate the solvent, forming a lipidic film on the vial walls. The residual solvent was removed placing the vials into a desiccator for 1 h. 1 mL of Milli-Q water (in case of SUVs for SLB formation) or PBS (for the binding of SUVs onto SLBs; different ratios of PBS and **PcSA** in PBS were used to obtain the desired %) was added. After performing 10 freeze-pump-thaw cycles, the vesicles were extruded through a 100 nm polycarbonate membrane (11 times; Whatman) and were stored in the fridge for two weeks.

Insertion Approach

DOPC-SUVs (0.5 mg/mL) in PBS were mixed with different concentrations of **PcSA** (0.6, 1.2, 3, 6, 12 and 30 µM) adjusting the volume to 1 mL of PBS pH= 7.4, being the same concentrations used for the formation of DOPC-SUVs containing different mol% of **PcSA** and were incubated for 4 h at room temperature under stirring. Vesicles were stored in the fridge and used within two weeks.

Particle Size Analysis

Particle size was recorded using DLS of 1 mL of DOPC-SUVs and DOPC-Pc SUVs (0.5 mg/mL) containing 0.1, 0.2, 0.5, 1, 2 and 5% of **PcSA** in PBS using a Nanotrac Wave particle size analyzer. The data are expressed as the average of three measurements, for a time of 120 s at 22°C. In the insertion experiment, the particle size of the resulting vesicles was measured using the same parameters mentioned before.

Spectroscopic Experiments

UV-Vis spectra were recorded using a Perkin Elmer Lambda 850 spectrophotometer. 1 mL of every sample of vesicles were used to record the data, in a Hellma 108-QS 1000 µL Semi-Micro Absorption Cell, 10 mm Light Path. Fluorescence spectra of the previous mentioned vesicles were recorded using a Perkin Elmer FL 6500 fluorescence spectrometer, using Hellma 105.250-QS 100 µL Ultra-Micro Fluorescence Cell with 100 µL of every sample and irradiating at 665 nm.

Z-potential Measurements

Z-potential values were obtained using a Malvern Instruments Zetasizer Nano ZS and a folded capillary Zeta Cell from Malvern Panalytical. Samples were obtained diluting 800 µl of vesicles (0.5 mg/mL) 20 times with ultra-purified water, and Z-potential values were expressed as an average of 3 measurements at 25°C of 800 µL of every sample.

QCM-D Measurements

For the fabrication of SLBs, vesicles (SUVs) were diluted to a concentration of 0.1 mg/ml in PBS directly before use. SLBs were obtained by flowing this solution on a cleaned and activated SiO_2 surface, after obtaining a stable baseline. The quality of the SLBs was monitored in situ by QCM-D (where high quality SLBs are defined by Δf = -24 ± 1 Hz and $\Delta D < 0.5\times10^{-6}$). QCM-D measurements were performed with a Qsense Analyser from Biolin Scientific, and SiO_2-coated sensors (QSX303, Biolin Scientific) were used throughout this work. Measurements were done at 22 ºC and operated with four parallel flow chambers, using two Ismatec peristaltic pumps with a flow rate of 20 µl/min. For every measurement, the fifth overtone was used for the normalized frequency ($\Delta f5$) and dissipation ($\Delta D5$). In a typical experiment, SLBs were formed on previously cleaned and activated SiO_2 sensors. Afterwards, solutions of SAv, of siglec-10 or siglec-10/PEG (1:1) mixtures, and then DOPC-SUVs or DOPC-Pc SUVs were added until a stable plateau was reached. Each step was followed by rinsing with PBS. The SLB and SAv steps were found reproducible within a standard deviation of 5% over multiple series. The siglec steps were found reproducible within a standard deviation of below 20%. For practical reasons, this % deviation could not be determined for the vesicle steps but based on previous experience,[41,43,45] we can assume the upper limit of the siglec (20% standard deviation) to hold also in this case.

SUPPORTING INFORMATION

Supporting Figures

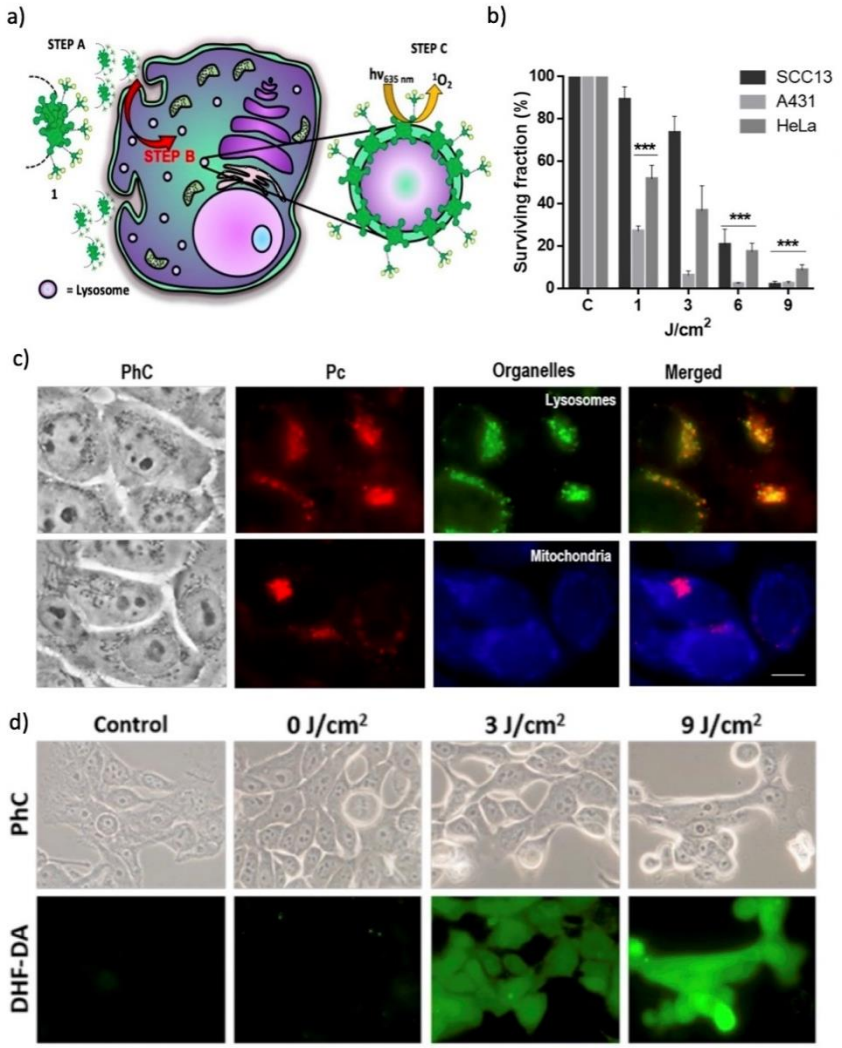

Figure S1. a) Schematic cartoon representing the mechanism of internalization and subcellular behavior of the **PcSA** photosensitizer, according to previous PDT studies by de la Escosura, Torres and coworkers,[1] which involve the following steps. STEP A: **PcSA** is aggregated outside the cell. STEP B: The incorporation into the lysosomes provokes the disruption of **PcSA** aggregation. STEP C: On-site activation of the photodynamic properties of **PcSA**. b) Graphic showing the survival of A431, HeLa and SCC-13 cells after 5 h of incubation of **PcSA** at a concentration of 0.5 μM and after different light doses. c) Subcellular localization of **PcSA** (10 μM) after 18 h of incubation in SCC-13 cells; PhC: phase contrast. Blue fluorescence is from mitochondria (after irradiation with UVA exciting lamp of 360-370 nm and the presence of MitoTracker), green fluorescence is from lysosomes (after irradiation with a blue exciting lamp of 450-490 nm and the presence of LysoTracker), and red fluorescence is from **PcSA** (after irradiation with green exciting light of 545 nm). Scale bar: 10 μm. A similar behavior was observed with the two other cell lines. d) ROS production within the cells, detected by DHF-DA fluorescent probe after PDT with **PcSA**, upon irradiation with different red light doses. The first column is a control without **PcSA**, the second column are the cells in the dark as a second control, and the third and fourth columns are the cells irradiated with different light doses. By fluorescence microscopy (λ_{ex} = 436 nm), it can be detected a fluorescence signal that indicates the production of ROS. PhC: phase contrast. Figure adapted with permission from ref. 1 (39 in the main text). Copyright 2021 Wiley.

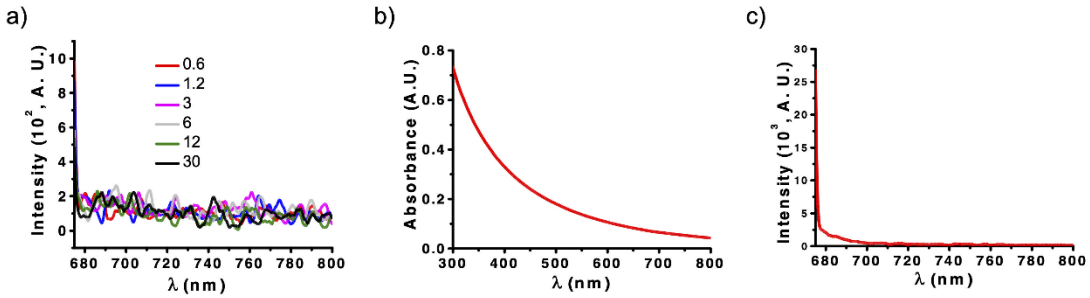

Figure S2. a) Fluorescence emission of **PcSA** in PBS, with concentrations (μM) equal to those employed for encapsulation in SUVs (irradiation at 665 nm). b) Scattering of DOPC SUVs (0.5 mg/mL) in PBS. c) Fluorescence spectra of DOPC SUVs, same conditions (irradiation at 665 nm).

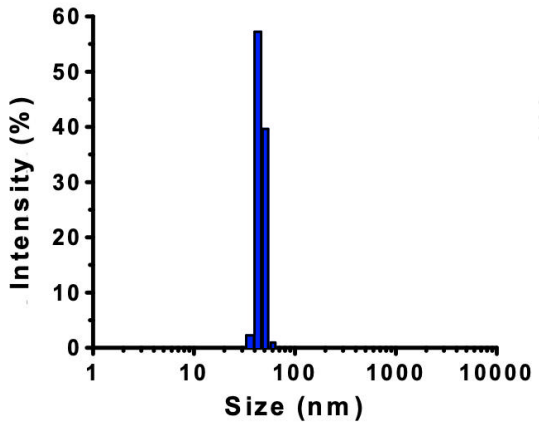

Figure S3. DLS size distribution of **PcSA** (5 μM) in PBS.

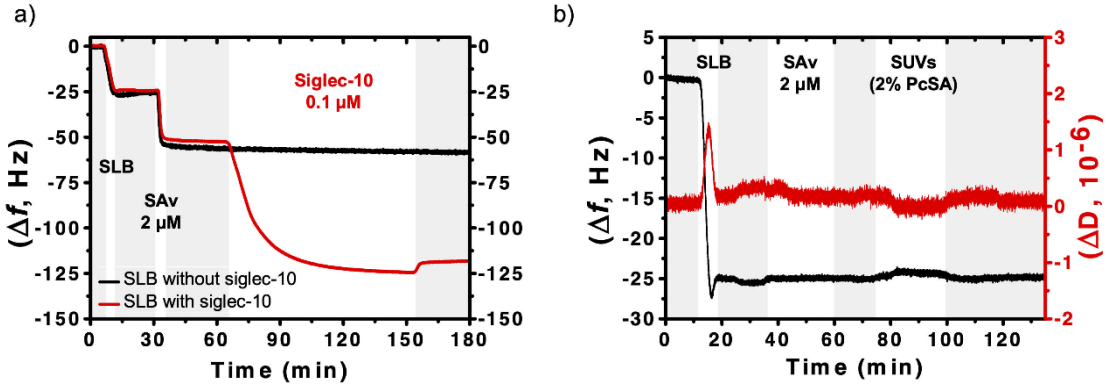

Figure S4. QCM-D measurements showing stepwise: a) Comparison of the frequency shift (Δ*f*) after the addition or not of biotinylated siglec-10 over a DOPC-based SLB displaying 2 % of DOPE-biotin. The frequency shift (Δ*f*) of the SLB displaying siglec-10 is represented in red, while the one corresponding to the SLB not displaying siglec-10 is represented in black. The 5th overtone was used in all the experiments. Grey shades represent PBS addition. b) Formation of a DOPC-based SLB, flow of SAv and DOPC-Pc SUVs containing 2 % of **PcSA** (dilution 1:100 - 1:2), with no interactions observed. The frequency shift (Δ*f*) is represented in black and the variation of dissipation is represented in red (ΔD).

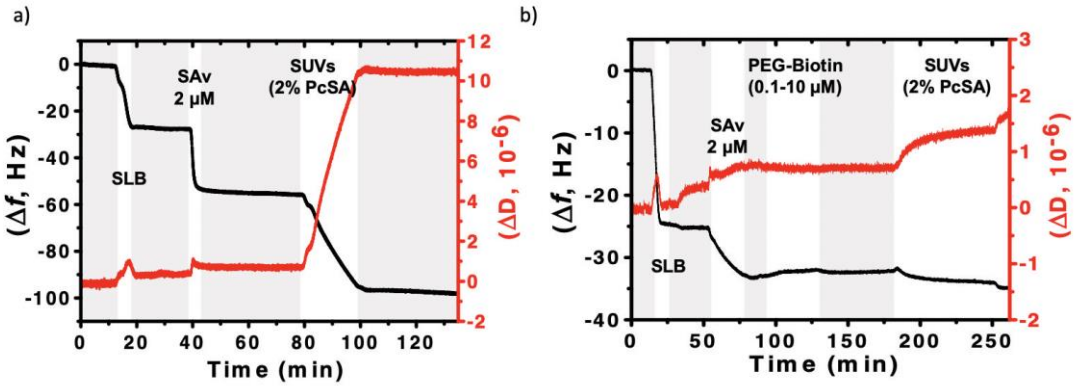

Figure S5. QCM-D measurements showing: a) formation of a SLB containing 2% of DOPE-biotin, adsorption of SAv and binding of DOPC-Pc SUVs containing 2 % of **PcSA** (dilution 1:10). b) Formation of a SLB containing 2% of DOPE-biotin, adsorption of SAv and PEG-biotin, and flow of DOPC-Pc SUVs containing 2 % of **PcSA** (dilution 1:5). The 5th overtone was used in all the experiments. The frequency shift (Δ*f*) is represented in black and the variation of dissipation is represented in red (ΔD). Grey shades represent PBS addition.

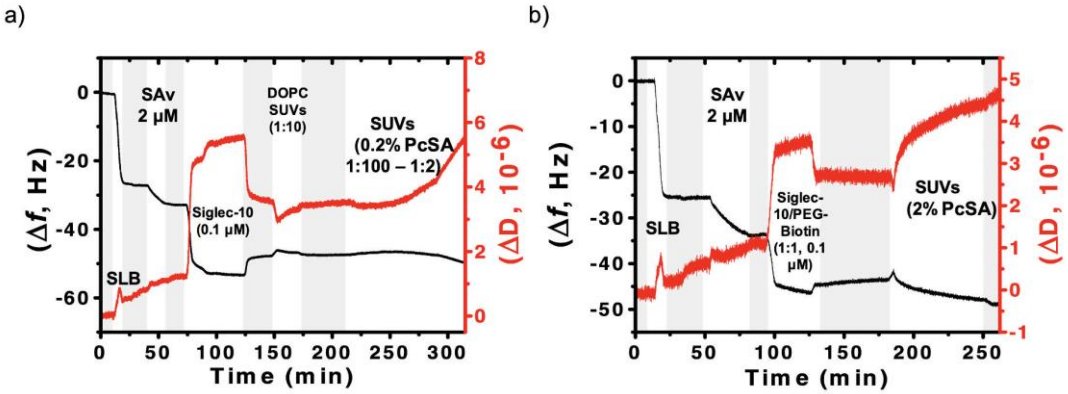

Figure S6. QCM-D measurements showing: a) Formation of DOPC SLB (with 0.2% of DOPE-biotin), followed by adsorption of SAv and biotinylated siglec-10, followed by flowing solutions with DOPC SUVs (without 1) and various dilutions of SUVs containing 0.2% of **PcSA**, both of which did not show any appreciable adsorption. b) Formation of DOPC SLB (with 0.2% of DOPE-biotin), followed by adsorption of SAv and siglec-10 (from a 1:1 mixture of PEG-biotin and biotinylated siglec-10), followed by flowing a solution with SUVs containing 2% **PcSA** (dilution 1:5) showing only minor adsorption. The 5th overtone was used in all the experiments. The frequency shift (Δ*f*) is represented in black and the variation of dissipation is represented in red (ΔD). Grey shades represent PBS addition.

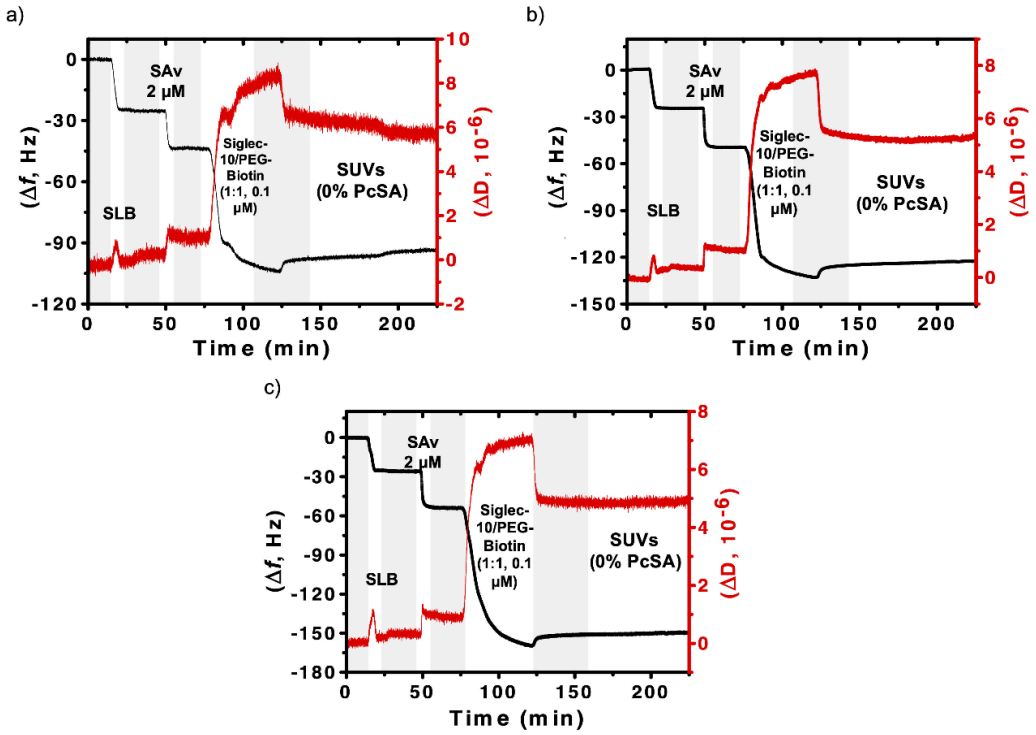

Figure S7. QCM-D measurements showing stepwise: Formation of a SLB, adsorption of SAv and a mixture (1:1) of PEG-biotin and biotinylated human siglec-10, and no binding of DOPC-Pc SUVs containing 0 % of **PcSA** (dilution 1:100 – 1:2), with a) SLB doped with 0.5 % of DOPE-biotin, b) SLB doped with 1 % of DOPE-biotin and c) SLB doped with 2 % of DOPE-biotin. The 5th overtone was used in all the experiments. The frequency shift (Δ*f*) is represented in black and the variation of dissipation is represented in red (ΔD). Grey shades represent PBS addition.

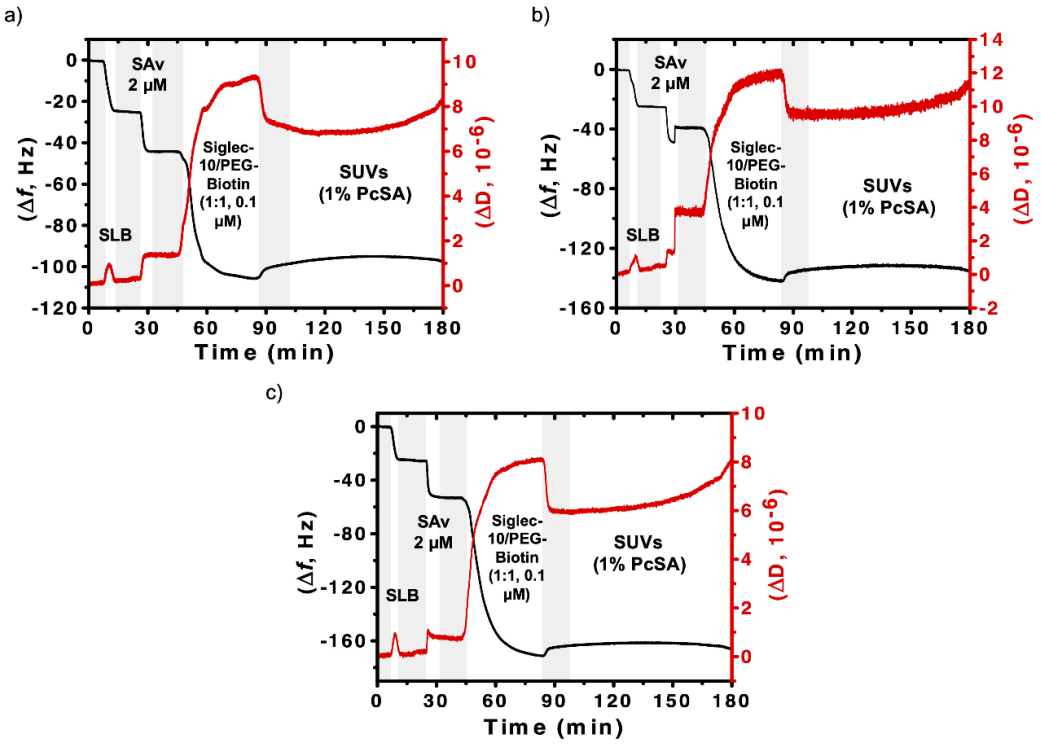

Figure S8. QCM-D measurements showing stepwise: Formation of a SLB, adsorption of SAv and a mixture (1:1) of PEG-biotin and biotinylated human siglec-10, and flow of DOPC-Pc SUVs containing 1 % of **PcSA** (dilution 1:100 – 1:2), with a) SLB doped with 0.5 % of DOPE-biotin, b) SLB doped with 1 % of DOPE-biotin and c) SLB doped with 2 % of DOPE-biotin. The 5th overtone was used in all the experiments. The frequency shift (Δ*f*) is represented in black and the variation of dissipation is represented in red (ΔD). Grey shades represent PBS addition.

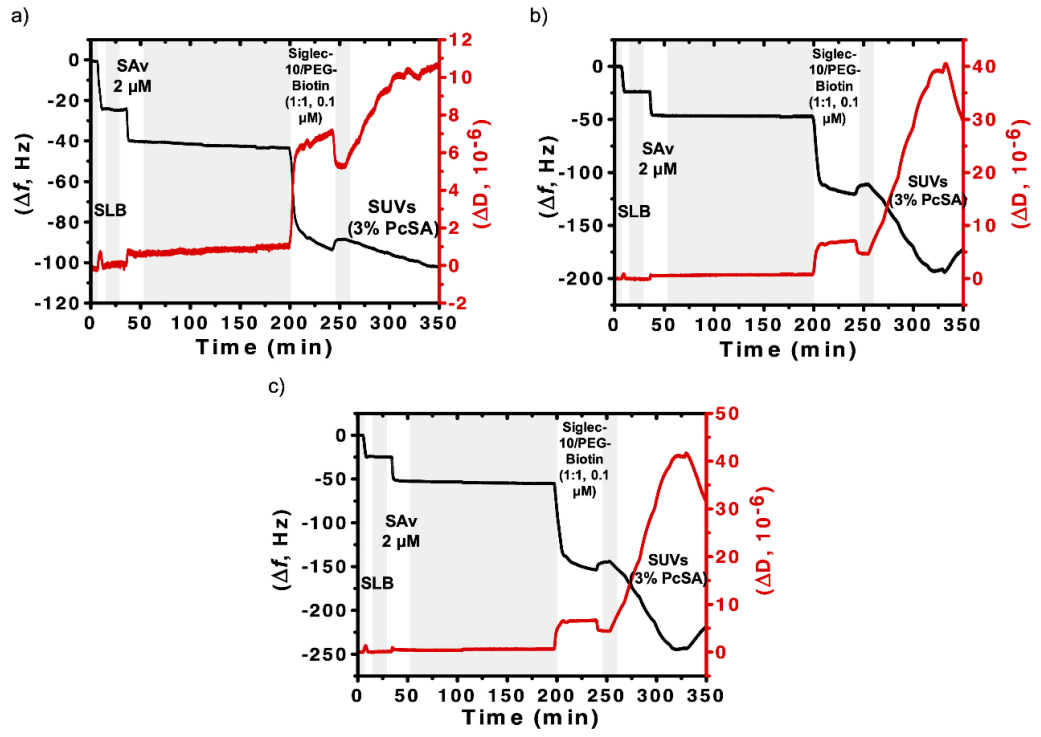

Figure S9. QCM-D measurements showing stepwise: Formation of a SLB, adsorption of SAv and a mixture (1:1) of PEG-biotin and biotinylated human siglec-10, and flow of DOPC-Pc SUVs containing 3 % of **PcSA** (dilution 1:100 – 1:2), with a) SLB doped with 0.5 % of DOPE-biotin, b) SLB doped with 1 % of DOPE-biotin and c) SLB doped with 2 % of DOPE-biotin. The 5th overtone was used in all the experiments. The frequency shift (Δ*f*) is represented in black and the variation of dissipation is represented in red (ΔD). Grey shades represent PBS addition.

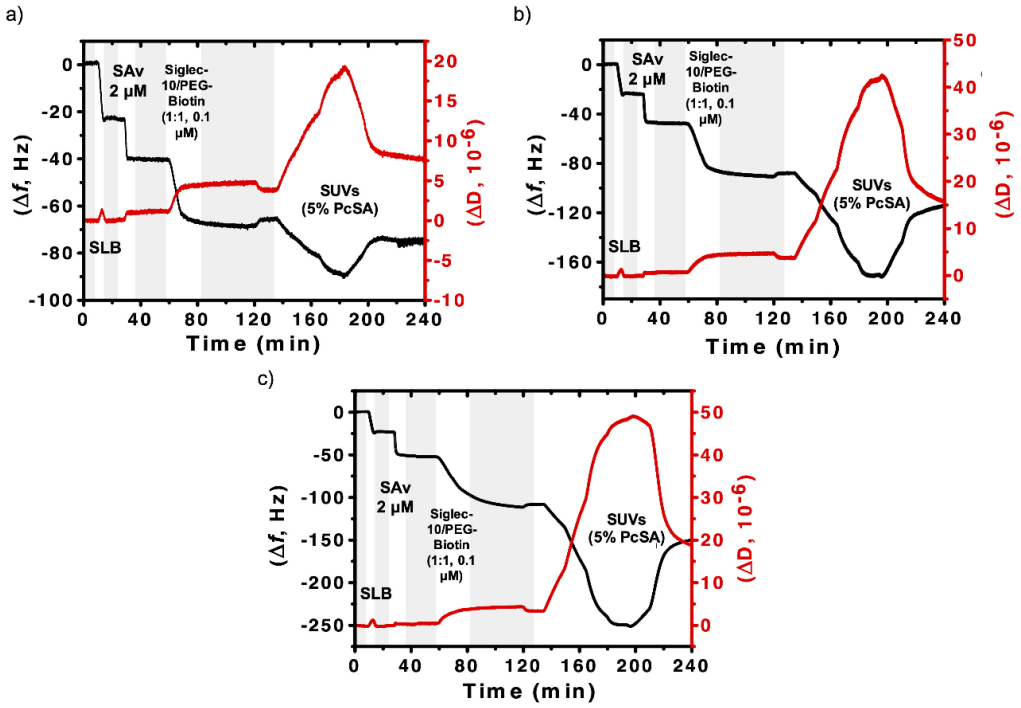

Figure S10. QCM-D measurements showing stepwise: Formation of a SLB, adsorption of SAv and a mixture (1:1) of PEG-biotin and biotinylated human siglec-10, and flow of DOPC-Pc SUVs containing 5 % of **PcSA** (dilution 1:100 – 1:2), with a) SLB doped with 0.5 % of DOPE-biotin, b) SLB doped with 1 % of DOPE-biotin and c) SLB doped with 2 % of DOPE-biotin. The 5th overtone was used in all the experiments. The frequency shift (Δf) is represented in black and the variation of dissipation is represented in red (ΔD). Grey shades represent PBS addition.

References

[1] V. Almeida-Marrero, M. Mascaraque, M. J. Vicente-Arana, A. Juarranz, T. Torres, A. de la Escosura, *Chem. Eur. J.* **2021**, *27*, 9634−9642.