Supporting information

Weak Multivalent Binding of Influenza Hemagglutinin Nanoparticles at a Sialoglycan-Functionalized Supported Lipid Bilayer

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Supporting figures

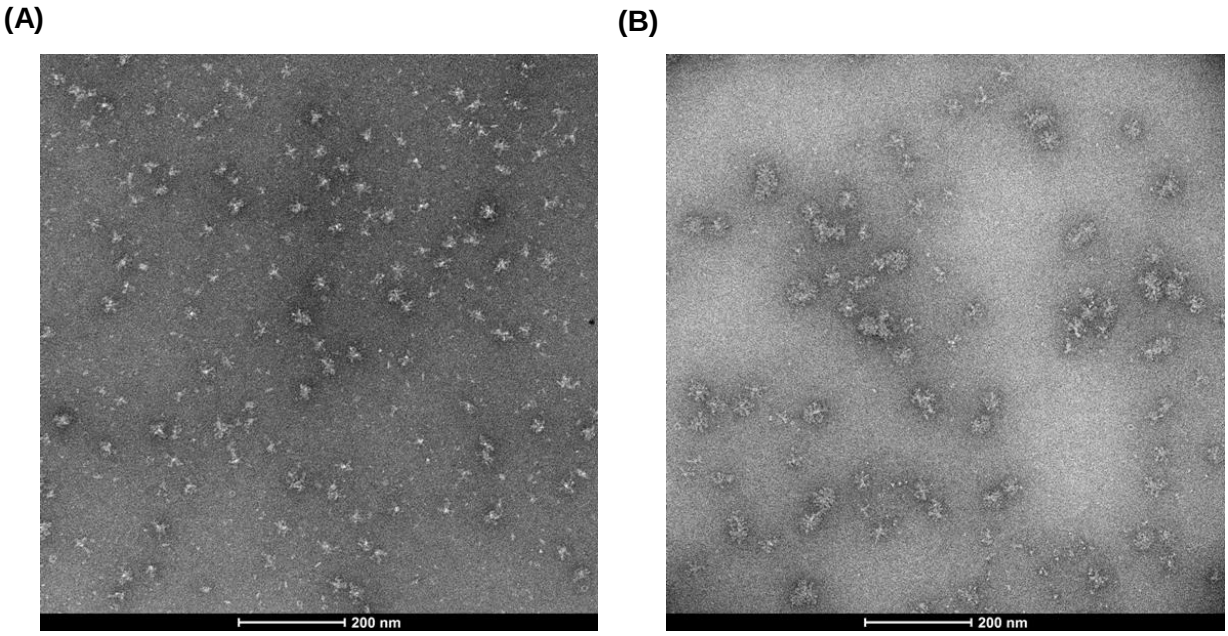

Figure S1. **Electron micrograph of HA trimer rosettes from Influenza virus embedded in Phospho-Tungstic-Acid (PTA, 1% (w/v), pH 7.4).** A) rHA rosettes from A/New Caledonia/20/99. B) rHA rosettes from A/Brisbane/59/07

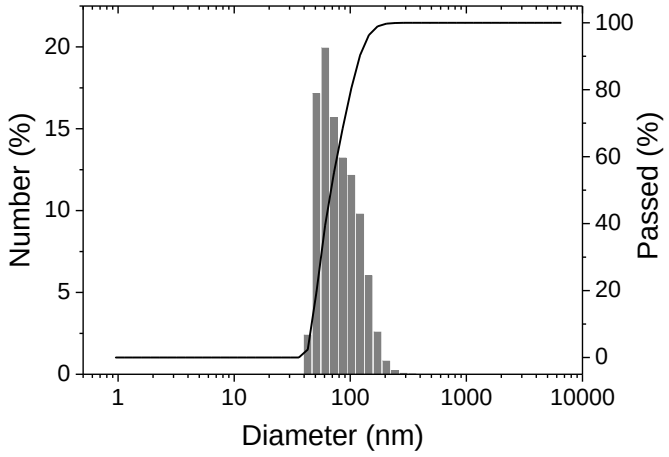

Figure S2: Dynamic light scattering (DLS) data of DOPC vesicles containing 5 mol% DOPE-biotin, extruded 11x through a membrane with a 100 nm pore size.

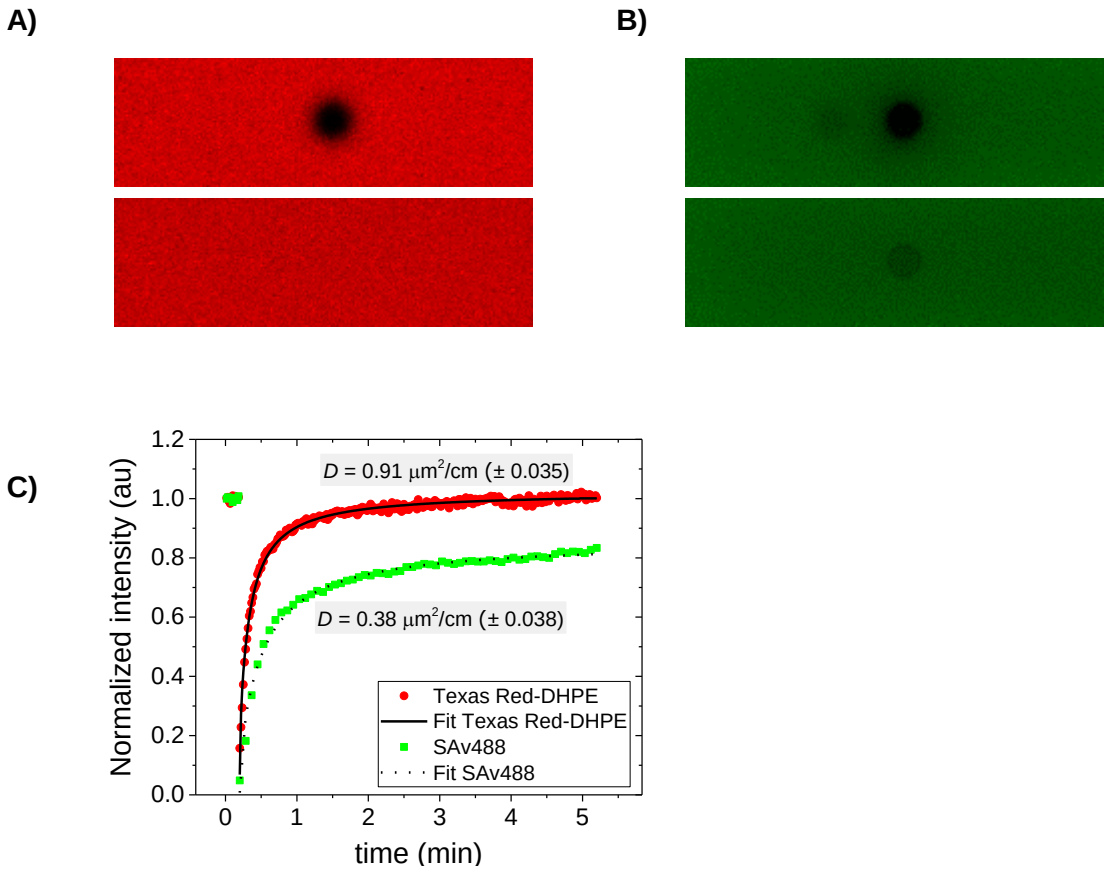

Figure S3. Fluorescence recovery after photobleaching (FRAP) by confocal microscopy of DOPC SLB with 0.2 mol% TR-DHPE and 1 mol% DOPE-biotin after 1 h incubation of 0.2 uM SAv488 and subsequent rinsing with HEPES buffer. A) red channel from TR-DHPE after photobleaching (top) and after 5 min recovery (bottom). B) green channel from SAv488 after photobleaching (top) and after 5 min recovery (bottom). C) Fluorescence intensity recovery profiles vs time of the Texas Red-DHPE and SAv488 after photobleaching.

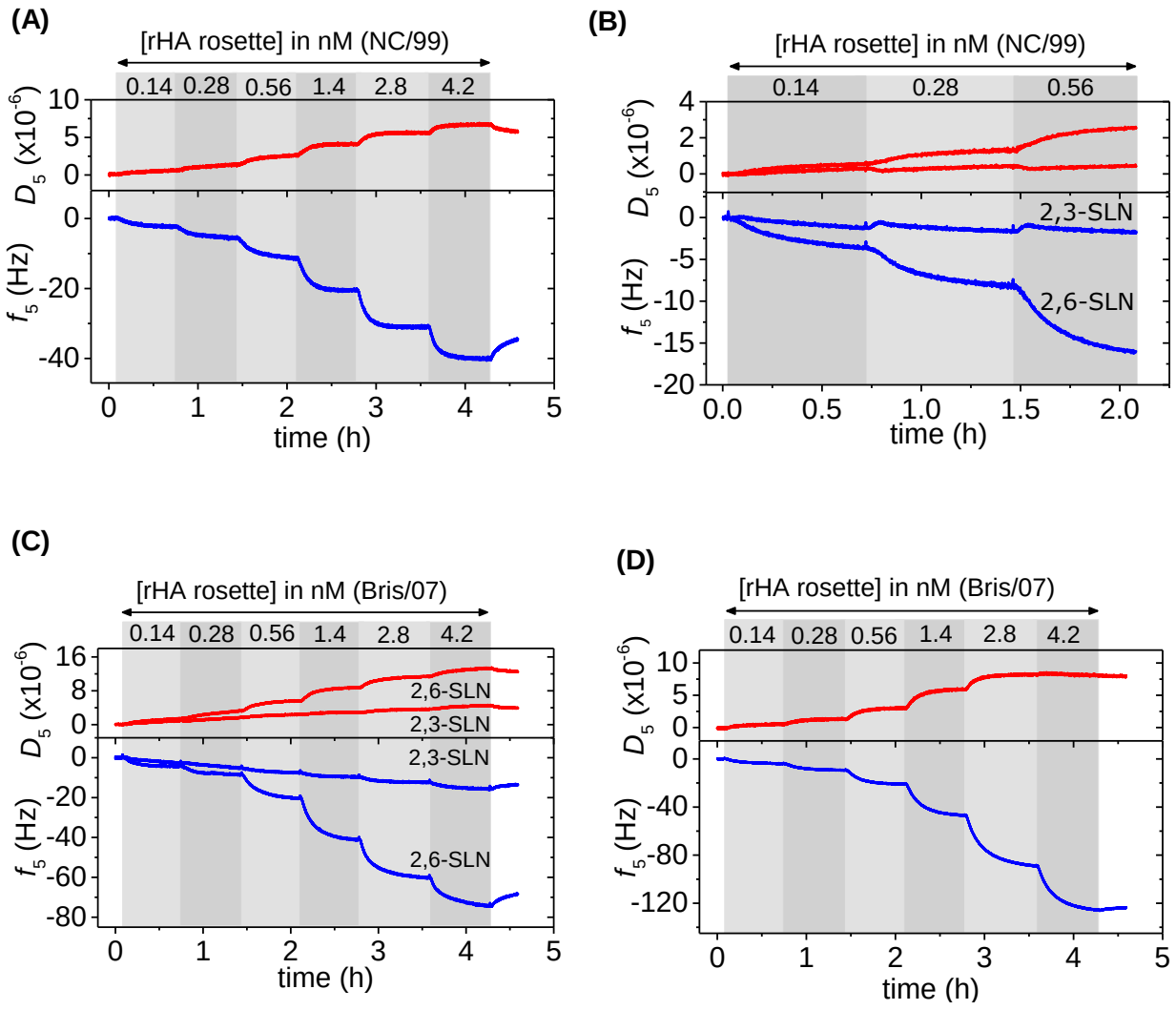

Figure S4: QCM-D binding profiles for titrations of rHA clusters onto SLB platforms: (A) NC/99 rHA rosettes at a 2,6-SLN surface with 0.4 mol% DOPE-biotin, (B) NC/99 HA rosettes at 2,3-SLN and 2,6-SLN surfaces with 0.4 mol% DOPE-biotin, (C) Bris/07 rHA rosettes at 2,3-SLN and 2,6-SLN surfaces with 0.4 mol% DOPE-biotin, and (D) Bris/07 HA cluster at a 2,6-SLN surface with 5 mol% DOPE-biotin. Grey areas indicate the binding steps and white areas indicate washing with HEPES saline buffer at pH 7.4. All steps were performed under flow.

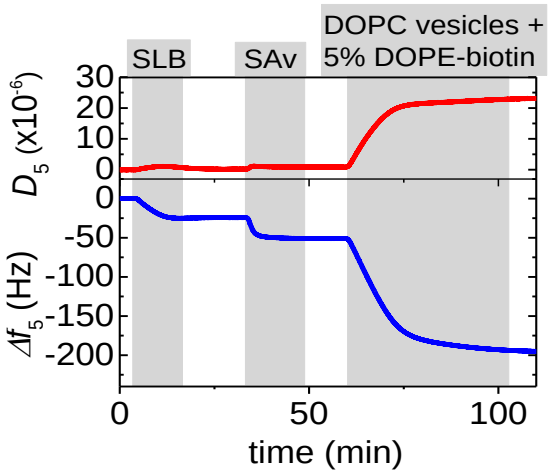

Figure S5: QCM-D binding profile of biotinylated lipid bilayer vesicles of 100 nm in diameter at SAv-modified SLB. The formation of DOPC SLB containing 2 mol% of DOPE-biotin was followed by absorption of SAv (1 μM) and subsequently by DOPC vesicles doped with 5 mol% of DOPE-biotin. Grey areas indicate the binding steps and white areas indicate buffer wash steps. All steps were performed under flow.

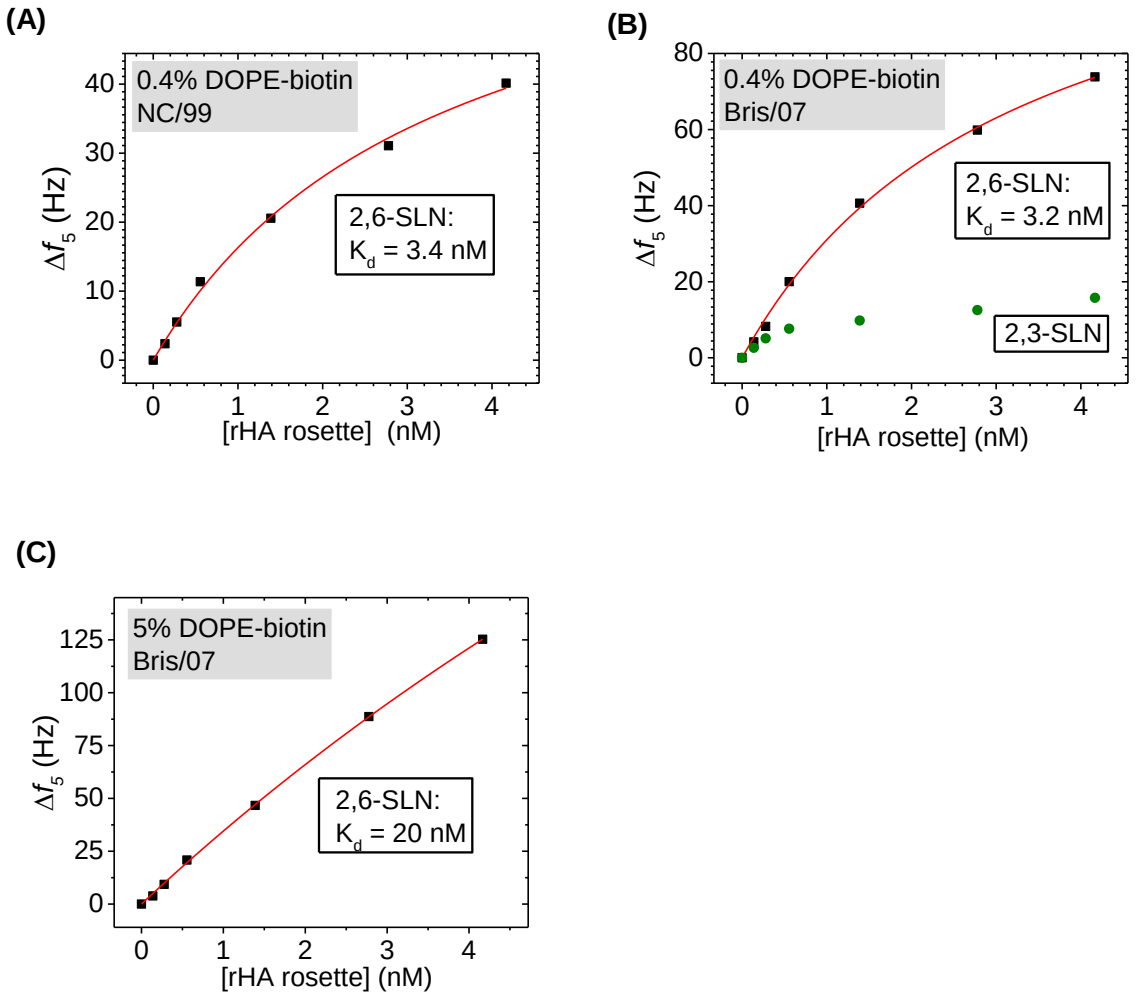

Figure S6: **Langmuir binding model fitted to the binding data** for: A) NC/99 at 0.4% DOPE-biotin in SLB with 2,6 SLN (see Figure S4A), B) Bris/07 at 0.4% DOPE-biotin in SLB with 2,6 SLN (see Figure S4C) and C) Bris/07 at 5% DOPE-biotin in SLB with 2,6 SLN (see Figure S4D). Dissociation constants K_d are given and plateau values were co-fitted with K_d. Plateau values obtained from the fits are 71, 130 and 739 for A, B and C, respectively.

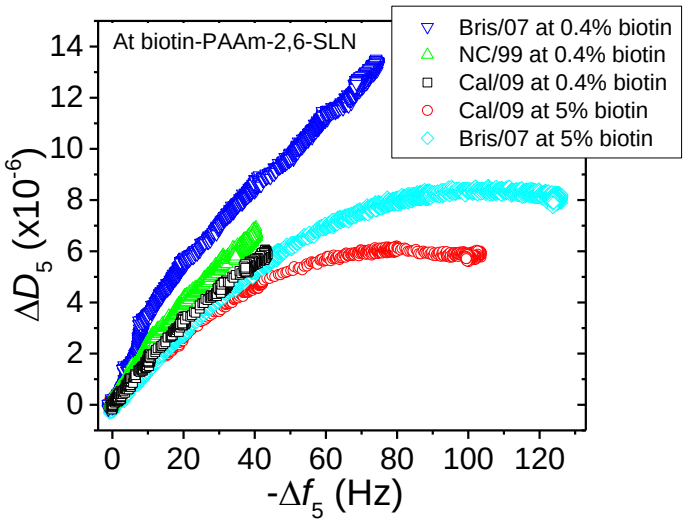

Figure S7. ΔD_5 as function of $-\Delta f_5$ for the rHA cluster titration step for the three tested clusters at surfaces with 0.4% or 5% DOPE-biotin, and with the 2,6-SLN receptor.

Calculation of the quantification of receptors on a surface

Considering that one DOPC lipid covers 0.725 nm^2 and, therefore, the lipid density in SLB is 1.38 molecule per nm^2 (= 2.3×10^{-10} mol/cm^2 = a), we obtain as follows:

- fraction DOPE-biotin = x;
- Θ(bt, SLB) = x × a = xa;
- Θ(SAv) = ½xa;
- Θ(bt, PAA) = xa;
- Θ(SLN, PAA) = 4xa (= ratio SLN/biotin in PAA).

Therefore at x = 0.1%: Θ(SLN) = 0.92 pmol/cm^2, *etc*.