SUPPLEMENTARY INFORMATION

Well-defined biomimetic surfaces to characterize glycosaminoglycan-mediated interactions on the molecular, supramolecular and cellular levels

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SUPPLEMENTARY FIGURES

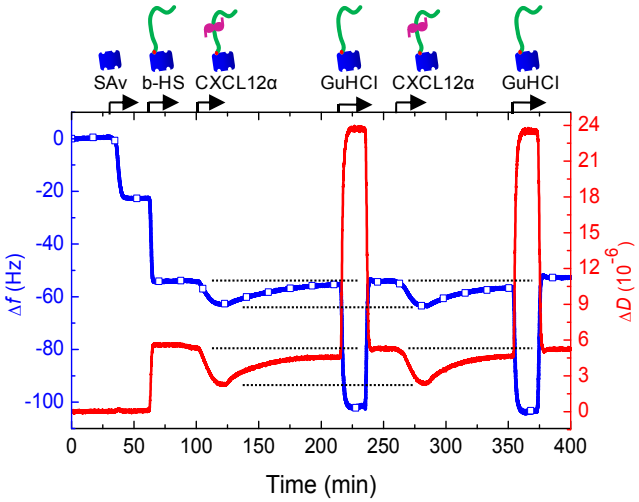

Figure S1. Regeneration of HS films by guanidine hydrochloride (GuHCl). QCM-D (Δ*f* – *blue lines with square symbols*, Δ*D* – *red lines*) was used to follow the surface functionalization on a gold-supported OEG monolayer and the effect of GuHCl on the model surface. Start and duration of each incubation step with different samples are indicated by an arrow; during all other times, the surface was exposed to working buffer. The assembly of the model surfaces, including loading with CXCL12α, was performed as in Fig. 2*A*. Upon exposure of the CXCL12α-loaded surface to 2 M GuHCl (GuHCl (Sigma Aldrich) was dissolved at 8 M in ultrapure water and then diluted in working buffer to the desired concentration), frequency and dissipation shifts recovered the values for a virgin HS film, indicating (i) total release of the protein, and (ii) that the HS film itself is not significantly affected by GuHCl. A second injection of CXCL12α generated the same shifts in frequency and dissipation as the first one, confirming that the surfaces can be effectively regenerated by 2 M GuHCl. *Horizontal black dashed lines* are provided to facilitate comparison of data at different times. The large changes in Δ*f* and Δ*D* observed during incubation with GuHCl are predominantly due to changes in the viscosity and density of the bulk solution owing to the presence of GuHCl and thus unrelated to surface processes.

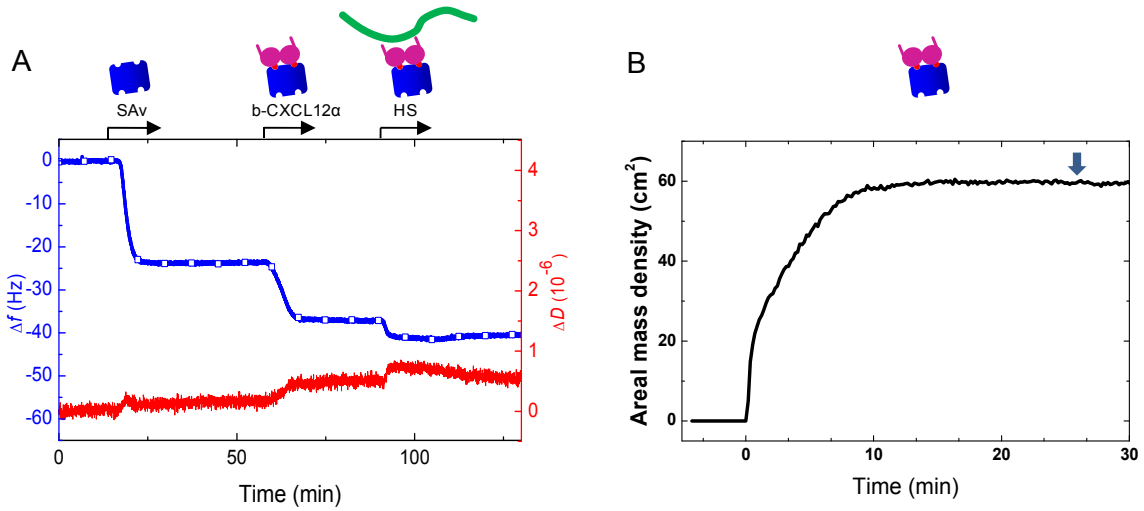

Figure S2. Assembly of surfaces presenting b-CXCL12α. (*A*) Functionalization of a gold-supported OEG monolayer was followed by QCM-D (Δ*f* – *blue lines with square symbols*, Δ*D* – *red lines*). Start and duration of incubation steps with different samples are indicated by an arrow; during all other times, the surface was exposed to working buffer. SAv was incubated at 20 µg/mL, b-CXCL12α at 5 µg/mL and HS at 50 µg/mL. b-CXCL12α bound stably with final shifts of Δf = -13 ± 0.5 Hz and $\Delta D = 0.4 \pm 0.1 \times 10^{-6}$. Assuming a density of 1.2 g/cm^3 for the b-CXCL12α film with trapped solvent, the frequency shift would correspond to a film thickness of approximately 2.0 nm, slightly lower than the dimensions of CXCL12α monomers (2.7 to 4 nm, depending on the exact orientation [1]). The QCM-D response is hence consistent with the formation of a monolayer of monomeric CXCL12α. HS (without biotin) bound readily to the CXCL12α monolayer, but not to a virgin SAv monolayer (Fig. 2*C*), confirming that the accessibility of the HS binding site was not obstructed by the immobilisation of CXCL12α through the C-terminal biotin. HS binding is partially reversible, as previously observed for CXCL12α bound to b-HS films (Fig. 2*A-B*). (*B*) Formation of a b-CXCL12α monolayer followed by SE. The surface was prepared as in *A*; incubation of b-CXCL12α started at 0 min, and the start of rinsing in working buffer is indicated by an arrow. From the molecular weights and maximal surface densities of SAv (60 kDa; 235 ng/cm^2, see Table 1) and b-CXCL12α (8.6 kDa; 59 ng/cm^2), we calculate a binding stoichiometry of 1.8. This confirms that biomolecules can be immobilized with a maximal stoichiometry close to two, provided they are small enough to avoid packing constraints, consistent with every immobilized SAv molecule exposing two binding sites as expected from the design of our immobilization platforms.

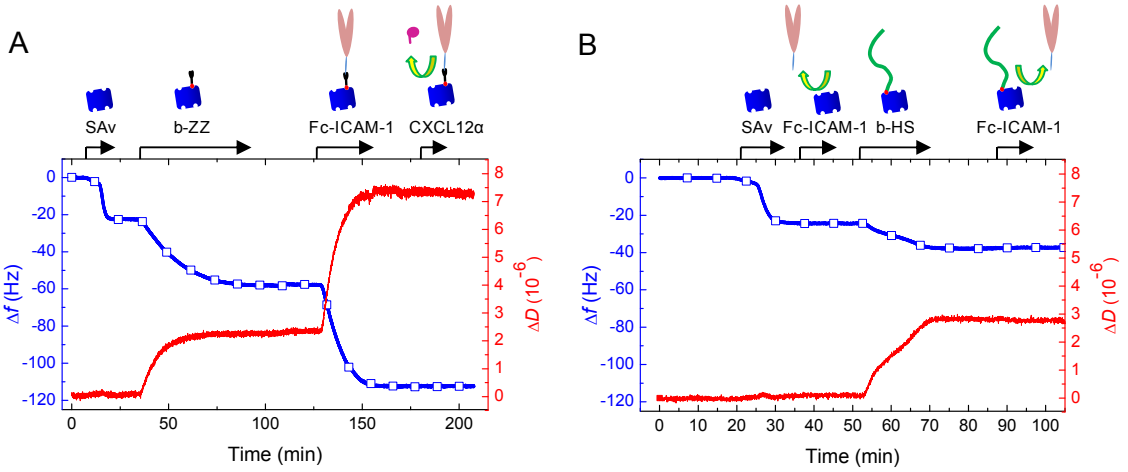

Figure S3. Assembly of surfaces presenting ICAM-1. Fc-ICAM-1 was immobilized in an oriented manner through a biotinylated linker molecule with a ZZ domain (b-ZZ) which recognizes the Fc-tag on ICAM-1. Functionalization of a gold-supported OEG monolayer was followed by QCM-D (Δ*f* – *blue lines with square symbols*, Δ*D* – *red lines*). Start and duration of incubation steps with different samples are indicated by an arrow; during all other times, the surface was exposed to working buffer. SAv was incubated as in Fig. 2*A*, b-ZZ at 0.05 µM, Fc-ICAM-1 at 0.2 µM, CXCL12α at 5 µg/mL and b-HS at 1 µg/mL. (*A*) The binding curves for b-ZZ and Fc-ICAM-1 saturated and binding was stable upon rinsing in working buffer, indicating formation of stable monolayers. The frequency shift at saturation for b-ZZ (-35.5 Hz) corresponds to a film thickness of approximately 6 nm (assuming a film density of 1.2 g/cm^3), consistent with the hydrodynamic diameter (7.1 nm; measured by dynmic light scattering) of b-ZZ. The lack of response for CXCL12α confirmed that ICAM-1 did not compromise the inertness of the surface against non-specific binding of chemokines. (*B*) In the absence of b-ZZ, Fc-ICAM-1 did not bind to a bare SAv monolayer nor to a SAv monolayer presenting b-HS (at approximately half-maximal coverage), confirming that Fc-ICAM-1 immobilization through b-ZZ is specific.

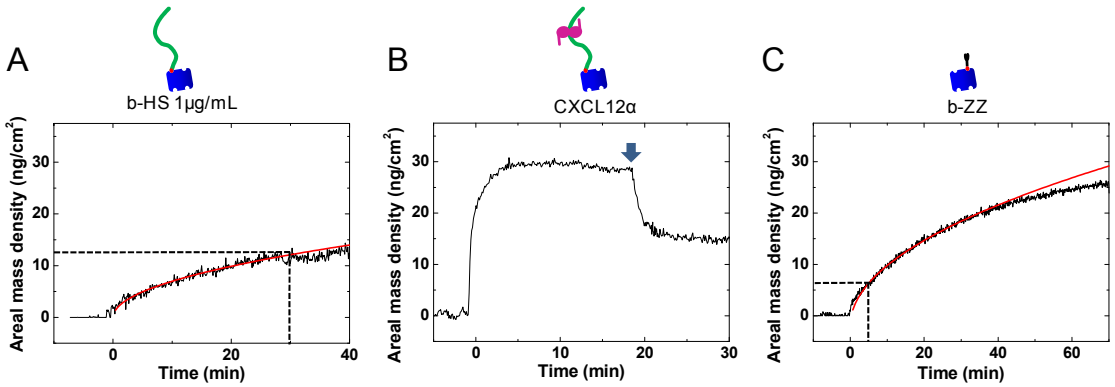

Figure S4. Tuning of biomolecular surface densities on SAv monolayers. Adsorption was followed by SE; each incubation step started at 0 min; the start of rinsing in working buffer is indicated by an arrow. (*A*) Adsorption of b-HS at a concentration of 1 µg/mL from still solution to a SAv-coated OEG monolayer. Only initial binding is shown, as adsorption did not reach saturation within reasonable times at such a low solution concentration; b-HS surface densities can be tuned by interrupting incubation at desired time points. For example, to create the surfaces used for Fig. 6*B*-*C*, b-HS was incubated for 30 min (indicated with *dotted lines*), reaching an areal mass density of 12.7 ± 1.3 ng/cm^2 (average value over 4 independent measurements, not shown), corresponding to 35 ± 5% of maximal coverage. (*B*) Representative data for the adsorption of CXCL12α at a concentration of 5 µg/mL on such a low density b-HS film. At equilibrium, the areal mass density of CXCL12α was 35 ± 4 ng/cm^2. (*C*) Adsorption of b-ZZ at a concentration of 0.05 µM from still solution to a SAv monolayer. Only initial binding is shown, as adsorption did not reach saturation within reasonable times at such a low solution concentration; b-ZZ coverage at saturation was found to be around 118.6 ± 0.2 ng/cm^2 using higher b-ZZ concentrations in solution (data not shown). To reach the b-ZZ surface density of 7 ng/cm^2, desired for Fig. 6*B*-*C* and corresponding to 6% of maximal coverage, b-ZZ was incubated for 5 min (indicated with *dotted lines*). Note that binding of b-HS and b-ZZ scales with the square root of incubation time (*red curves* are fits with square-root dependence) provided that the surface density is sufficiently low. The square-root dependence is expected for mass-transport limited binding, and indicates that surface coverages can be tuned by varying the incubation time (with square-root dependence, as shown) and/or the incubation concentration (with linear dependence, not shown). See ref. [2] for details.

SUPPLEMENTARY REFERENCES

1. Veldkamp CT, Ziarek JJ, Su J, Basnet H, Lennertz R, Weiner JJ, et al. Monomeric structure of the cardioprotective chemokine SDF-1/CXCL12. Protein Sci 2009;18:1359–69.
2. Hermens WT, Benes M, Richter R, Speijer H. Effects of flow on solute exchange between fluids and supported biosurfaces. Biotechnol Appl Biochem 2004;39:277–84.