

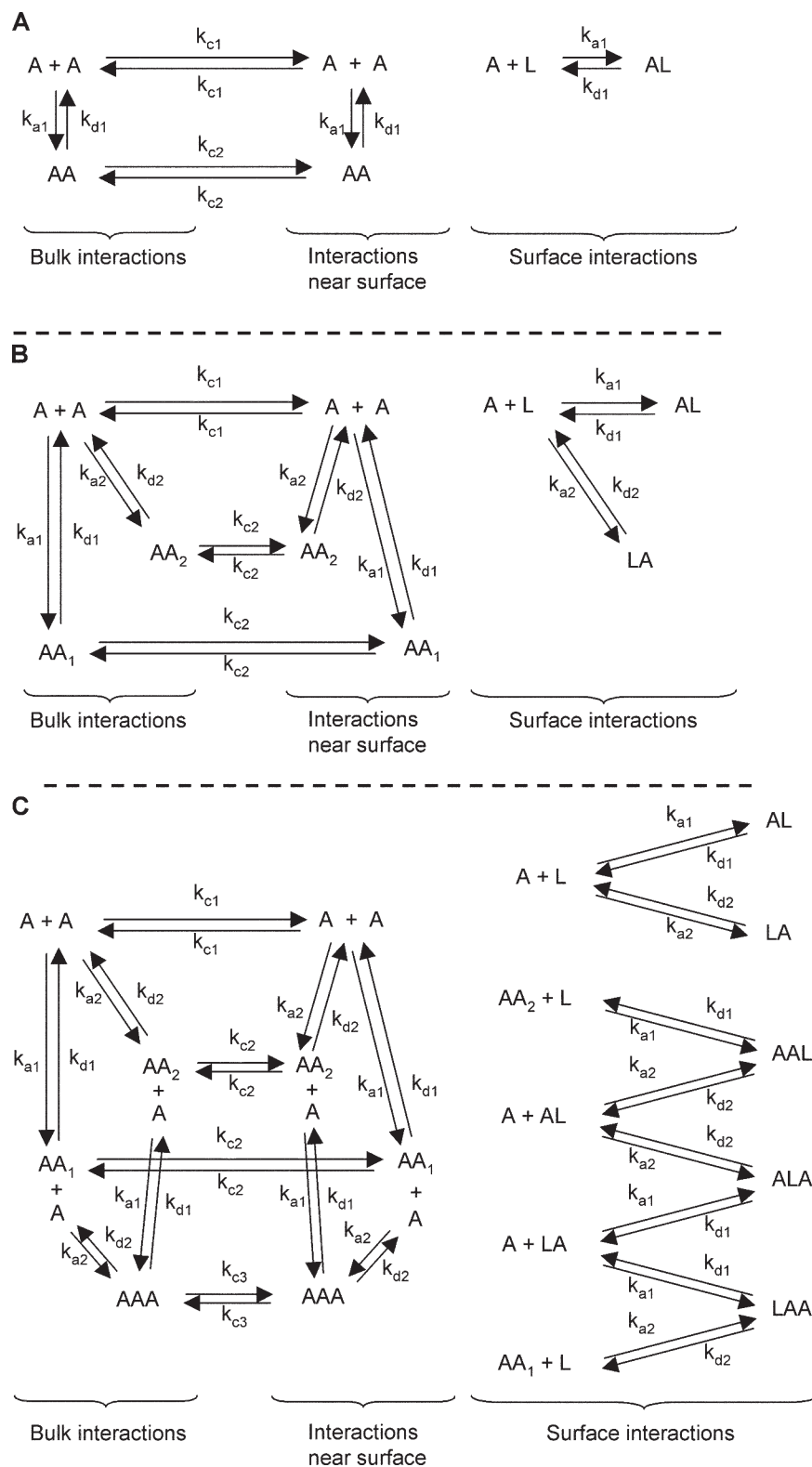
Klaile et al., <http://www.jcb.org/cgi/content/full/jcb.200904149/DC1>

Figure S1. **Reaction schemes for homophilic binding interactions in a BIAcore flow cell.** The same kind of molecules occur both as analytes in the soluble phase and as immobilized ligands in the solid phase. (A) Formation of one class of dimers by one homophilic binding site. (B) Formation of two classes of

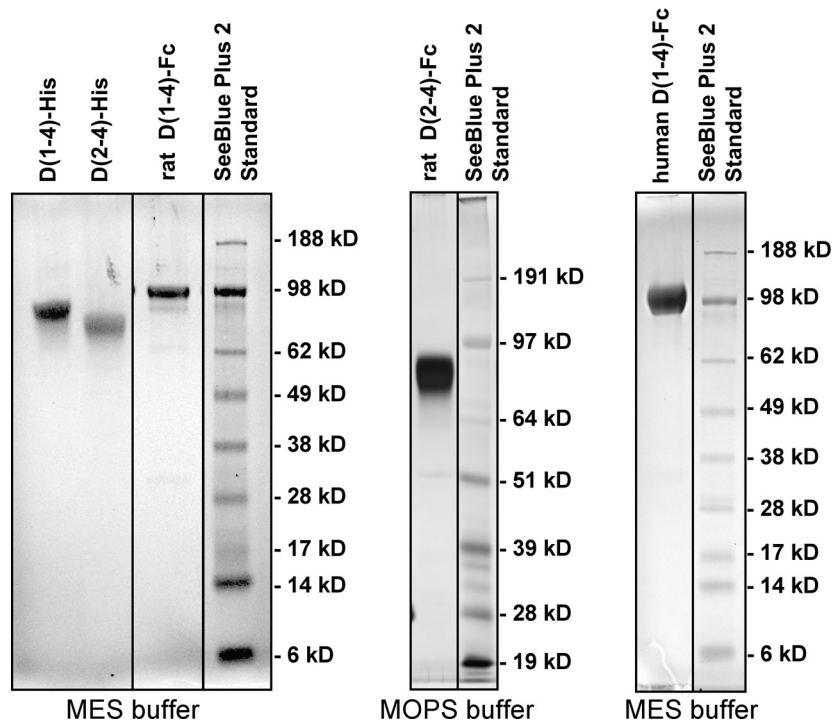
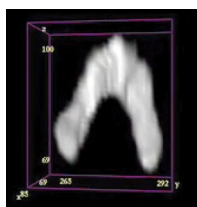
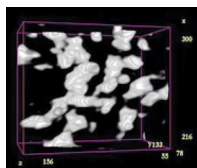


Figure S2. **SDS-PAGE of purified recombinant CEACAM1 ectodomains.** Purified rat CEACAM1 D(1–4)-His, D(2–4)-His, D(1–4)-Fc, D(2–4)-Fc, and human CEACAM1 D(1–4)-Fc were separated on a NuPAGE Novex Bis-Tris gel (4–12%; Invitrogen) in NuPAGE-MES-SDS or NuPAGE-MOPS-SDS running buffer (Invitrogen) under reducing conditions and stained with Coomassie brilliant blue. Black lines indicate that intervening lanes have been spliced out.

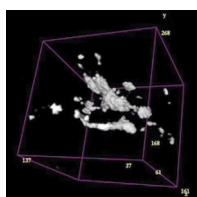
dimers (types 1 and 2) by two different homophilic binding sites. (C) Formation of two classes of dimers (types 1 and 2) and one class of trimers by two different homophilic binding sites. The association and dissociation reactions underlying the different complex formations are characterized by a set of kinetic rate constants,  $k_{a1}/k_{d1}$  and  $k_{a2}/k_{d2}$ , respectively. These binding reactions occur both between the soluble molecules in the fluid phase and with the immobilized molecules at the surface. In order for the surface interactions to take place, the soluble molecules in the bulk phase must be transported to the surface. This mass transport process is characterized by a mass transport coefficient,  $k_c$ . Because the monomers, dimers, and trimers are of different sizes, they have different mass transport coefficients,  $k_{c1}$ ,  $k_{c2}$ , and  $k_{c3}$ , respectively. (C) In the trimer model, an asymmetry is introduced because the immobilized ligand makes up one of the three components of the trimer that is formed on the surface. Therefore, two types of dimers and three types of trimers contribute to the SPR-based signal. These complexes are the following: AL, type 1 dimer; LA, type 2 dimer; AAL, A-type 2-A-type 1-L; ALA, A-type 1-L-type 2-A; and LAA, L-type 2-A-type 1-A. A, analyte; L, ligand.



Video 1. **D(1-4)-His molecules analyzed by molecular tomography.** 3D reconstructions of D(1-4)-His molecules presented and described in detail in Fig. 4 (A-H) are shown in the same order, rotating around one axis. Monomeric D(1-4)-His adopting an extended (Fig. 4, A and B), kinked (Fig. 4 C), or back-folded (Fig. 4 D) form. (Fig. 4 E) D(1-4)-His dimer, interacting exclusively via the D1 domains (C-dimer; see Results). (Fig. 4, F and G) D(1-4)-His dimers interacting via three (Fig. 4 F) or four (Fig. 4 G) of their Ig domains (A-dimers; see Results). (Fig. 4 H) D(1-4)-His trimer consisting of a monomer binding via its D1 domain to an A-dimer. The units of the coordinates are pixels, and the pixel size is 5.74 Å.



Video 2. **Proteoliposomes analyzed by molecular tomography.** Free surfaces of Ni-NTA liposomes with attached D(1-4)-His or D(2-4)-His ectodomains presented in Fig. 6 (B and C) are shown in the same order, rotating around one axis. The units of the coordinates are pixels, and the pixel size is 5.74 Å.



Video 3. **D(1-4)-His molecules bridging two liposomes analyzed by molecular tomography.** An overview and details of the monomeric bridge (C-dimer) presented in Fig. 6 E and the heptameric bridge presented in Fig. 6 F are shown. The units of the coordinates are pixels, and the pixel size is 5.74 Å.