

Supporting Information for

**Using Covalent Dimers of Human Carbonic Anhydrase II as a Model for
Bivalency in Immunoglobulins**

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Calculation of the number of ligands accessible to (CA)₂'s on the surface.

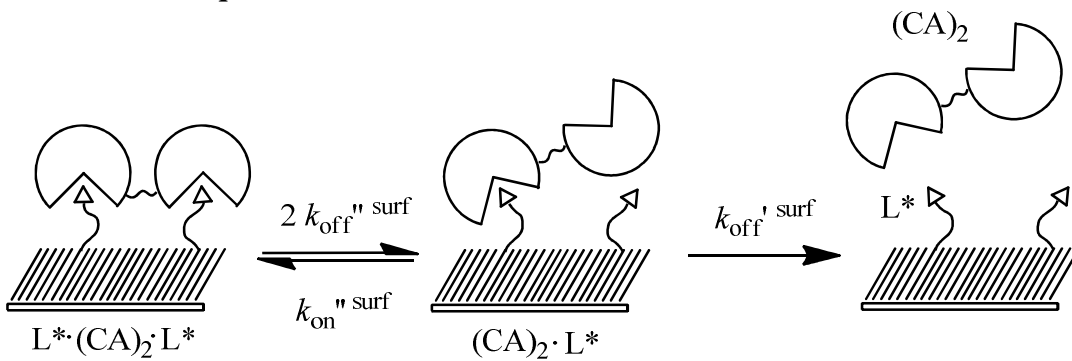
The maximum number of ligands accessible to the binding sites of (CA)₂ is the number of ligands found in a circle of the SAM with a radius equal to the distance between the binding sites of (CA)₂. The minimum distance between the binding sites of (CA)₂ (4.2 nm) is twice the radius of CA (2.1 nm). The number of lattice sites, n , covered by a molecule of radius r , adsorbed on a hexagonal lattice characterized by a lattice vector of length a , is given by eq S1 in which $k = r_{\text{protein}} / a$. For (CA)₂ adsorbed on the SAM having lattice sites arranged on a hexagonal lattice, $a = 0.5$ nm and $r_{\text{protein}} = 4.2$ nm (i.e., $k = 4.2 \text{ nm} / 0.5 \text{ nm} = 8.4$) yield $n \sim 238$ (eq S1).¹

$$n = 3k(k + 1) + 1 \quad (\text{S1})$$

Roughly 5 are the ligands accessible to (CA)₂, which results from multiplying n by the mole fraction—that is, 238 lattice sites per CA dimer * 0.02 ligands per lattice site ~ 5 ligands per CA dimer.

(1) Stankowski, S. *Biochim. Biophys. Acta* **1984**, 777, 167-182.

Derivation of Equation 13



The rate of dissociation of (CA)₂ from the SAM is described by three differential equations (eq S2-S4).

$$\frac{d[\text{L}^* \cdot (\text{CA})_2 \cdot \text{L}^*]}{dt} = -2k_{\text{off}}'' \text{ surf} [\text{L}^* \cdot (\text{CA})_2 \cdot \text{L}^*] + k_{\text{on}}'' \text{ surf} [(\text{CA})_2 \cdot \text{L}^*] \quad (\text{S2})$$

$$\frac{d[(CA)_2 \cdot L^*]}{d t} = 2k_{\text{off}}''^{\text{surf}} [L^* \cdot (CA)_2 \cdot L^*] - k_{\text{on}}''^{\text{surf}} [(CA)_2 \cdot L^*] - k_{\text{off}}'^{\text{surf}} [(CA)_2 \cdot L^*] \quad (\text{S3})$$

$$\frac{d[(CA)_2]}{d t} = k_{\text{off}}'^{\text{surf}} [(CA)_2 \cdot L^*] \quad (\text{S4})$$

Application of the steady state approximation to $[CA_2 \cdot L^*]$.

$$\frac{d[(CA)_2 \cdot L^*]}{d t} = 0 \quad (\text{S5})$$

Then solving equation S3 for $[CA_2 \cdot L^*]$

$$[(CA)_2 \cdot L^*]^* = \frac{2k_{\text{off}}''^{\text{surf}} [L^* \cdot (CA)_2 \cdot L^*]}{k_{\text{on}}''^{\text{surf}} + k_{\text{off}}'^{\text{surf}}} \quad (\text{S6})$$

The signal in response units (RU) is proportional to concentration of $(CA)_2$ in solution.

$$\frac{dRU}{d t} = -\frac{d[(CA)_2]}{d t} = -k_{\text{off}}'^{\text{surf}} [(CA)_2 \cdot L^*] \quad (\text{S7})$$

Substitution of equation S6 into S7 yields equation S8.

$$-\frac{d[(CA)_2]}{d t} = -k_{\text{off}}'^{\text{surf}} \frac{2k_{\text{off}}''^{\text{surf}} [L^* \cdot (CA)_2 \cdot L^*]}{k_{\text{on}}''^{\text{surf}} + k_{\text{off}}'^{\text{surf}}} \quad (\text{S8})$$

The observed rate constant $k_{\text{off}}^{\text{avidity,surf}}$ is equal to function of rate constants in S8 (eq S9).

$$k_{\text{off}}^{\text{avidity,surf}} = \frac{k_{\text{off}}'^{\text{surf}} 2k_{\text{off}}''^{\text{surf}}}{k_{\text{on}}''^{\text{surf}} + k_{\text{off}}'^{\text{surf}}} \quad (\text{S9})$$

When $k_{\text{on}}''^{\text{surf}} \gg k_{\text{off}}'^{\text{surf}}$ the denominator becomes $k_{\text{on}}''^{\text{surf}}$. Replacing the ratio of $k_{\text{off}}'^{\text{surf}}$ to $k_{\text{on}}''^{\text{surf}}$ by $K_d''^{\text{surf}}$ according to the definition $K_d = k_{\text{off}}' / k_{\text{on}}''$ yields eq S10, which corresponds to equation 13 of the main text.

$$k_{\text{off}}^{\text{avidity,surf}} \approx 2 K_d''^{\text{surf}} k_{\text{off}}'^{\text{surf}} \quad (\text{S10})$$

Scheme S1. Synthesis of $(CA)_2$'s from double mutants of HCAII and commercially available thiol-selective cross-linkers. A solution of HCAII double mutant in 10mM phosphate buffer at pH 7.4 was treated with aliquots of a solution of crosslinker in DMF.

