Supporting Information for

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

Eric T. Mack, Phillip W. Snyder, Raquel Perez-Castillejos, and George M. Whitesides*

Department of Chemistry and Chemical Biology, Harvard University

12 Oxford Street, Cambridge, MA 02138

* Correspondence: gwhitesides@gmwgroup.harvard.edu

Table of Contents

Calculation of the number of ligands accessible to $(CA)_2$'s on the surface.

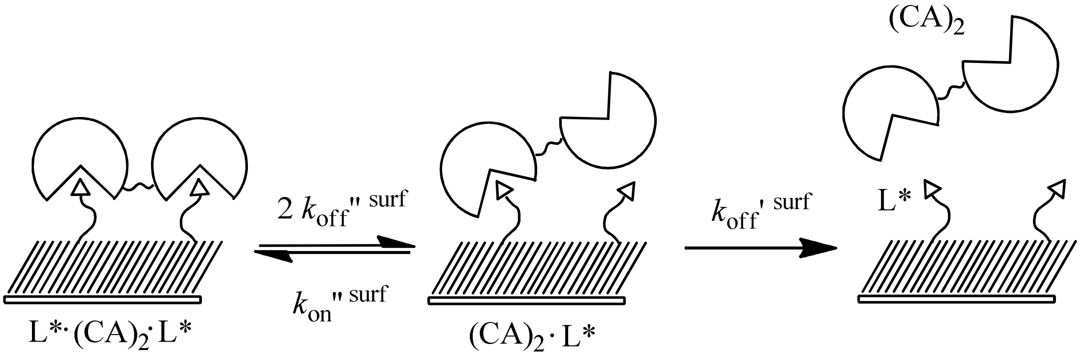
The maximum number of ligands accessible to the binding sites of $(CA)_2$ is the number of ligands found in a circle of the SAM with a radius equal to the distance between the binding sites of $(CA)_2$. The minimum distance between the binding sites of $(CA)_2$ (4.2 nm) is twice the radius of CA (2.1 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 $k = r_{\mathrm{protein}} / a$. For $(CA)_2$ adsorbed on the SAM having lattice sites arranged on a hexagonal lattice, $a = 0.5$ nm and $r_{\mathrm{protein}} = 4.2$ nm (i.e., $k = 4.2$ nm / 0.5 nm = 8.4) yield $n \sim 238$ (eq S1).[1]

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

Roughy 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).

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

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

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

Application of the steady state approximation to $[\mathrm{CA}_2{\cdot}\,\mathrm{L}^*]$.

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

Then solving equation S3 for $[\mathrm{CA}_2{\cdot}\,\mathrm{L}^*]$

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

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

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

Substitution of equation S6 into S7 yields equation S8.

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

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

$$k_{\mathrm{off}}{}^{avidity,surf} = \frac{k_{\mathrm{off}}{}'^{\,surf}\;2\,k_{\mathrm{off}}{}''^{\,surf}}{k_{\mathrm{on}}{}''^{\,surf} + k_{\mathrm{off}}{}'^{\,surf}} \tag{S9}$$

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

$$k_{\mathrm{off}}{}^{\mathrm{avidity,surf}} \approx 2\,K_{\mathrm{d}}{}''^{\,surf}\;k_{\mathrm{off}}{}'^{\,surf} \tag{S10}$$

Scheme S1. Synthesis of $(CA)_2$'s from double mutants of HCAII and commericially 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.