Online Supplement

In vitro **selection and characterization of DARPins and Fab fragments for the co-crystallization of membrane proteins: The Na^+-citrate symporter CitS as an example**

Thomas Huber[1], Daniel Steiner[1], Daniela Röthlisberger[1,2] and Andreas Plückthun[1,3]

[1] Department of Biochemistry, University of Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland

[2] present address: Department of Biochemistry, University of Washington, Seattle, WA 98195, USA

[3] to whom correspondence should be addressed:

Email: plueckthun@bioc.unizh.ch

Tel: +41-44-635 55 70

Fax: +41-44-635 57 12

Derivation of equations for the affinity determination

The two equilibria of CitS and DARPin in the assay solution can be described by Eq. 1, when assuming a 1:1 interaction.

$$C + A \overset{K_{D1}}{\rightleftharpoons} CA \quad \text{and} \quad C_b + A \overset{K_{D2}}{\rightleftharpoons} C_bA \quad \text{(Eq. 1)}$$

Here C is the his-tagged CitS ($_{His}$CitS) in solution, A is the myc-tagged DARPin (DARPin$_{myc5}$) and C_b is the bound AVI-tagged CitS (CitS$_{AVI}$) present on the beads.

The equilibria are characterized by the following basic relationships (Eq. 2 to 6):

$$K_{D1} = \frac{[C][A]}{[CA]} \text{ (Eq. 2)} \qquad K_{D2} = \frac{[C_b][A]}{[C_bA]} \text{ (Eq. 3)}$$

$$[C]_t = [C] + [CA] \text{ (Eq. 4)} \qquad [A]_t = [A] + [CA] + [C_bA] \text{ (Eq. 5)}$$

$$[C_b]_t = [C_b] + [C_bA] \text{ (Eq. 6)}$$

where $[C]$ is the concentration of free $_{His}$CitS, $[C]_t$ is the total concentration of added $_{His}$CitS, $[C_b]$ is the uncomplexed amount of CitS$_{AVI}$, present on beads, $[C_b]_t$ is the total amount of CitS$_{AVI}$ present on the beads, $[A]$ is the concentration of free DARPin$_{myc}$, $[A]_t$ is the total concentration of added DARPin$_{myc}$, and $[CA]$ and $[C_bA]$ are the concentrations of the corresponding complexes. All bead-bound species are treated like molecules in solution and therefore molar concentrations were used. K_{D1} and K_{D2} are the dissociation constants of the two equilibria. We assume here that the dissociation constants K_{D1} and K_{D2} are the same (therefore, named K_D). Using the mass balances in Eq. 2 and Eq. 3, we obtain:

$$K_D = \frac{([C]_t - [CA])([A]_t - [CA] - [C_b A])}{[CA]} \qquad \text{(Eq. 7)}$$

$$K_D = \frac{([C_b]_t - [C_b A])([A]_t - [CA] - [C_b A])}{[C_b A]} \qquad \text{(Eq. 8)}$$

From these two equations, it follows that

$$[CA] = \frac{[C_b A][C]_t}{[C_b]_t} \qquad \text{(Eq. 9)}$$

Combining Eq. 8 and Eq. 9 will give Eq. 10:

$$K_D = \frac{([C_b]_t - [C_b A])\left([A]_t - \frac{[C_b A][C]_t}{[C_b]_t} - [C_b A]\right)}{[C_b A]} \qquad \text{(Eq. 10)}$$

Solving Eq. 10 for $[C_bA]$ will yield Eq. 11 (solution of the quadratic equation).

$$[C_b A] = \frac{([A]_t + [C]_t + [C_b]_t + K_D) - \sqrt{([A]_t + [C]_t + [C_b]_t + K_D)^2 - 4\left(\frac{[C]_t}{[C_b]_t} + 1\right)[C_b]_t [A]_t}}{2\left(\frac{[C]_t}{[C_b]_t} + 1\right)}$$

(Eq. 11)

The measured ECL signal is proportional to the concentration of bound complex $[C_bA]$. Taking also a term of background binding (BG) into account we obtain Eq. 12.

$$ECL = Const. \cdot [C_b A] + BG \qquad \text{(Eq. 12)}$$

where *Const.* is simply a proportionality constant which is obtained from the fit and relates the measured ECL signal to bound complexes. We can then express the measured ECL signal by Eq. 13.

$$ECL = \frac{Const.}{2\left(\frac{[C]_t}{[C_b]_t} + 1\right)}\left\{([A]_t + [C]_t + [C_b]_t + K_D) - \sqrt{([A]_t + [C]_t + [C_b]_t + K_D)^2 - 4\left(\frac{[C]_t}{[C_b]_t} + 1\right)[C_b]_t [A]_t}\right\} + BG$$

(Eq. 13)

We thus fit the parameters K_D, $[A]_t$, *Const.* and *BG*. Even though $[A]_t$ should be known, possible errors in the concentration of active molecules make it advisable to fit this term as well.