

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**

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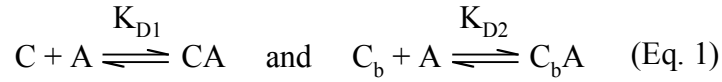
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Derivation of equations for the affinity determination

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



Here C is the his-tagged CitS (HisCitS) in solution, A is the myc-tagged DARPin ($\text{DARPin}_{\text{myc5}}$) 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):

$$K_{D1} = \frac{[C][A]}{[CA]} \quad (\text{Eq. 2})$$

$$K_{D2} = \frac{[C_b][A]}{[C_bA]} \quad (\text{Eq. 3})$$

$$[C]_t = [C] + [CA] \quad (\text{Eq. 4})$$

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

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

where $[C]$ is the concentration of free HisCitS , $[C]_t$ is the total concentration of added HisCitS , $[C_b]$ 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 $\text{DARPin}_{\text{myc}}$, $[A]_t$ is the total concentration of added $\text{DARPin}_{\text{myc}}$, 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:

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

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

From these two equations, it follows that

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

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

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

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

$$[C_bA] = \frac{([A]_t + [C]_t + [C_b]_t + K_D) - \sqrt{([A]_t + [C]_t + [C_b]_t + K_D)^2 - 4 \left(\frac{[C]_t}{[C_b]_t} + 1 \right) [C_b]_t [A]_t}}{2 \left(\frac{[C]_t}{[C_b]_t} + 1 \right)} \quad (\text{Eq. 11})$$

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.

$$ECL = Const. \cdot [C_bA] + BG \quad (\text{Eq. 12})$$

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.

$$ECL = \frac{Const.}{2 \left(\frac{[C]_t}{[C_b]_t} + 1 \right)} \left\{ ([A]_t + [C]_t + [C_b]_t + K_D) - \sqrt{([A]_t + [C]_t + [C_b]_t + K_D)^2 - 4 \left(\frac{[C]_t}{[C_b]_t} + 1 \right) [C_b]_t [A]_t} \right\} + BG \quad (\text{Eq. 13})$$

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.