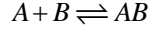


## Supplementary Material

### Derivation of the quadratic equation used to fit the binding data



$$K = \frac{[A]*[B]}{[AB]} = \frac{([A]_0 - [AB]) * ([B]_0 - [AB])}{[AB]}, \text{ where } [A]_0 \text{ and } [B]_0 \text{ are total or initial concentrations, } K \text{ is the } K_d$$

$$[AB] * K = ([A]_0 - [AB]) * ([B]_0 - [AB])$$

$$[AB] * K = ([A]_0 * [B]_0) - ([AB] * [A]_0) - ([AB] * [B]_0) + [AB]^2$$

$$[AB]^2 - ([A]_0 + [B]_0 + K) * [AB] + [A]_0 * [B]_0 = 0, \text{ which is the form of}$$

$$ax^2 + bx + c = 0 \text{ where } a = 1, b = -([A]_0 + [B]_0 + K), c = [A]_0 * [B]_0, \text{ thus}$$

$$[AB] = \left( ([A]_0 + [B]_0 + K) - \sqrt{([A]_0 + [B]_0 + K)^2 - 4 * [A]_0 * [B]_0} \right) / 2$$

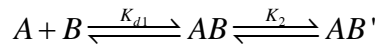
During the titration experiments, we kept the total concentration of one component constant ( $c$ ) while changing the concentration of the other component ( $x$ ). We followed the signal given by our  $AB$  complex (CaM-ME) from  $x = 0$ , that is the fluorescence of the free CaM ( $s$ ). The actual signal is relativized by an amplitude coefficients ( $Amp$ ). Substituting the above considerations to the quadratic equation solved for the binding reaction of A and B:

$$[AB] = s + Amp * \left( (c + x + K) - \sqrt{(c + x + K)^2 - 4 * c * x} \right) / 2$$

Division of both sides by the total concentration of the constant component will result in an amplitude ( $Amp$ ), which measures the total signal change independent of concentration.  $y$  then will equal the bound fraction of the component monitored by the signal.

$$y = s + Amp * \left( (c + x + K) - \sqrt{(c + x + K)^2 - 4 * c * x} \right) / 2 * c$$

### Description of the origin of Equation 2



$$K_{d,1} = \frac{[A]*[B]}{[AB]} \rightarrow [AB] = \frac{[A]*[B]}{K_{d,1}}$$

$$K_2 = \frac{[AB']}{[AB]} \rightarrow [AB'] = K_2 * [AB]$$

Because we observe the species  $AB$  and  $AB'$ ,  $K_{eq}$  can be defined as:

$$K_{eq} = \frac{[A]*[B]}{[AB] + [AB']} = \frac{[A]*[B]}{\frac{[A]*[B]}{K_{d,1}} + K_2 * \frac{[A]*[B]}{K_{d,1}}} = \frac{K_{d,1}}{1 + K_2}$$

### Description of the origin of Equation 3

$C$ : concentration of the chaser

$L$ : concentration of the ligand to be chased off

$$K_C = \frac{E^*C}{EC} \rightarrow EC = \frac{E^*C}{K_C}$$

$$K_L = \frac{E^*L}{EL} \rightarrow EL = \frac{E^*L}{K_L}$$

$$\frac{EC}{EC + EL + E} = \frac{\frac{E^*C}{K_C}}{\frac{E^*C}{K_C} + \frac{E^*L}{K_L} + E} = \frac{\frac{C}{K_C}}{\frac{C}{K_C} + \frac{L}{K_L} + 1} = \frac{C}{C + \left(\frac{K_C^*L}{K_L} + K_C\right)}$$

$$K_{d,app} = \frac{K_C^*L}{K_L} + K_C \quad \text{if } L \text{ is saturating, then } K_{d,app} = \frac{K_C^*L}{K_L}$$

