Supplementary Material

Derivation of the quadratic equation used to fit the binding data

$$A + B \rightleftharpoons AB$$

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

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

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

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

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

$$A + B \underset{}{\overset{K_{d1}}{\rightleftharpoons}} AB \underset{}{\overset{K_2}{\rightleftharpoons}} AB'$$

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

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

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

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

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

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

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