

Supplementary data 1. Diffusion limit

The rate constant we found for the association rate of the N-lobe binding sites in the R-state is extremely fast ($3.2 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$). To ascertain whether this was theoretically possible, we calculated the diffusion limited reaction speed for CaM and Ca^{2+} based on the rate of intermolecular collisions. Using the Smoluchowski theory and the Einstein–Smoluchowski relation^{26,27}:

$$k_{diffusion} = 4\pi(r_A + r_B)(D_A + D_B)N_A \times 10^3 \quad \text{M}^{-1} \text{s}^{-1}$$

$$D = \frac{k_B T}{6\pi\eta r} \quad \text{m}^2 \text{s}^{-1}$$

$$R_{\text{Ca}^{2+}} = 1.14 \text{ \AA}$$

$$r_{\text{CaM}} = 25 \text{ \AA}$$

$$N_A = 6.022 \times 10^{23} \text{ mol}^{-1}$$

$$k_B = 1.3807 \times 10^{-23} \text{ JK}^{-1}$$

$$T = 308 \text{ K}$$

$$\eta_{\text{water},308\text{K}} = 7.25 \times 10^{-4} \text{ Pa s}$$

We find a rate limit of $5.6 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ for our conditions (35°C in aqueous solution), indicating that our determined rate of $3.2 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ is theoretically possible.