Electronic Supplementary Information

Structural plasticity of calmodulin on the surface of CaF_2 nanoparticles preserves its biological function

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Supplementary Methods

Protein expression and purification

Expression was performed by adding 0.4 mM IPTG to 1 liter of BL21(DE3)[pET12b-AtCaM1] bacterial culture grown in LB medium containing 100 μg/mL ampicillin to exponential phase and incubating at 30 °C for 4-5 hours. Bacterial cells were pelletted and resuspended in 50 mL of extraction buffer (50 mM Tris-HCl pH 7.5, 2 mM EDTA) and frozen. After thawing, 1 mM DTT and 0.1 mg/mL lysozyme were added; the lysate was incubated 30 minutes in ice and sonicated briefly to shear the bacterial DNA. Cell debris was removed by centrifugation at 15,000 xg for 30 min at 4°C. A first precipitation step was obtained by adding ammonium sulphate at 55% saturation. After precipitate removal by centrifugation at 27,000 xg for 30 min at 4°C, few drops of 10% H_2SO_4 were added to the final pH value of 4. The precipitated protein was collected by another centrifugation step and resuspended in 15 mL of 50 mM Tris-HCl pH 7.5, 1 mM DTT. The sample was then dialyzed extensively firstly against double-distilled water and then against dialysis buffer (10 mM Tris-HCl, 100 mM NaCl, 0.5 mM EGTA, 1 mM DTT). The clarified sample was charged with 5 mM $CaCl_2$ and applied to a Phenyl-Sepharose (GE Healthcare) column equilibrated with Equilibration/Wash buffer I (50 mM Tris-HCl pH 7.5, 0.5 mM $CaCl_2$, 1 mM DTT). The column was washed with Equilibration/Wash buffer I and with Wash buffer II (50 mM Tris-HCl pH 7.5, 0.5 mM $CaCl_2$, 1 mM DTT, 200 mM NaCl). CaM1 was eluted with Elution buffer (50 mM Tris-HCl pH 7.5, 1 mM EGTA, 1

mM DTT). The purity of CaM1 was estimated by SDS-PAGE analysis and the protein concentration was determined by the Bradford assay.[1]

Estimate of the size-dependent protein coverage of NP surface and concentration adjustments

The maximum number of proteins bound to each NP depends on the surface of the NP accessible to proteins and on the surface of the NP occupied by a bound protein. Based on the simplest geometric model of interaction and considering all the objects as rigid spheres, the maximal stoichiometric protein/NP ratio to ensure a minimal presence of unbound proteins in solution is:

$$N_{CaM1}^{max} = \frac{4\pi(r_{NP} + r_{CaM1})^2}{\pi r_{CaM1}^{\ 2}} \qquad (1)$$

where $N_{CaM1}{}^{max}$ is the maximum number of CaM1 molecules that can occupy the surface of the NP, r_{NP} is the radius of the NP and r_{CaM1} is the radius of CaM1. In other words, the geometric problem is that of occupying the augmented surface area of a sphere by $N_{CaM1}{}^{max}$ circles, each of area $\pi r_{CaM1}{}^2$.

Also, assuming that the density of CaF_2 NPs is equal to that of bulk CaF_2 (ρ = 3.18 g/cm^3), the molecular weight of CaF_2 NPs (*NP* in the equation) was calculated as follows:

$$MW_{NP} = \frac{4\pi r_{NP}^3}{3} \cdot \rho_{CaF_2} \cdot N_A \qquad (2)$$

For example, in the presence of NPs with a diameter of 25 nm, $N_{CaM1}{}^{max}$ = 144, and the molecular weight of CaF_2 NPs is 15.5 *10^6 g/mol. For near-UV CD spectra with reasonable signal-to-noise ratio, approximately 59 μM (~1 mg mL^{-1}) CaM1 is required, so that 0.41 μM is the concentration of saturating CaF_2 NPs (6.35 mg mL^{-1}) which, under the maximal binding geometric assumption, guarantees the amount of free CaM1 to keep low.

Thermal denaturation profiles

Thermal denaturation of CaM1 both alone and in the presence of CaF_2 NPs was monitored from 20-96 °C using the same conditions and concentration range as for far-UV spectra. The ellipticity signal at 208 nm (θ_{208}) was recorded at a scan rate of 1 °C min^{-1} and a response time of 4 sec, using a 0.1 cm quartz cuvette. Thermally denatured samples were cooled to 25 °C and, after 5-15 min, the far-UV CD spectrum was recorded to check for residual structures (results not shown).

Calculation of the Ca^{2+} concentration within the nanocrystal of the synthesized CaF_2 NPs

Cell volume = 163.13 $Å^3$ Number of Ca^{2+} within the unit cell = Z = 4

4/163.13 $Å^3$ = 2.45 $\cdot 10^{-2}$ ions/ $Å^3$ = 2.45 $\cdot$ 10^{25} ions/L = 2.45 $\cdot$ 10^{25}/ N_A mol/L (M) ~ 41 M

Dynamic Light Scattering experiments

DLS measurements were performed with a Zetasizer Nano-S (Malvern Instruments) using a polystyrene low volume disposable sizing cuvette (ZEN0112). Viscosity and Refractive Index were set at 0.8872 cP and 1.330 respectively (default values for water), temperature was set to 25 °C with 2 min equilibration time. The measurement angle was 173° backscatter and the analysis model was set to multiple narrow modes. For each measurement a minimum of 7 determinations were performed, each consisting of 13-15 repetitions. Buffers (5 mM Tris-HCl, pH 7.5, 150 mM KCl, adjusted with $CaCl_2$ or EGTA), were filtered through a Jet Biofilm 0.22 mm membrane, while protein-only solutions were filtered through an Anotop 10 filter (Whatman, 0.02 μm).

Supplementary Figures

Figure S1

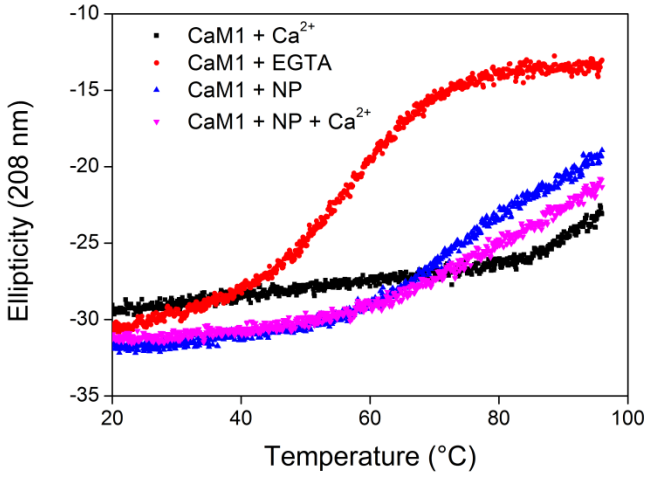

Figure S1. Thermal denaturation profiles of CaM1 in the presence and absence of CaF_2 NPs. Thermal denaturation profiles of ~12 µM CaM1 in the presence of 1 mM EGTA or Ca^{2+}, with or without incubation with 1.4 mg mL^{-1} CaF_2 NPs. Thermal denaturation was followed by monitoring the ellipticity signal at 208 nm over 20-96 °C.

Figure S2

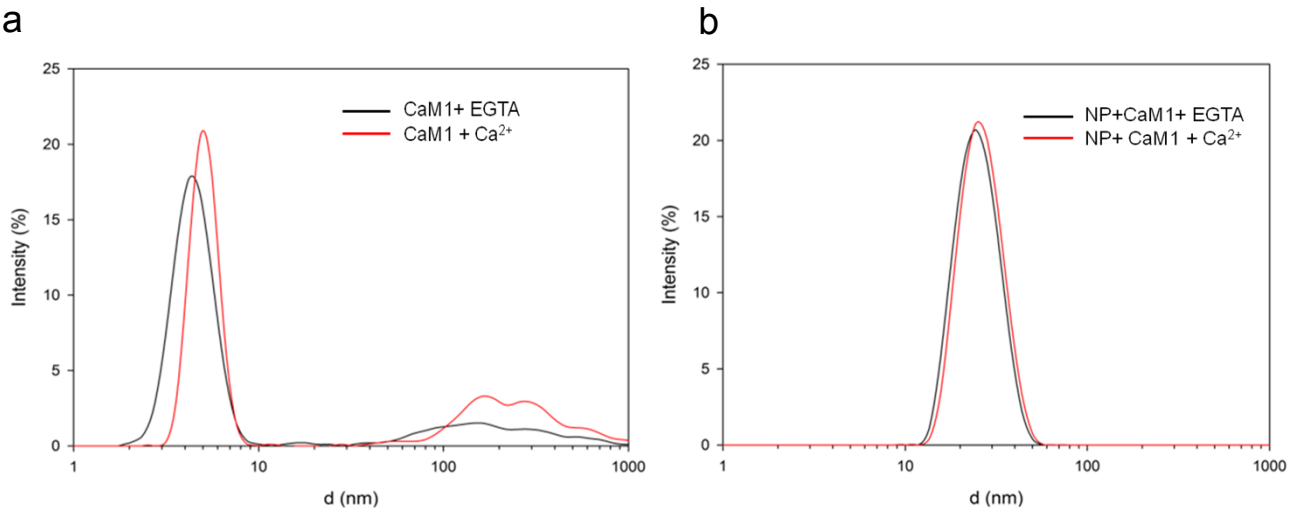

Figure S2. Hydrodynamic diameter measurements of CaM1 upon binding of Ca^{2+} and upon interaction with CaF_2 NPs. All measurements were performed at T= 25 °C in 5 mM Tris-HCl pH 7.5, 150 mM KCl buffer. a) Size distributions of 40 µM CaM1 in the presence of 1 mM EGTA and 1 mM Ca^{2+}. Hydrodynamic diameter of CaM1 in the presence of saturating EGTA is 4.5 ± 0.3 nm while in the presence of saturating Ca^{2+} is 5.1 ± 0.3 nm. b) Size distributions of 60 µM CaM1 incubated with 7 mg

mL^{-1} CaF_2 NPs in the presence of equal amounts of saturating EGTA or Ca^{2+}. Hydrodynamic diameter of NPs + CaM1 mixture is 26.8 ± 0.7 nm in the presence of saturating Ca^{2+} and 25.5 ± 0.5 nm in the presence of saturating EGTA .

Figure S3

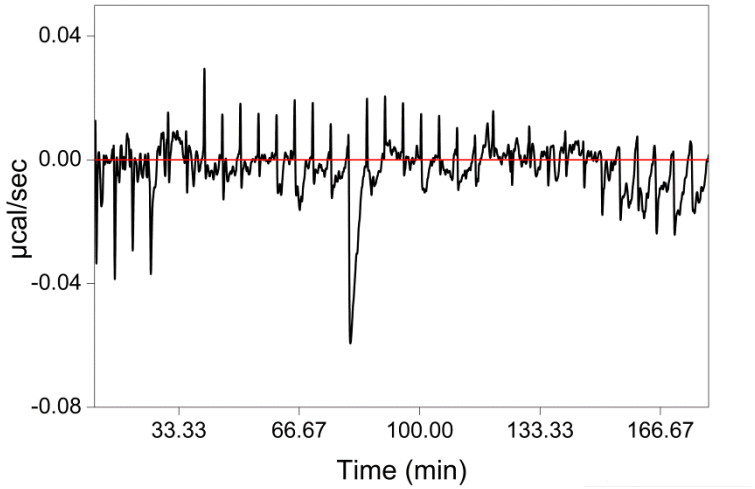

Figure S3. ITC raw data for titration of CaM1 EE105-141QQ in a solution of CaF_2 NPs in 5 mM Tris-HCl, 150 mM KCl, pH 7.5 at 25 °C. The particle concentration in the cell (0.2 mL) was 240 nM, and the protein concentration in the syringe was 190 µM.

Figure S4

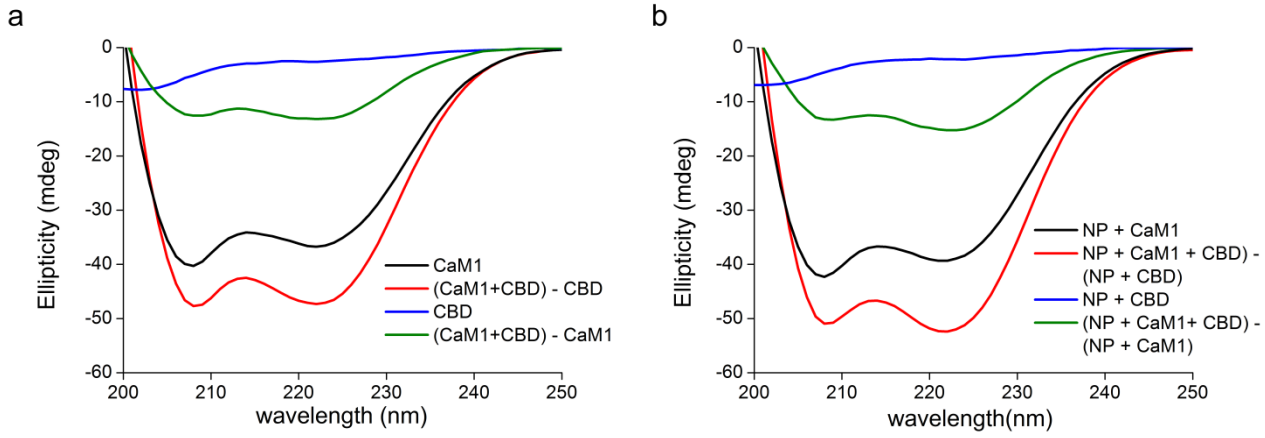

Figure S4. Far-UV CD spectra of CaM1, CBD peptide, and CaM1-CBD complex in the absence of added Ca^{2+} without (a) and with (b) CaF_2 NPs. All measurements were performed at 25 °C in 5 mM Tris-HCl pH 7.5, 150 mM KCl buffer. For the far-UV CD spectra 12 µM CaM1 and 24 µM peptide were used, alone (a) and incubated (b) with 1.4 mg mL^{-1} CaF_2 NPs.

1. Bradford, M. M., A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Anal Biochem* **1976,** *72*, 248-54.