Electronic Supplementary Information

"Nanodevice-induced conformational and functional changes in a prototypical calcium sensor protein"

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Supplementary Methods

Protein expression and purification

Rec was expressed in *E. coli* strain BL21 (DE3) (Novagen) containing plasmids encoding for Rec (pET11a-Rec) and N-myristoyl transferase (yNMT1) from *S. cerevisiae* (pBB131- yNMT1). The cell culture was grown to exponential phase in LB medium containing ampicillin (100 μg/ml) for pET11a-Rec and kanamycin (30 μg/ml) for pBB13- yNMT1 and induction was done with 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) for 3 h at 37 °C. To allow N-myristoylation of Rec, myristic acid (50 μg/ml in 50% ethanol) was added to the culture at an OD_{600} of 0.4. Bacterial cells were harvested by centrifugation and resuspended in 50 mM Tris-HCl pH 8, Protease Inhibitor Cocktail (Sigma), 0.1 mg/ml lysozyme and DNAse (5U/ml). After incubation of the lysate for 30 minutes at 30°C, 1 mM DTT was added. Then, cell debris was removed by centrifugation and the supernatant was charged with 5 mM $CaCl_2$ and loaded onto a Phenyl-Sepharose (GE Healthcare) column equilibrated with 50 mM Tris-HCl pH 7.5, 0.5 mM $CaCl_2$, 1 mM DTT). Rec was eluted with buffer 50 mM Tris-HCl pH 7.5, 2 mM EGTA, 1 mM DTT and the elution fractions were analyzed by SDS-PAGE. Protein concentration was determined by the Bradford assay.[1] Purified proteins were dialyzed against decalcified NH_4HCO_3 buffer, lyophilized and stored at -80° C until use.

Nanoparticle synthesis, structural and spectroscopic characterization

A proper amount of metal chlorides (3.5 mmol) with Ca/Yb/Er=0.78/0.20/0.02 metal molar ratio were dissolved in 25 mL of deionized water containing 20 mmol of potassium citrate. Then 3 mL of NH_4F water solution (3.5M) were added. The resulting clear solution was treated in a Teflon-lined autoclave at 190 °C for 6 hours. NPs were collected by centrifugation at 7000 rpm for 10 minutes and redispersed in water, saline solutions. The colloidal solutions under investigation are stable for several days. As demonstrated by Wang et al.[2] lanthanide ions as doping agents can be used in order to tune the growth and the size of the nanoparticles, as desired.

X-ray powder diffraction (XRPD) pattern was measured using a Thermo ARL X'TRA powder diffractometer, equipped with a Cu anode X-ray source (Kα, λ =1.5418 Å), using a Peltier Si(Li) cooled solid state detector. The patterns was collected with a scan rate of 0.04 °/s in the 5°-90° 2θ range. The phase identifications were performed with the PDF-4+ 2011 database provided by the International Centre for Diffraction Data (ICDD). Polycrystalline samples were ground in a mortar and then put in a low-background sample holder for the data collection.

The upconversion fluorescence measurements of the colloidal water solutions were carried out by excitation with a 1 W diode laser at 980 nm (CNILASER). The emission signal was analyzed by a half-meter monochromator (HR460, Jobin Yvon) equipped with a 1200 lines/mm grating and

detected with a CCD detector (Spectrum One, Jobin Yvon) or with a photomultiplier. The spectral resolution of the emission spectra was 0.1 nm.

The X-ray diffraction pattern and the upconversion emission spectrum of the synthesized CaF_2 NPs is reported in **Figure S3**.

Preparation of liposomes

A mixture of 2 mg of lipids (40% w/w phosphatidylethanolamine (PE), 40% w/w phosphatidylcholine (PC), 15% w/w phosphatidylserine (PS), and 5% w/w cholesterol (Cho), corresponding to the lipid composition in bovine ROS)[3] was dissolved in $CHCl_3$ and dried down by vacuum in a speed-vac concentrator. The sample was resuspended in 1 ml of decalcified and filtered buffer (5 mM Tris-HCl 150 mM KCl pH=7.5), sonicated for 30 min (Labsonic 1510, Braun; 100W), and extruded 20 times through a polycarbonate filter (Whatman, pore diameter= 100 nm).

Supplementary Figures

Figure S1

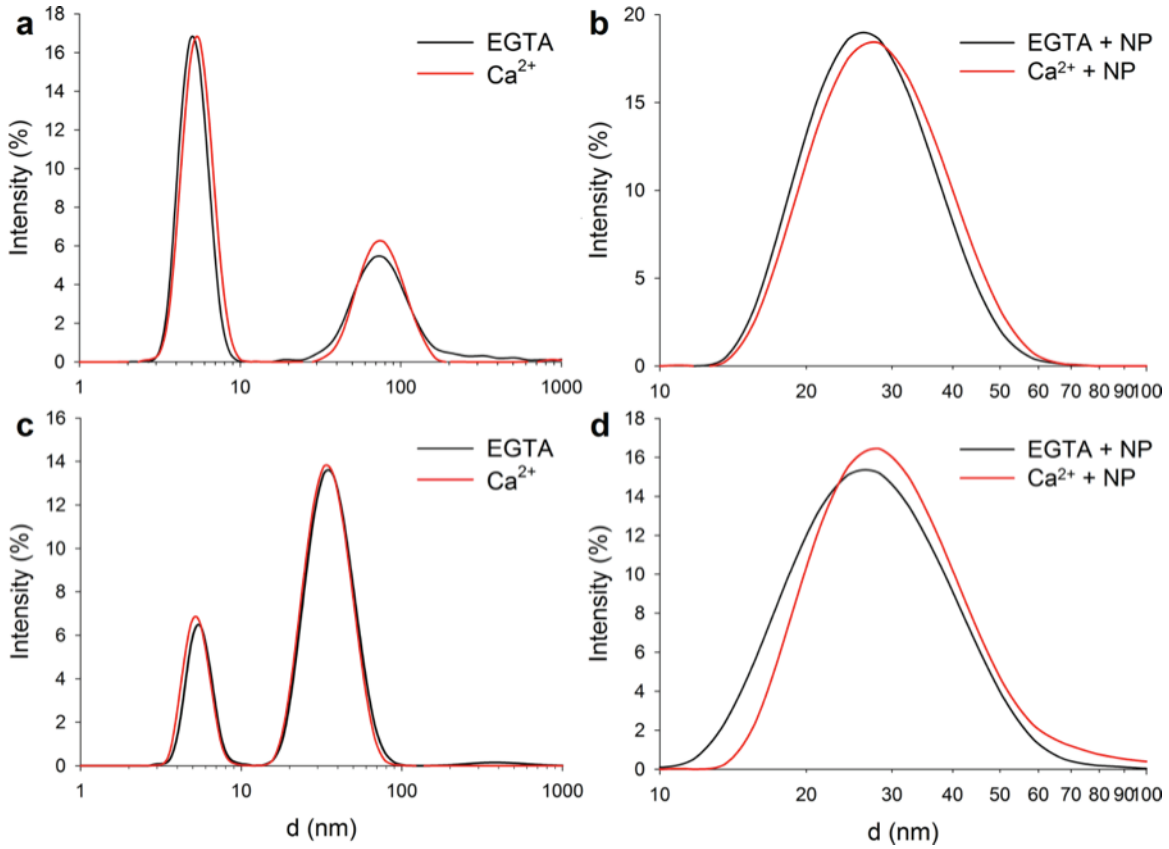

Figure S1. Hydrodynamic diameter measurements of mRec and nmRec upon binding of Ca^{2+} and upon interaction with CaF_2 NPs. All measurements were performed at T= 25°C in 5 mM Tris/HCl pH 7.5, 150 mM KCl buffer. a) Size distributions of 43 μM mRec in the presence of 200 μM EGTA and 1 mM Ca^{2+}. b) Size distributions of 30 μM mRec incubated with 5 mg/mL CaF_2 NPs in the presence of equal amounts (230 μM) of saturating EGTA or Ca^{2+}. c) Size distributions of 29 μM nmRec in the presence of equal amounts (230 μM) of saturating EGTA or Ca^{2+}. d) Size distributions of 29 μM nmRec incubated with 5 mg/mL CaF_2 NPs in the presence of equal amounts (230 μM) of saturating EGTA or Ca^{2+}. Data obtained by DLS measurements reporting the intensity of scattered light as % of the total area. Quantitative size measurements are reported in **Table 2**.

Figure S2

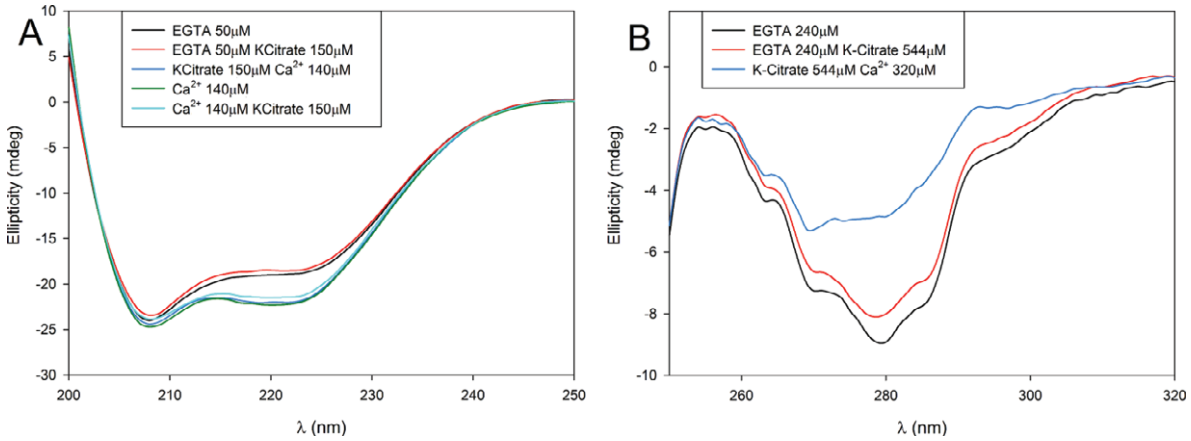

Figure S2. Structural changes occurring in mRec upon binding of Ca^{2+} and upon interaction with K-Citrate. All measurements were performed at T= 25 °C in 5 mM Tris/HCl pH 7.5, 150 mM KCl buffer. A) Far-UV CD spectra of 6 µM mRec in the presence of 50 µM EGTA or 140 µM Ca^{2+} both in the absence and in the presence of 150 µM K-Citrate. B) Near-UV CD spectra of 30 µM mRec in the presence of 240 µM EGTA both in the absence and in the presence of 544 µM K-Citrate, and in the presence of 320 µM Ca^{2+}. The $\theta_{222}/\theta_{208}$ ratio for all are reported in **Table S1**.

Figure S3

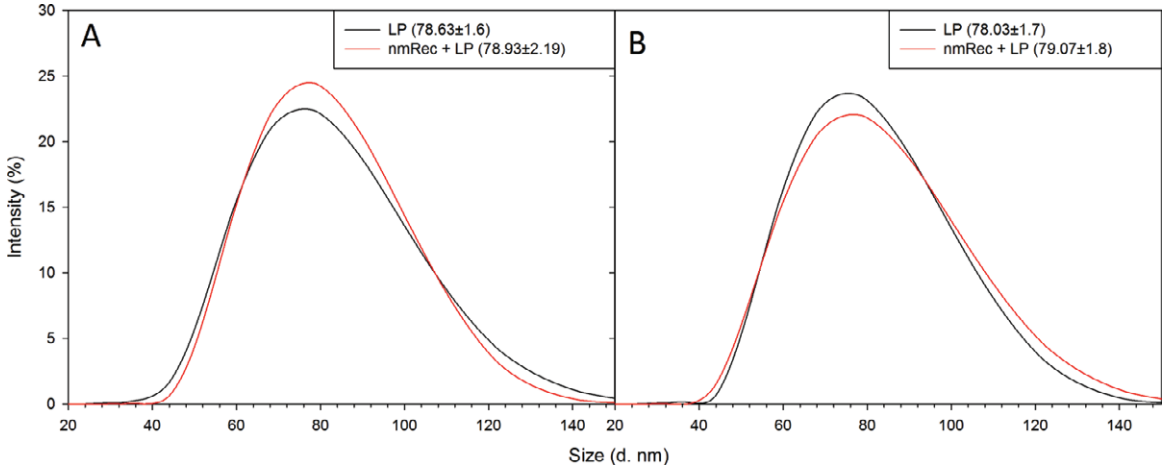

Figure S3. Hydrodynamic diameter measurements of nmRec upon interaction with ROS-like liposomes, in the absence and in the presence of Ca^{2+}. All measurements were performed at T= 25°C in 5 mM Tris/HCl pH 7.5, 150 mM KCl buffer. A) Size distributions of 30 µM nmRec incubated with 20 nM LP in the presence of 240 µM EGTA. B) Size distributions of 30 µM nmRec incubated with 20 nM LP in the presence of 240 µM Ca^{2+}. Data obtained by DLS measurements reporting the intensity of scattered light as % of the total area (**Table 2**)

Figure S4

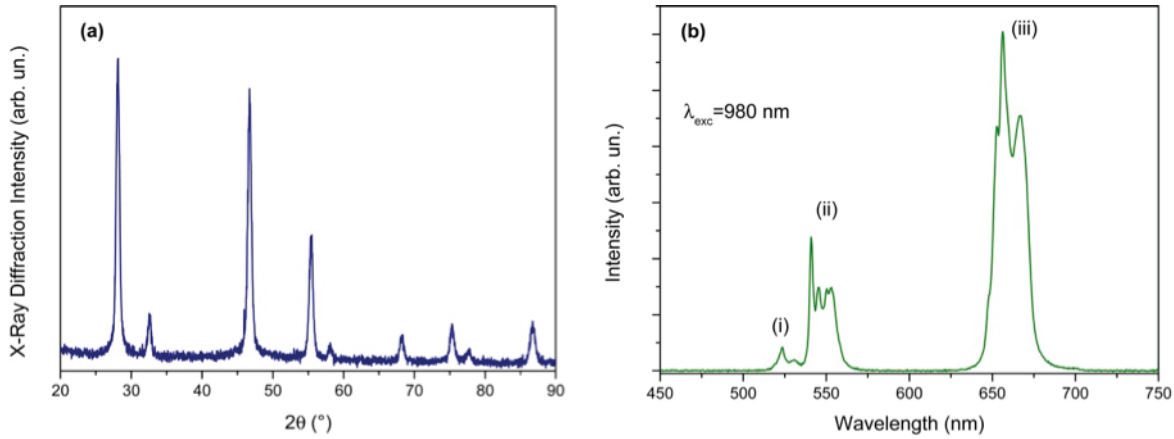

Figure S4: (a) XRD pattern of CaF_2 NPs powder compatible with the presence of fluorite-like crystal structure (single phase); (b) UC emission spectrum (λ_{exc}=980 nm) of colloidal solution (about 1% wt in H_2O) of CaF_2 NPs obtained after heat treatment at 190°C for 6 h and doped with Er^{3+}/Yb^{3+} [Er^{3+} transitions: (i) $^2H_{11/2}\rightarrow{}^4I_{15/2}$; (ii) $^4S_{3/2}\rightarrow{}^4I_{15/2}$; (iii) $^4F_{9/2}\rightarrow{}^4I_{15/2}$].

Figure S5

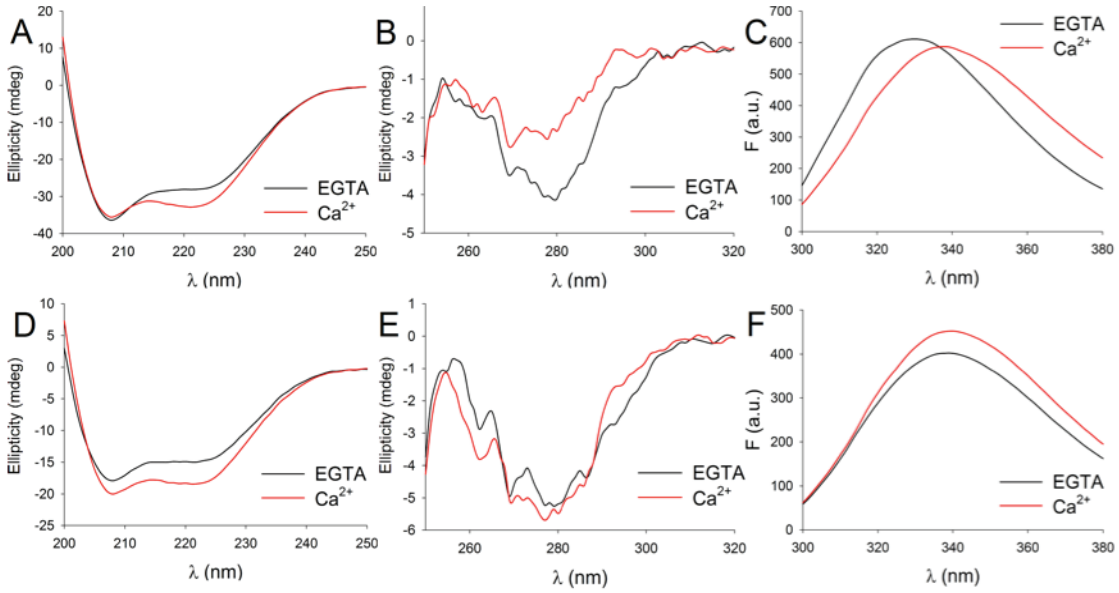

Figure S5. Structural effects of Rec-liposome interactions in the presence and in the absence of Ca^{2+}. All measurements were performed at T= 25 °C in 5 mM Tris/HCl pH 7.5, 150 mM KCl buffer. A) Far-UV CD spectra of 5.8 μM mRec incubated with 4 nM ROS-like liposomes, in the presence of 600 μM EGTA or 800 μM Ca^{2+}. The $\theta_{222}/\theta_{208}$ ratio for both forms is reported in **Table 1**. B) Near-UV CD spectra of 24.1 μM mRec incubated with 20 nM ROS-like liposomes, in the presence of 240 μM EGTA or 630 μM Ca^{2+}. C) Intrinsic Trp fluorescence emission spectrum (λ^{exc}= 290 nm) of 0.28 μM mRec incubated with 0.25 nM ROS-like liposomes, in the presence of 30 μM EGTA or 180 μM Ca^{2+}. Wavelengths of maximal emission are reported in **Table 1**. D) Same as in A), with of 5.4 μM nmRec. E) Same as in B, with 26.7 μM nmRec. F) Same as in C, with 0.29 μM nmRec .

Supplementary Tables

Table S1. $\theta_{222}/\theta_{208}$ ratio of mRec in the presence of K-Citrate

	Θ_{222}	Θ_{208}	$\Theta_{222}\backslash\ \Theta_{208}$
mRec EGTA	-18.9	-24.0	0.79
mRec EGTA + KCitrate	-18.5	-23.4	0.79
mRec EGTA + KCitrate + Ca^{2+}	-22.0	-24.4	0.90
mRec EGTA + Ca^{2+}	-22.2	-24.7	0.90
mRec EGTA + Ca^{2+} + KCitrate	-21.4	-23.9	0.90

Supplementary References

1. Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, *Anal Biochem 72*, 248-254.
2. Wang, F., Han, Y., Lim, C. S., Lu, Y., Wang, J., Xu, J., Chen, H., Zhang, C., Hong, M., and Liu, X. (2010) Simultaneous phase and size control of upconversion nanocrystals through lanthanide doping, *Nature 463*, 1061-1065.
3. Anderson, R. E., and Maude, M. B. (1970) Phospholipids of bovine outer segments, *Biochemistry 9*, 3624-3628.