

Figure 2: Fluorescence emission spectra of CaM34/110-AEDANS/DAB. The doubly labeled CaM mutant, CaM34/110-AEDANS/DAB (1.0 μ M) was mixed with 0.5 mM EGTA (+EGTA), which promotes a rigid, extended conformation, 1 mM CaCl₂ (+Ca²⁺), which promotes a more flexible conformation, 1mM CaCl₂ and 18 μ M helix H (+helix H), or with 1 mM CaCl₂ and 2 μ M CyaA1-412 (+CyaA). In a control experiment using CaM34/110 labeled only with the donor, minimal change in fluorescence emission was observed indicating that the changes in the fluorescent signal reflect changes in the efficiency of energy transfer from the donor (AEDANS) to the acceptor (DAB)⁷.

Methods: The expression and purification of recombinant CaM were performed as described previously¹. To express the adenylyl cyclase domain (amino acids 1-412) of CyaA, pET-CyaA1-412 (kindly provided by Dr. Emanuel Hanski at Hebrew Univ.-Hadassah Medical School) were transformed into *E. coli* B834(DE3) cells harboring pREP4. The resulting cells were cultured at the optimized condition for CyaA expression (19-hour induction at 24 °C with 30 μ M IPTG) and the high-speed supernatant of *E. coli* lysate was prepared without denaturant. Recombinant CyaA1-412 with greater than 95% purity was obtained by fractionation through a Ni-chelating column followed by a Q-sepharose column, concentrated to greater than 10 mg/ml, and stored at -80 °C. The identity of CyaA1-412 was confirmed by its molecular weight (46,216 daltons based on mass spectrometry, 32 daltons higher than its ideal mass) and its CaM-activated adenylyl cyclase activity. The yield was around 12 mg from each liter of *E. coli* culture. To perform the FRET experiment, CaM34/110, a human CaM mutant in which T34 and T110 were both changed to cysteine was randomly labeled with a fluorescence donor, N-iodoacetyl-N'-(5-sulfo-1-naphthyl)ethylenediamine (1,5-IAEDANS) and a non-fluorescence acceptor, 4-dimethylaminophenylazophenyl-4'-maleimide (DAB) and purified by HPLC. The steady-state fluorescence emission of CaM34/110-AEDANS/DAB was recorded as described¹. The dissociation constant between CaM and CyaA and between CaM and the EF helix H peptide was determined by titration of donor-acceptor labeled CaM34/110 with CyaA1-412 protein and EF H peptide, respectively¹.

1. Drum, C. L. et al. An extended conformation of calmodulin induces interactions between the structural domains of adenylyl cyclase from *Bacillus anthracis* to promote catalysis. *J Biol Chem* **275**, 36334-40 (2000).

Figure 3: Effect of YbCl₃ on the adenylyl cyclase activity of EF. The adenylyl cyclase assay was performed under conditions that mimicked the solution in EF-CaM crystals (1 mM MgCl₂, 1:1.25 molar ratio of EF to CaM, and low pH (6.5)). The experiment is done in triplicate. At 0.3 mM YbCl₃ concentration which was used to generate EF-CaM crystal, YbCl₃ had a marginal effect on the activity of EF. At higher than 2.5 mM YbCl₃ concentration, the assay solution formed macroscopic precipitates, resulting in drastically reduced activity.

Supplemental table

Crystallographic and refinement statistics			
A. Crystallographic data			
Crystal	EF-CH ₆ -CaM-3'dATP*	H ₆ -EF	EF-CH ₆ -CaM
Space group	I222	P2 ₁ 2 ₁ 2	I222
a (Å)	117.60	50.47	116.73
b (Å)	167.44	203.60	167.31
c (Å)	343.48	74.03	344.30
X-Ray source	APS, 14-BM-C	APS, 14-BM-C	NSLS X-25
Resolution (Å)	2.75	2.6	2.95
completeness (%) (last shell)	91.6 (57.2)	98.5 (95.4)	98.8 (98.1)
R-sym (last shell)	9.7 (26.4)	8.7 (27.3)	6.1 (26.6)
I/σ (last shell)	4.6 (2.8)	5.6 (1.7)	18.5 (4.1)
Redundancy (last shell)	10.9 (3.3)	6.5 (4.8)	16.0 (5.5)
B. Refinement			
Resolution (Å)	20-2.75	30-2.6	30-2.95
R-factor/free (%)	21.5/28.6	22.8/27.6	27.8/31.5
Bond length (Å)	0.005	0.011	0.011
Bond angle (degree)	1.8	1.55	1.6

* H₆-EF and EF-CH₆ are tagged with hexahistidine at the N- and C-terminal end, respectively.