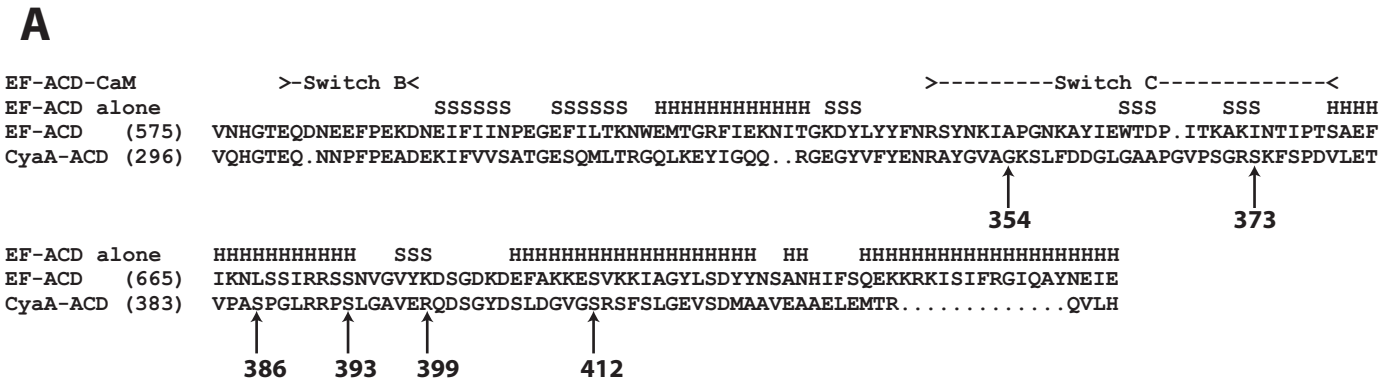
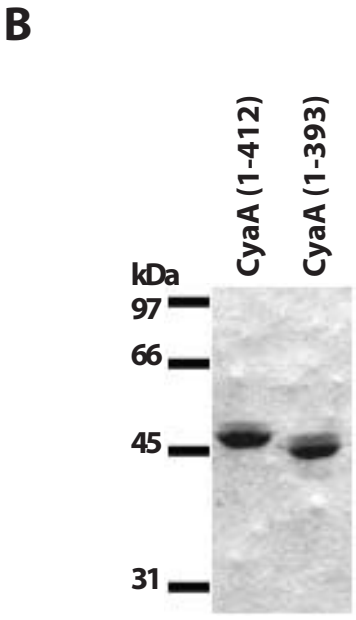
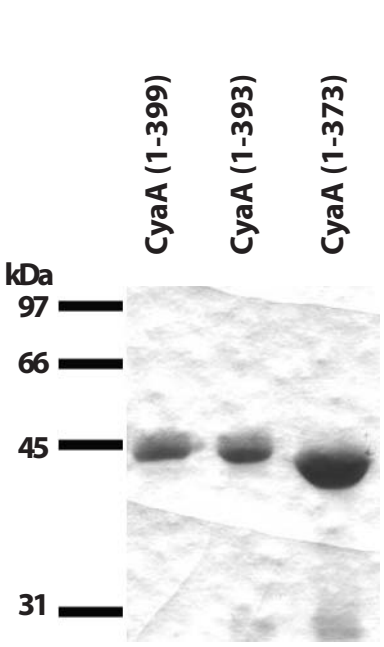
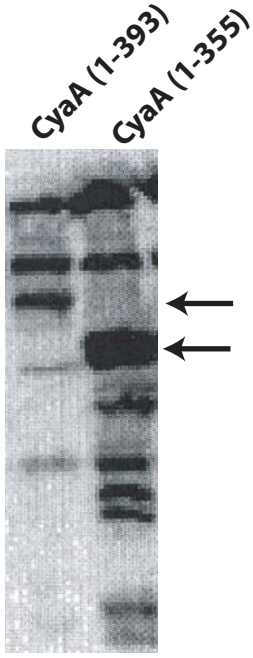
Supplemental Figure 1



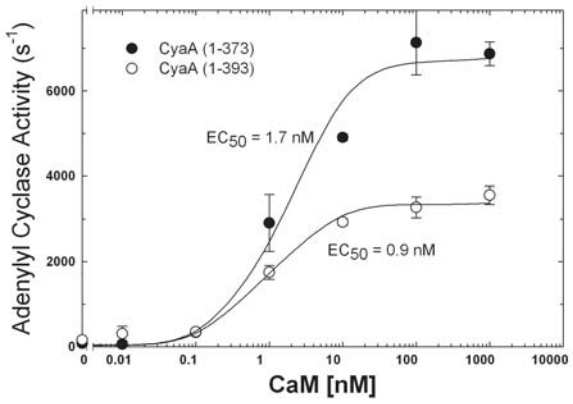






E

CyaA-ACD constructs	Specific Activity (µmol/min/mg)
Purified proteins	
CyaA (1-393)	3,376
CyaA (1-373)	6,779
Lysates	
CyaA (1-412)	26
CyaA (1-399)	31
CyaA (1-393)	69
CyaA (1-355)	0.07



Characterization of CyaA-ACD truncation mutants. (A) Schematic diagram of the CyaA-ACD termination site. A comparison of EF and CyaA-ACD with the secondary structure of EF-ACD is shown on top, and the last residue of the CyaA-ACD mutants is indicated on the bottom. These mutants were designed based on sequence comparison between EF-ACD and CyaA-ACD, and the secondary structure of EF-ACD. Switch B and switch C are defined according to the structures of EF-ACD and CaM-bound EF-ACD-CaM (Drum et al., 2002, *Nature*, 415, 396-402). (B) Purified CyaA (1-412) and CyaA (1-393) proteins on an SDS-PAGE gel. (C) Purified CyaA(1-399), CyaA (1-393) and CyaA (1-373) proteins on an SDS-PAGE gel. Two μg of protein was loaded in both B and C and stained with Coomassie blue. (D) Immunoblot analysis of lysates of CyaA (1-393) and CyaA (1-355). Rabbit polyclonal antibody against CyaA-ACD was used. The expression of CyaA (1-355) is comparable to that of CyaA (1-393). No observable immunoblot or detable adenylyl cyclase activity could be found for CyaA-ACD (1-386) (not shown). (E) Adenylyl cyclase activities of CyaA-ACD mutants. Experiments were performed in the presence of 100 μg *E. coli* lysate or 5 ng purified protein, 10 mM CaM, 5 mM ATP, 10 mM Mg^{2+} and 1mM Ca^{2+}. (F) CaM-dependent adenylyl cyclase acitvities of CyaA (1-393) and CyaA (1-373). Five ng purified protein, 5 mM ATP, 10 mM Mg^{2+}, 1mM Ca^{2+} and indicated CaM were included in the experiments.