Supporting Information

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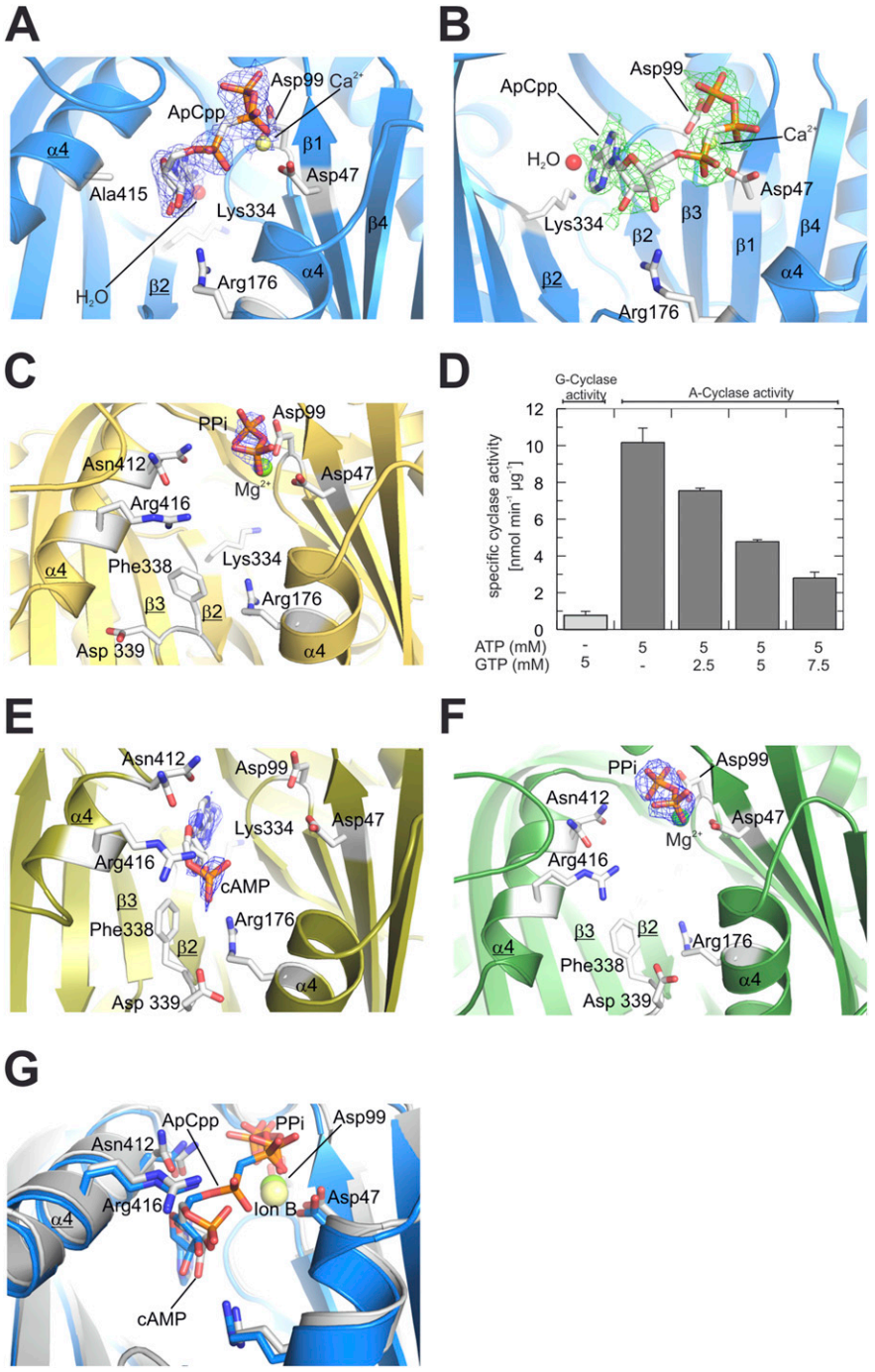

Fig. S1. Crystal structures of soluble adenylyl cyclase (sAC) catalytic domains (sAC-cat) complexes with α,β-methylene–ATP (ApCpp) and products. (*A*) sAC-cat active site with the ATP analog colored according to atom type and overlaid with $2F_o$-F_c density contoured at 1σ. (*B*) sAC-cat active site with the ATP analog and Ca^{2+} colored according to atom type and overlaid with F_o-F_c omit density contoured at 2σ. (*C*) sAC-cat active site after reaction with ATP and Mg^{2+} resulting in pyrophosphate (PP_i) density, with the ligand colored according to atom type and overlaid with $2F_o$-F_c density contoured at 1σ. (*D*) sAC-cat guanyl cyclase (GC) activity (light gray) and its AC activity at a constant ATP concentration in the presence of increasing amounts of GTP (gray). Error bars indicate SD ($n = 2$). (*E*) sAC-cat active site soaked with cAMP, with the ligand colored according to atom type and overlaid with $2F_o$-F_c density contoured at 1σ. (*F*) sAC-cat active site soaked with PP_i, with the ligand colored according to atom type and overlaid with $2F_o$-F_c density contoured at 1σ. (*G*) Overlay of the active sites of sAC-cat soaked with both products cAMP and PP_i (gray) and of the sAC-cat/ApCpp complex (blue), with relevant residues shown in stick presentation.

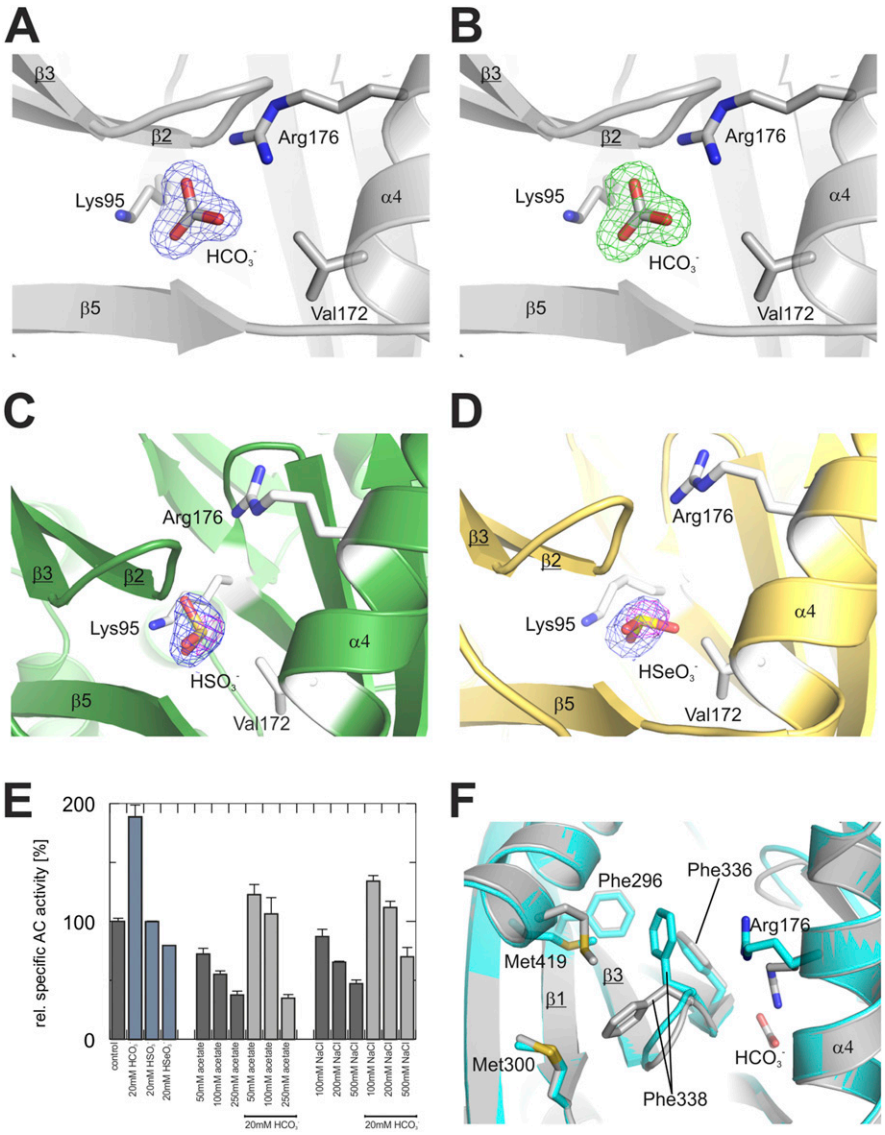

Fig. S2. Crystal structures of apo sAC-cat soaked with bicarbonate (HCO_3^-) and related anions. (*A*) HCO_3^- binding site in sAC-cat apo, with $2F_o$-F_c density for the ligand contoured at 1σ. The closest residues are shown as sticks. (*B*) Regulatory site in sAC-cat soaked with HCO_3^-, with F_o-F_c omit density for the ligand contoured at 3σ. (*C*) $HSeO_3^-$ bound to sAC-cat with $2F_o$-F_c density (blue) for the ligand contoured at 1σ and the anomalous scattering signal (magenta) at 4σ. $HSeO_3^-$ is colored according to atom type. (*D*) HSO_3^- bound to sAC-cat (ligand colored according to atom type), with $2F_o$-F_c density (blue) for the HSO_3^- contoured at 1σ and the anomalous scattering signal (magenta) at 3σ. (*E*) Influence of HCO_3^-, HSO_3^-, and $HSeO_3^-$ on sAC activity. Acetate and chloride ions at high concentrations inhibit basal and stimulated sAC activity. Error bars indicate SD ($n = 2$). rel., relative. (*F*) Overlay of active site regions of apo sAC (cyan) and the sAC/bicarbonate complex (gray) shows the movement of Phe338.

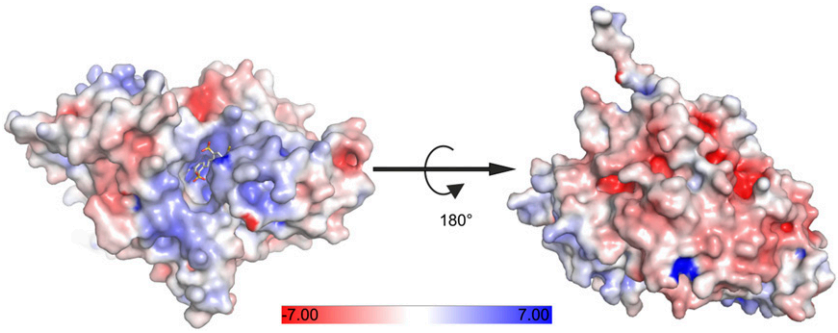

Fig. S3. Electrostatic potential of the sAC surface. Electrostatic potential color-coded on the front (*Left*) and back (*Right*) of the sAC surface shows the negatively charged backside and the positive potential at the active site entrance. The 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS) is shown in sticks and colored according to atom type.

Table S1. Diffraction data, phasing, and refinement statistics

sAC-cat complex	apo	apo, thiomersal soak	ApCpp	apo, bicarbonate soak	apo, biselenite soak
PDB code	4CLF		4CLK	4CLL	4CLY
Space group	$P6_3$	$P6_3$	$P6_3$	$P6_3$	$P6_3$
Unit cell constants	a = b = 99.7 Å, c = 97.9 Å	a = b = 99.7 Å, c = 98.1 Å	a = b = 100.3 Å, c = 97.5 Å	a = b = 99.8 Å, c = 98.1 Å	a = b = 100.2 Å, c = 97.5 Å
Resolution	44.45–1.70 Å	86.57–1.80 Å	48.77–2.20 Å	49.03–1.70 Å	48.79–2.05 Å
Unique reflections*	60,638 (5,058)	101,373 (8,047)†	28,304 (1,852)	60,918 (5,089)	68,658 (13,047)†
$\langle I/\sigma(I)\rangle$	14.9 (1.9)	13.5 (2.5)	10.6 (1.7)	10.8 (2.4)	18.4 (4.2)
Completeness	100% (100%)	100% (100%)	100% (100%)	100% (100%)	100% (100%)
R_{meas}‡	7.4% (94.3%)	7.5% (55.1%)	17.0% (117.9%)	10.8% (69.1%)	9.0% (58.6%)
Heavy atom sites	—	2	—	—	1
BAYES CC	—	31.5%	—	—	—
FOM	—	0.35	—	—	—
Refinement resolution	1.70 Å		2.20 Å	1.70 Å	2.05 Å
Reflections used for refinement	57,557		26,864	57,822	33,106
Protein atoms	3,740		3,662	3,751	3,714
Solvent atoms	286		168	319	263
Ligand atoms	10		53	72	29
rmsd					
Bond lengths	0.019 Å		0.019 Å	0.019 Å	0.019 Å
Bond angles	2.0°		2.0°	1.7°	2.0°
Average B-factor	29.0 $Å^2$		35.0 $Å^2$	23.5 $Å^2$	38.6 $Å^2$
Final R_{cryst}/R_{free}§,¶	16.5%/20.5%		17.6%/23.6%	16.2%/19.8%	15.7%/19.7%

ApCpp, α,β-methylene–ATP; BAYES CC, Bayesian correlation coefficient; FOM, figure of merit; PDB, Protein Data Bank; R_{cryst}, residual factor for diffraction data used in crystallographic refinement; R_{free}, residual factor for diffraction data omitted from crystallographic refinement; R_{meas}, residual factor for individual measurements; sAC-cat, soluble adenylyl cyclase catalytic domains.

*Numbers in parentheses are for the outermost shell.

†Data with anomalous signal, Bijvoet-related reflections not merged.

‡$R_{meas} = \frac{\sum_h \sqrt{\frac{nh}{nh-1}} \sum_i^{nh} |\hat{I}_h - I_{h,i}|}{\sum_h \sum_i^{nh} I_{h,i}}$, with $\hat{I}_h = \frac{1}{nh} \sum_i^{nh} I_{h,i}$.

§R-factor $= \frac{\sum ||F_{obs}| - k|F_{calc}||}{\sum |F_{obs}|}$; $|F_{obs}|$ is the observed and $|F_{calc}|$ the calculated structure factor amplitude.

¶R_{free} was calculated from 5% of measured reflections omitted from refinement.

Table S2. Diffraction data and refinement statistics

sAC-cat complex	ApCpp, bisulfite soak	apo, bisulfite soak	apo, ATP/Mg^{2+} soak	apo, cAMP soak	apo, $cAMP/PP_i$ soak
PDB code	4CLW	4CM2	4CLU	4CLP	4CLT
Space group	$P6_3$	$P6_3$	$P6_3$	$P6_3$	$P6_3$
Unit cell constants	a = b = 100.6 Å, c = 97.2 Å	a = b = 100.2 Å, c = 97.7 Å	a = b = 100.7 Å, c = 97.3 Å	a = b = 100.6 Å, c = 97.1 Å	a = b = 99.8 Å, c = 98.1 Å
Resolution	87.10–2.15 Å	86.73–1.80 Å	87.21–1.90 Å	87.11–1.90 Å	87.44–1.95 Å
Unique reflections*	58,807 (3,829)†	101,606 (7,993)†	44,046 (3,269)	43,918 (3,242)	40,997 (2,970)
<I/σ(I)>	20.3 (4.8)	19.7 (2.5)	13.4 (2.5)	13.5 (2.0)	11.3 (2.1)
Completeness	98% (97%)	100% (99%)	100% (100%)	100% (100%)	100% (100%)
R_{meas}‡	7.2% (50.8%)	7.8% (75.5%)	9.0% (75.7%)	9.6% (89.8%)	10.3% (72.2%)
Refinement resolution	2.15 Å	1.80 Å	1.90 Å	1.90 Å	1.95 Å
Reflections used for refinement	28,395	48,894	41,832	41,710	38,932
Protein atoms	3,638	3,757	3,684	3,678	3,673
Solvent atoms	147	244	202	209	106
Ligand atoms	66	31	39	92	60
rmsd					
Bond lengths	0.019 Å	0.019 Å	0.019 Å	0.019 Å	0.019 Å
Bond angles	2.0°	2.0°	2.0°	2.0°	2.0°
Average B-factor	48.4 $Å^2$	30.0 $Å^2$	34.6 $Å^2$	35.6 $Å^2$	31.8 $Å^2$
Final R_{cryst}/R_{free}§,¶	16.2%/20.3%	16.2%/20.5%	16.0%/20.5%	16.1%/20.7%	17.3%/22.0%

PP_i, pyrophosphate.

*Numbers in parentheses are for the outermost shell.

†Data with anomalous signal, Bijvoet-related reflections not merged.

‡$R_{meas} = \frac{\sum_h \sqrt{\frac{nh}{nh-1}} \sum_i^{nh} \left| \hat{I}_h - I_{h,i} \right|}{\sum_h \sum_i^{nh} I_{h,i}}$, with $\hat{I}_h = \frac{1}{nh} \sum_i^{nh} I_{h,i}$.

§R-factor $= \frac{\sum ||F_{obs}| - k|F_{calc}||}{\sum |F_{obs}|}$; $|F_{obs}|$ is the observed and $|F_{calc}|$ the calculated structure factor amplitude.

¶R_{free} was calculated from 5% of measured reflections omitted from refinement.

Table S3. Diffraction data and refinement statistics

sAC-cat complex	apo, DIDS	apo, PP_i soak	ApCpp, bicarbonate soak
PDB code	4CLZ	4CLS	4CM0
Space group	$P6_3$	$P6_3$	$P6_3$
Unit cell constants	a = b = 100.5 Å, c = 97.4 Å	a = b = 100.9 Å, c = 97.6 Å	a = b = 100.2 Å, c = 95.3 Å
Resolution	87.04–1.90 Å	86.68–1.85 Å	88.50–3.20 Å
Unique reflections*	85,010 (6,250)[†]	47,385 (3,628)	9,419 (431)
<I/σ(I)>	13.6 (2.4)	9.0 (1.58)	17.0 (2.7)
Completeness	100% (100%)	100% (100%)	100% (100%)
R_{meas}[‡]	8.8% (73.8%)	10.4% (88.7%)	12.1% (87.2%)
Refinement resolution	1.90 Å	1.85 Å	3.20 Å
Reflections used for refinement	41,806	45,014	8,952
Protein atoms	3,692	3,665	3,613
Solvent atoms	283	162	—
Ligand atoms	141	29	33
rmsd			
Bond lengths	0.019 Å	0.019 Å	0.019 Å
Bond angles	2.0°	2.0°	2.0°
Average B-factor	32.8 $Å^2$	35.6 $Å^2$	61.7 $Å^2$[‖]
Final R_{cryst}/R_{free}[§,¶]	16.1%/20.0%	17.4%/21.4%	25.0%/29.0%

DIDS, 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid.

*Numbers in parentheses are for the outermost shell.

[†]Data with anomalous signal, Bijvoet-related reflections not merged.

[‡]$R_{meas} = \frac{\sum_h \sqrt{\frac{nh}{nh-1}} \sum_i^{nh} |\hat{I}_h - I_{h,i}|}{\sum_h \sum_i^{nh} I_{h,i}}$, with $\hat{I}_h = \frac{1}{nh} \sum_i^{nh} I_{h,i}$.

[§]R-factor $= \frac{\sum ||F_{obs}| - k|F_{calc}||}{\sum |F_{obs}|}$; $|F_{obs}|$ is the observed and $|F_{calc}|$ the calculated structure factor amplitude.

[¶]R_{free} was calculated from 5% of measured reflections omitted from refinement.

[‖]Refinement with overall temperature factor.