Supporting Information

Liu *et al*. 10.1073/pnas.0708850105

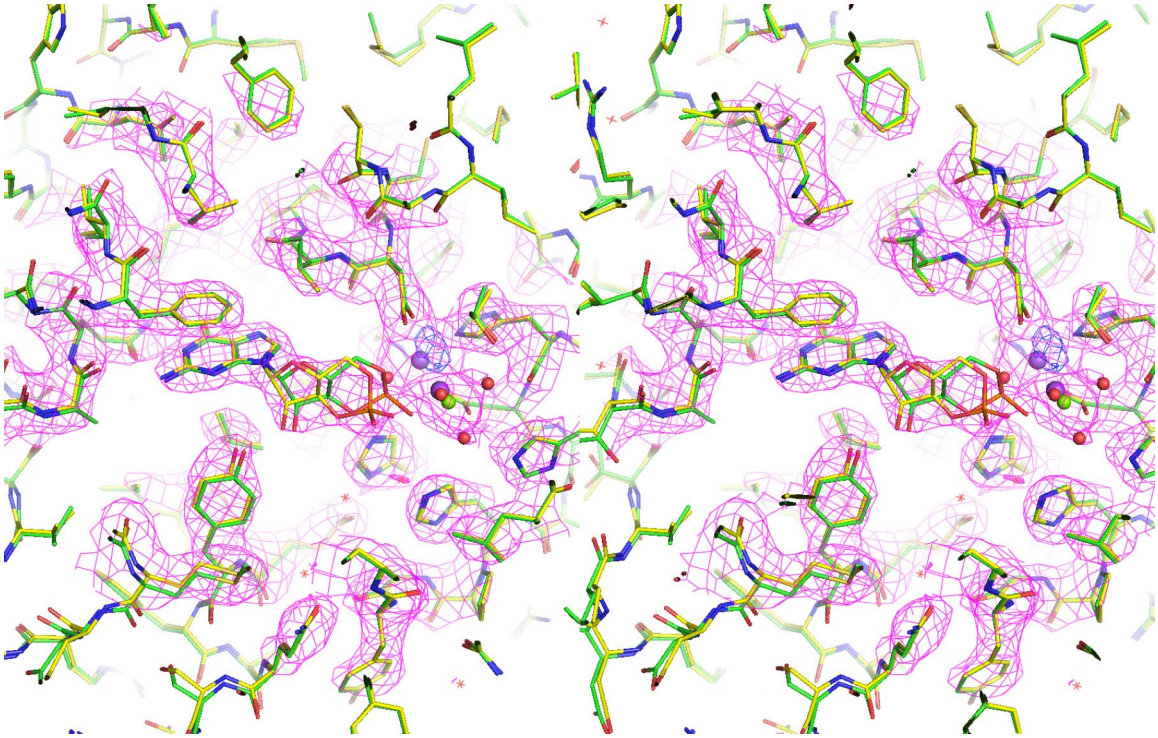

Fig. S1. Crystalline PDE9 was unable to completely hydrolyze cGMP in the absence of divalent cations in 2 days (1mM cGMP). The strong positive $F_{o,original} - F_{o,cGMP+no\ ion}$ electron density (blue, 10 σ) indicates significant exchange of the original divalent cations (green) by Na^+ (blue purple). The final refined $E_{Na,Na}S$ complex model (yellow carbons) are embedded in the initial 2Fo-Fc maps calculated before ligands and water molecules were built (pink, 1.2 σ), and was superimposed with the *ES* complex model discussed in Fig. 1*C* (green carbons).

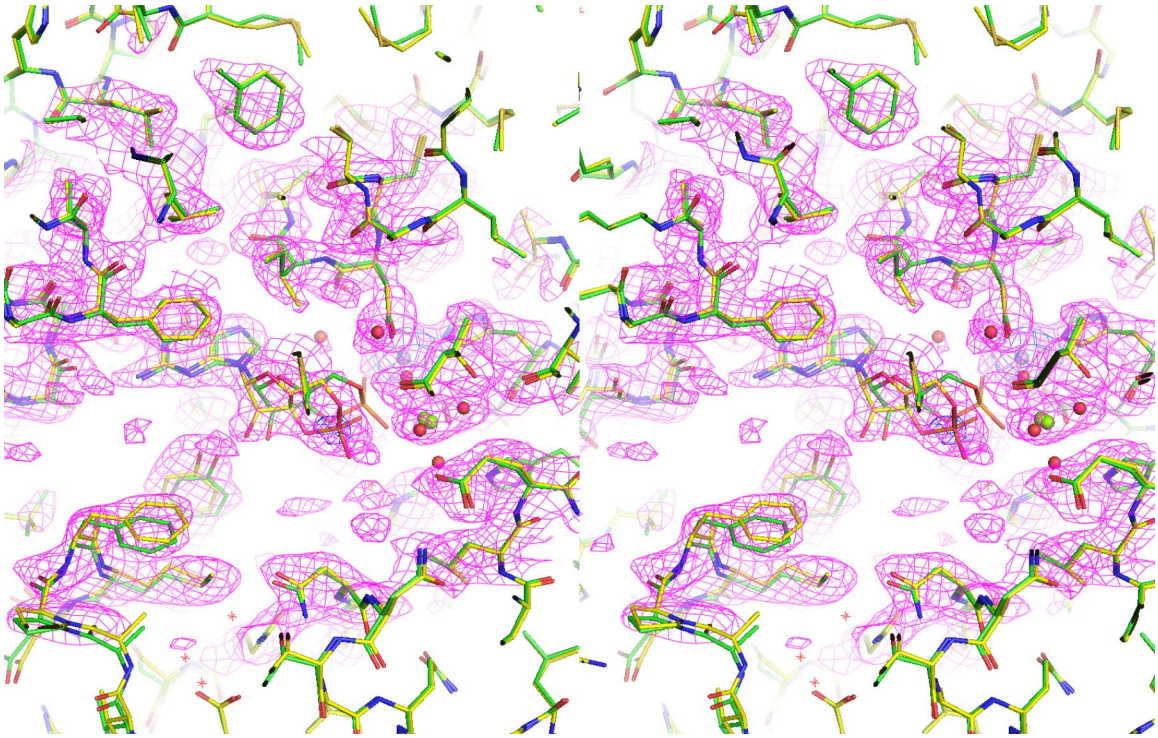

Fig. S2. Crystalline PDE9 was unable to completely hydrolyze cGMP in the presence of Zn^{2+} in 2 days (1mM cGMP + 2mM $ZnCl_2$). The strong positive $F_o - F_{o,cGMP+no\ ion}$ electron density (blue, 10 σ) indicates the full occupancies of Zn^{2+}, as well as the precise location of the phosphorus. The final refined $E_{Zn,Zn}S$ complex model (yellow carbons) are embedded in the initial 2Fo-Fc map calculated before ligands and water molecules were built (pink, 1.2 σ), and was superimposed with the *ES* complex model discussed in Fig. 1*C* (green carbons).

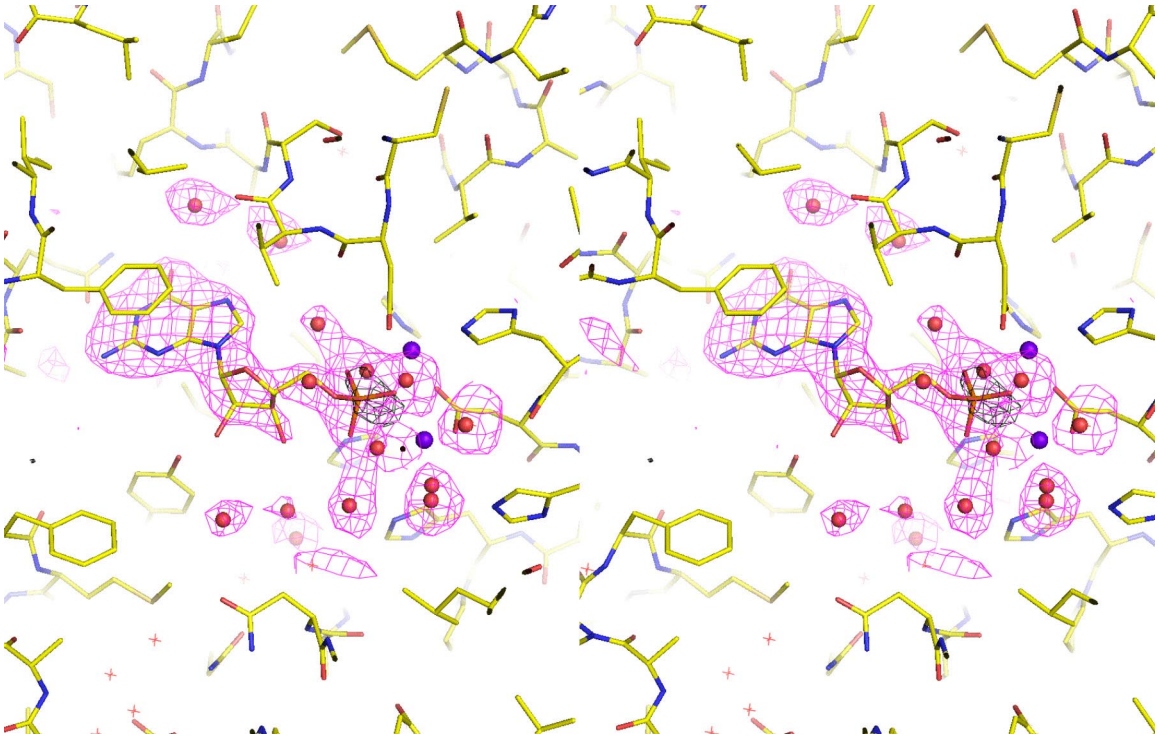

Fig. S3. The final refined model of *E*+*P* complex (sticks model, yellow carbon) embedded in the initial Fo-Fc electron density (2σ) before ligand and waters were included. The ribose and phosphates in the *E*+*P* complexes, if kept full occupancies during refinements, would have average B factors 40% higher than those of the bases, and would result in strong negative Fo-Fc electron densities around the phosphate (−4σ, black). In the final *E*+*P* model, the hydrolysis center in the *E*+*P* complex was modeled 50% occupied by the partially ordered ribose and phosphate and 50% unliganded. With this model, no strong difference electron densities were observed.

Table S1. Ligands used in soaking, X-ray data collection, and refinement statistics

	Complex						
	ES	*EP*	$E_{Na,Na}S$	$E_{Zn,Zn}S$	*E*+*P*	*E*+*P*	*E*+*P*
Ligand	20 mM cGMP	20 mM cGMP	1 mM cGMP	1 mM cGMP	1 mM cGMP	1 mM cGMP	20 mM GMP
Divalent metal ion	2 mM $MnCl_2$	$MnCl_2$+ $MgCl_2$	None	0.2–2 mM $ZnCl_2$	200 μM $MgCl_2$	500 μM $MnCl_2$	20 mM $MgCl_2$
Soaking time	1 min	Brief (2 h)	2 days	2 days	2 days	2 days	2 days
Resolution (Å)	2.7	2.3	2.3	2.3	2.4	2.15	2.4
R merge (%)	9.6 (59)	7.7 (43)	8.5 (68)	9.0 (54)	10.4 (59)	5.8 (46)	10.4 (36)
I/σ	15 (2.0)	18.9 (3.7)	23 (2.5)	19 (2.7)	28 (3.0)	29 (3.3)	19 (2.2)
Completeness (%)	99.9(100)	99.4(100)	98.7(94.7)	99.8(100)	99.8(100)	96.8(97.2)	91(55)
Redundancy	6.2 (6.3)	4.0 (4.0)	7.0 (6.5)	6.1 (6.1)	6.9 (5.5)	4.6 (4.6)	7.9 (5.1)
R factor (%)	19.1 (32)	18.8 (23)	18.5 (28)	18.1 (28)	18.4 (30)	17.5 (23)	17.3 (28)
R free (%)	22.0 (33)	21.2 (28)	20.5 (34)	20.5 (30)	21.1 (30)	19.8 (25)	22 (35)
Bond length deviation (Å)	0.023	0.012	0.013	0.013	0.017	0.016	0.022
Bond angle deviation (°)	1.9	1.2	1.3	1.2	1.4	1.4	2.0
Ligand in monomer A	cGMP	GMP	cGMP	cGMP	GMP	GMP	GMP
Ligand in monomer B	IBMX	IBMX	IBMX	IBMX/ nucleotides	IBMX	IBMX	IBMX/GMP

The *EP* complex experiments were repeated with 20 mM cGMP + 20 mM $MnCl_2$ + 20 mM $MgCl_2$ for a few seconds, 1 min, 1 h, or 2 h or with 2 mM cGMP + 2 mM $MnCl_2$ for 2 min. The $E_{Zn,Zn}S$ complex experiment was done with 0.2 or 2 mM $ZnCl_2$. The *E*+*P* complex experiments with $MgCl_2$ or $MnCl_2$ were repeated twice. Only the highest resolution statistics of the same experiments are included in the table.

Other Supporting Information Files SI Appendix

SI Appendix