Supplemental Information

Fluorescence modeling and global fitting was done by solving the differential equations for the mechanism in Equation 9 using the scientist package. The rate constants and assumptions regarding Mg^{2+} dependence of the fluorescence enhancement are summarized in (Table SI). We initially modeled the data (Figure SIA) using the following assumptions: each step of the reaction subsequent to substrate binding is irreversible, each intermediate reversibly binds Mg^{2+}; the rate of binding and subsequent steps are zero in the absence of Mg^{2+} and the FRET signal of the enzyme-substrate intermediates are the same in the presence and absence of Mg^{2+}. The data time and Mg^{2+} dependence of the fluorescence signal in (Figure 3a) are very different than the simulated data in (Figure SIA). The fit was not improved by assuming that the fluorescence enhancement only occurred with Mg^{2+} bound to enzyme substrate complex (not shown). We subsequently relaxed the kinetic model to allow the individual steps of the mechanism to be reversible. This model produced a FRET signal, shown in (Figure S1B), which had time and Mg^{2+} dependence very similar to those observed experimentally. All of the steps of the reaction depend upon Mg^{2+} and the amplitudes of each of the steps are similar to those in (Figure 3a). The major differences in the rate constants in models A and B are that in model B the conversion between the first and second enzyme substrate constant is reversible with an equilibrium constant near one, the rate constant for the rate constant between the second and third substrate intermediates is increased from 0.25 to 0.4 s^{-1} and the equilibrium of the final slow step of the reaction is also near one. We also tested a model using the same rate constants but in which FRET requires bound Mg^{2+} (Figure SIC). In this case the overall amplitudes of the signal were strongly dependent upon Mg^{2+} concentration, which was not observed experimentally. We also simulated a series of models in which only the initial enzyme substrate complex (Figure SID), the first and second enzyme substrate complexes (Figure SIE) or all three enzyme substrate complexes (Figure SIE) reversibly bound Mg^{2+} and the fluorescence enhancement is the same in the presence and absence of Mg^{2+}. The time dependence of the fluorescence in SID is flat for the first 100 ms at the lowest Mg^{2+} concentration and the total signal amplitude has a larger Mg^{2+} dependence than the experimental data. Figures SIE and SIF provide better simulations of the data but SIE lacks the Mg^{2+} dependence of the rates of steps 3-5 and SIF does a relatively poor job of modeling the slower parts of the time course. The simulation in SIB best models the data. Model B may not be a unique fit to the kinetic data but it provides a good fit which is consistent with the observed data. The rate constants of all of the steps are dependent upon Mg^{2+} whereas the amplitudes show relatively little dependence upon Mg^{2+} concentration.

Summary Of Parameters Used In Simulations Of Pde5 Mant-Cgmp Kinetic Models

Step(i)		Model in Figure SI						K_{iMg}[a]
		A[b]	B[b]	C[b]	D[b]	E[b]	F[b]	
1	$k_1(\mu M^{-1}s^{-1})$	20$^{(Mg)}$	20$^{(Mg)}$	20$^{(Mg)}$	20$^{(Mg)}$	20$^{(Mg)}$	20$^{(Mg)}$	8
	$K_{-1}(s^{-1})$	310$^{(Mg,+)}$	310$^{(Mg)}$	310$^{(Mg,-)}$	310$^{(-)}$	310$^{(Mg)}$	310$^{(Mg)}$	
2	$K_2(s^{-1})$	25 $^{(Mg,+)}$	15 $^{(Mg,+)}$	15$^{(Mg,-)}$	15$^{(-)}$	15$^{(Mg,+)}$	15$^{(Mg,+)}$	7
	$K_{-2}(s^{-1})$	0	10$^{(Mg,+)}$	10$^{(Mg,-)}$	10$^{(-)}$	10$^{(-)}$	10$^{(Mg,+)}$	
3	$K_3(s^{-1})$	0.24$^{(Mg,+)}$	0.4$^{(Mg,+)}$	0.4$^{(Mg,-)}$	0.4$^{(-)}$	0.4$^{(-)}$	0.4$^{(Mg,+)}$	5
	$K_{-3}(s^{-1})$	0	.02$^{(Mg,+)}$	.02$^{(Mg,-)}$	.02$^{(-)}$	.02$^{(-)}$	.02$^{(-)}$	
4	$K_4(s^{-1})$	.03$^{(Mg,+)}$	.03$^{(Mg,+)}$	.03$^{(Mg,-)}$	.03$^{(-)}$	.03$^{(-)}$	.03$^{(-)}$	10
	$K_{-4}(s^{-1})$	0	.0001$^{(Mg,+)}$	.0001$^{(Mg,-)}$	.0001$^{(-)}$	.0001$^{(-)}$	.0001$^{(-)}$	
5	$K_5(s^{-1})$	0.007$^{(Mg,+)}$	.003$^{(Mg,+)}$	.003$^{(Mg,-)}$	.003$^{(-)}$	.003$^{(-)}$	.003$^{(-)}$	3
	$K_{-5}(s^{-1})$	0	.003$^{(Mg,+)}$	.003$^{(Mg,-)}$	.003$^{(-)}$	.003$^{(-)}$	.003$^{(-)}$	

[a]Dissociation constant for Mg^{2+} from the indicated intermediate. K

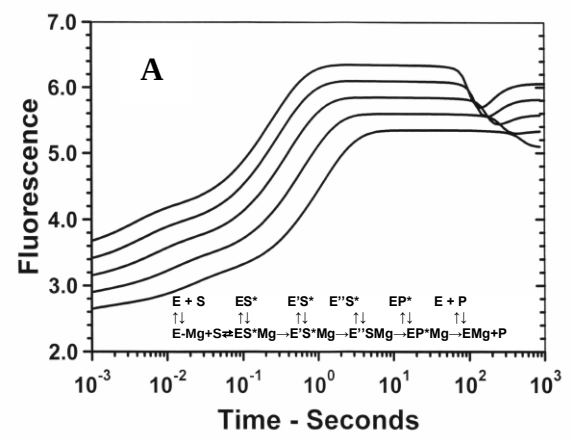
[b]Rate constants used in simulations B-D were obtained by global fitting in which all steps were allowed to be reversible. Rate constants used in simulation A used the rate constants from Table 1 for steps 2-5 and the same rates constants for substrate binding as used in B-D.

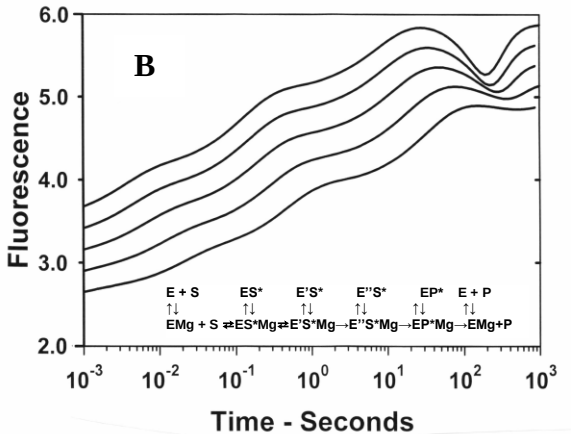
$^{(Mg)}$Denotes reaction steps in which Mg^{2+} rapidly binds and dissociates with the dissociation constant indicated in the right had column.

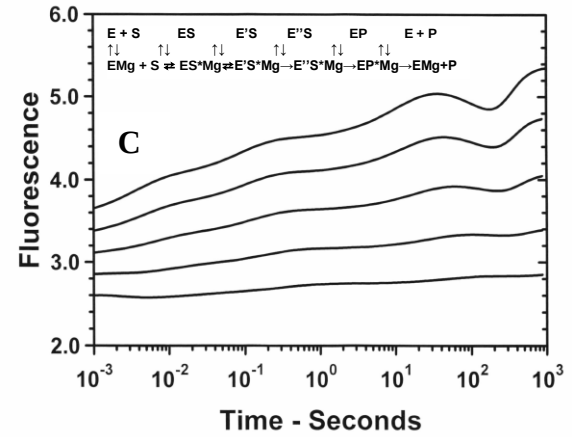
$^{(+)}$Intermediates in which the enzyme-nucleotide FRET signal occurs equally in the presence and absence of Mg^{2+}.

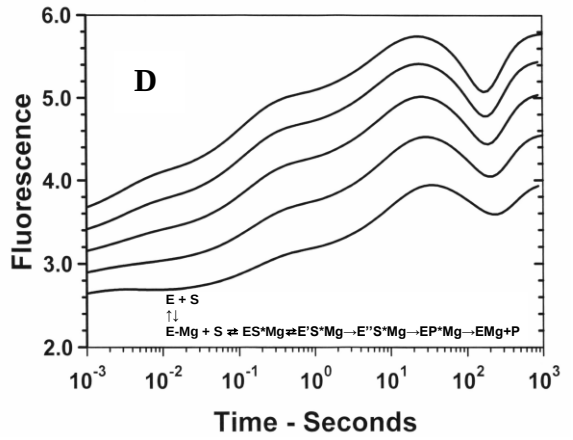
$^{(-)}$Intermediates in which the enzyme-nucleotide FRET signal requires Mg^{2+} binding.

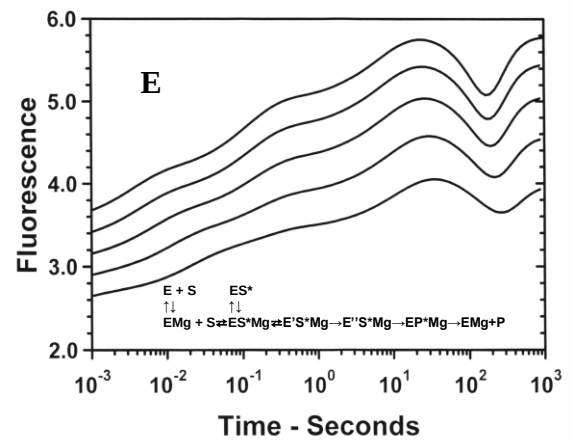
SI Figure

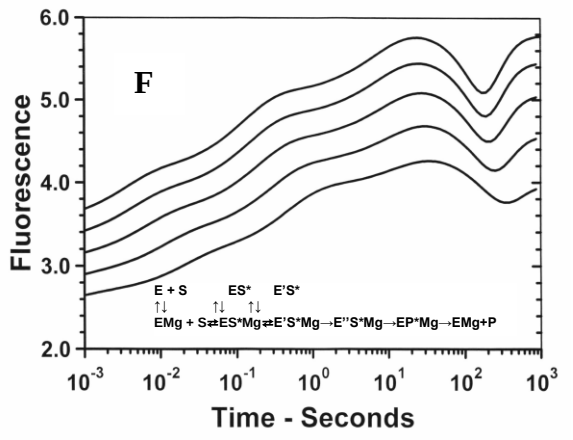

A
Fluorescence
Time - Seconds
E + S ES* E'S* E''S* EP* E + P
E-Mg+S⇄ES*Mg→E'S*Mg→E''SMg→EP*Mg→EMg+P


B
Fluorescence
Time - Seconds
E + S ES* E'S* E''S* EP* E + P
EMg + S ⇄ES*Mg⇄E'S*Mg→E''S*Mg→EP*Mg→EMg+P


C
Fluorescence
Time - Seconds
E + S ES E'S E''S EP E + P
EMg + S ⇄ ES*Mg⇄E'S*Mg→E''S*Mg→EP*Mg→EMg+P


D
Fluorescence
Time - Seconds
E + S
E-Mg + S ⇄ ES*Mg⇄E'S*Mg→E''S*Mg→EP*Mg→EMg+P


E
Fluorescence
Time - Seconds
E + S ES*
EMg + S⇄ES*Mg⇄E'S*Mg→E''S*Mg→EP*Mg→EMg+P


F
Fluorescence
Time - Seconds
E + S ES* E'S*
EMg + S⇄ES*Mg⇄E'S*Mg→E''S*Mg→EP*Mg→EMg+P