Supplementary Information for:

One and Two Metal Ion Catalysis: Global Single Turnover Kinetic Analysis of PvuII Endonuclease Mechanism†

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Table S1. Measured Dissociation Rate Constants for DNA Binding to PvuII Endonuclease.[a]

Metal Ion/Concentration	Rate constant (s^{-1})	Technique
Metal free	3.4e-3	Fluorescence anisotropy
10 mM Ca(II)	3.9e-4	Fluorescence anisotropy
0.3 mM Ca(II)	1.13e-3	Fluorescence anisotropy
1 mM Mg(II)	1.8e-3	DNA cleavage trap
0.3 mM Mg(II)	4.0e-4	DNA cleavage trap

[a]Data taken from Ref. (*1*).

Table S2. Dissociation Rate Constants (k_{off}) for Metal Ion Binding.

Protein	Metal ion	k_{off} (s^{-1})
Calmodulin[a]	Mg(II)	2700, 6600
Phospholipase A2[b]	Ca(II)	1000
proPLA2[b]	Ca(II)	3000
Troponin C[c]	Ca(II)	600
Troponin C[d]	Mg(II)	>230
Parvalbumin[e]	Ca(II)	>3e5

[a]Ref. (*2*). [b](*3, 4*). [c](*5*). [d](*6*). [e](*7*)

Table S3. Summary of Kinetic Parameters from Single Turnover Studies[a]

Parameter	Value	Source
k_0	3.3e3 $M^{-1}s^{-1}$	(*1*)
k_{-0}	0.001 s^{-1}	(*1*)
K_0	300 nM	(*1*)
k_1	4.7e7 $M^{-2}s^{-1}$	This study
k_{-1}	1000 s^{-1}	Table S2, this study
K_1	4.6 mM	This study
k_2	3.7e4 $M^{-1}s^{-1}$	This study
k_{-2}	0.001 s^{-1}	(*1*)
K_2	27 nM	This study
k_3	1.1e8 $M^{-2}s^{-1}$	This study
k_{-3}	1000 s^{-1}	Table S2, this study
K_3	3.1 mM	This study
k_3'	7.7e7 $M^{-2}s^{-1}$	This study
k_{-3}'	190 s^{-1}	This study
K_3'	1.6 mM	This study
k_4	2e5 $M^{-1}s^{-1}$	This study
k_{-4}	0.001 s^{-1}	(*1*)
K_4	5 nM	This study
k_5	1.1 s^{-1}	This study
k_6	0.011 s^{-1}	This study

[a]See **Fig. 6** and **Fig. 8** for definitions of parameters.

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