SUPPORTING INFORMATION

Location of Inhibitor Binding Sites in the Human Inducible Prostaglandin E Synthase, MPGES1

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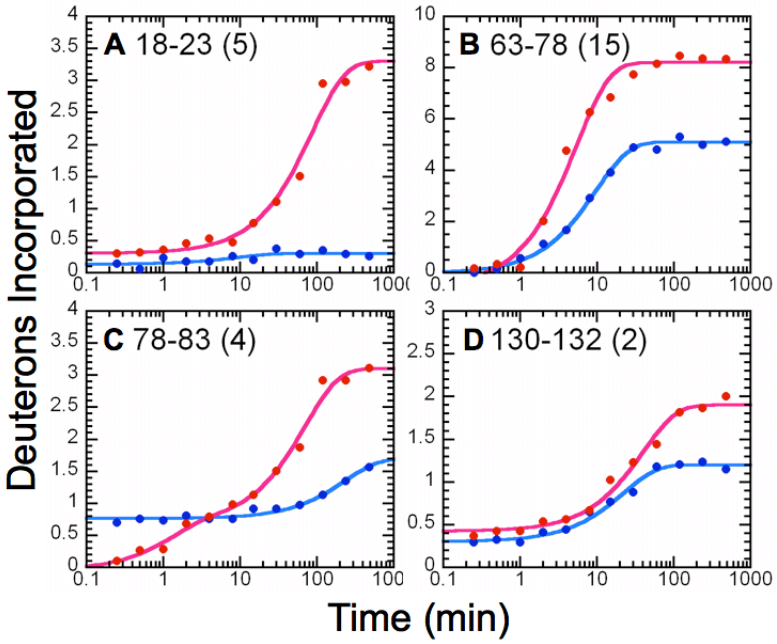
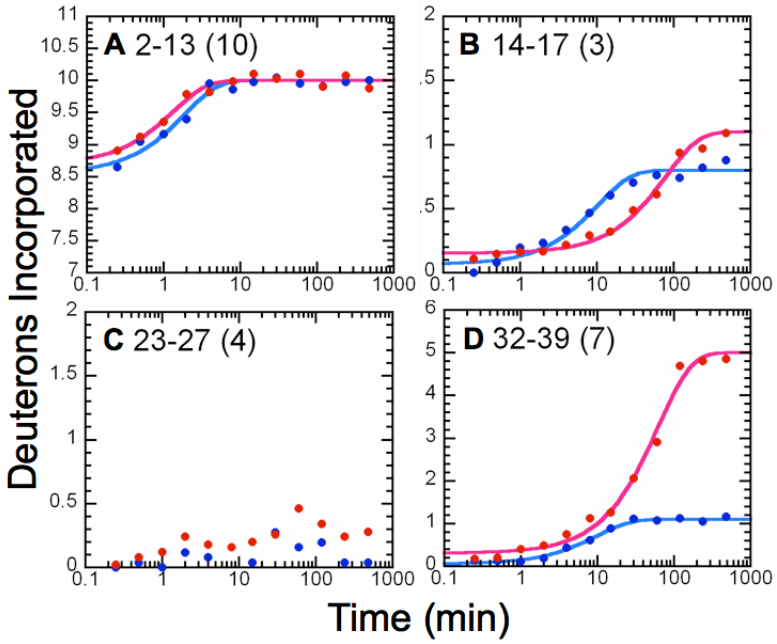
Figure S1. Peptic peptide map of MPGES1. White bars, with corresponding residue numbers, represent peptides analyzed for deuterium incorporation. Gray bars indicate the transmembrane helices, as determined by electron crystallography from 2D crystals.



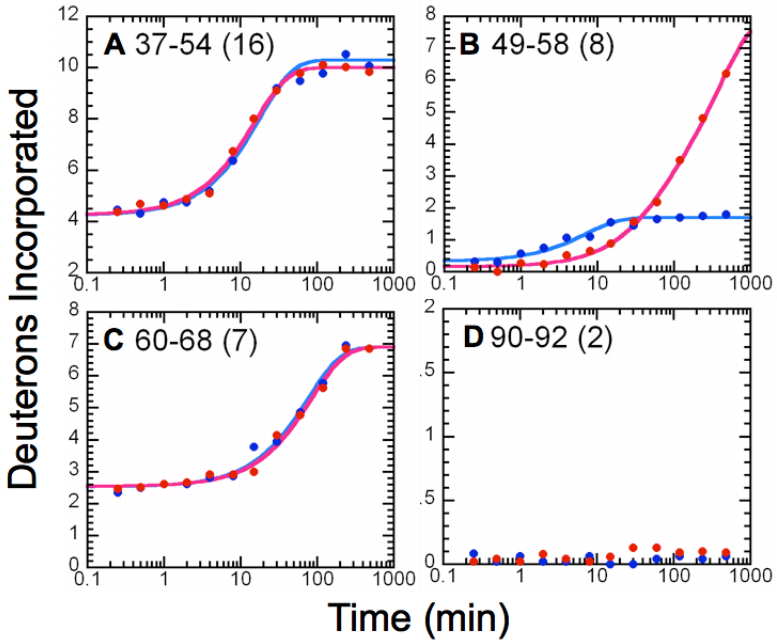
Peptide/ Inhibitor	A_{fast} (D)	A_1 (D)	k_1 (min^{-1})	A_2 (D)	k_2 (min^{-1})	A_3 (D)	k_3 (min^{-1})
18-23 (5)							
none	0	5	$\leq 1 \times 10^{-4}$				
1	0.3	3.00 ± 0.07	0.0115 ± 0.0012	1.70 ± 0.07	$\leq 1 \times 10^{-4}$		
63-78 (15)							
none	0	5.11 ± 0.09	0.103 ± 0.006	10.00 ± 0.12	$\leq 1 \times 10^{-4}$		
1	0	8.73 ± 0.33	0.185 ± 0.021	7.05 ± 0.31	$\leq 1 \times 10^{-4}$		
78-83 (4)							
none	0.8	0.94 ± 0.02	0.00428 ± 0.00038	2.30 ± 0.13	$\leq 1 \times 10^{-4}$		
1	0	0.72 ± 0.19	0.802 ± 0.053	2.42 ± 0.12	0.0141 ± 0.0018	0.86 ± 0.12	$\leq 1 \times 10^{-4}$
130-132 (2)							
none	0.3	0.90 ± 0.02	0.0454 ± 0.0046	0.75 ± 0.05	$\leq 1 \times 10^{-4}$		
1	0.5	1.48 ± 0.03	0.0248 ± 0.0024				

Figure S2. Amide H/D exchange kinetic profiles for MPGES1•GSH. Shown are the average kinetic profiles for deuterium incorporation as a function of time for MPGES1 complexed with either GSH (blue) or GSO_3^- (red), with the number of exchangeable amide protons for each peptide in parentheses. The kinetic data were analyzed as described in the main text.



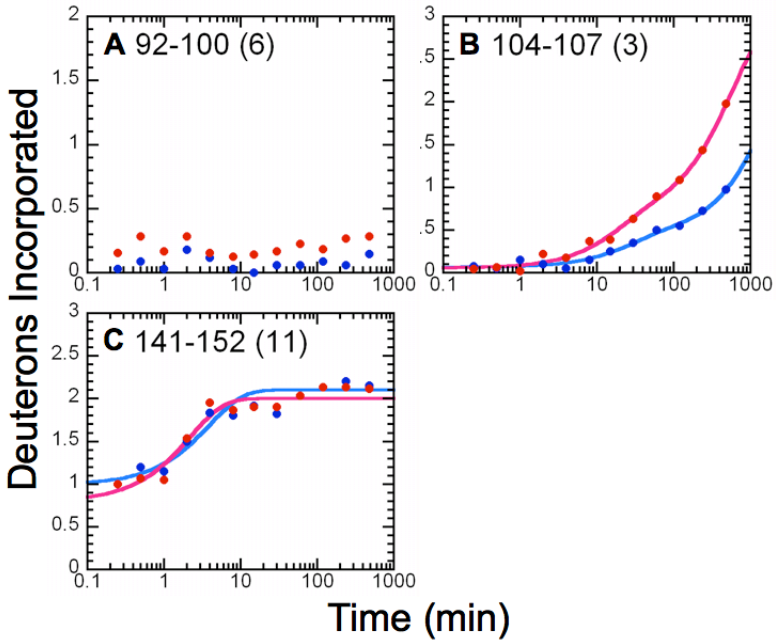
Peptide/ Inhibitor	A_{fast} (D)	A_1 (D)	k_1 (min^{-1})	A_2 (D)	k_2 (min^{-1})	A_3 (D)	k_3 (min^{-1})
2-13 (10)							
none	8.6	1.44 ± 0.10	0.536 ± 0.087				
1	8.7	1.31 ± 0.11	0.747 ± 0.132				
14-17 (3)							
none	0	0.74 ± 0.03	0.0971 ± 0.0142	2.29 ± 0.03	≤ 1 x 10^{-4}		
1	0	0.95 ± 0.02	0.0127 ± 0.0011	2.06 ± 0.10	≤ 1 x 10^{-4}		
23-27 (4)							
none	0	4	≤ 1 x 10^{-4}				
1	0	4	≤ 1 x 10^{-4}				
32-39 (7)							
none	0	1.05 ± 0.03	0.102 ± 0.009	5.90 ± 0.04	≤ 1 x 10^{-4}		
1	0.3	4.69 ± 0.09	0.161 ± 0.0013	2.00 ± 0.09	≤ 1 x 10^{-4}		

Figure S3. Amide H/D exchange kinetic profiles for MPGES1•GSH. Shown are the average kinetic profiles for deuterium incorporation as a function of time for MPGES1 complexed with either GSH (blue) or GSO_3^- (red), with the number of exchangeable amide protons for each peptide in parentheses. The kinetic data were analyzed as described in the main text.



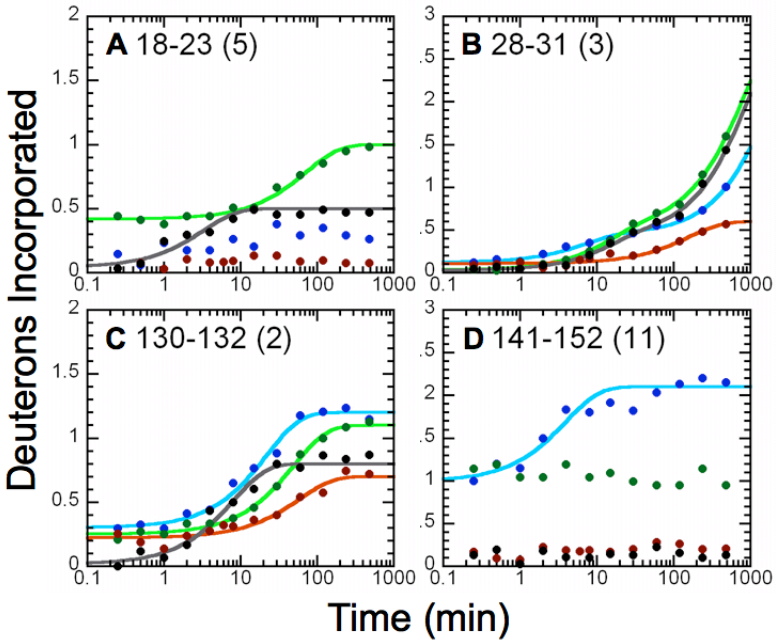
Peptide/ Inhibitor	A_{fast} (D)	A_1 (D)	k_1 (min^{-1})	A_2 (D)	k_2 (min^{-1})	A_3 (D)	k_3 (min^{-1})
37-54 (16)							
none	3.9	5.90 ± 0.15	0.0601 ± 0.0054	6.18 ± 0.26	$\leq 1 \times 10^{-4}$		
1	4.2	5.75 ± 0.11	0.0649 ± 0.0045	5.96 ± 0.21	$\leq 1 \times 10^{-4}$		
49-58 (8)							
none	0.2	1.37 ± 0.08	0.141 ± 0.025	6.44 ± 0.07	$\leq 1 \times 10^{-4}$		
1	0.1	1.89 ± 0.46	0.0210 ± 0.0065	5.95 ± 0.47	$\leq 2.54 \times 10^{-3}$		
60-68 (7)							
none	2.6	4.37 ± 0.08	0.0131 ± 0.0011				
1	2.6	4.36 ± 0.07	0.0119 ± 0.0009				
90-92 (2)							
none	0	2	$\leq 1 \times 10^{-4}$				
1	0	2	$\leq 1 \times 10^{-4}$				

Figure S4. Amide H/D exchange kinetic profiles for MPGES1. Shown are the average kinetic profiles for deuterium incorporation as a function of time for MPGES1 complexed with either GSH (blue) or GSO_3^- (red), with the number of exchangeable amide protons for each peptide in parentheses. The kinetic data were analyzed as described in the main text.



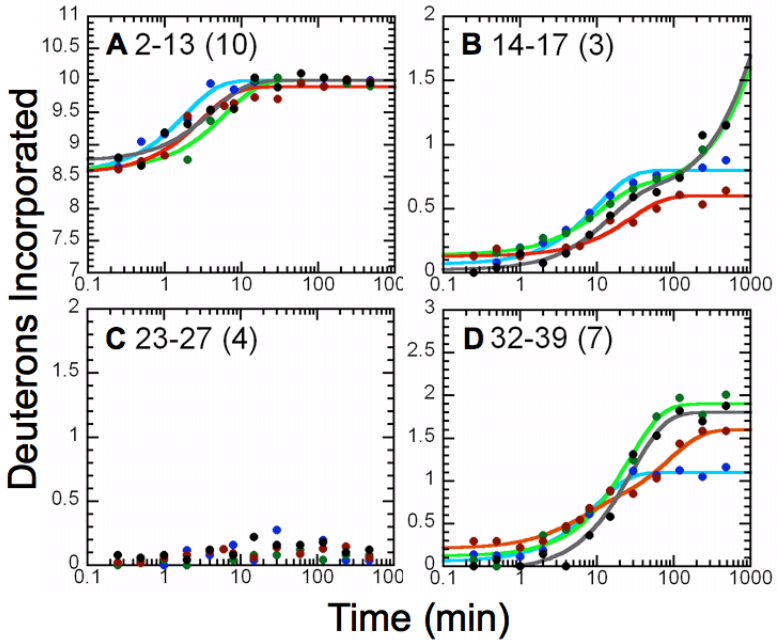
Peptide/ Inhibitor	A_{fast} (D)	A_1 (D)	k_1 (min^{-1})	A_2 (D)	k_2 (min^{-1})	A_3 (D)	k_3 (min^{-1})
92-100 (6)							
none	0	6	$\leq 1 \times 10^{-4}$				
1	0	6	$\leq 1 \times 10^{-4}$				
104-107 (3)							
none	0	0.37 ± 0.05	0.0365 ± 0.0021	2.56 ± 0.05	$\leq 4.90 \times 10^{-4}$		
1	0	0.60 ± 0.07	0.0535 ± 0.0051	2.35 ± 0.07	$\leq 1.72 \times 10^{-3}$		
141-152 (11)							
none	0.8	1.11 ± 0.11	0.254 ± 0.029	9.06 ± 0.06	$\leq 1 \times 10^{-4}$		
1	0.7	1.27 ± 0.12	0.364 ± 0.037	9.03 ± 0.06	$\leq 1 \times 10^{-4}$		

Figure S5. Amide H/D exchange kinetic profiles for MPGES1. Shown are the average kinetic profiles for deuterium incorporation as a function of time for MPGES1 complexed with either GSH (blue) or GSO_3^- (red), with the number of exchangeable amide protons for each peptide in parentheses. The kinetic data were analyzed as described in the main text.



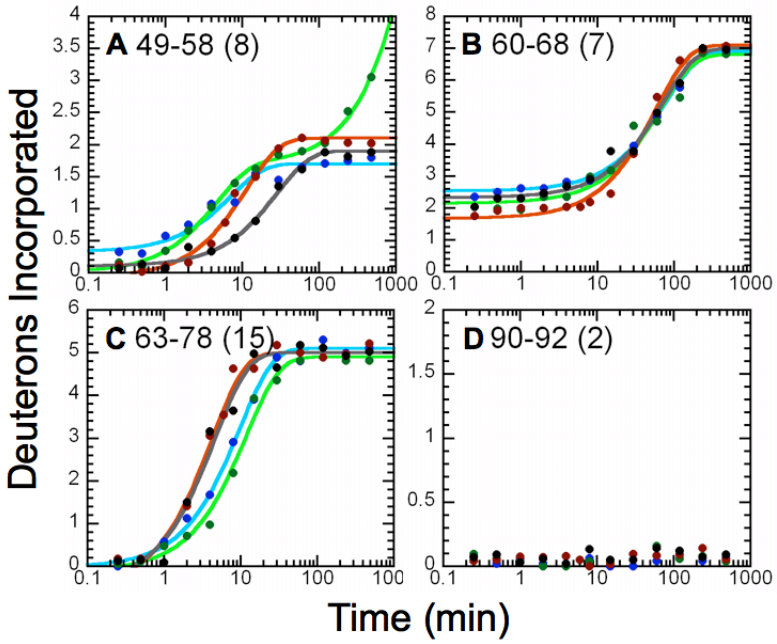
Peptide/ Inhibitor	A_{fast} (D)	A_1 (D)	k_1 (min^{-1})	A_2 (D)	k_2 (min^{-1})	A_3 (D)	K (min^{-1})
18-23 (5)							
none	0	5	$\leq 1 \times 10^{-4}$				
2	0	0.46 ± 0.04	0.303 ± 0.070	4.54 ± 0.02	$\leq 1 \times 10^{-4}$		
3	0	5	$\leq 1 \times 10^{-4}$				
4	0.3	0.58 ± 0.01	0.0139 ± 0.0014	4.11 ± 0.05	$\leq 1 \times 10^{-4}$		
28-31 (3)							
none	0.1	0.33 ± 0.03	0.141 ± 0.015	2.55 ± 0.02	$\leq 5.12 \times 10^{-4}$		
2	0	0.40 ± 0.04	0.0687 ± 0.0170	2.57 ± 0.04	$\leq 1.05 \times 10^{-3}$		
3	0	0.49 ± 0.01	0.00637 ± 0.00078	2.65 ± 0.09	$\leq 1 \times 10^{-4}$		
4	0	0.48 ± 0.04	0.0686 ± 0.0130	2.48 ± 0.03	$\leq 1.19 \times 10^{-3}$		
130-132 (2)							
none	0.3	0.90 ± 0.02	0.0454 ± 0.0046	0.75 ± 0.05	$\leq 1 \times 10^{-4}$		
2	0	0.78 ± 0.04	0.125 ± 0.019	1.21 ± 0.04	$\leq 1 \times 10^{-4}$		
3	0.1	0.48 ± 0.02	0.0172 ± 0.0031	1.37 ± 0.10	$\leq 1 \times 10^{-4}$		
4	0.1	0.85 ± 0.01	0.0202 ± 0.0013	0.95 ± 0.05	$\leq 1 \times 10^{-4}$		
141-152 (11)							
none	0.8	1.11 ± 0.11	0.254 ± 0.029	9.06 ± 0.06	$\leq 1 \times 10^{-4}$		
2	0	11	$\leq 1 \times 10^{-4}$				
3	0	11	$\leq 1 \times 10^{-4}$				
4	1.1	9.92 ± 0.03	$\leq 1 \times 10^{-4}$				

Figure S6. Amide H/D exchange kinetic profiles for MPGES1. Shown are the average kinetic profiles for deuterium incorporation as a function of time for the MPGES1•GSH complex in the absence of an inhibitor (blue) and in the presence of compound **2** (black), **3** (red), or **4** (green). The kinetic data were analyzed as described in the main text.



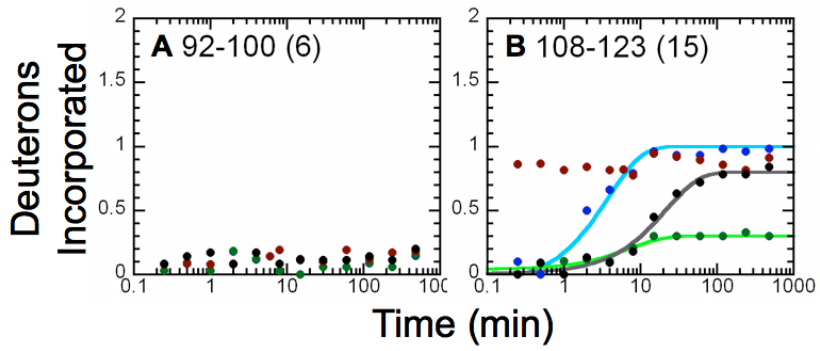
Peptide/ Inhibitor	A_{fast} (D)	A_1 (D)	k_1 (min^{-1})	A_2 (D)	k_2 (min^{-1})	A_3 (D)	k_3 (min^{-1})
2-13 (10)							
none	8.6	1.44 ± 0.10	0.536 ± 0.087				
2	8.7	1.26 ± 0.11	0.230 ± 0.055				
3	8.6	1.35 ± 0.11	0.321 ± 0.063				
4	8.6	1.39 ± 0.07	0.158 ± 0.025				
14-17 (3)							
none	0	0.74 ± 0.03	0.0971 ± 0.0142	2.29 ± 0.03	$\leq 1 \times 10^{-4}$		
2	0	0.59 ± 0.07	0.0723 ± 0.0134	2.39 ± 0.07	$\leq 6.05 \times 10^{-4}$		
3	0	0.47 ± 0.03	0.0358 ± 0.0077	2.48 ± 0.05	$\leq 1 \times 10^{-4}$		
4	0.1	0.51 ± 0.02	0.0965 ± 0.0113	2.35 ± 0.02	$\leq 5.18 \times 10^{-4}$		
23-27 (4)							
none	0	4	$\leq 1 \times 10^{-4}$				
2	0	4	$\leq 1 \times 10^{-4}$				
3	0	4	$\leq 1 \times 10^{-4}$				
4	0	4	$\leq 1 \times 10^{-4}$				
32-39 (7)							
none	0	1.05 ± 0.03	0.102 ± 0.009	5.90 ± 0.04	$\leq 1 \times 10^{-4}$		
2	0	1.85 ± 0.05	0.0345 ± 0.0038	5.22 ± 0.15	$\leq 1 \times 10^{-4}$		
3	0.2	0.44 ± 0.13	0.186 ± 0.011	0.95 ± 0.14	0.0115 ± 0.0029	5.61 ± 0.14	$\leq 1 \times 10^{-4}$
4	0	1.78 ± 0.05	0.0382 ± 0.0041	5.15 ± 0.13	$\leq 1 \times 10^{-4}$		

Figure S7. Amide H/D exchange kinetic profiles for MPGES1. Shown are the average kinetic profiles for deuterium incorporation as a function of time for the MPGES1-GSH complex in the absence of an inhibitor (blue) and in the presence of compound **2** (black), **3** (red), or **4** (green). The kinetic data were analyzed as described in the main text.



Peptide/ Inhibitor	A_{fast} (D)	A_1 (D)	k_1 (min^{-1})	A_2 (D)	k_2 (min^{-1})	A_3 (D)	k_3 (min^{-1})
49-58 (8)							
none	0.2	1.37 ± 0.08	0.141 ± 0.025	6.44 ± 0.07	≤ 1 x 10^{-4}		
2	0	1.80 ± 0.04	0.0354 ± 0.0030	6.16 ± 0.10	≤ 1 x 10^{-4}		
3	0	2.17 ± 0.06	0.0886 ± 0.0075	5.90 ± 0.08	≤ 1 x 10^{-4}		
4	0	1.68 ± 0.06	0.217 ± 0.022	6.31 ± 0.04	≤ 5.20 x 10^{-4}		
60-68 (7)							
none	2.6	4.37 ± 0.08	0.0131 ± 0.0011				
2	2.3	4.68 ± 0.10	0.0141 ± 0.0013				
3	1.6	5.43 ± 0.09	0.0172 ± 0.0013				
4	2.3	4.66 ± 0.15	0.0153 ± 0.0021				
63-78 (15)							
none	0	5.11 ± 0.09	0.103 ± 0.006	10.00 ± 0.12	≤ 1 x 10^{-4}		
2	0	5.43 ± 0.23	0.215 ± 0.026	10.03 ± 0.21	≤ 1 x 10^{-4}		
3	0	5.53 ± 0.21	0.180 ± 0.018	10.00 ± 0.19	≤ 1 x 10^{-4}		
4	0	5.00 ± 0.13	0.0844 ± 0.0073	10.02 ± 0.20	≤ 1 x 10^{-4}		
90-92 (2)							
none	0	2	≤ 1 x 10^{-4}				
2	0	2	≤ 1 x 10^{-4}				
3	0	2	≤ 1 x 10^{-4}				
4	0	2	≤ 1 x 10^{-4}				

Figure S8. Amide H/D exchange kinetic profiles for MPGES1. Shown are the average kinetic profiles for deuterium incorporation as a function of time for the MPGES1-GSH complex in the absence of an inhibitor (blue) and in the presence of compound **2** (black), **3** (red), or **4** (green). The kinetic data were analyzed as described in the main text.

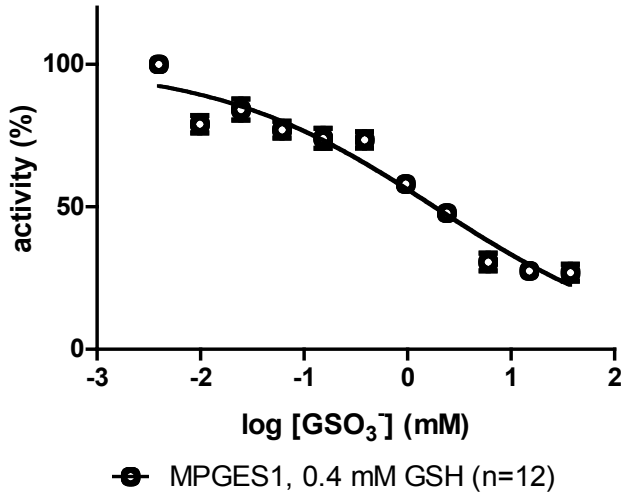


Peptide/ Inhibitor	A_{fast} (D)	A_1 (D)	k_1 (min^{-1})	A_2 (D)	k_2 (min^{-1})	A_3 (D)	k_3 (min^{-1})
92-100 (6)							
none	0	6	$\leq 1 \times 10^{-4}$				
2	0	6	$\leq 1 \times 10^{-4}$				
3	0	6	$\leq 1 \times 10^{-4}$				
4	0	6	$\leq 1 \times 10^{-4}$				
108-123 (15)							
none	0	1.07 ± 0.07	0.261 ± 0.029	14.06 ± 0.05	$\leq 1 \times 10^{-4}$		
2	0	0.79 ± 0.03	0.0460 ± 0.0055	14.24 ± 0.05	$\leq 1 \times 10^{-4}$		
3	0.8	14.15 ± 0.02	$\leq 1 \times 10^{-4}$				
4	0	0.26 ± 0.02	0.139 ± 0.033	14.69 ± 0.02	$\leq 1 \times 10^{-4}$		

Figure S9. Amide H/D exchange kinetic profiles for MPGES1. Shown are the average kinetic profiles for deuterium incorporation as a function of time for the MPGES1-GSH complex in the absence of an inhibitor (blue) and in the presence of compound **2** (black), **3** (red), or **4** (green). The kinetic data were analyzed as described in the main text.

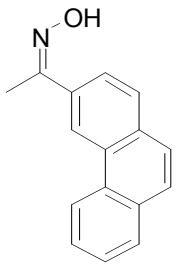
Figure S10. GSO_3^- IC_{50}.

[total protein] 15 µg/ml, [PGH_2] 10 µM, [GSH] 0.4 mM, [GSO_3^-] from 0.004 to 37.5 mM, n=12

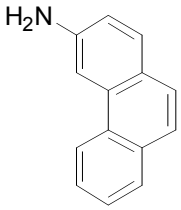


log IC_{50}	0.26
IC_{50} (mM)	**1.8**
Hill Slope	-0.4085
95% Confidence Interval	
log IC_{50}	0.15 to 0.36
IC_{50} (mM)	**1.4 to 2.3**

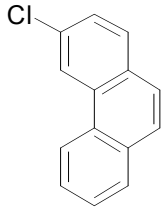
Synthesis of MF63 (3)



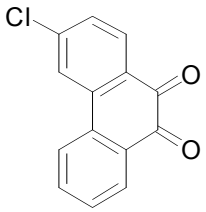
Synthesis of 1-(3-phenanthryl)ethanone oxime. To a solution of 1-(3-phenanthryl)ethanone in 25 mL of ethanol was added 4 g of hydroxylamine hydrochloride. The reaction mixture was heated to reflux followed by the addition of 7 mL of pyridine. After 3 h, the reaction was cooled down to room temperature and the solvent was removed *in vacuo*. Ice was added to the residue and the mixture was stirred for 1 h. The resulting off-white solid was filtered and washed with water. 5.02 g of the product (94 %) was obtained by recrystallization from ether.
^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 8.93 (s, 1H), 8.74 (d, J = 8.2 Hz, 1H), 7.92 (d, J = 7.2 Hz, 1H), 7.90 (dd, J = 8.4, 14.0 Hz, 2H), 7.76 (dd, J = 8.8, 14.8 Hz, 2H), 7.71-7.60 (m, 2H), 2.49 (s, 3H); LCMS, single peak, 1.51 min, m/e, 236.28 (M+1).



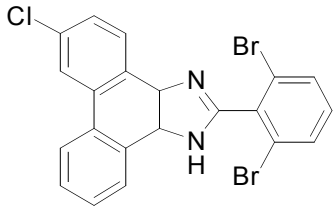
Synthesis of 3-phenanthrylamine. To 60 g of polyphosphoric acid was added 5.02 g of 1-(3-phenanthryl)ethanone oxime at room temperature. The reaction mixture was stirred at 100 ^{o}C for 2 h, cooled down to room temperature followed by the addition of ice. Stirred 30 minutes, filtered and washed with water. This white solid was then placed in 50 mL of methanol and 4 mL of concentrated HCl. The reaction was refluxed overnight, cooled down to room temperature and concentrated down. A mixture of ethyl acetate/water was added to the residue and the resulting solution was made basic with 10 N KOH. The aqueous layer was extracted with ethyl acetate and combined organic layers were washed with water, brine, dried over $MgSO_4$ and solvent was removed under reduced pressure to afford 3.74g (90%) of 3-phenanthrylamine as a beige solid. LCMS, single peak, 1.09 min, m/e, 194.24 (M+1).



Synthesis of 3-chlorophenanthrene. 3.39 g of $CuCl_2$ and 4.84 mL of *t*-butyl nitrite were dissolved in 10 mL of acetonitrile. The 3-phenanthrylamine (3.74 g) was added over 10 minutes as a solution in 6 mL of acetonitrile. The reaction was stirred for 45 minutes at 65 ^oC, cooled down to room temperature followed by the addition of 100 mL of 1 N HCl. The aqueous layer was extracted with dichloromethane and combined organic layers were washed with water, brine, dried over $MgSO_4$ and solvent was remove under reduced pressure. The residue was purified by flash chromatography on silica gel (hexane/ethyl acetate = 7/3) to produce 2.53 g (63%) of 3-chlorophenanthrene as a white solid. ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm) 8.65 (d, *J* = 1.6 Hz, 1H), 8.60 (d, *J* = 8.0 Hz, 1H), 7.90 (dd, *J* = 1.6, 8.0 Hz, 1H), 7.82 (d, *J* = 8.4 Hz, 1H), 7.75-7.61 (m, 4H), 7.55 (dd, *J* = 2.0, 8.8 Hz, 1H); LCMS, single peak, 1.75 min, m/e, 213.67 (M+1).

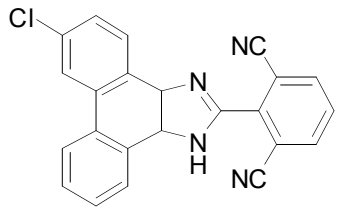


Synthesis of 3-chlorophenanthrene-9,10-dione. To a solution of 2.53 g of 3-chlorophenanthrene in 70 mL of acetic acid was added 4.75 g of CrO_3. The reaction was stirred for 2 h at 100 ^oC, cooled down to room temperature and poured into 300 mL of water. The suspension was stirred for 1h, filtered and washed with water. The residue was dried under high vacuum to afford 2.5 g (86%) of 3-chlorophenanthrene-9,10-dione. ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm) 8.21 (d, *J* = 8.0 Hz, 1H), 8.14 (d, *J* = 8.4 Hz, 1H), 8.00-7.95 (m, 2H), 7.75 (t, *J* = 8.0 Hz, 1H), 7.53 (t, *J* = 8.0 Hz, 1H), 7.44 (dd, *J* = 1.6, 8.0 Hz, 1H); LCMS, single peak, 1.47 min, m/e, 243.66 (M+1).



Synthesis of 6-chloro-2-(2,6-dibromophenyl)-3a,11b-dihydro-1H-phenanthro[9,10-d]imidazole. To a solution of 0.613 g of 3-chlorophenanthrene-9,10-dione in 25 mL of

acetic acid was added 0.8 g of ammonium bicarbonate followed by 1.0 g of 2,6-dibromobenzaldehyde. The reaction was stirred overnight at 130 ^{o}C, cooled down to room temperature, and poured into the 40 mL of water. The residue was filtered, washed with water, and purified by flash column chromatography (hexane/ethyl acetate = 6/4) to afford 0.75 g (61%) of white solid product. LCMS, single peak, 1.55 min, m/e, 486.90 (M+1).



MF63

Synthesis of MF63. To a solution of 0.75 g of 6-chloro-2-(2,6-dibromophenyl)-3a,11b-dihydro-1H-phenanthro[9,10-d]imidazole was added 0.348 g of CuCN. The reaction was stirred overnight at 80 ^{o}C, cooled down to room temperature, poured into a mixture of 40 mL of water, 40 mL of ethyl acetated and 5.5 mL of concentrated ammonium hydroxide, and stirred for 1 h at room temperature. The aqueous layer was extracted with ethyl acetate and the combined organic layers were washed with 10% ammonium hydroxide, water, brine, dried over $MgSO_4$ and solvent was removed under reduced pressure. The residue was purified by flash column chromatography (hexane/ethyl acetate = 5/5) to afford 0.346 g (59%) of 2-(6-chloro-3a,11b-dihydro-1H-phenanthro[9,10-d]imidazol-2-yl) isophthalo-nitrile. ^{1}H NMR (DMSO, 400 MHz) δ (ppm) 14.32 (s, 1H), 9.04-8.88 (m, 2H), 8.60-8.36 (m, 4H), 7.98 (t, J = 8.0 Hz, 1H), 7.90-7.76 (m, 2H), 7.75-7.67 (m, 1H); LCMS, single peak, 1.54 min, m/e, 381.06 (M+1).