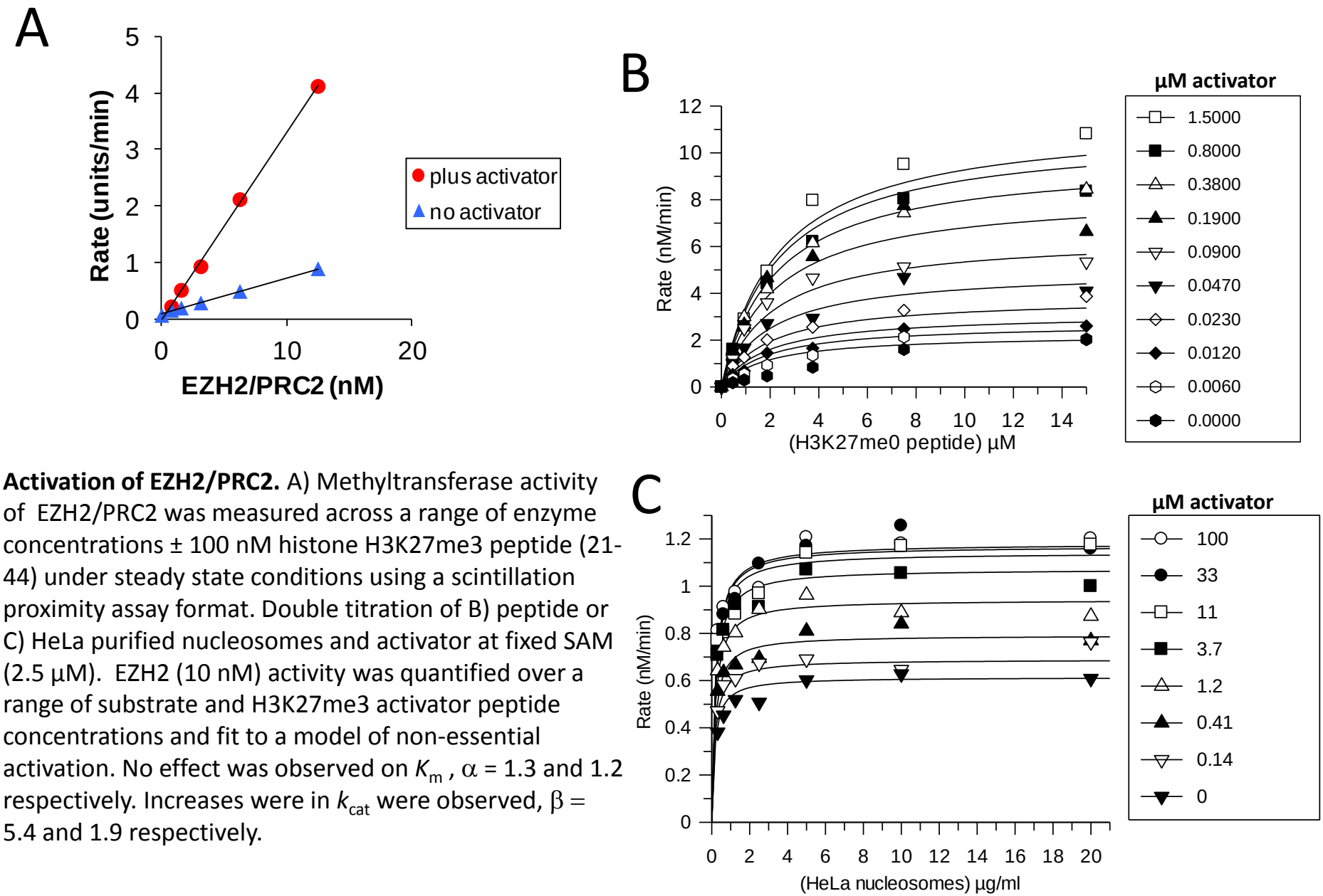
Long Residence Time Inhibition of EZH2 in Activated Polycomb Repressive Complex 2

Glenn S. Van Aller, Melissa Baker Pappalardi, Heidi M. Ott, Elsie Diaz, Martin Brandt, Benjamin J. Schwartz, William H. Miller, Dashyant Dhanak, Michael T. McCabe, Sharad K. Verma, Caretha L. Creasy, Peter J. Tummino and Ryan G. Kruger

Supplementary Information

Supplementary Figure 1



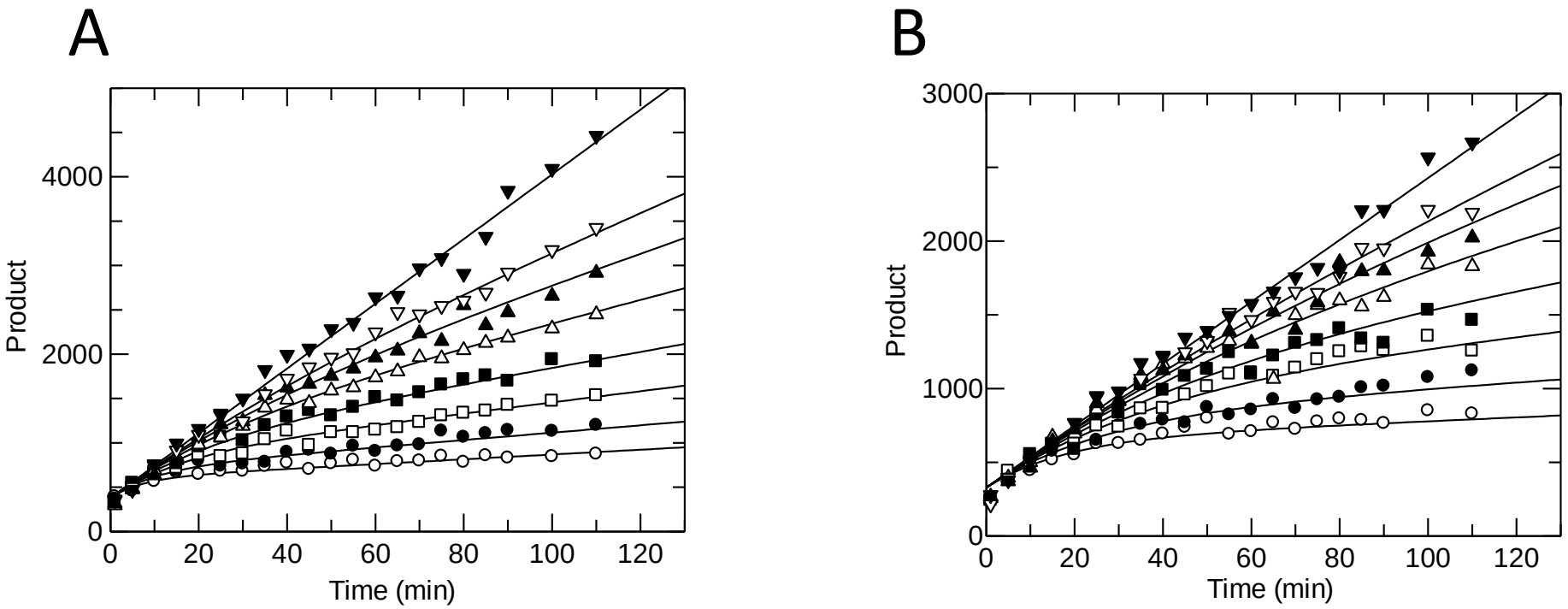
Activation of EZH2/PRC2. A) Methyltransferase activity of EZH2/PRC2 was measured across a range of enzyme concentrations ± 100 nM histone H3K27me3 peptide (21-44) under steady state conditions using a scintillation proximity assay format. Double titration of B) peptide or C) HeLa purified nucleosomes and activator at fixed SAM (2.5 µM). EZH2 (10 nM) activity was quantified over a range of substrate and H3K27me3 activator peptide concentrations and fit to a model of non-essential activation. No effect was observed on K_m , α = 1.3 and 1.2 respectively. Increases were in k_{cat} were observed, β = 5.4 and 1.9 respectively.

Supplementary Table 1

EZH2/PRC2	K_a app (nM)	β, as defined in Figure 1B
Wild type	86	7.4
A677G	33	3.1
Y641F	5.7	4.5

Apparent activation constants (K_a^{app}) for mutant EZH2. Peptide substrate (H3 21-44) substrate was fixed at K_m for each mutant or wild type EZH2. Methyltransferase activity was quantified over a range of H3K27me3 activator peptide concentrations and fit to Eq 1 assuming alpha = 1.

Supplementary Figure 2



EZH2 reaction progress curves. Time course data using A) wild type (230 pM) or B) Y641F (220 pM) mutant EZH2, for uninhibited (▼), and inhibited reactions at 0.5 nM (▽), 0.8 nM (▲), 1.2 nM (△), 2 nM (■), 3 nM (□), 5 nM (●), and 8 nM (○), of GSK126. Enzymes were pre-activated with 1 µM K27me3 peptide and the reactions were run at 10 µM peptide substrate and 0.3 µM SAM. Progress curve data were fit globally to Eq 2.

Supplementary Table 2. Summary of kinetic parameters for GSK126 using activated EZH2/PRC2

EZH2/PRC2	k_{on} (min^{-1})	k_{off} (min^{-1})	$t_{1/2}$ (min)	Residence time (min)	K_i (nM)	K_i^* (nM)
Wild type	0.20 ± 0.02	0.016 ± 0.005	44 ± 14	64 ± 20	4.7 ± 0.5	0.37 ± 0.07
A677G	0.11 ± 0.04	0.013 ± 0.001	54 ± 6	77 ± 8	0.87 ± 0.2	0.093 ± 0.01
Y641F	0.18 ± 0.01	0.024 ± 0.001	30 ± 1	43 ± 1	1.8 ± 0.4	0.21 ± 0.04

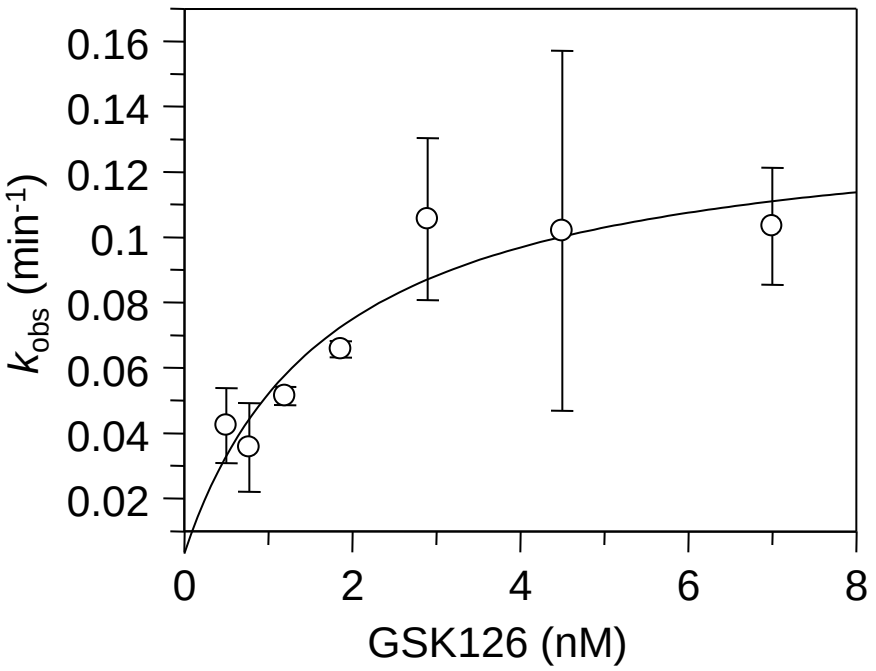
Forward progress curves data were fit globally as described in the methods to determine k_{on}, k_{off}, K_i and K_i* using allosterically activated PRC2 containing wild type or mutant EZH2. Rapid dilution experiments were used to confirm reversibility and to confirm k_{off} for GSK126.

Supplementary Table 3

Reconstituted NCPs	K_a (μM)	β	α	K_M (nM)	k_{cat}(min^{-1})	k_{cat}/K_m(min^{-1} nM^{-1}) x10^{-3}
unmarked	3.8 ± 1.8	11 ± 1	2.1 ± 0.3	21 ± 1	0.017 ± 0.001	0.83 ± 0.12
heterodimer H3K27me3	0.9 ± 0.5	8.4 ± 0.3	1.9 ± 0.3	16 ± 2	0.020 ± 0.001	1.3 ± 0.28

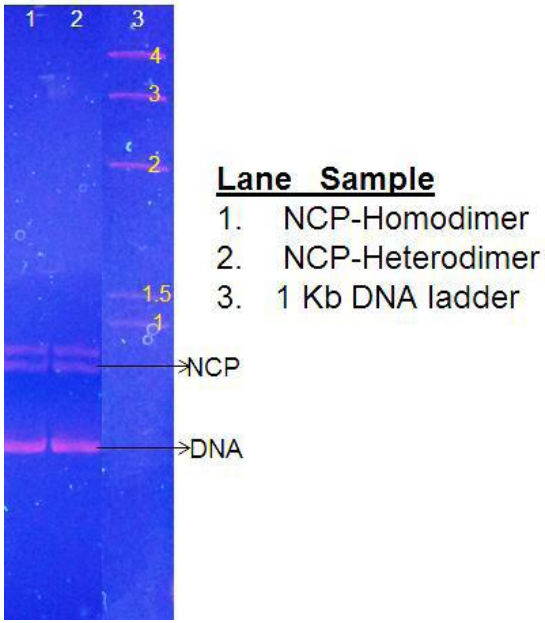
- **Activation of EZH2/PRC2 using reconstituted NCPs.** Results were compiled from double titration experiments using activator peptide and recombinant NCPs without epigenetic modification or NCPs with a mixture of H3K_c27me3 and umarked H3. The data shown are an average ± standard deviation from 2 independent experiments. Figure 1C and 1D show plots of representative data and Figure 1B shows a scheme describing the model of activation.

Supplementary Figure 3



Observed rate constant of inactivation as a function of inhibitor concentration. Reaction progress curve data were fit to equation 2 in the presence of the indicated concentrations of GSK126. EZH2 (230 pM) was pre-activated with 1 µM K27me3 peptide and the reaction was run at 10 µM peptide substrate and 0.3 µM SAM.

Supplementary Figure 4



Electrophoretic Mobility Shift Assay. Ethidium bromide-stained 0.8% agarose gel electrophoresis of the final nucleosome core particles (NCP) used in Figure 1.