Nucleosome Binding Alters the Substrate Bonding Environment of Histone H3 Lysine 36 Methyltransferase NSD2

Myles B. Poulin,[†] Jessica L. Schneck,[‡] Rosalie E. Matico,[‡] Wangfang Hou,[‡] Patrick J. McDevitt,[‡] Marc Holbert,[‡] and Vern L. Schramm*[,†]

[†]Department of Biochemistry, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461, United States

[‡]Biological Sciences, Platform Technology and Science, GlaxoSmithKline, Collegeville, PA 19426, United States

Supporting Information

A. Supporting Information Figure S1-S4

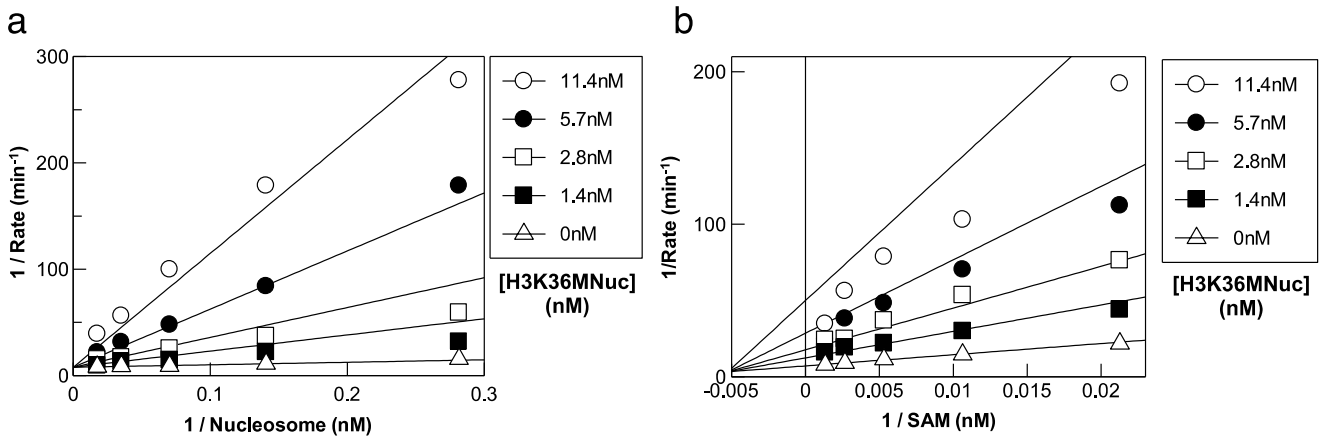


Figure S1. Inhibition kinetics for H3K36MNuc. Kinetics for the inhibition of NSD2 were measured with multiple concentrations of [H3K36MNuc]. Inhibition by H3K36MNuc was measured with varying concentrations of recombinant human polynucleosome (a) or SAM (b) and K_i values where determined by global fitting to the kinetics data. Double reciprocal plots indicate NSD2 inhibition by H3K36MNuc is competitive against nucleosome (K_{is} = 0.63 ± 0.15 nM) (a) and uncompetitive against SAM (b) (K_{is} = 1.0 ± 0.3 nM, K_{ii} = 1.9 ± 0.3 nM). A K_M = 14.3 ± 0.9 nM for recombinant human polynucleosome and a K_M of 203 ± 13 nM for SAM were measured under the same conditions.

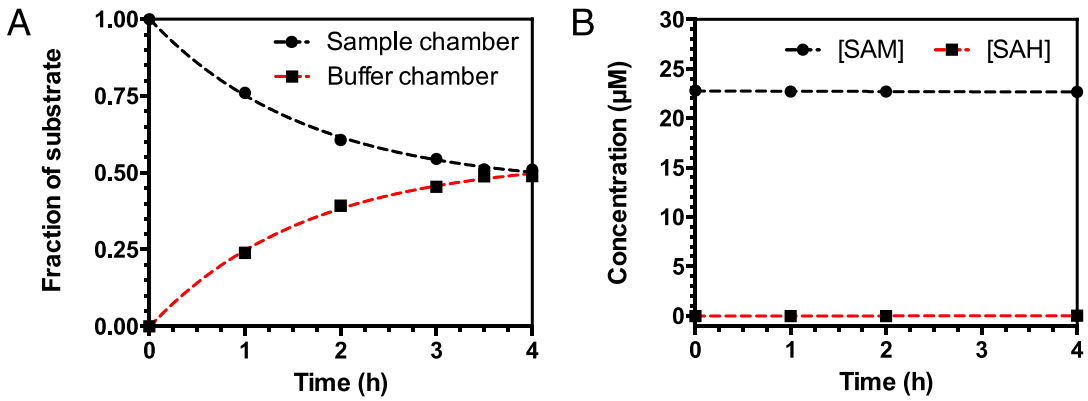


Figure S2. Rapid equilibrium dialysis. (A) The fraction of radio labeled substrate in both the sample and buffer wells of a RED device were measured as a function of time to determine the minimum time required to reach equilibrium. (B) The concentration of SAM and SAH were monitored overtime for incubation mixtures including H3K36S nucleosome. No SAH production was observed over the course of BIE incubations.

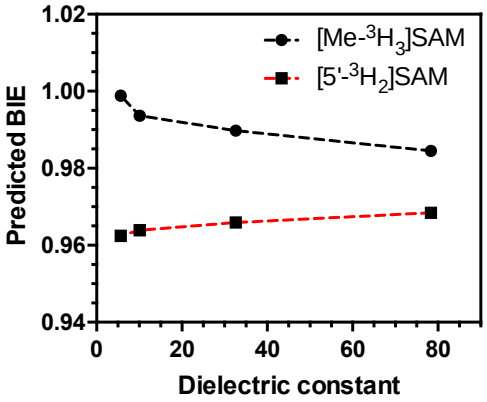


Figure S3. Calculated effect of dielectric environment on [Me-$^{3}H_3$]SAM and [5′-$^{3}H_2$]SAM BIEs. Equilibrium BIEs were calculated from the scaled vibrational frequencies from energy minimized SAM "free" state structure and SAM "bound" state structures calculated using water (ε = 78.4), methanol (ε = 32.6), dichloroethane (ε = 10.1), or chlorobenzene (ε = 5.7) as an implicit solvent model (PCM).

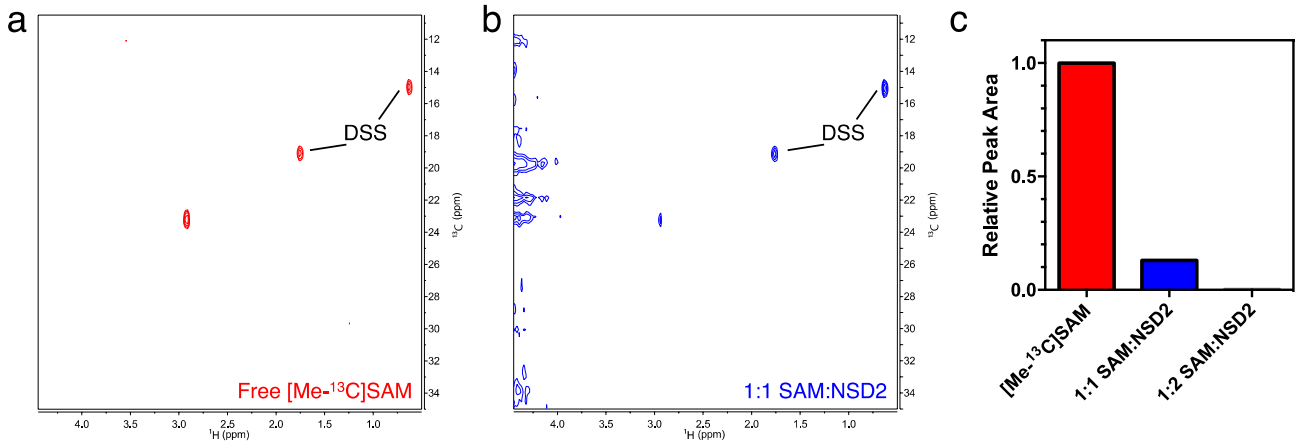


Figure S4. NMR of free [Me-^{13}C]SAM and NSD2•[Me-^{13}C]SAM complex show no change in Me–$^{1}H_3$ chemical shifts. Two-dimensional HSQC spectra for [Me-^{13}C]SAM free in solution (a) or in 1:1 complex with NSD2 protein (b) shows a 0.02 ppm change in ^{1}H chemical shift. A decrease in relative peak area of [Me-^{13}C]SAM compared to internal DSS standard is seen with increasing NSD2 concentration (c).

B. Supporting Information Tables S1

Table S1. Calculated BIEs for [Me-$^3H_3$]SAM and [5′-$^3H_2$]SAM in implicit solvents with varying dielectric constants.

Solvent model in bound state	*Dielectric constant (ε)*	*Calculated [Me-$^3H_3$]SAM BIE*	*Calculated [5′-$^3H_2$]SAM BIE*
chlorobenzene	5.697	0.999	0.962
dichloroethane	10.125	0.994	0.964
acetone	20.493	0.994	0.964
methanol	32.613	0.990	0.966
water	78.355	0.984	0.968

Table S2. Calculated BIEs for [Me-$^3H_3$]SAM interactions with NSD2 F1117, R1138 and Y1179.

Y1179 O···H distance (Å)	*R1138 O···H distance (Å)*	*F1117 O···H distance (Å)*	*Calculated BIE*
2.30	2.28	2.26	1.021
2.50	2.50	2.50	1.069
2.30	2.30	2.30	1.030
2.20	2.20	2.20	0.984
2.10	2.10	2.10	0.945
1.95	1.95	1.95	0.887
1.85	1.85	1.85	0.853
1.80	1.80	1.80	0.843
1.75	1.75	1.75	0.858

Table S3. Calculated BIEs for [Me-$^3H_3$]SAM with restriction of the average Me C–H bond lengths.

Average C–H bond length in unbound structure	*Change relative to unbound structure*	*Average C–H bond length in bound structure*	*Calculated BIE*
1.0904 Å	1.000	1.0904 Å	0.994
1.0904 Å	0.995	1.0849 Å	0.956
1.0904 Å	0.990	1.0795 Å	0.896
1.0904 Å	0.980	1.0686 Å	0.772
1.0904 Å	0.970	1.0577 Å	0.652
1.0904 Å	0.960	1.0468 Å	0.552
1.0904 Å	0.950	1.0359 Å	0.480

Table S4. Calculated BIEs for [Me-$^3H_3$]SAM with restriction of single Me C–H bond lengths.

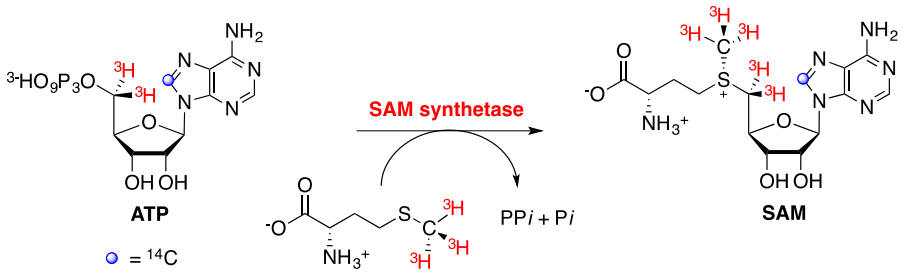
C–H bond length in unbound structure	*Change relative to unbound structure*	*C–H bond length in bound structure*	*Calculated BIE*
1.0908 Å	1.00	1.0908 Å	0.994
1.0908 Å	0.99	1.0799 Å	0.977
1.0908 Å	0.97	1.0581 Å	0.880
1.0908 Å	0.95	1.0362 Å	0.786
1.0908 Å	0.90	0.9817 Å	0.635

C. Supporting Information Methods.

General methods. All chemical reagents were purchased from commercial sources and used without further purification unless otherwise indicated. L-[Me-$^{3}H_3$]methionine (specific activity: 70-85 Ci/mmol) was purchased from PerkinElmer Inc., D-[6-$^{3}H_2$]glucose (50 Ci/mmol) and [8-^{14}C]adenine (47 mCi/mmol) were purchased from American Radio Labeled Chemicals Inc. L-[Me-^{13}C]methionine was purchased from Cambridge Isotope Laboratories, Inc. Scintillation counting was performed using a Tri-Carb 2910 TR and using Ultima Gold^{TM} liquid scintillation cocktail from PerkinElmer for all measurements. Human recombinant histone H3K36M polynucleosome (H3K36MNuc) were obtained as a generous gift from GlaxoSmithKline and contain an average of 9 histone octamers assembled per 2710 bp linearized plasmid DNA. NSD2 (985-1365) *N*-terminal truncation mutant and *Escherichia coli* SAM synthetase enzymes were expressed and purified as previously described.[1, 2] For NSD2 (985-1365), bound SAM was removed by partial unfolding with 2 M urea as previously described.[1]

Preparation of human recombinant H3K36M containing polynucleosome. Recombinant human histone H3 containing a K36M point mutation was expressed in *E. coli* and purified from inclusion bodies according to previous reports.[3, 4] Recombinant human histone proteins were assembled into octamers by combining equimolar concentrations of recombinant human histone H2A, H2B, H3K36M and H4 protein under denaturing conditions in 6M guanidinium HCl followed by step dialysis in decreasing concentrations of NaCl over a period of several days. The renatured histone octamers were purified by size exclusion chromatography on a column of Superdex 200. Final H3K36M containing polynucleosome were prepared by salt dialysis as previously described,[3, 4] using histone octamers and linearized pUC57 plasmid DNA at a 10:1 molar ratio.

Synthesis of radio-labeled SAM. [Me-^{13}C]SAM, [Me-$^{3}H_3$]SAM, [5′-$^{3}H_3$]SAM and [8-^{14}C]SAM were synthesized enzymatically from labeled [Me-^{13}C]methionine, [Me-$^{3}H_3$]methionine, [5′-$^{3}H_2$]ATP or [8-^{14}C]ATP using *E. coli* SAM synthetase as described previously.[1]



Inhibition of NSD2 by H3K36M containing polynucleosome. Steady-state kinetic parameters were obtained using a filter-binding assay, which measured di-methylation of human H3K36-recombinant polynucleosomes from [Me-$^3H_3$]SAM. Initial rate studies of full-length NSD2 were preformed in 25 μL solutions containing 50mM TrisHCl, pH 7.5, 5 mM $MgCl_2$, 30 mM NaCl, 6% glycerol, 0.002% Tween-20, 5 mM DTT, 1-5 nM NSD2, 3.6-57 nM recombinant nucleosomes, 6-375 nM [Me-$^3H_3$]SAM/6-375 nM unlabeled-SAM, and 0-11.4 nM H3K36MNuc, in half-area, 96-well plates. Reactions were quenched at 60min with 25 μL 300 μM unlabeled-SAM in dH2O, and reaction mixtures were transferred to 96-well Millipore MSDEN6B50 filtration binding plates. After a 15 min incubation, filter wells were washed three times with 100 μL of 50 mM Phosphate Buffer, pH 7.5, and then incubated at 50°C for 30 min. MicroScint-20 (50 μL) was added to each dried well, and the amount of [Me-$^3H_3$]-Nucleosome was quantified by scintillation counting on a TopCount instrument (Perkin-Elmer). Dead-end inhibition studies conforming to competitive and noncompetitive inhibition were fitted to Eq (S1) and (S2) in Grafit 7.0.2 (Erithacus Software Ltd.), respectively:

$$v = VA/[K_a(1+I/K_{is}) + A] \quad \text{(S1)}$$

$$v = VA/[K_a(1 + I/K_{is}) + A(1+I/K_{ii})] \quad \text{(S2)}$$

where K_{is} and K_{ii} are respectively the apparent slope and intercept inhibition constants, A is the concentration of varied substrate, V is the maximal velocity, I is the concentration of inhibitor, and K_a is the K_M for the varied substrate.

Measurement of [Me-$^3H_3$]SAM and [5′-$^3H_2$]SAM BIEs. BIEs were measured via a competitive radiolabel approach using rapid equilibrium dialysis (RED). Incubation mixtures for the binary complex contained 4 μM NSD2 and 20 μM of SAM (total of [8-^{14}C]SAM and [Me-$^3H_3$]SAM or [5′-$^3H_2$]SAM and cold carrier) in a 1:1 ratio of ^{3}H:^{14}C counts per minute (CPM), in 50 mM Tris-HCl (pH 9.0 @ 25 °C) containing 5 mM $MgCl_2$, 2mM DTT and 8% glycerol. Each 100 μL incubation mixture was dialyzed against 300 μL of buffer in a 8 kDa molecular weight cutoff RED device (Thermo Fischer Scientific). The incubation mixture was added to the red sample well of the RED device surrounded by a cylinder of dialysis membrane, and the dialysis buffer was added to the attached buffer well.

The time required to reach equilibrium was determined using control incubations containing only labeled SAM and buffer ran in the same way. Aliquots of 10 μL were removed from both the sample and buffer wells after 1, 2, 3, 3.5 and 4 h, mixed with 10 mL of liquid scintillation fluid (PerkinElmer) and counted for 10 cycles (10 min per cycle). The fraction of labeled SAM in the sample and buffer wells reached equilibrium after 3.5–4 h as shown in Figure S1A. The ratio of ^{3}H:^{14}C CPM also remained constant in both the sample and buffer wells indicating there is no isotope effect for dialysis in the absence of enzyme.

BIE incubations were dialyzed for 4.5 h at which time 50 μL was removed from each sample and buffer well, diluted to 500 μL with water, and mixed with 10 mL of Ultima Gold liquid scintillation fluid (PerkinElmer). Each sample was counted in dual channel format for 10 cycles of 10 min per cycle in a in a Tri-Carb 2910 TR scintillation counter (PerkinElmer). Spectral deconvolution was performed using a [8-^{14}C]SAM standard prepared in the same way as BIE samples using Eq S3 Eq S4, where $^{3}H_{cpm}$ and $^{14}C_{cpm}$ are the total cpm for each isotope, channel1 and channel2 are the total cpm in each channel and *r* is the ratio of cpm in channel 1 to channel 2 for the ^{14}C standard. BIEs were calculated using Eq S5, where $^{14}C_S$ and $^{3}H_S$ are the ^{14}C and ^{3}H counts in the sample well and $^{14}C_B$ and $^{3}H_B$ are the ^{14}C and ^{3}H counts in the buffer well, respectively.

$$^{3}H_{cpm} = \text{channel1} - (r \times \text{channel2}) \quad \text{(S3)}$$

$$^{14}C_{cpm} = \text{channel2} \times (1 + r) \quad \text{(S4)}$$

$$\text{BIE} = \frac{^{14}C_S / ^{14}C_B - 1}{^{3}H_S / ^{3}H_B - 1} \quad \text{(S5)}$$

BIE measurements for the SAM•NSD2•Nucleosome ternary complex included 5 μM H3K36MNuc in the incubation mixture to mimic native nucleosome substrate. The fraction of SAM bound did not change upon H3K36MNuc addition, indicating that the dissociation constant is unchanged. We observed no SAH formation in incubations with H3K36MNuc over the course of 4 h incubations (Figure S2B). BIEs were determined as above.

Two-dimensional HSQC of [Me-^{13}C]SAM. All NMR experiments were performed on a Bruker DRX 600-MHz NMR spectrometer equipped with a triple resonance cryoprobe. Spectra were recorded at 298 K and referenced to internal 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS). Chemical shift assignments for free [Me-^{13}C]SAM was confirmed by measuring the two-dimensional heteronuclear single quantum coherence spectra on a sample containing 5 μM [Me-^{13}C]SAM and 500 μM DSS in 50 mM Tris-HCl (pH 9.0 @ 25 °C), 5 mM $MgCl_2$, 2mM DTT and 10 % D_2O. SAM ^{13}C and ^{1}H chemical

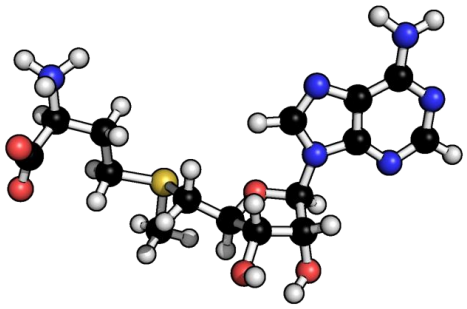
shifts measured under these conditions are in good agreement with previously published values measured under similar conditions.[5] Samples for the NSD2•[Me-^{13}C]SAM bound complex contained 5 μM or 10 μM NSD2 (980-1365), 5 μM [Me-^{13}C]SAM and 500 μM DSS in the same buffer and were measured under the same conditions. A change in the methyl group ^{1}H chemical shift of 0.02 ppm was observed with no new resonances. The relative peak area for [Me-^{13}C]SAM methyl group resonance relative to internal DSS standard is shown in Figure S3c.

Computational methods. *General*. DFT calculations for the free state and bound state conformations of SAM were modeled using the M062X functional and 6-31G(d) basis set as implemented in Gaussian 09,[6] using the harmonic approximation. Equilibrium BIEs were calculated from the scaled vibrational frequencies of optimized SAM free state and bound state structures using the ISOEFF98 program,[7] in an analogous manner to those described in Stratton *et al.*[8] Starting coordinates for the SAM "bound" state conformation were obtained from the crystal structure of NSD1 (pdb 3OOI)[9] since no structure of NSD2 was available.

Calculation of the SAM free state. Initial coordinates for the SAM "free" state were obtained from the predicted low energy conformer in the PubChem 3D database[10] (PubChem CID 34756) and further optimized using water as an implicit solvent (polarizable continuum model, PCM).

Atomic coordinates for free state of SAM in water:

S	-2.19681	-0.69556	-1.46582
O	0.69900	-1.15055	-0.80554
O	0.02942	-2.91025	2.20005
O	2.06982	-3.49951	0.55834
O	-6.53360	-0.13070	0.25237
O	-6.18513	1.40932	1.86617
N	2.61429	0.00817	-0.16286
N	2.79388	2.20257	0.27545
N	4.96770	-0.53881	-0.48793
N	-5.20666	3.18671	0.27466
N	6.38009	1.39392	-0.22715
N	5.58551	3.50260	0.32606
C	-0.20539	-1.77278	0.10467
C	0.55297	-1.85556	1.43083
C	1.97595	-2.14201	0.92260
C	2.03278	-1.31313	-0.36477
C	-1.47772	-0.95465	0.18932
C	-3.88267	-0.14035	-1.05552
C	3.95396	0.29879	-0.22205
C	1.97760	1.18705	0.14011
C	-3.85941	1.26631	-0.46618
C	-2.52798	-2.38632	-2.00392
C	4.04397	1.65999	0.05178
C	-5.28186	1.80861	-0.29945
C	5.34457	2.20901	0.04247
C	-6.09863	0.94539	0.70450
C	6.13504	0.09810	-0.47089
H	-0.42352	-2.79557	-0.22663
H	0.51272	-0.89736	1.96118

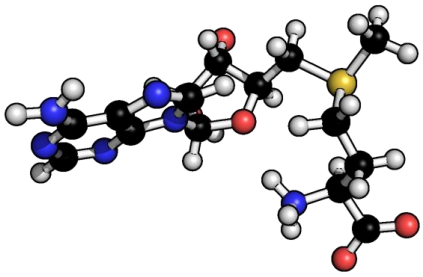


H	2.76076	-1.85002	1.62990
H	2.63300	-1.81904	-1.12570
H	-2.22132	-1.46533	0.80819
H	-1.27516	0.04454	0.58192
H	-4.32141	-0.87477	-0.37928
H	-4.41355	-0.16411	-2.01196
H	0.31145	-2.78937	3.12065
H	1.52670	-3.98829	1.20391
H	0.90139	1.22925	0.23775
H	-3.36994	1.26550	0.51451
H	-3.29864	1.93424	-1.12909
H	-2.98233	-2.94658	-1.18512
H	-1.58122	-2.82590	-2.31781
H	-3.20347	-2.31277	-2.85701
H	-5.79044	1.86446	-1.26234
H	-5.29744	3.06133	1.29876
H	-4.34193	3.68709	0.05074
H	7.01275	-0.50796	-0.68357
H	6.50771	3.87148	0.13575
H	4.81636	4.15796	0.34087
H	-5.99236	3.76684	-0.03224

Influence of SAM bound state dielectric environment on calculated BIEs. Energy minimized structures for the SAM bound state were calculated including water (ε = 78.4), methanol (ε = 32.6), acetone (ε = 20.4), dichloroethane (ε = 10.1) and chlorobenze (ε = 5.7) as implicit solvent models (PCM). No constraints on geometry were imposed.

Atomic coordinated for SAM bound state in water:

N	2.10698	-1.97169	-0.73220
C	3.45862	-1.49132	-0.31335
C	4.50157	-2.51621	-0.85713
O	4.08236	-3.26156	-1.77463
C	3.54896	-1.27983	1.18878
C	2.60598	-0.21717	1.74497
S	3.04134	1.43220	1.07096
C	3.58169	2.31105	2.55110
C	1.42879	2.21211	0.75172
C	0.80223	1.73349	-0.54265
O	0.32508	0.38092	-0.42176
C	-0.43772	2.56383	-0.91852
O	-0.13057	3.74094	-1.60488
C	-1.24192	1.55412	-1.75119
O	-0.79447	1.55091	-3.09060
C	-0.94993	0.23624	-1.02634
O	5.63601	-2.45716	-0.34974
N	-5.70301	-1.40978	-0.09525
C	-5.02162	-1.22154	-1.23443
N	-3.77642	-0.78228	-1.39664
C	-3.19892	-0.51951	-0.21467
C	-3.76808	-0.66204	1.04643
C	-5.09745	-1.13813	1.07536
N	-5.76161	-1.35819	2.22328
N	-2.87615	-0.29625	2.03486
C	-1.79946	0.05838	1.37935
N	-1.92899	-0.05146	0.01520
H	2.26162	-2.49733	-1.60863
H	1.42985	-1.20826	-0.86655
H	1.71194	-2.63321	-0.05600
H	3.62151	-0.55002	-0.85043
H	4.58745	-1.02862	1.41769
H	3.32556	-2.21935	1.70789
H	2.68205	-0.13895	2.83122
H	1.56014	-0.39021	1.47840
H	4.47529	1.79576	2.90596
H	2.78634	2.28897	3.29645
H	3.82195	3.33078	2.24996



H	1.65238	3.27957	0.65567
H	0.78360	2.04604	1.61960
H	1.53672	1.77938	-1.35632
H	-0.99313	2.82835	-0.01238
H	0.02661	3.48087	-2.53117
H	-2.30921	1.77871	-1.77381
H	0.05297	1.08115	-3.16679
H	-0.93833	-0.60948	-1.71983
H	-5.56594	-1.45976	-2.14531
H	-6.75323	-1.55093	2.17974
H	-5.39079	-1.00026	3.09255
H	-0.87603	0.39593	1.82813

Atomic coordinated for SAM bound state in methanol:

N	2.10472	-1.99316	-0.72641
C	3.44939	-1.49168	-0.30772
C	4.50667	-2.49970	-0.85882
O	4.09250	-3.24788	-1.77631
C	3.54032	-1.28373	1.19437
C	2.59464	-0.22333	1.75014
S	3.03415	1.42519	1.07697
C	3.57093	2.30572	2.55750
C	1.42490	2.20899	0.75177
C	0.80215	1.73264	-0.54517
O	0.32931	0.37787	-0.43050
C	-0.43950	2.56162	-0.91958
O	-0.13344	3.74116	-1.60141
C	-1.23977	1.55237	-1.75617
O	-0.78584	1.55262	-3.09334
C	-0.94807	0.23398	-1.03254
O	5.64089	-2.42344	-0.35552
N	-5.69980	-1.41010	-0.09357
C	-5.01865	-1.22672	-1.23351
N	-3.77311	-0.78880	-1.39795
C	-3.19561	-0.52186	-0.21718
C	-3.76402	-0.65926	1.04466
C	-5.09394	-1.13447	1.07585
N	-5.75819	-1.34762	2.22423
N	-2.87163	-0.29029	2.03150
C	-1.79538	0.06160	1.37453
N	-1.92500	-0.05332	0.01046
H	2.27464	-2.52517	-1.59748
H	1.41782	-1.24087	-0.87035
H	1.71685	-2.65488	-0.04649
H	3.59656	-0.54598	-0.84205
H	4.57851	-1.03017	1.42247
H	3.32099	-2.22461	1.71274
H	2.66610	-0.14602	2.83689
H	1.55008	-0.39570	1.47781
H	4.46280	1.79047	2.91666
H	2.77278	2.28616	3.29996
H	3.81346	3.32467	2.25551
H	1.65062	3.27622	0.65703
H	0.77587	2.04448	1.61717
H	1.53772	1.78434	-1.35771
H	-0.99606	2.82168	-0.01273
H	0.01584	3.48603	-2.53043
H	-2.30728	1.77522	-1.78300
H	0.06001	1.07999	-3.16716
H	-0.93772	-0.61115	-1.72685
H	-5.56338	-1.46810	-2.14326
H	-6.74790	-1.54991	2.18314
H	-5.38370	-0.99624	3.09440
H	-0.87171	0.39988	1.82231

Atomic coordinated for SAM bound state in acetone:

N	2.10190	-2.00453	-0.72297
C	3.44270	-1.49216	-0.30367
C	4.50732	-2.49119	-0.86012
O	4.09405	-3.23974	-1.77764

C	3.53419	-1.28769	1.19841
C	2.58781	-0.22792	1.75390
S	3.03098	1.41908	1.07932
C	3.56751	2.30203	2.55863
C	1.42399	2.20655	0.75152
C	0.80220	1.73247	-0.54664
O	0.33217	0.37623	-0.43648
C	-0.44083	2.56099	-0.91849
O	-0.13547	3.74279	-1.59593
C	-1.23835	1.55276	-1.75862
O	-0.77911	1.55665	-3.09395
C	-0.94723	0.23342	-1.03675
O	5.64103	-2.40589	-0.35912
N	-5.69690	-1.41138	-0.09237
C	-5.01569	-1.23210	-1.23272
N	-3.76995	-0.79484	-1.39870
C	-3.19306	-0.52396	-0.21885
C	-3.76110	-0.65702	1.04352
C	-5.09138	-1.13183	1.07620
N	-5.75629	-1.33874	2.22464
N	-2.86881	-0.28453	2.02916
C	-1.79294	0.06535	1.37126
N	-1.92212	-0.05438	0.00720
H	2.28227	-2.54049	-1.59065
H	1.41038	-1.25798	-0.87283
H	1.71730	-2.66632	-0.04138
H	3.58146	-0.54377	-0.83575
H	4.57257	-1.03411	1.42608
H	3.31647	-2.22943	1.71587
H	2.65658	-0.15042	2.84091
H	1.54384	-0.39910	1.47812
H	4.45827	1.78686	2.92053
H	2.76815	2.28551	3.29992
H	3.81186	3.31999	2.25470
H	1.65148	3.27358	0.65771
H	0.77302	2.04334	1.61578
H	1.53759	1.78862	-1.35923
H	-0.99778	2.81688	-0.01056
H	0.00570	3.49252	-2.52758
H	-2.30600	1.77438	-1.78872
H	0.06470	1.08052	-3.16658
H	-0.93776	-0.61076	-1.73233
H	-5.56029	-1.47674	-2.14163
H	-6.74412	-1.54978	2.18517
H	-5.37895	-0.99326	3.09576
H	-0.86934	0.40481	1.81836

Atomic coordinated for SAM bound state in dichloroethane:

N	2.10042	-2.02700	-0.70837
C	3.43232	-1.48925	-0.29084
C	4.51174	-2.46926	-0.86045
O	4.09717	-3.21704	-1.77803
C	3.52920	-1.28857	1.21012
C	2.58140	-0.23002	1.76468
S	3.02624	1.41332	1.08153
C	3.55761	2.30638	2.55700
C	1.42077	2.20121	0.74535
C	0.80142	1.72370	-0.55267
O	0.33839	0.36447	-0.44549
C	-0.44522	2.54824	-0.92346
O	-0.14197	3.73267	-1.59512
C	-1.23431	1.53794	-1.76832
O	-0.76184	1.54470	-3.09871
C	-0.94437	0.22012	-1.04376
O	5.64428	-2.36593	-0.36544
N	-5.69935	-1.39702	-0.08860
C	-5.01830	-1.22632	-1.22986
N	-3.76965	-0.79744	-1.39898

C	-3.19120	-0.52625	-0.22063
C	-3.75746	-0.65157	1.04295
C	-5.09143	-1.11757	1.07851
N	-5.75777	-1.31054	2.22686
N	-2.86144	-0.28234	2.02650
C	-1.78491	0.05885	1.36675
N	-1.91618	-0.06370	0.00228
H	2.30544	-2.56958	-1.56977
H	1.39808	-1.29332	-0.86839
H	1.72555	-2.69094	-0.02375
H	3.55095	-0.53680	-0.82094
H	4.56848	-1.03361	1.43310
H	3.31743	-2.23097	1.72888
H	2.64789	-0.14860	2.85179
H	1.53824	-0.40228	1.48572
H	4.44816	1.79597	2.92610
H	2.75541	2.29397	3.29551
H	3.80175	3.32287	2.24777
H	1.64850	3.26814	0.64750
H	0.76628	2.04339	1.60826
H	1.53622	1.78579	-1.36578
H	-1.00444	2.79763	-0.01482
H	-0.01636	3.49009	-2.53111
H	-2.30236	1.75567	-1.80809
H	0.07904	1.06330	-3.16567
H	-0.93447	-0.62533	-1.73807
H	-5.56520	-1.47090	-2.13731
H	-6.74136	-1.54013	2.19217
H	-5.36861	-0.99060	3.10210
H	-0.85856	0.39286	1.81237

Atomic coordinated for SAM bound state in chlorobenzene:

N	1.75615	-2.20052	-0.53267
C	3.13016	-1.66263	-0.29579
C	4.12254	-2.69073	-0.94752
O	3.57715	-3.47380	-1.76178
C	3.41993	-1.39866	1.16909
C	2.61350	-0.26517	1.79768
S	3.13051	1.34706	1.07814
C	3.68728	2.25354	2.53751
C	1.56559	2.21072	0.72781
C	0.91477	1.77606	-0.56878
O	0.40119	0.43588	-0.48301
C	-0.30688	2.65812	-0.89703
O	0.03081	3.84398	-1.54641
C	-1.15390	1.70055	-1.74743
O	-0.71836	1.73242	-3.08939
C	-0.89706	0.35324	-1.06629
O	5.30685	-2.57823	-0.60656
N	-5.58253	-1.42503	-0.05117
C	-4.91298	-1.26045	-1.19965
N	-3.68183	-0.78819	-1.38664
C	-3.11262	-0.46120	-0.21968
C	-3.66877	-0.57132	1.04976
C	-4.98299	-1.08939	1.10450
N	-5.63685	-1.27388	2.26105
N	-2.78613	-0.13623	2.01850
C	-1.72794	0.22908	1.34473
N	-1.85524	0.05807	-0.01525
H	1.89067	-2.82461	-1.35939
H	1.06916	-1.45921	-0.70468
H	1.42549	-2.78860	0.23908
H	3.19829	-0.74052	-0.88495
H	4.49155	-1.19860	1.24361
H	3.23242	-2.30617	1.75488
H	2.79564	-0.19209	2.87244
H	1.53637	-0.35395	1.62807
H	4.57026	1.73549	2.91386
H	2.88746	2.26927	3.27895
H	3.94947	3.26000	2.20927
H	1.84817	3.26475	0.62902
H	0.90456	2.09595	1.59331

H	1.64076	1.83106	-1.39037
H	-0.83698	2.90679	0.03017
H	0.06920	3.63320	-2.49769
H	-2.21452	1.95523	-1.75123
H	0.09719	1.21744	-3.20139
H	-0.92975	-0.46833	-1.78862
H	-5.45323	-1.55135	-2.09718
H	-6.60934	-1.54761	2.23746
H	-5.25448	-0.91883	3.12550
H	-0.81639	0.61572	1.77662

Influence of interactions with active site amino acids on calculated BIEs. To mimic the active site environment of NSD2, atoms for the backbone carbonyls of F1117 and R1138 and the side chain of Y1179 were included as shown in Figure S4. Initial coordinates for each amino acid were derived from the crystallographic position of the equivalent amino acids in the structure of NSD1 (pdb 3OOI).[9] A single atom from each amino acid residue was fixed in its crystallographic position and the positions of the remaining atoms were optimized. To reduce calculation time, bound SAM was simplified to the ethyl-ehtyl-methyl sulfonium ion. The average O–H distance between the SAM Me group hydrogen and the carbonyl oxygen of F1117 and R1138 or side chain oxygen of Y1179 where constrained to 2.50, 2.30, 2.20, 2.10, 1.95, 1.85, 1.80 and 1.75 Å with no other geometric constraints.

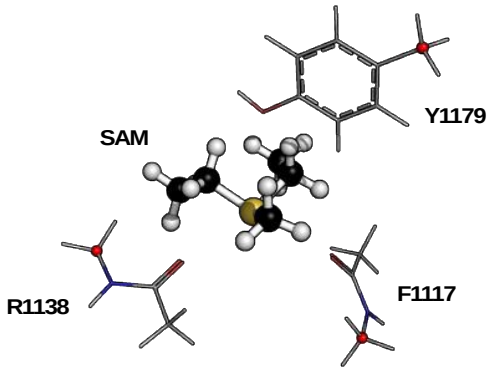


Figure S4. Bound state model of SAM including "methyltransfer pore" amino acid residue interactions. Calculations included atoms for the backbone carbonyls of F1117 and R1138 and the side chain of Y1179. Atoms locked in their crystallographic position are shown as red spheres. BIEs were calculated for models where the average O–H distance between the SAM Me group hydrogen and the carbonyl oxygen of F1117 and R1138 or side chain oxygen of Y1179 where constrained to 2.50, 2.30, 2.20, 2.10, 1.95, 1.85, 1.80 and 1.75 Å. No other constraints on geometry where imposed. Calculated equilibrium BIE are summarized in Figure 2 and Table S2.

Atomic coordinated for SAM bound state no constraint on O–H distances:

C	-2.45248	-1.61584	2.45598
C	-1.25215	-1.52511	1.52458
S	-1.10387	0.17511	0.86675
C	-0.33855	-0.10371	-0.74327
C	0.30750	0.79536	1.84208
C	-0.07599	0.97269	3.30485
O	-3.12138	-1.45283	-0.74381
C	-3.92098	-1.97367	-1.52481
C	-4.04439	-1.50874	-2.95689
N	-4.71348	-2.99750	-1.14890
C	-4.69374	-3.49408	0.21127
O	-0.62635	2.86295	-0.19819
C	-0.91949	4.03679	0.03811

C	-0.75422	4.61758	1.42541
N	-1.40094	4.87038	-0.90303
C	-1.64857	4.46082	-2.27299
O	1.99182	-1.85216	0.76809
C	3.21631	-1.51453	0.22486
C	4.25910	-2.42851	0.14505
C	3.38274	-0.21684	-0.25326
C	5.47161	-2.03932	-0.41893
C	4.59944	0.15206	-0.81220
C	5.66553	-0.74787	-0.90660
C	6.97889	-0.32271	-1.51079
H	-3.36235	-1.37540	1.90115
H	-2.53589	-2.63540	2.84292
H	-2.36764	-0.93860	3.31002
H	-1.39797	-2.15455	0.64510
H	-0.30011	-1.76848	2.00409
H	-0.03433	0.87868	-1.10646
H	-1.12962	-0.52834	-1.36284
H	0.50187	-0.79014	-0.62601
H	0.54411	1.74574	1.35953
H	1.12487	0.08092	1.71113
H	-0.96276	1.60329	3.41764
H	0.75215	1.45649	3.82881
H	-0.26080	0.01468	3.79877
H	-3.08588	-1.66301	-3.45943
H	-4.25296	-0.43662	-2.95803
H	-4.82483	-2.02702	-3.51700
H	-5.38948	-3.36615	-1.80103
H	-5.30111	-2.87006	0.87555
H	-5.05884	-4.52130	0.25707
H	-3.66293	-3.47887	0.57094
H	-1.37385	4.04173	2.11817
H	0.28890	4.50523	1.73217
H	-1.03177	5.67096	1.49664
H	-1.62594	5.81806	-0.63777
H	-1.26666	3.44763	-2.38905
H	-1.13225	5.12520	-2.96977
H	-2.72170	4.47240	-2.49012
H	2.02574	-2.78138	1.04052
H	4.13024	-3.44179	0.51930
H	2.56536	0.49523	-0.18870
H	6.28109	-2.76143	-0.47947
H	4.72405	1.16512	-1.18644
H	6.83693	0.09401	-2.51262
H	7.46248	0.44747	-0.90105
H	7.66866	-1.16606	-1.59171

Atomic coordinated for SAM bound state with O–H distances of 2.50 Å:

C	-2.28722	-1.67634	2.39188
C	-1.16070	-1.56767	1.37391
S	-1.08297	0.13241	0.70276
C	-0.34582	-0.14275	-0.92212
C	0.35136	0.79128	1.61837
C	0.02019	0.97833	3.09238
O	-3.24471	-1.55141	-0.66309
C	-4.07441	-2.06928	-1.41330
C	-4.25303	-1.60196	-2.83902
N	-4.85265	-3.09373	-1.00930
C	-4.77990	-3.59814	0.34674
O	-0.82163	2.92469	-0.19309
C	-1.09302	4.07722	0.14710
C	-0.93541	4.52504	1.58416
N	-1.54879	5.00410	-0.71754
C	-1.80175	4.72210	-2.11851
O	2.14668	-1.89535	0.71442
C	3.36845	-1.51651	0.19586
C	4.47578	-2.35472	0.23840
C	3.46767	-0.25363	-0.38255
C	5.68459	-1.92315	-0.30155
C	4.68233	0.15983	-0.91452
C	5.81255	-0.66265	-0.88411
C	7.12628	-0.18735	-1.44903

H	-3.24048	-1.45945	1.90469
H	-2.31849	-2.69657	2.78498
H	-2.15399	-0.99593	3.23691
H	-1.36521	-2.20062	0.50915
H	-0.17094	-1.79573	1.77906
H	-0.01492	0.82846	-1.29082
H	-1.13839	-0.54187	-1.55433
H	0.48369	-0.84540	-0.82897
H	0.54904	1.74273	1.12056
H	1.17742	0.09070	1.46743
H	-0.87467	1.59213	3.23064
H	0.85741	1.48569	3.57830
H	-0.12478	0.02265	3.60323
H	-3.32100	-1.77459	-3.38390
H	-4.44158	-0.52626	-2.83187
H	-5.06645	-2.10614	-3.36400
H	-5.55507	-3.45834	-1.63518
H	-5.37708	-2.99017	1.03473
H	-5.12396	-4.63255	0.39435
H	-3.73835	-3.56518	0.67225
H	-1.63333	3.95529	2.20410
H	0.07935	4.29156	1.91653
H	-1.12042	5.59023	1.73563
H	-1.76603	5.92510	-0.36610
H	-1.38489	3.74002	-2.33761
H	-1.32011	5.47176	-2.74995
H	-2.87740	4.71278	-2.32281
H	2.22071	-2.80603	1.03623
H	4.39832	-3.34245	0.68773
H	2.59775	0.39539	-0.41848
H	6.54465	-2.58611	-0.26724
H	4.75463	1.14563	-1.36713
H	6.99065	0.27790	-2.42967
H	7.58973	0.55726	-0.79307
H	7.83128	-1.01430	-1.56228

Atomic coordinated for SAM bound state with O–H distances of 2.30 Å:

C	-2.44389	-1.61083	2.45139
C	-1.24830	-1.52605	1.51329
S	-1.09959	0.17130	0.84787
C	-0.33527	-0.11824	-0.76095
C	0.31510	0.79297	1.81759
C	-0.06480	0.97733	3.28040
O	-3.13488	-1.45675	-0.73625
C	-3.93729	-1.97988	-1.51274
C	-4.07114	-1.51463	-2.94377
N	-4.72353	-3.00695	-1.13246
C	-4.69257	-3.50450	0.22720
O	-0.64022	2.86853	-0.19937
C	-0.93168	4.04012	0.04911
C	-0.77342	4.60407	1.44425
N	-1.40693	4.88579	-0.88456
C	-1.65134	4.49172	-2.25967
O	1.99817	-1.86012	0.75321
C	3.22445	-1.51795	0.21692
C	4.27441	-2.42489	0.15235
C	3.38536	-0.22272	-0.26966
C	5.48860	-2.03102	-0.40472
C	4.60383	0.15099	-0.82147
C	5.67715	-0.74174	-0.90029
C	6.99278	-0.31097	-1.49545
H	-3.35629	-1.37188	1.90011
H	-2.52647	-2.62856	2.84328
H	-2.35377	-0.92997	3.30198
H	-1.40049	-2.15855	0.63713
H	-0.29413	-1.76961	1.98852
H	-0.01996	0.85848	-1.12940
H	-1.12798	-0.53725	-1.38191
H	0.49905	-0.81165	-0.64083
H	0.55201	1.74119	1.33104
H	1.13089	0.07650	1.68802
H	-0.94993	1.61039	3.39230

H	0.76552	1.46171	3.80036
H	-0.25047	0.02190	3.77891
H	-3.11600	-1.66785	-3.45302
H	-4.28075	-0.44273	-2.94312
H	-4.85489	-2.03356	-3.49863
H	-5.40210	-3.37808	-1.78048
H	-5.29572	-2.88181	0.89656
H	-5.05614	-4.53220	0.27471
H	-3.65911	-3.48831	0.57922
H	-1.41182	4.03234	2.12335
H	0.26356	4.47009	1.76279
H	-1.03342	5.66139	1.52313
H	-1.63199	5.83053	-0.60920
H	-1.26477	3.48172	-2.38749
H	-1.13723	5.16691	-2.94758
H	-2.72428	4.50121	-2.47783
H	2.03535	-2.78786	1.03018
H	4.14980	-3.43631	0.53301
H	2.56212	0.48355	-0.21757
H	6.30381	-2.74756	-0.45340
H	4.72407	1.16200	-1.20260
H	6.85489	0.11381	-2.49439
H	7.47321	0.45445	-0.87718
H	7.68354	-1.15315	-1.58007

Atomic coordinated for SAM bound state with O–H distances of 2.20 Å:

C	-2.51097	-1.56519	2.48485
C	-1.28861	-1.48904	1.58141
S	-1.10906	0.20782	0.92202
C	-0.34746	-0.08366	-0.68748
C	0.30331	0.80867	1.90746
C	-0.09163	0.99594	3.36593
O	-3.08332	-1.41400	-0.76683
C	-3.87989	-1.94184	-1.54652
C	-3.99939	-1.48704	-2.98210
N	-4.67237	-2.96366	-1.16570
C	-4.65703	-3.45008	0.19815
O	-0.53136	2.86454	-0.19297
C	-0.82347	4.04910	-0.01493
C	-0.64483	4.70048	1.33855
N	-1.31059	4.83337	-0.99454
C	-1.55581	4.35748	-2.34311
O	1.89210	-1.85925	0.73904
C	3.12860	-1.54153	0.20972
C	4.13573	-2.48956	0.08268
C	3.34332	-0.22922	-0.20499
C	5.36215	-2.11928	-0.46365
C	4.57335	0.12027	-0.74687
C	5.60467	-0.81375	-0.88763
C	6.93106	-0.41009	-1.47790
H	-3.40569	-1.31800	1.90867
H	-2.61366	-2.58191	2.87455
H	-2.43899	-0.88438	3.33737
H	-1.41790	-2.12026	0.70053
H	-0.35083	-1.73958	2.08473
H	-0.04569	0.89881	-1.05323
H	-1.14732	-0.51208	-1.29451
H	0.49082	-0.77202	-0.56262
H	0.56001	1.75352	1.42412
H	1.11042	0.08140	1.78459
H	-0.96674	1.64441	3.46779
H	0.74027	1.46398	3.89818
H	-0.30125	0.04284	3.85966
H	-3.03675	-1.63581	-3.47820
H	-4.21734	-0.41679	-2.99110
H	-4.77235	-2.01543	-3.54313
H	-5.34497	-3.33864	-1.81774
H	-5.25817	-2.81495	0.85754
H	-5.03380	-4.47272	0.25136
H	-3.62626	-3.44347	0.55847
H	-1.23148	4.14266	2.07329
H	0.40782	4.63117	1.62506

H	-0.94980	5.74830	1.36516
H	-1.53126	5.79426	-0.77715
H	-1.19440	3.33180	-2.40193
H	-1.01992	4.97249	-3.06992
H	-2.62647	4.37829	-2.57143
H	1.89301	-2.80017	0.97051
H	3.96847	-3.51456	0.40673
H	2.55363	0.50946	-0.10479
H	6.14367	-2.86758	-0.56104
H	4.73664	1.14548	-1.06990
H	6.80692	-0.00039	-2.48518
H	7.41632	0.35933	-0.86885
H	7.61078	-1.26287	-1.54345

Atomic coordinated for SAM bound state with O–H distances of 2.10 Å:

C	-2.57512	-1.51735	2.52247
C	-1.33442	-1.45093	1.64388
S	-1.12071	0.24526	0.99312
C	-0.36509	-0.05123	-0.61843
C	0.29502	0.81913	1.98923
C	-0.10650	1.00809	3.44560
O	-3.03716	-1.35888	-0.80039
C	-3.83092	-1.88912	-1.58167
C	-3.94025	-1.44213	-3.02037
N	-4.62940	-2.90534	-1.19937
C	-4.62579	-3.38291	0.16767
O	-0.41642	2.85143	-0.19120
C	-0.70472	4.04493	-0.07365
C	-0.50734	4.76660	1.24051
N	-1.20114	4.77667	-1.08830
C	-1.45096	4.23354	-2.41008
O	1.78186	-1.85674	0.71573
C	3.03024	-1.56553	0.19786
C	3.99896	-2.54643	0.02843
C	3.29512	-0.24601	-0.16128
C	5.23868	-2.20145	-0.50407
C	4.53783	0.07765	-0.69012
C	5.53149	-0.88958	-0.87326
C	6.86960	-0.51530	-1.45613
H	-3.45643	-1.25668	1.93163
H	-2.69779	-2.53439	2.90558
H	-2.51158	-0.84088	3.37921
H	-1.45070	-2.07702	0.75741
H	-0.41046	-1.71447	2.16587
H	-0.06392	0.93550	-0.97539
H	-1.17686	-0.47674	-1.21404
H	0.46770	-0.74664	-0.48870
H	0.57271	1.76015	1.50962
H	1.09045	0.07900	1.86765
H	-0.96605	1.67750	3.54393
H	0.73232	1.45203	3.98761
H	-0.34428	0.05789	3.93234
H	-2.97134	-1.58397	-3.50596
H	-4.16807	-0.37394	-3.03657
H	-4.70292	-1.98004	-3.58642
H	-5.29815	-3.28297	-1.85385
H	-5.22186	-2.73587	0.82001
H	-5.01798	-4.39955	0.22439
H	-3.59690	-3.38857	0.53353
H	-1.06654	4.23734	2.01641
H	0.55270	4.72776	1.50477
H	-0.82901	5.80957	1.22163
H	-1.41303	5.74940	-0.92084
H	-1.11645	3.19708	-2.40968
H	-0.89491	4.79225	-3.16675
H	-2.51901	4.26834	-2.64867
H	1.74577	-2.80576	0.90820
H	3.79161	-3.57687	0.30911
H	2.53396	0.51736	-0.02982
H	5.99106	-2.97429	-0.63355
H	4.74165	1.10863	-0.96854
H	6.76172	-0.12717	-2.47402

H	7.35740	0.26190	-0.85948
H	7.53903	-1.37776	-1.49554

Atomic coordinated for SAM bound state with O–H distances of 1.95 Å:

C	-2.64944	-1.45155	2.59917
C	-1.39494	-1.39689	1.73986
S	-1.13731	0.29679	1.09979
C	-0.39915	-0.01018	-0.51864
C	0.29323	0.83625	2.09364
C	-0.09864	1.03137	3.55153
O	-2.97135	-1.27541	-0.84686
C	-3.75533	-1.81499	-1.63220
C	-3.85416	-1.37628	-3.07395
N	-4.55087	-2.83293	-1.25058
C	-4.55713	-3.30281	0.11917
O	-0.24977	2.81438	-0.20561
C	-0.56280	4.00748	-0.15819
C	-0.35813	4.81340	1.10423
N	-1.09721	4.66083	-1.20599
C	-1.35386	4.03199	-2.48777
O	1.63507	-1.83604	0.66417
C	2.90116	-1.57745	0.17023
C	3.83211	-2.59046	-0.01947
C	3.22017	-0.25834	-0.14366
C	5.09008	-2.27826	-0.52837
C	4.48028	0.03164	-0.65064
C	5.43685	-0.96821	-0.85489
C	6.79217	-0.63227	-1.42041
H	-3.51956	-1.16663	2.00277
H	-2.79690	-2.47091	2.96729
H	-2.58507	-0.78699	3.46518
H	-1.50452	-2.01354	0.84564
H	-0.48521	-1.68190	2.27569
H	-0.09559	0.98394	-0.85999
H	-1.23222	-0.42905	-1.09506
H	0.42321	-0.72011	-0.37920
H	0.59418	1.77009	1.61225
H	1.07146	0.07892	1.96538
H	-0.92907	1.73565	3.65504
H	0.75814	1.43480	4.09716
H	-0.37737	0.08853	4.03127
H	-2.87820	-1.50829	-3.54786
H	-4.09484	-0.31101	-3.09787
H	-4.60407	-1.92616	-3.64551
H	-5.20983	-3.22040	-1.90927
H	-5.14769	-2.64481	0.76532
H	-4.96570	-4.31298	0.17596
H	-3.53048	-3.32246	0.49089
H	-0.89165	4.32072	1.92134
H	0.70682	4.81189	1.35024
H	-0.70369	5.84597	1.02747
H	-1.31953	5.63977	-1.09915
H	-1.05171	2.98833	-2.41136
H	-0.77606	4.51667	-3.27882
H	-2.41860	4.08231	-2.73811
H	1.56163	-2.78809	0.83039
H	3.58188	-3.61989	0.22803
H	2.48708	0.52952	0.00302
H	5.81411	-3.07507	-0.67342
H	4.72702	1.06168	-0.89556
H	6.70984	-0.26732	-2.44943
H	7.28334	0.15044	-0.83412
H	7.44599	-1.50748	-1.42765

Atomic coordinated for SAM bound state with O–H distances of 1.85 Å:

C	-2.72505	-1.36619	2.66397
C	-1.46209	-1.33976	1.81588
S	-1.15251	0.34495	1.17622
C	-0.43304	0.01472	-0.44651
C	0.29620	0.84757	2.16290
C	-0.08137	1.05067	3.62329
O	-2.94300	-1.20444	-0.87923
C	-3.71536	-1.75192	-1.67121
C	-3.80482	-1.31572	-3.11406
N	-4.50531	-2.77538	-1.29436
C	-4.51883	-3.24264	0.07629
O	-0.11087	2.77672	-0.23211
C	-0.43740	3.96786	-0.22440
C	-0.22836	4.82115	1.00536
N	-0.99473	4.57454	-1.28783
C	-1.25670	3.89568	-2.54273
O	1.52953	-1.82038	0.63411
C	2.80781	-1.59021	0.15550
C	3.71332	-2.62626	-0.03162
C	3.16348	-0.27741	-0.14462
C	4.98415	-2.34378	-0.52526
C	4.43613	-0.01775	-0.63726
C	5.36789	-1.04105	-0.83947
C	6.73600	-0.73930	-1.39342
H	-3.58383	-1.05566	2.06364
H	-2.90229	-2.38327	3.02526
H	-2.65032	-0.70758	3.53372
H	-1.57753	-1.95168	0.91891
H	-0.56578	-1.65033	2.36041
H	-0.11299	1.00961	-0.77968
H	-1.28621	-0.39161	-1.00904
H	0.37376	-0.71549	-0.29644
H	0.61989	1.77310	1.67886
H	1.05581	0.07246	2.02714
H	-0.88403	1.78529	3.73364
H	0.79212	1.41793	4.16788
H	-0.39469	0.11667	4.09928
H	-2.82168	-1.43291	-3.57677
H	-4.06170	-0.25431	-3.14166
H	-4.53979	-1.87761	-3.69331
H	-5.15399	-3.17130	-1.95820
H	-5.10036	-2.57486	0.72041
H	-4.94375	-4.24609	0.13219
H	-3.49344	-3.27935	0.45028
H	-0.75566	4.35750	1.84345
H	0.83781	4.83209	1.24519
H	-0.57886	5.84862	0.89185
H	-1.22237	5.55558	-1.21706
H	-0.98239	2.84894	-2.41766
H	-0.66027	4.32856	-3.35013
H	-2.31780	3.96173	-2.80462
H	1.42932	-2.77219	0.78861
H	3.43381	-3.65029	0.20676
H	2.44906	0.52811	-0.00031
H	5.68884	-3.15811	-0.66854
H	4.71210	1.00699	-0.87290
H	6.67383	-0.40579	-2.43459
H	7.22823	0.05519	-0.82439
H	7.37858	-1.62236	-1.36426

Atomic coordinated for SAM bound state with O–H distances of 1.80 Å:

C	-2.76314	-1.33927	2.68855
C	-1.49465	-1.31886	1.84875
S	-1.16525	0.36477	1.21643
C	-0.45114	0.03309	-0.40862
C	0.28863	0.84939	2.20404
C	-0.08459	1.04877	3.66597
O	-2.92308	-1.16894	-0.89572

C	-3.68508	-1.72072	-1.69516
C	-3.76133	-1.28617	-3.13911
N	-4.47376	-2.74722	-1.32484
C	-4.49882	-3.21227	0.04650
O	-0.05660	2.76040	-0.23714
C	-0.38053	3.95249	-0.25583
C	-0.16824	4.83286	0.95394
N	-0.93930	4.53548	-1.33156
C	-1.20456	3.82874	-2.57029
O	1.47616	-1.80632	0.61786
C	2.76105	-1.59177	0.14849
C	3.65109	-2.64058	-0.04109
C	3.13826	-0.28234	-0.13910
C	4.92905	-2.37437	-0.52501
C	4.41789	-0.03922	-0.62256
C	5.33462	-1.07541	-0.82727
C	6.70998	-0.79212	-1.37310
H	-3.61571	-1.01832	2.08467
H	-2.95151	-2.35673	3.04310
H	-2.68837	-0.68576	3.56213
H	-1.60894	-1.92546	0.94789
H	-0.60488	-1.64028	2.39784
H	-0.12538	1.03146	-0.73314
H	-1.31225	-0.36882	-0.96704
H	0.35066	-0.70513	-0.25430
H	0.62118	1.77374	1.72363
H	1.04195	0.06903	2.06222
H	-0.87734	1.79324	3.78138
H	0.79396	1.40065	4.21251
H	-0.41055	0.11605	4.13606
H	-2.77397	-1.40366	-3.59267
H	-4.01798	-0.22480	-3.17009
H	-4.49087	-1.84891	-3.72437
H	-5.11379	-3.14735	-1.99455
H	-5.07927	-2.53892	0.68567
H	-4.93221	-4.21219	0.10077
H	-3.47633	-3.25698	0.42756
H	-0.70476	4.39573	1.80050
H	0.89673	4.83782	1.19892
H	-0.50696	5.86097	0.81354
H	-1.16382	5.51864	-1.28330
H	-0.94623	2.78155	-2.41770
H	-0.59792	4.23196	-3.38546
H	-2.26332	3.90294	-2.83932
H	1.35978	-2.75815	0.76149
H	3.35421	-3.66177	0.18832
H	2.43508	0.53280	0.00698
H	5.62192	-3.19849	-0.67023
H	4.71124	0.98276	-0.84893
H	6.65991	-0.47744	-2.42083
H	7.20177	0.00952	-0.81397
H	7.34657	-1.67854	-1.32203

Atomic coordinated for SAM bound state with O–H distances of 1.75 Å:

C	-2.75969	-1.29773	2.79288
C	-1.54094	-1.29957	1.88125
S	-1.17435	0.38326	1.27048
C	-0.49303	0.05691	-0.37007
C	0.30984	0.81657	2.23668
C	-0.02031	0.99229	3.71172
O	-2.93625	-1.10803	-0.92826
C	-3.71349	-1.65210	-1.71845
C	-3.82935	-1.19331	-3.15194
N	-4.48299	-2.69205	-1.34566
C	-4.46319	-3.18349	0.01664
O	0.02240	2.74043	-0.23147
C	-0.30327	3.93198	-0.26855
C	-0.08742	4.83235	0.92557

N	-0.86923	4.49529	-1.35088
C	-1.13712	3.76768	-2.57698
O	1.41305	-1.81197	0.53187
C	2.70970	-1.60703	0.08970
C	3.58938	-2.66499	-0.09691
C	3.10919	-0.29859	-0.17023
C	4.88053	-2.40912	-0.55014
C	4.40227	-0.06604	-0.62286
C	5.30950	-1.11126	-0.82377
C	6.69958	-0.83986	-1.33808
H	-3.63409	-0.92430	2.25367
H	-2.96812	-2.31869	3.12528
H	-2.60955	-0.67784	3.68098
H	-1.73247	-1.87147	0.97055
H	-0.63563	-1.67428	2.36814
H	-0.15323	1.05823	-0.68078
H	-1.37075	-0.32593	-0.92123
H	0.30044	-0.69790	-0.23199
H	0.65185	1.74293	1.76707
H	1.04424	0.02517	2.05911
H	-0.80229	1.74164	3.86356
H	0.87658	1.32616	4.23944
H	-0.34099	0.05345	4.17268
H	-2.85525	-1.30603	-3.63450
H	-4.08332	-0.13095	-3.15816
H	-4.57687	-1.74448	-3.72534
H	-5.13498	-3.08887	-2.00572
H	-5.01175	-2.51625	0.68945
H	-4.90470	-4.18010	0.06629
H	-3.42815	-3.24895	0.35925
H	-0.63224	4.41692	1.77782
H	0.97634	4.83060	1.17535
H	-0.41487	5.86106	0.76418
H	-1.09278	5.47936	-1.31911
H	-0.89353	2.72029	-2.40329
H	-0.52080	4.14632	-3.39676
H	-2.19324	3.85046	-2.85378
H	1.27424	-2.76603	0.63647
H	3.27425	-3.68518	0.11171
H	2.41266	0.52326	-0.02899
H	5.56535	-3.24033	-0.69329
H	4.71387	0.95502	-0.82804
H	6.67765	-0.54416	-2.39234
H	7.17821	-0.02924	-0.78067
H	7.33254	-1.72649	-1.25466

Influence of interactions of SAM C–S bond length on calculated BIEs. The C–S bond length in the bound state structure of SAM was constrained to 1.810, 1.815, 1.820 and 1.850 Å and energy minimized using an implicit acetone solvent model (PCM).

Atomic coordinated for SAM bound state with C–S bond length of 1.810 Å:

N	2.10128	-2.00488	-0.72257
C	3.44229	-1.49278	-0.30364
C	4.50645	-2.49236	-0.85999
O	4.09265	-3.24101	-1.77721
C	3.53413	-1.28779	1.19834
C	2.58814	-0.22754	1.75361
S	3.03139	1.41893	1.07779
C	3.56857	2.30496	2.56186
C	1.42449	2.20682	0.75053
C	0.80230	1.73261	-0.54740
O	0.33220	0.37642	-0.43684
C	-0.44077	2.56111	-0.91907
O	-0.13561	3.74287	-1.59664
C	-1.23854	1.55282	-1.75893

O	-0.77982	1.55657	-3.09439
C	-0.94725	0.23354	-1.03698
O	5.64031	-2.40734	-0.35928
N	-5.69678	-1.41138	-0.09153
C	-5.01583	-1.23210	-1.23204
N	-3.77017	-0.79475	-1.39834
C	-3.19300	-0.52376	-0.21863
C	-3.76075	-0.65681	1.04385
C	-5.09096	-1.13180	1.07688
N	-5.75548	-1.33876	2.22553
N	-2.86823	-0.28442	2.02931
C	-1.79249	0.06544	1.37115
N	-1.92203	-0.05415	0.00713
H	2.28150	-2.54136	-1.59001
H	1.40998	-1.25818	-0.87277
H	1.71649	-2.66616	-0.04060
H	3.58129	-0.54464	-0.83612
H	4.57262	-1.03444	1.42573
H	3.31620	-2.22926	1.71621
H	2.65732	-0.14958	2.84055
H	1.54404	-0.39877	1.47832
H	4.45880	1.78890	2.92353
H	2.76747	2.28748	3.30114
H	3.81239	3.32256	2.25654
H	1.65215	3.27379	0.65648
H	0.77368	2.04382	1.61495
H	1.53750	1.78851	-1.36019
H	-0.99749	2.81705	-0.01102
H	0.00575	3.49252	-2.52824
H	-2.30619	1.77448	-1.78864
H	0.06403	1.08056	-3.16728
H	-0.93783	-0.61072	-1.73246
H	-5.56064	-1.47675	-2.14083
H	-6.74327	-1.55006	2.18647
H	-5.37785	-0.99324	3.09652
H	-0.86878	0.40490	1.81803

Atomic coordinated for SAM bound state with C–S bond length of 1.815 Å:

N	2.10006	-2.00502	-0.72293
C	3.44135	-1.49361	-0.30405
C	4.50498	-2.49373	-0.86045
O	4.09089	-3.24196	-1.77787
C	3.53337	-1.28868	1.19793
C	2.58797	-0.22792	1.75322
S	3.03180	1.41807	1.07655
C	3.57007	2.30663	2.56483
C	1.42511	2.20682	0.75022
C	0.80239	1.73339	-0.54774
O	0.33230	0.37718	-0.43765
C	-0.44081	2.56211	-0.91846
O	-0.13592	3.74425	-1.59550
C	-1.23895	1.55428	-1.75856
O	-0.78089	1.55885	-3.09425
C	-0.94732	0.23458	-1.03749
O	5.63882	-2.40948	-0.35956
N	-5.69589	-1.41241	-0.09084
C	-5.01529	-1.23292	-1.23153
N	-3.76983	-0.79512	-1.39816
C	-3.19249	-0.52385	-0.21859
C	-3.75989	-0.65704	1.04403
C	-5.08991	-1.13253	1.07741
N	-5.75408	-1.33960	2.22624
N	-2.86729	-0.28419	2.02925
C	-1.79188	0.06617	1.37082
N	-1.92167	-0.05367	0.00685
H	2.28002	-2.54178	-1.59025
H	1.40918	-1.25794	-0.87328
H	1.71488	-2.66594	-0.04083
H	3.58086	-0.54557	-0.83656
H	4.57201	-1.03588	1.42525
H	3.31496	-2.23004	1.71579
H	2.65747	-0.14977	2.84011

H	1.54372	-0.39879	1.47823
H	4.45975	1.78957	2.92615
H	2.76757	2.28846	3.30247
H	3.81361	3.32384	2.25826
H	1.65305	3.27378	0.65658
H	0.77450	2.04361	1.61472
H	1.53727	1.78970	-1.36077
H	-0.99713	2.81759	-0.01002
H	0.00502	3.49444	-2.52731
H	-2.30662	1.77594	-1.78761
H	0.06292	1.08289	-3.16788
H	-0.93815	-0.60931	-1.73343
H	-5.56022	-1.47787	-2.14017
H	-6.74181	-1.55125	2.18742
H	-5.37639	-0.99379	3.09708
H	-0.86824	0.40615	1.81745

Atomic coordinated for SAM bound state with C–S bond length of 1.820 Å:

N	2.10003	-2.00494	-0.72335
C	3.44157	-1.49412	-0.30448
C	4.50470	-2.49500	-0.86045
O	4.09018	-3.24349	-1.77748
C	3.53350	-1.28879	1.19745
C	2.58829	-0.22766	1.75240
S	3.03232	1.41795	1.07493
C	3.57145	2.30889	2.56757
C	1.42571	2.20731	0.74962
C	0.80227	1.73396	-0.54803
O	0.33224	0.37774	-0.43772
C	-0.44110	2.56267	-0.91819
O	-0.13650	3.74486	-1.59526
C	-1.23948	1.55485	-1.75808
O	-0.78188	1.55956	-3.09392
C	-0.94752	0.23508	-1.03726
O	5.63863	-2.41098	-0.35973
N	-5.69588	-1.41251	-0.09069
C	-5.01544	-1.23246	-1.23138
N	-3.77003	-0.79449	-1.39796
C	-3.19256	-0.52371	-0.21835
C	-3.75979	-0.65751	1.04428
C	-5.08977	-1.13312	1.07761
N	-5.75375	-1.34083	2.22643
N	-2.86712	-0.28499	2.02955
C	-1.79177	0.06563	1.37113
N	-1.92172	-0.05359	0.00712
H	2.27981	-2.54206	-1.59049
H	1.40953	-1.25757	-0.87402
H	1.71442	-2.66543	-0.04109
H	3.58166	-0.54635	-0.83732
H	4.57218	-1.03623	1.42483
H	3.31476	-2.22997	1.71551
H	2.65786	-0.14925	2.83927
H	1.54401	-0.39848	1.47752
H	4.46039	1.79061	2.92873
H	2.76733	2.29017	3.30335
H	3.81501	3.32569	2.25987
H	1.65386	3.27421	0.65586
H	0.77547	2.04427	1.61444
H	1.53677	1.79027	-1.36141
H	-0.99709	2.81802	-0.00953
H	0.00418	3.49512	-2.52712
H	-2.30717	1.77645	-1.78676
H	0.06181	1.08343	-3.16791
H	-0.93836	-0.60866	-1.73337
H	-5.56048	-1.47698	-2.14006
H	-6.74146	-1.55258	2.18766
H	-5.37595	-0.99547	3.09740
H	-0.86810	0.40543	1.81781

Atomic coordinated for SAM bound state with C–S bond length of 1.850 Å:

N	1.95657	-2.09792	-0.53050
C	3.33047	-1.57340	-0.26962
C	4.33744	-2.60914	-0.86480
O	3.83940	-3.40436	-1.69707
C	3.56555	-1.27832	1.20104
C	2.68819	-0.17055	1.77619
S	3.11953	1.43759	1.00101
C	3.69428	2.40839	2.46719
C	1.50531	2.21954	0.68849
C	0.84586	1.70844	-0.57553
O	0.34692	0.36944	-0.40620
C	-0.38827	2.54742	-0.95049
O	-0.07375	3.71389	-1.64956
C	-1.20416	1.53503	-1.76613
O	-0.75339	1.50524	-3.10372
C	-0.92851	0.22706	-1.01746
O	5.51313	-2.50586	-0.47637
N	-5.70374	-1.33917	-0.07426
C	-5.01763	-1.17767	-1.21432
N	-3.76576	-0.75851	-1.38048
C	-3.18816	-0.48692	-0.20110
C	-3.76020	-0.60322	1.06098
C	-5.09713	-1.05937	1.09376
N	-5.76783	-1.24865	2.24174
N	-2.86383	-0.23797	2.04580
C	-1.78141	0.09147	1.38749
N	-1.91129	-0.03425	0.02455
H	2.05903	-2.69605	-1.37010
H	1.27170	-1.34617	-0.68069
H	1.61873	-2.70006	0.22743
H	3.41705	-0.65805	-0.86768
H	4.62323	-1.02758	1.31472
H	3.38994	-2.18316	1.79464
H	2.84632	-0.04851	2.84962
H	1.62109	-0.32611	1.59477
H	4.58705	1.90068	2.83239
H	2.89508	2.41619	3.20831
H	3.92945	3.40812	2.10392
H	1.73283	3.28202	0.55154
H	0.87620	2.08923	1.57377
H	1.56421	1.72081	-1.40529
H	-0.93351	2.82421	-0.04162
H	0.05703	3.44727	-2.57815
H	-2.26878	1.77049	-1.79529
H	0.08810	1.02414	-3.17143
H	-0.92266	-0.62886	-1.69882
H	-5.56328	-1.42195	-2.12272
H	-6.75814	-1.44737	2.20111
H	-5.38780	-0.90331	3.11170
H	-0.85265	0.41857	1.83316

Influence of interactions of SAM C–H bond length restriction on calculated BIEs. The average Me group C–H bond lengths in the bound state structure of SAM were constrained to be 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 % shorter then equilibrium bond lengths (Figure 2b and Table S3). Structures for each were minimized using an implicit acetone solvent model (PCM). Energy-minimized structures with C–H bond lengths constrained by ≥1.0 % contained a single imaginary frequency and thus may not represent true stationary points on the energy surface for this simplified model, but are meant to mimic the effect of constraint imposed by the enzyme. BIEs were calculated from the scaled vibrational frequencies for all normal modes using Isoeff98 as described above.

Atomic coordinated for SAM bound state with 0.5 % restriction of C–H bond length:

N	2.10129	-2.00479	-0.72294
C	3.44210	-1.49245	-0.30365
C	4.50661	-2.49157	-0.86015
O	4.09302	-3.24033	-1.77736
C	3.53365	-1.28793	1.19842
C	2.58739	-0.22797	1.75377
S	3.03102	1.41895	1.07934
C	3.56831	2.30124	2.55933
C	1.42429	2.20688	0.75159
C	0.80254	1.73269	-0.54660
O	0.33225	0.37655	-0.43622
C	-0.44032	2.56132	-0.91875
O	-0.13479	3.74296	-1.59637
C	-1.23798	1.55306	-1.75876
O	-0.77882	1.55665	-3.09408
C	-0.94708	0.23380	-1.03663
O	5.64048	-2.40615	-0.35953
N	-5.69659	-1.41182	-0.09226
C	-5.01531	-1.23272	-1.23260
N	-3.76970	-0.79510	-1.39862
C	-3.19298	-0.52365	-0.21880
C	-3.76111	-0.65649	1.04353
C	-5.09123	-1.13175	1.07627
N	-5.75608	-1.33852	2.22476
N	-2.86897	-0.28367	2.02918
C	-1.79307	0.06617	1.37128
N	-1.92217	-0.05374	0.00724
H	2.28178	-2.54092	-1.59054
H	1.40985	-1.25820	-0.87298
H	1.71657	-2.66638	-0.04124
H	3.58089	-0.54406	-0.83575
H	4.57207	-1.03449	1.42609
H	3.31578	-2.22959	1.71596
H	2.65582	-0.15056	2.84080
H	1.54346	-0.39900	1.47776
H	4.45511	1.78833	2.91776
H	2.77303	2.28311	3.29696
H	3.81044	3.31442	2.25714
H	1.65204	3.27383	0.65760
H	0.77312	2.04395	1.61573
H	1.53805	1.78849	-1.35911
H	-0.99730	2.81745	-0.01092
H	0.00675	3.49249	-2.52790
H	-2.30559	1.77487	-1.78883
H	0.06501	1.08056	-3.16663
H	-0.93759	-0.61051	-1.73206
H	-5.55977	-1.47775	-2.14149
H	-6.74381	-1.55004	2.18543
H	-5.37882	-0.99265	3.09577
H	-0.86957	0.40590	1.81838

Atomic coordinated for SAM bound state with 1.0 % restriction of C–H bond length:

N	2.10053	-2.00461	-0.72387
C	3.44147	-1.49294	-0.30414
C	4.50567	-2.49241	-0.86055
O	4.09208	-3.24066	-1.77819
C	3.53272	-1.28880	1.19802
C	2.58665	-0.22863	1.75332
S	3.03123	1.41824	1.07944
C	3.56995	2.29943	2.56013
C	1.42490	2.20719	0.75234
C	0.80287	1.73360	-0.54596
O	0.33227	0.37752	-0.43593
C	-0.43984	2.56260	-0.91782
O	-0.13410	3.74432	-1.59520
C	-1.23782	1.55473	-1.75801
O	-0.77883	1.55863	-3.09339
C	-0.94710	0.23516	-1.03635
O	5.63938	-2.40772	-0.35945
N	-5.69584	-1.41317	-0.09283
C	-5.01439	-1.23383	-1.23304
N	-3.76901	-0.79548	-1.39883

C	-3.19274	-0.52349	-0.21891
C	-3.76109	-0.65647	1.04330
C	-5.09093	-1.13253	1.07579
N	-5.75594	-1.33949	2.22416
N	-2.86943	-0.28293	2.02910
C	-1.79358	0.06745	1.37141
N	-1.92226	-0.05278	0.00735
H	2.28103	-2.54083	-1.59141
H	1.40951	-1.25767	-0.87413
H	1.71526	-2.66601	-0.04229
H	3.58081	-0.54450	-0.83601
H	4.57116	-1.03579	1.42604
H	3.31436	-2.23052	1.71526
H	2.65450	-0.15158	2.84040
H	1.54278	-0.39915	1.47678
H	4.45251	1.78828	2.91503
H	2.77879	2.28011	3.29415
H	3.81041	3.30769	2.25967
H	1.65310	3.27407	0.65866
H	0.77368	2.04429	1.61642
H	1.53833	1.78944	-1.35850
H	-0.99669	2.81867	-0.00988
H	0.00723	3.49402	-2.52682
H	-2.30538	1.77675	-1.78789
H	0.06491	1.08240	-3.16620
H	-0.93771	-0.60888	-1.73210
H	-5.55847	-1.47938	-2.14202
H	-6.74354	-1.55161	2.18463
H	-5.37911	-0.99323	3.09519
H	-0.87041	0.40782	1.81870

Atomic coordinated for SAM bound state with 2.0 % restriction of C–H bond length:

N	2.09852	-2.00480	-0.72412
C	3.43977	-1.49404	-0.30432
C	4.50337	-2.49416	-0.86072
O	4.08939	-3.24208	-1.77846
C	3.53109	-1.29018	1.19789
C	2.58570	-0.22937	1.75310
S	3.03178	1.41705	1.07916
C	3.57303	2.29741	2.56049
C	1.42605	2.20755	0.75295
C	0.80359	1.73472	-0.54547
O	0.33232	0.37884	-0.43566
C	-0.43872	2.56440	-0.91714
O	-0.13246	3.74604	-1.59442
C	-1.23729	1.55705	-1.75745
O	-0.77859	1.56109	-3.09293
C	-0.94710	0.23713	-1.03621
O	5.63708	-2.41026	-0.35949
N	-5.69435	-1.41570	-0.09298
C	-5.01257	-1.23697	-1.23309
N	-3.76760	-0.79744	-1.39882
C	-3.19216	-0.52350	-0.21896
C	-3.76094	-0.65565	1.04315
C	-5.09030	-1.13303	1.07559
N	-5.75567	-1.33916	2.22389
N	-2.87017	-0.27990	2.02892
C	-1.79445	0.07102	1.37130
N	-1.92233	-0.05101	0.00733
H	2.27879	-2.54148	-1.59143
H	1.40817	-1.25732	-0.87474
H	1.71258	-2.66563	-0.04235
H	3.57977	-0.54563	-0.83604
H	4.56970	-1.03793	1.42601
H	3.31199	-2.23179	1.71500
H	2.65310	-0.15246	2.84019
H	1.54178	-0.39906	1.47618
H	4.44712	1.79036	2.90918
H	2.78999	2.27653	3.28718
H	3.81015	3.29551	2.26243
H	1.65489	3.27430	0.65959
H	0.77473	2.04479	1.61692

H	1.53905	1.79035	-1.35804
H	-0.99537	2.82067	-0.00913
H	0.00843	3.49579	-2.52612
H	-2.30477	1.77954	-1.78706
H	0.06499	1.08465	-3.16606
H	-0.93802	-0.60666	-1.73228
H	-5.55598	-1.48418	-2.14202
H	-6.74306	-1.55223	2.18416
H	-5.37961	-0.99138	3.09466
H	-0.87194	0.41307	1.81865

Atomic coordinated for SAM bound state with 3.0 % restriction of C–H bond length:

N	2.09846	-2.00453	-0.72442
C	3.43969	-1.49400	-0.30425
C	4.50329	-2.49436	-0.86022
O	4.08939	-3.24260	-1.77774
C	3.53048	-1.29023	1.19802
C	2.58474	-0.22953	1.75287
S	3.03126	1.41688	1.07931
C	3.57388	2.29650	2.56155
C	1.42578	2.20792	0.75327
C	0.80362	1.73520	-0.54539
O	0.33226	0.37934	-0.43564
C	-0.43853	2.56488	-0.91756
O	-0.13201	3.74630	-1.59512
C	-1.23706	1.55741	-1.75781
O	-0.77813	1.56109	-3.09322
C	-0.94710	0.23763	-1.03626
O	5.63689	-2.41050	-0.35872
N	-5.69356	-1.41738	-0.09286
C	-5.01154	-1.23936	-1.23294
N	-3.76679	-0.79921	-1.39873
C	-3.19188	-0.52374	-0.21897
C	-3.76096	-0.65502	1.04310
C	-5.09005	-1.13315	1.07561
N	-5.75565	-1.33851	2.22392
N	-2.87073	-0.27774	2.02877
C	-1.79505	0.07326	1.37115
N	-1.92240	-0.05027	0.00725
H	2.27889	-2.54147	-1.59155
H	1.40839	-1.25689	-0.87544
H	1.71209	-2.66506	-0.04260
H	3.58000	-0.54564	-0.83596
H	4.56898	-1.03793	1.42655
H	3.31126	-2.23193	1.71491
H	2.65125	-0.15294	2.84002
H	1.54103	-0.39915	1.47522
H	4.43942	1.79386	2.90461
H	2.79842	2.27382	3.28033
H	3.80748	3.28450	2.26590
H	1.65474	3.27463	0.66001
H	0.77405	2.04521	1.61689
H	1.53937	1.79077	-1.35771
H	-0.99538	2.82144	-0.00975
H	0.00896	3.49578	-2.52673
H	-2.30451	1.78001	-1.78766
H	0.06538	1.08446	-3.16612
H	-0.93802	-0.60630	-1.73217
H	-5.55451	-1.48785	-2.14179
H	-6.74291	-1.55217	2.18409
H	-5.38006	-0.98963	3.09444
H	-0.87290	0.41636	1.81845

Atomic coordinated for SAM bound state with 4.0 % restriction of C–H bond length:

N	2.09712	-2.00457	-0.72496
C	3.43866	-1.49482	-0.30478
C	4.50169	-2.49580	-0.86072
O	4.08735	-3.24390	-1.77816
C	3.52956	-1.29122	1.19750
C	2.58432	-0.23010	1.75244

S	3.03167	1.41610	1.07893
C	3.57698	2.29488	2.56175
C	1.42662	2.20847	0.75417
C	0.80397	1.73641	-0.54454
O	0.33236	0.38060	-0.43516
C	-0.43805	2.56647	-0.91628
O	-0.13136	3.74800	-1.59357
C	-1.23689	1.55941	-1.75674
O	-0.77813	1.56353	-3.09221
C	-0.94705	0.23926	-1.03579
O	5.63540	-2.41236	-0.35943
N	-5.69271	-1.41871	-0.09348
C	-5.01045	-1.24048	-1.23339
N	-3.76593	-0.79961	-1.39888
C	-3.19152	-0.52360	-0.21901
C	-3.76089	-0.65504	1.04291
C	-5.08971	-1.13392	1.07511
N	-5.75555	-1.33940	2.22327
N	-2.87121	-0.27699	2.02878
C	-1.79555	0.07453	1.37141
N	-1.92241	-0.04927	0.00750
H	2.27728	-2.54175	-1.59200
H	1.40752	-1.25653	-0.87611
H	1.71029	-2.66474	-0.04306
H	3.57956	-0.54651	-0.83641
H	4.56816	-1.03942	1.42608
H	3.30989	-2.23286	1.71432
H	2.65074	-0.15380	2.83957
H	1.54055	-0.39920	1.47471
H	4.43382	1.79614	2.89885
H	2.80951	2.27109	3.27307
H	3.80759	3.27257	2.26828
H	1.65610	3.27507	0.66121
H	0.77490	2.04582	1.61775
H	1.53958	1.79204	-1.35698
H	-0.99474	2.82292	-0.00834
H	0.00928	3.49769	-2.52529
H	-2.30431	1.78218	-1.78638
H	0.06533	1.08685	-3.16536
H	-0.93804	-0.60431	-1.73213
H	-5.55302	-1.48944	-2.14235
H	-6.74270	-1.55354	2.18319
H	-5.38041	-0.99012	3.09383
H	-0.87376	0.41826	1.81896

Atomic coordinated for SAM bound state with 5.0 % restriction of C–H bond length:

N	2.09654	-2.00440	-0.72556
C	3.43810	-1.49498	-0.30505
C	4.50106	-2.49611	-0.86086
O	4.08678	-3.24411	-1.77841
C	3.52867	-1.29169	1.19729
C	2.58345	-0.23055	1.75222
S	3.03129	1.41573	1.07920
C	3.57770	2.29376	2.56302
C	1.42651	2.20873	0.75480
C	0.80406	1.73719	-0.54423
O	0.33252	0.38131	-0.43536
C	-0.43796	2.56730	-0.91586
O	-0.13126	3.74899	-1.59289
C	-1.23678	1.56043	-1.75658
O	-0.77804	1.56486	-3.09206
C	-0.94691	0.24009	-1.03599
O	5.63469	-2.41290	-0.35932
N	-5.69180	-1.42000	-0.09346
C	-5.00950	-1.24201	-1.23338
N	-3.76516	-0.80065	-1.39895
C	-3.19098	-0.52388	-0.21914
C	-3.76042	-0.65501	1.04278
C	-5.08906	-1.13440	1.07506
N	-5.75495	-1.33957	2.22324
N	-2.87104	-0.27607	2.02857
C	-1.79548	0.07568	1.37117

N	-1.92213	-0.04882	0.00729
H	2.27674	-2.54153	-1.59262
H	1.40711	-1.25620	-0.87671
H	1.70943	-2.66457	-0.04379
H	3.57932	-0.54660	-0.83646
H	4.56725	-1.04008	1.42618
H	3.30874	-2.23341	1.71385
H	2.64952	-0.15476	2.83938
H	1.53974	-0.39936	1.47412
H	4.42565	1.79923	2.89504
H	2.81762	2.26866	3.26623
H	3.80532	3.26125	2.27212
H	1.65614	3.27532	0.66224
H	0.77443	2.04590	1.61802
H	1.53980	1.79312	-1.35653
H	-0.99472	2.82355	-0.00790
H	0.00927	3.49889	-2.52468
H	-2.30421	1.78318	-1.78615
H	0.06548	1.08829	-3.16532
H	-0.93792	-0.60326	-1.73259
H	-5.55187	-1.49161	-2.14229
H	-6.74202	-1.55404	2.18318
H	-5.38001	-0.98972	3.09367
H	-0.87390	0.42002	1.81867

D. Supporting Information References.

(1) Poulin, M. B.; Schneck, J. L.; Matico, R. E.; McDevitt, P. J.; Huddleston, M. J.; Hou, W.; Johnson, N. W.; Thrall, S. H.; Meek, T. D.; Schramm, V. L., *Proc. Natl. Acad. Sci. U. S. A.* **2016,** *113*, 1197.
(2) Markham, G. D.; Hafner, E. W.; Tabor, C. W.; Tabor, H., *J. Biol. Chem.* **1980,** *255*, 9082.
(3) Luger, K.; Rechsteiner, T. J.; Richmond, T. J., *Methods Enzymol.* **1999,** *304*, 3.
(4) Dyer, P. N.; Edayathumangalam, R. S.; White, C. L.; Bao, Y.; Chakravarthy, S.; Muthurajan, U. M.; Luger, K., *Methods Enzymol.* **2004,** *375*, 23.
(5) Stolowitz, M. L.; Minch, M. J., *J. Am. Chem. Soc.* **1981,** *103*, 6015.
(6) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery Jr., J. A.; Peralta, J. E.; Ogliaro, F.; Bearpark, M. J.; Heyd, J.; Brothers, E. N.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A. P.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, N. J.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, Ö.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. 2009, *Gaussian 09*, Gaussian, Inc.: Wallingford, CT.
(7) Anisimov, V.; Paneth, P., *J. Math. Chem.* **1999,** *26*, 75.
(8) Stratton, C. F.; Namanja-Magliano, H. A.; Cameron, S. A.; Schramm, V. L., *ACS Chem. Biol.* **2015,** *10*, 2182.
(9) Qiao, Q.; Li, Y.; Chen, Z.; Wang, M.; Reinberg, D.; Xu, R. M., *J. Biol. Chem.* **2011,** *286*, 8361.
(10) Kim, S.; Bolton, E. E.; Bryant, S. H., *J. Cheminform.* **2013,** *5*, 1.