Equatorial Active Site Compaction and Electrostatic Reorganization in Catechol-O-methyltransferase

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Experimental Details

Materials
Isotopically-labelled compounds: $^{15}$N-labelled ammonium chloride (99%), $^{13}$C$_6$H$_7$-labelled D-Glucose (U-$^{13}$C$_6$, 99%; 1,2,3,4,5,6,6-d$_7$; 97-98%) and deuterium oxide (99.8%) were purchased from Goss Scientific. 3,5-dinitrocatechol (DNC), S-adenosyl-L-methionine (SAM) and sinefungin (5′-deoxy-5′-(1,4-diamino-4-carboxybutyl)adenosine) were purchased with the highest purity available from Sigma-Aldrich (Dorset, UK) and used as received.

Protein expression and purification
Expression and purification of human soluble catechol-$O$-methyltransferase (S-COMT) for NMR studies was performed as described previously. Protein purification for crystallography involved two more steps following nickel affinity chromatography. Fractions showing S-COMT content, observed as a peak in UV absorbance at 280 nm after affinity chromatography, were pooled and loaded onto a HiPrep desalting 26/10 column. The column was washed with at least 1.5 column volumes of filtered and degassed water and equilibrated with 2 column volumes of filtered and degassed buffer (50 mM Tris buffer pH 8 containing 20 mM NaCl). Proteins were then eluted and loaded onto an anion exchange HiTrap Q FF column using 50 mM Tris buffer pH 8 as a baseline buffer. The fractions were then eluted with 50 mM Tris-HCl, 1 M NaCl, pH 8 and the fractions containing S-COMT were concentrated as described previously.

Crystallogenesis
The sitting drop vapour diffusion technique was used to grow crystals of two complexes of human S-COMT with 3,5-dinitrocatechol (DNC), Mg$^{2+}$ and either S-adenosyl-L-methionine (SAM) or its derivative sinefungin. The purified protein was collected, diluted (1 μM) and mixed with SAM/sinefungin (10 μM) and DNC (10 μM) overnight at 4 °C through gentle rocking. The COMT protein complexes were then concentrated to a final concentration of 6 mg/mL protein with bound DNC and SAM/sinefungin ligand in a 50 mM Tris buffer (with 100 mM NaCl, 10 mM DTT, 2mM MgCl2) at pH 7.4. S-COMT:DNC:Mg$^{2+}$:sinefungin complex crystals were grown by mixing 200 nL of protein with an equal volume of reservoir solution comprising 0.1 M amino acid stock (0.2 M L-Na-glutamate; 0.2 M alanine (racemic); 0.2M glycine; 0.2 M lysine HCl (racemic); 0.2 M serine (racemic)), 0.1 M imidazole; MES monohydrate (acid) pH 6.5, 50% v/v precipitant mix (25% v/v MPD; 25% PEG 3350) Morpheus condition H4. S-COMT:DNC:Mg$^{2+}$:SAM complex crystals were grown from a reservoir comprising 0.1 M alcohols mix (0.2 M 1,6-hexanediol; 0.2 M 1-butanediol; 0.2 M 1,2-propanediol; 0.2 M 2-propanol; 0.2 M 1,4-butanediol; 0.2 M 1,3-propanediol), 0.1 M imidazole; MES monohydrate (acid) pH 6.5, 50% v/v precipitant mix (25% v/v MPD; 25% PEG 3350) Morpheus condition D4. All drops were set using a Mosquito (TTP) pipetting robot and incubated at 4 °C. Single crystals suitable for X-ray analysis were flash frozen by plunge freezing in liquid nitrogen.

Crystallography data collection and structure determination
Data were collected from single cryogenically frozen crystals at Diamond Light Source, Oxfordshire, and full details of data collection and refinement statistics are presented in Table S1. Structures were subsequently solved by molecular replacement in Phaser. All models were subsequently completed and refined using iterative cycles of rebuilding and refinement in COOT and Phenix.refine. Validation with Molprobity was integrated into the iterative rebuilding and refinement cycle. Final models with R and Rf of 0.1452 & 0.1798 for the S-COMT:SAM:DNC:Mg$^{2+}$ complex and 0.1169 & 0.1492 for the S-
COMT:sinefungin:DNC:Mg$^{2+}$ complex have been deposited in the protein data bank, with accession codes 6I3C and 6I3D, respectively.

NMR experiments
All NMR measurements were performed at 298 K, using standard pulse sequences on an 800 MHz Bruker Avance III NMR spectrometer with TCI cryoprobe equipped with Z gradients and TopSpin software version 3.2 housed in the Manchester Institute of Biotechnology. NMR samples containing 0.5 mM human S-COMT, 2.5 mM MgCl$_2$, 5 mM DNC and 5 mM SAM or 5 mM sinefungin in 50 mM Tris-HCl buffer, 50 mM NaCl, 10 mM DTT, 2 mM NaN$_3$, pH 7.5 were loaded into 5-mm diameter NMR tubes. $^2$H$_2$O was added to the protein samples (10% v/v) to allow a deuterium lock and 0.5% v/v trimethylsilyl propanoic acid (TSP) was added as a reference signal. For the backbone $^1$H, $^{13}$C and $^{15}$N resonance assignment, transverse relaxation-optimised spectroscopy (TROSY)-based 3D HNCACB, HN(CO)CACB, HNCO, HN(CA)CO, HNCA and HN(CO)CA spectra were acquired using a non-uniform sampling strategy (Table S2). All NMR spectra were analysed using CCPNMR Analysis version 2.4.6. Backbone resonances were assigned using standard triple resonance methodology. Prediction of solution secondary structure from the backbone chemical shifts using the TALOS+ webserver was in good agreement with published X-ray crystal structures (Figure S4). In total, 97% of all backbone resonances were assigned, with 205 and 204 out of a possible 215 residues assigned in $^1$H-$^{15}$N TROSY spectra of the SAM and sinefungin complexes, respectively. Backbone assignments for the two complexes are very similar, the only significant differences are located in the SAM/sinefungin pocket and the DNC loops (Figure 3 and Figure S3).

DFT cluster models
DFT calculations were performed in Gaussian 09 revision D.01, on models constructed from the S-COMT:SAM:DNC:Mg$^{2+}$ X-ray crystal structure. Any cleavage between C$\alpha$ and C$\beta$ atoms was capped with a methyl group and cleavage at the backbone amide with a hydrogen atom. All calculations employed the B3LYP functional. Natural charges were calculated using the natural bond orbital (NBO) analysis implemented in Gaussian 09. To generate an approximate transition-state structure for the SAM:catechol reactions, partially relaxed scans (i.e. with the same atoms were fixed as during the minimizations) of the C-O distance between the transferring methyl and acceptor catechol oxygen were performed until the maximum potential energy on the adiabatic surface was obtained with a certainty of $\pm$0.02 Å along the C-O distance. The larger ~580-atom cluster models include all amino acids with at least one heavy atom within a radius of 10 Å of the Mg$^{2+}$ ion, as well as the water molecule ligating the Mg$^{2+}$ and one additional crystallographic water molecule (Fig. S6). Geometry optimization was performed using the 6-31G(d) basis set with all protein heavy atoms fixed during optimization. Single-point calculations were performed using the larger 6-311G+(d,p) basis set applied to the SAM, DNC, Mg$^{2+}$ and oxygen atoms ligated to the Mg$^{2+}$ in order to correct energies and to perform the NBO analysis. The smaller ~220-atom models comprised primarily the first-shell amino acids around the Mg$^{2+}$ and DNC. Specifically, the model contained SAM or sinefungin, DNC, Mg$^{2+}$, one crystallographic water molecule in proximity to the Mg$^{2+}$ ion and the amino acid residues labelled in Fig. S7. Geometry optimization was performed using the 6-31G(d,p) basis set with some C$\alpha$ or equivalent atoms fixed as shown in Fig. S7. The sinefungin calculations also required the C2 atom of the sinefungin adenine moiety to be fixed. All calculations were performed using Grimme’s D3 dispersion correction and a polarizable continuum solvent with generic solvent ($\varepsilon$=4.0) and single-point calculations were performed using the 6-
311G+(d,p) basis set applied to all atoms in order to correct energies and to perform the NBO analysis. The apparent free energy barrier for the SAM:catechol reaction was determined from normal mode (frequency) calculations of the reactant state and TS. These calculations were performed using the same method as the geometry optimization and only one significant (>20 cm⁻¹) imaginary frequency was observed in the TS calculation.

**MD simulations**

MD simulations were carried out using the Amber 14 force field in Gromacs 5.1. DNC, SAM and sinefungin GAFF parameters were generated using RESP fitting to HF/6-31G(d) structures using Antechamber (Table S5). For SAM*, the S1, methyl and surrounding atoms were given the charges of the corresponding sinefungin atoms, with the charge of the extra sinefungin hydrogen distributed between these atoms. Octahedral Mg²⁺ bonding was parametrised using a DFT model built from the SAM:DNC crystal structure (Figure S15), which includes the Mg²⁺, the active site water molecule, dopamine and amino acids D141, D168, N169 and E198 which hydrogen bonds to the hydroxyl of the catecholate. Note that dopamine was used as these parameters were initially prepared for other simulations and consistency of parameters across simulations were desired, but this is expected to have a minor effect on the Mg²⁺ bonding. After energy minimization at the B3LYP/6-311+G(d,p) level of theory with a PCM continuum model (ε=80), bond force constants were obtained from frequency calculations, while the angle force constants were obtained from fitting harmonic potentials to the energies from relaxed scans of ±10°.

Prior to MD simulations, the system was solvated in a TIP3P water box of at least 10 Å with counter-ions generated in AmberTools. All simulations used the leap-frog integrator and the following parameters were used: 10 Å van der Waals and electrostatic cutoffs, particle mesh Ewald for long-range electrostatics, LINCS bonding restraints and periodic boundary conditions. After steepest descent energy minimisation, the solvent was equilibrated with 100 ps constant volume and 100 ps constant pressure simulations with a 10 kJ mol⁻¹ Å⁻² harmonic position constraint applied to the protein and substrates, after which all constraints were removed for the production run. Figures S14 and S16 show that there are no significant changes in active site geometry during the 50-60 ns sampling and that the simulation results are consistent with the crystal structures.

**Constrained MD.** Restraints were applied to the 20 residues with the largest difference in Φ and Ψ derived from TALOS-N between the SAM and sinefungin. Starting from the coordinates and velocities after 10 ns of unconstrained MD, restraints were increased gradually every 200 ps with force constants of 20, 50 and 75 kJ mol⁻¹ rad⁻² and finally 100 kJ mol⁻¹ rad⁻² for the 500 ns analysis run.

**Energy decomposition.** For a given residue X, the energy during the MD simulation can be recalculated with zero partial charges on the atoms of X, as well as swapping the charge of SAM for sinefungin and vice-versa. In this way, the electrostatic stabilization of sinefungin over SAM by residue X can be calculated:

\[
\Delta E(X) = [E_{\text{Tot}}(q_X=0) - E_{\text{Tot}}]\text{SAM} - [E_{\text{Tot}}(q_X=0) - E_{\text{Tot}}]\text{sinefungin}
\]

where \(\Delta E(X)\) is the electrostatic stabilization energy of residue X, \(E_{\text{Tot}}\) is the system energy and \(q_X =0\) indicates that all partial charges of X have been set to 0.
Table S1. X-ray data collection and refinement statistics for both complexes described in this study.

|                           | COMT:SAM:DNC:Mg²⁺ | COMT:Sinefungin:DNC:Mg²⁺ |
|---------------------------|--------------------|--------------------------|
| Wavelength (Å)            | 0.9282             | 0.9795                   |
| Resolution range (Å)      | 39.13 - 1.336      | 37.02 - 1.42             |
|                           | (1.384 - 1.336)    | (1.471 - 1.42)           |
| Space group               | P 1 2 1 1          | P 1 2 1 1                |
| Unit cell parameters      |                    |                          |
| a (Å)                     | 43.694             | 42.961                   |
| b (Å)                     | 61.749             | 75.797                   |
| c (Å)                     | 46.071             | 64.345                   |
| Total reflections         | 157981 (12486)     | 249222 (20113)           |
| Unique reflections        | 49463 (4891)       | 76895 (7577)             |
| Multiplicity              | 3.2 (2.5)          | 3.2 (2.7)                |
| Completeness (%)          | 99.50 (99.15)      | 99.26 (98.25)            |
| Mean I/sigma(I)           | 8.25 (2.06)        | 14.80 (4.22)             |
| Wilson B-factor (Å²)      | 10.33              | 12.6                     |
| R-merge                   | 0.08128 (0.3631)   | 0.04946 (0.3015)         |
| R-meas                    | 0.09735 (0.4585)   | 0.05904 (0.3751)         |
| R-pim                     | 0.05295 (0.2766)   | 0.03189 (0.22)           |
| CC1/2                     | 0.994 (0.824)      | 0.998 (0.86)             |
| CC*                       | 0.999 (0.95)       | 1 (0.962)                |
| Reflections used in       | 49425 (4889)       | 76889 (7577)             |
| refinement                |                    |                          |
| Reflections used for R-free | 2468 (212)     | 3719 (351)               |
| R-work                    | 0.1452 (0.1916)    | 0.1169 (0.1696)          |
| R-free                    | 0.1798 (0.2439)    | 0.1492 (0.2320)          |
| CC(work)                  | 0.970 (0.935)      | 0.979 (0.947)            |
| CC(free)                  | 0.950 (0.921)      | 0.971 (0.855)            |
| Number of non-hydrogen    | 1941               | 4216                     |
| macromolecules            | 1727               | 3621                     |
| ligands                   | 15                 | 84                       |
| solvent                   | 199                | 511                      |
| Protein residues          | 217                | 434                      |
| RMS(bonds)                | 0.007              | 0.011                    |
| RMS(angles)               | 0.94               | 1.16                     |
| Ramachandran favored (%)  | 97.17              | 96.74                    |
| Ramachandran allowed (%)  | 2.83               | 3.26                     |
| Ramachandran outliers (%) | 0                  | 0                        |
| Rotamer outliers (%)      | 0.53               | 1                        |
| Clashscore                | 0.58               | 4.84                     |
| Average B-factor          | 15.83              | 19.88                    |
| macromolecules            | 14.04              | 17.07                    |
| ligands                   | 11.86              | 13.56                    |
| solvent                   | 31.7               | 40.82                    |
**Figure S1.** Left, schematic representation of the anti-parallel β-strand interactions between protein chains in the dimeric S-COMT:sinefungin:DNC:Mg\(^{2+}\) X-ray crystal structure. Hydrogen bonds are represented as solid blue lines and non-bonded contacts represented as striped red lines (the width of the striped line is proportional to the number of atomic contacts). Residue colors represent the chemical nature of the amino acid: blue – positive, red – negative, green – neutral, purple – aromatic. Rendered using PDBsum.\(^{20}\) Right, X-ray crystal structure of the COMT:sinefungin:DNC:Mg\(^{2+}\) complex showing in red those residues identified using PDBsum. Interactions are listed below in the accompanying table on the following page.
Figure S1 continued.

Hydrogen bonds
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| Atom no. | Name  | Residue  | Chain | Distance |
|----------|-------|----------|-------|----------|
| 1.       | O     | ARG      | A     | 2.72     |
| 2.       | C     | ARG      | A     | 2.92     |
| 3.       | N     | HIS      | A     | 2.82     |
| 4.       | O     | HIS      | A     | 2.82     |
| 5.       | N     | GLN      | A     | 3.03     |
| 6.       | O     | NE2 GLN  | A     | 2.95     |

Non-bonded contacts
-------------------

| Atom no. | Name  | Residue  | Chain | Distance |
|----------|-------|----------|-------|----------|
| 1.       | CA    | ARG      | A     | 3.71     |
| 2.       | C     | ARG      | A     | 3.76     |
| 3.       | CB    | ARG      | A     | 3.59     |
| 4.       | O     | ARG      | A     | 3.62     |
| 5.       | O     | ARG      | A     | 2.72     |
| 6.       | CB    | ARG      | A     | 3.54     |
| 7.       | CG    | ARG      | A     | 3.63     |
| 8.       | OE2   | GLU      | A     | 3.65     |
| 9.       | O     | CYS      | A     | 3.52     |
| 10.      | O     | CYS      | A     | 2.92     |
| 11.      | O     | CYS      | A     | 3.64     |
| 12.      | O     | CYS      | A     | 3.86     |
| 13.      | O     | CYS      | A     | 3.40     |
| 14.      | CA    | THR      | A     | 3.14     |
| 15.      | C     | THR      | A     | 3.46     |
| 16.      | CB    | THR      | A     | 3.75     |
| 17.      | CG2   | THR      | A     | 3.69     |
| 18.      | CG2   | THR      | A     | 3.38     |
| 19.      | CD    | TYR      | A     | 3.86     |
| 20.      | N     | HIS      | A     | 2.82     |
| 21.      | O     | HIS      | A     | 3.18     |
| 22.      | O     | HIS      | A     | 3.48     |
| 23.      | O     | HIS      | A     | 3.87     |
| 24.      | O     | HIS      | A     | 3.53     |
| 25.      | O     | HIS      | A     | 2.82     |
| 26.      | O     | HIS      | A     | 3.78     |
| 27.      | CD    | TYR      | A     | 3.73     |
| 28.      | CE1   | TYR      | A     | 3.80     |
| 29.      | N     | GLN      | A     | 3.03     |
| 30.      | CA    | GLN      | A     | 3.74     |
| 31.      | CB    | GLN      | A     | 3.45     |
| 32.      | CD    | GLN      | A     | 3.70     |
| 33.      | OE1   | GLN      | A     | 3.76     |
| 34.      | OE1   | GLN      | A     | 3.60     |
| 35.      | OE1   | GLN      | A     | 3.50     |
| 36.      | OE1   | GLN      | A     | 3.68     |
| 37.      | NE2   | GLN      | A     | 2.95     |

Number of hydrogen bonds: 6
Number of non-bonded contacts: 37
**Figure S2.** X-ray crystal structure distances between DNC and SAM or sinefungin. The electron density is also shown.
Table S2– Key reactant distances ($R$, Å) and angles ($A$, °) in selected X-ray crystal structures of COMT ternary complexes.

| Ternary complex$^a$ | $R$(D-A) | $R$(X-A) | $R$(D-X) | $A$(D-X-A) | $A$(X-A-C$\lambda$) | Res.$^b$ | PDB  |
|----------------------|----------|----------|----------|-------------|----------------|---------|------|
| human, SAM, DNC      | 4.57     | 2.81     | 1.78     | 169         | 114            | 1.34    | This work  |
| human, singefungin,   | 4.20,    | 2.71,    | 1.51,    | 170,        | 115,           | 1.42    | This work  |
| DNC                  | 4.22     | 2.74     | 1.50     | 169         | 117            |         |      |
| human, SAM, DNC      | 4.36     | 2.57     | 1.81     | 169         | 121            | 2.8     | 3A73         |
| human, SAM, DNC      | 4.51     | 2.71     | 1.82     | 170         | 116            | 1.98    | 3BWM         |
| human V108M, SAM, DNC| 4.53     | 2.73     | 1.81     | 174         | 115            | 1.30    | 3BWY         |
| rat, SAM, DNC        | 4.37     | 2.63     | 1.75     | 173         | 119            | 2.00    | 1VID         |
| rat, SAM, tolcapone  | 4.26     | 2.50     | 1.80     | 166         | 116            | 1.85    | 3S68         |
| HR, singefungin,     | 3.96     | 2.48     | 1.51     | 165         | 118            | 2.20    | 4PYL         |
| tolcapone            |          |          |          |             |                |         |      |

$^a$The ternary complexes of human, rat or humanized rat (HR) COMT contained bound SAM or singefungin, Mg$^{2+}$ and either DNC (3,4-dinitrocatechol) or tolcapone. $^b$Resolution (Å) of the X-ray crystal structure. Structures with resolution ≥2.0 Å are highlighted in red.
Figure S3. 2D $^1$H-$^{15}$N TROSY spectrum of the S-COMT:sinefungin:DNC:Mg$^{2+}$ complex recorded at pH 7.5 and 298 K. The assignments of backbone amide resonances are indicated by residue type and sequence number. Acquisition parameters are given in Table S2.
Table S3. Selected acquisition parameter settings for the acquisition of NMR spectra of the S-COMT complexes.

| Complex 1/Complex 2<sup>a</sup> | <sup>1</sup>H-<sup>15</sup>N TROSY | HNCA CB | HN(CO)CACB | HNCO |
|--------------------------------|-----------------------------|--------|------------|------|
|<sup>1</sup>H TD<sup>b</sup>    | 5998/5998                  | 2048/2048 | 2048/2048 | 2048/2048 |
|<sup>1</sup>H AQ (ms)           | 149.9/149.9                | 85.2/51.2 | 85.2/51.2 | 85.2/51.2 |
|<sup>15</sup>N TD               | 256/512                    | 78/78    | 78/78     | 88/88 |
|<sup>15</sup>N AQ (ms)          | 47.8/90.2                  | 14.6/13.7 | 14.6/13.7 | 16.4/15.5 |
|<sup>13</sup>C TD               | -/-                        | 450/450  | 450/450    | 106/106 |
|<sup>13</sup>C AQ (ms)          | -/-                        | 16.0/14.0 | 16.0/14.0 | 16.4/16.4 |

<sup>a</sup> Complex 1: S-COMT:SAM:DNC:Mg<sup>2+</sup>; Complex 2: S-COMT:sinefungin:DNC:Mg<sup>2+</sup>

<sup>b</sup> TD: the number of time domain data points obtained; AQ: acquisition time.

Figure S4. Backbone secondary structure prediction of the S-COMT:sinefungin:DNC:Mg<sup>2+</sup> complex derived with TALOS+ using the backbone <sup>1</sup>H<sub>N</sub>, <sup>15</sup>N, <sup>13</sup>Cα, <sup>13</sup>Cβ and <sup>13</sup>C′ chemical shifts. The secondary structure prediction is shown as red bars for α-helices and blue bars for β-strands, with the height of the bars representing the probability of the secondary structure assigned by the software. The secondary structure of the S-COMT:SAM:DNC:Mg<sup>2+</sup> complex crystal structure PDB: 3BWM<sup>9</sup> is reported below in the same color representation.
Figure S5. Continued on next page.
Figure S5. Differences in HN, CA, CB, C’ and rescaled N-H plane hypotenuse NMR chemical shifts (ppm) between the S-COMT:SAM:DNC:Mg^{2+} complex and the S-COMT:sinefungin: DNC:Mg^{2+} complexes represented as a graph (left) and putty diagram (right). Active site residues (up to 4 Å from ligands position in the crystal structure) are shown by blue bars. Putty diagram colored from low to high difference in NMR shift (white to red) and selected active site residues are labelled.
**Figure S6.** Energy minimized larger (~580 atom) DFT cluster models of COMT active site. (a) The whole SAM-catechol model and close-ups, without hydrogen atoms, of: (b) SAM:catechol for the reactant (dark green carbon atoms) and transition state (light green carbon atoms); (c) SAM:DNC (dark green carbon atoms) and sinefungin:DNC (light green carbon atoms); (d) SAM:DNC (dark green carbon atoms) and sinefungin:DNC with NH$_2$ in place of NH$_3^+$ (light green carbon atoms).

**Figure S7.** Energy minimized smaller (~220 atom) DFT models of COMT active site (without hydrogen atoms for clarity): (a) SAM:catechol for the reactant (dark green carbon atoms) and transition state (light green carbon atoms), with fixed atoms designated by asterisks; (b) SAM:DNC (dark green carbon atoms) and sinefungin:DNC (light green carbon atoms).
Table S4. Selected distances and charges in the ~580 atom DFT cluster models shown in Figure S6.

|                         | SAM:catechol:Mg2⁺ | DNC:Mg2⁺,b | DNC:Mg2⁺,b |
|-------------------------|-------------------|------------|------------|
|                         | reactant          | TS         | SAM        | sinefungin |
| R(D-A)c, Å              | 4.65              | 4.45       | 4.67       | 4.37       |
| R(X-A)c, Å              | 2.84              | 2.08       | 2.86       | 2.91       |
| R(D-X)c, Å              | 1.82              | 2.38       | 1.82       | 1.52       |
| q(D)c                   | 0.82              | 0.44       | 0.83       | 0.16       |
| q(A)c                   | -0.89             | -0.82      | -0.77      | -0.79      |
| q(XH3)c                 | 0.05              | 0.32       | 0.05       | 0.49       |

aReactant state and approximate TS in a model containing SAM, Mg²⁺ and catechol in place of DNC. The TS geometry was obtained from a relaxed scan with step size of ΔR(X-A) = 0.025 Å; bReactant state models containing DNC, Mg²⁺ and SAM or sinefungin; cDistances (R) and angles (A) are equivalent to those given in Table 1; q(D), q(A) and q(XH3) are the natural charge on the donor, acceptor and summed over the transferring group, respectively.

Table S5. Partial atomic charges (q) for selected SAM and sinefungin atoms from RESP fitting and the corresponding SAM* atoms used in MD simulations.

| Name | q(SAM) | q(SAM*) | Name | q(sinefungin) |
|------|--------|---------|------|---------------|
| C3   | -0.11873| -0.07224| C3   | -0.07224      |
| C4   | -0.04721| -0.19952| C4   | -0.19952      |
| S1   | 0.246273| 0.396006| C5   | 0.396006      |
| C5   | -0.15283| -0.71315| N2   | -0.71315      |
| H9   | 0.126886| 0.398943| H10  | 0.398943      |
| H10  | 0.126886| 0.398943| H11  | 0.398943      |
| H11  | 0.126886| 0.398943| H12  | 0.398943      |
| C6   | -0.28994| -0.3937 | C10  | -0.3937       |
| C7   | 0.04939 | 0.210796| C11  | 0.231796      |
| H14  | 0.120147| 0.050759| H17  | 0.051759      |
| H12  | 0.19498 | 0.126416| H18  | 0.131416      |
| H13  | 0.19498 | 0.126416| H19  | 0.131416      |
| H7   | 0.09019 | 0.074431| H9   | 0.101726      |
| H8   | 0.09019 | 0.074431| H7   | 0.075389      |
| H5   | 0.106928| 0.04724 | H8   | 0.075389      |
| H6   | 0.106928| 0.04724 | H5   | 0.04824       |
|      |        |         | H6   | 0.04824       |
Figure S8. Continued on next page.
Figure S8. Comparison between backbone torsion angles values Phi (Φ) and Psi (Ψ) [°] for S-COMT:SAM:DNC:Mg\textsuperscript{2+} complex derived from NMR data, MD and crystallography. Phi (Φ) and Psi (Ψ) torsion angles for NMR data were predicted by uploading the backbone \textsuperscript{1}H\textsubscript{N}, \textsuperscript{15}N, \textsuperscript{13}C\textsubscript{α}, \textsuperscript{13}C\textsubscript{β} and \textsuperscript{13}C\textsuperscript{'} chemical shifts to the TALOS-N webserver.\textsuperscript{19} Phi (Φ) and Psi (Ψ) torsion angles for crystallography were extracted from pdb files using the WHAT IF web server.\textsuperscript{21}
Figure S9. Continued on next page.
Figure S9. Comparison between backbone torsion angles values Phi (Φ) and Psi (Ψ) [°] for S-COMT:sinefungin:DNC:Mg²⁺ complex derived from NMR data, MD and crystallography (two chains for crystallographic dimer presented). Phi (Φ) and Psi (Ψ) torsion angles for NMR data were predicted by uploading the backbone ¹HΝ, ¹⁵Ν, ¹³Cα, ¹³Cβ and ¹³C’ chemical shifts to the TALOS-N webserver.¹⁹ Phi (Φ) and Psi (Ψ) torsion angles for crystallography were extracted from pdb files using the WHAT IF web server.²¹
Figure S10. \( R(\text{donor–C}_\alpha) \) from MD simulations for residues with significant changes in chemical shifts for the SAM (black), sinefungin (blue) and SAM* (red) simulations, where D refers to \( S_1 \) of SAM and the analogous carbon of sinefungin.
Figure S11. Selected active site distances for the residues shown (a), for the SAM (black), sinefungin (blue) and SAM* (red) MD simulations: M40C\(_\alpha\)–D141C\(_\alpha\) (b), M40C\(_\alpha\)–Y68C\(_\alpha\) (c), S\(_1\)–Y68C\(_\alpha\) (d), S\(_1\)–Y68C\(_Z\) (e) and M40C\(_\gamma\)–Y68C\(_Z\) (f). In sinefungin there is a greater M40–Y68 compaction than in SAM* compared to SAM (c) and as M40 pushes against Y68 the S\(_1\)–Y68C\(_Z\) distance (e) increases more than the S\(_1\)–Y68C\(_\alpha\). Since the M40 sidechain is pushing against the Y68 sidechain, the M40C\(_\gamma\)–Y68C\(_Z\) distance does not increase.
**Figure S12.** Active site compaction during unconstrained MD simulations (a,b) and SAM (c,d) and sinefungin (e,f) simulations performed with NMR-derived dihedral restraints. For the unconstrained simulation the black and blue lines are for the SAM and sinefungin simulations, respectively; for the restrained simulations the black and blue lines are for the SAM and sinefungin restraints, respectively.

**Figure S13.** Electrostatic stabilization energy ($\Delta E$) of sinefungin over SAM by the polar active site residues nearest the SAM methyl or sinefungin NH$_3^+$ for MD simulations of SAM (black line), SAM* (red line) and sinefungin (blue line).
Figure S14. (a-c) Donor-acceptor distances for SAM, SAM* and sinefungin, respectively, for the 50 ns MD simulations used in the analysis; (d) donor-acceptor distance distributions from the same SAM (black line), SAM* (red line) and sinefungin (blue line) MD simulations. (e) D141Cα-M40Cα distance vs donor-acceptor distance for the SAM (black), SAM* (red) and sinefungin (blue) simulations.

Note that the donor–acceptor distance distributions in (a) shows the average distance to be longer in the SAM* simulation than the SAM simulation. This is primarily due to a change in the S1-C-Oα angle, from an average of 163.5° in the SAM simulation to 170.3° in the SAM* simulation. There is little difference (< 0.1Å) between the average C–Oα distances found in each of these simulations as this distance is very close to the van der Waals limit, so the more linear angle forces the donor–acceptor further apart.

Figure S15. DFT model used for calculating the octahedral Mg^{2+} bonding parameters. Dopamine was used as these parameters were initially prepared for other simulations and consistency of parameters across simulations were desired.
Figure S16. RMSD and selected active site distances for unrestrained MD simulations of the SAM (A,D,G), SAM* (B,E,H) and Sinefungin (C,F,I).
Table S6. Coordinates for DFT model for calculating the octahedral Mg$^{2+}$-bonding parameters (charge = -2, spin multiplicity = 1).

| Atom | X          | Y          | Z          |
|------|------------|------------|------------|
| C    | 3.051529000 | -4.814252000 | 0.308025000 |
| H    | 3.987902000 | -4.411197000 | 0.703557000 |
| H    | 3.269242000 | -5.665449000 | -0.336028000 |
| C    | 2.308504000 | -3.719620000 | -0.444807000 |
| O    | 1.955708000 | -2.704804000 | 0.231242000 |
| O    | 2.089813000 | -3.873594000 | -1.677005000 |
| C    | 4.507028000 | -0.252471000 | 0.520641000 |
| H    | 5.596881000 | -0.230710000 | 0.501249000 |
| H    | 4.150470000 | -1.265494000 | 0.713457000 |
| C    | 3.919334000 | 0.278480000 | -0.786528000 |
| C    | 4.507028000 | -0.252471000 | 0.520641000 |
| H    | 5.596881000 | -0.230710000 | 0.501249000 |
| H    | 4.150470000 | -1.265494000 | 0.713457000 |
| C    | 3.919334000 | 0.278480000 | -0.786528000 |
| C    | 4.507028000 | -0.252471000 | 0.520641000 |
| H    | 5.596881000 | -0.230710000 | 0.501249000 |
| H    | 4.150470000 | -1.265494000 | 0.713457000 |
| C    | 3.919334000 | 0.278480000 | -0.786528000 |
| C    | 4.507028000 | -0.252471000 | 0.520641000 |
| H    | 5.596881000 | -0.230710000 | 0.501249000 |
| H    | 4.150470000 | -1.265494000 | 0.713457000 |
| C    | 3.919334000 | 0.278480000 | -0.786528000 |
| C    | 4.507028000 | -0.252471000 | 0.520641000 |
| H    | 5.596881000 | -0.230710000 | 0.501249000 |
| H    | 4.150470000 | -1.265494000 | 0.713457000 |
| C    | 3.919334000 | 0.278480000 | -0.786528000 |
| C    | 4.507028000 | -0.252471000 | 0.520641000 |
| H    | 5.596881000 | -0.230710000 | 0.501249000 |
| H    | 4.150470000 | -1.265494000 | 0.713457000 |
| C    | 3.919334000 | 0.278480000 | -0.786528000 |
| C    | 4.507028000 | -0.252471000 | 0.520641000 |
| H    | 5.596881000 | -0.230710000 | 0.501249000 |
| H    | 4.150470000 | -1.265494000 | 0.713457000 |
| C    | 3.919334000 | 0.278480000 | -0.786528000 |
| C    | 4.507028000 | -0.252471000 | 0.520641000 |
| H    | 5.596881000 | -0.230710000 | 0.501249000 |
| H    | 4.150470000 | -1.265494000 | 0.713457000 |
| C    | 3.919334000 | 0.278480000 | -0.786528000 |
| C    | 4.507028000 | -0.252471000 | 0.520641000 |
| H    | 5.596881000 | -0.230710000 | 0.501249000 |
| H    | 4.150470000 | -1.265494000 | 0.713457000 |
| C    | 3.919334000 | 0.278480000 | -0.786528000 |
| C    | 4.507028000 | -0.252471000 | 0.520641000 |
| H    | 5.596881000 | -0.230710000 | 0.501249000 |
| H    | 4.150470000 | -1.265494000 | 0.713457000 |
| C    | 3.919334000 | 0.278480000 | -0.786528000 |
| C    | 4.507028000 | -0.252471000 | 0.520641000 |
| H    | 5.596881000 | -0.230710000 | 0.501249000 |
| H    | 4.150470000 | -1.265494000 | 0.713457000 |
| C    | 3.919334000 | 0.278480000 | -0.786528000 |
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