

# 1 Ligand Parameters

The structures for the parameterized ligands and the PNMT cofactor are given in Figure S1. The PDB atom names are highlighted in this figure. The corresponding atom types and partial charges for each atom are given in Tables S1-3.

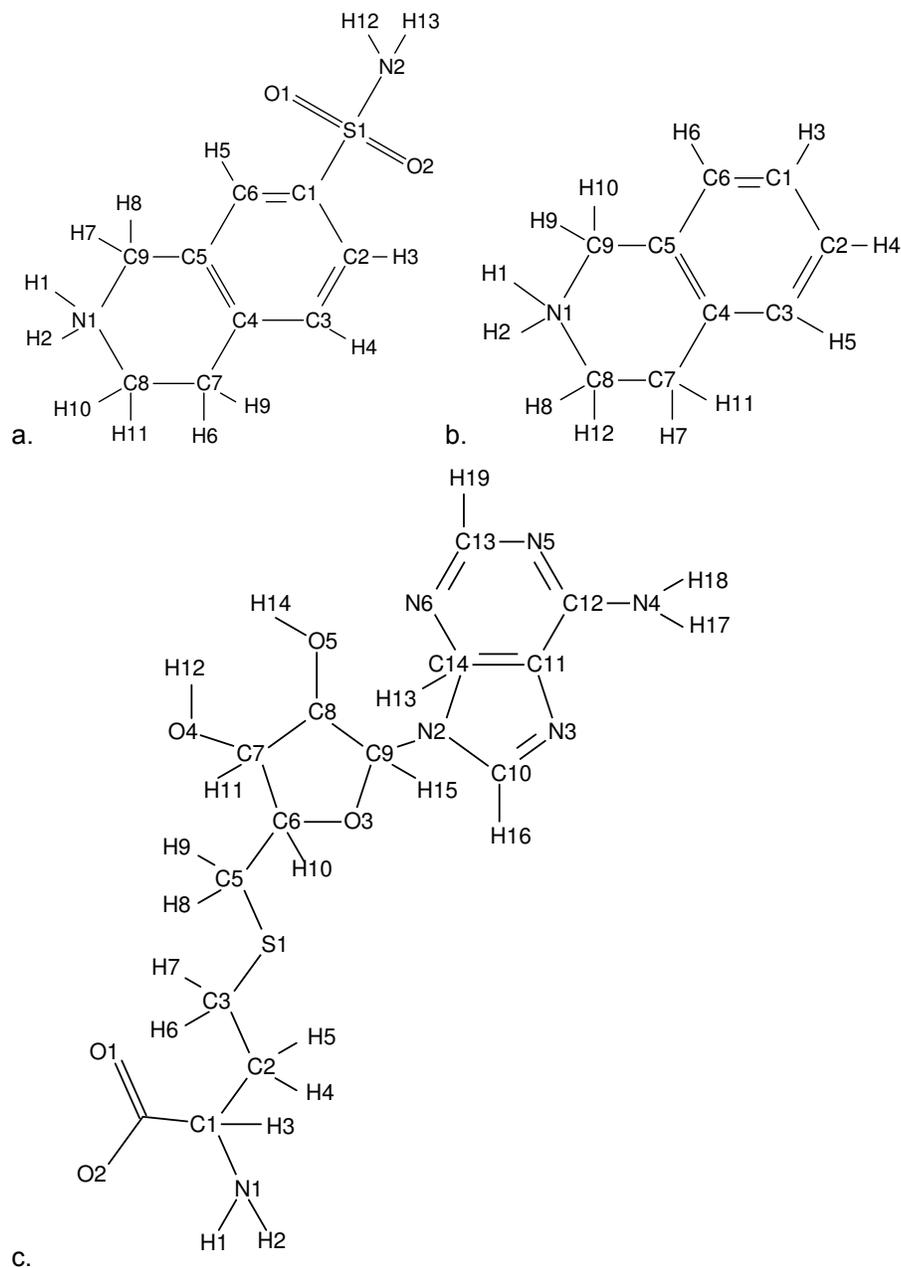


Figure S 1: The structures for (a) 7-sulfamoyl-1,2,3,4-tetrahydroisoquinolinium, (b) 1,2,3,4-tetrahydroisoquinolinium, and (c) the cofactor S-adenosyl-L-homocysteine with the PDB atom names listed.

Atom Name	Atom Type	Partial Charge
O1	o	-0.573668
S1	sy	1.232538
O2	o	-0.573668
N2	n3	-1.037186
H12	hn	0.469850
H13	hn	0.469850
C1	ca	-0.028732
C6	ca	-0.216911
H5	ha	0.200250
C2	ca	-0.129610
H3	ha	0.216974
C3	ca	-0.225853
H4	ha	0.183828
C4	ca	0.053522
C5	ca	0.065648
C9	c3	-0.178191
H7	hx	0.172836
H8	hx	0.172836
N1	n4	-0.313324
H1	hn	0.348583
H2	hn	0.348583
C8	c3	-0.090633
H10	hx	0.141132
H11	hx	0.141132
C7	c3	0.017190
H6	hc	0.066512
H9	hc	0.066512

Table S 1: GAFF Atom types and partial charges for 7-sulfamoyl-1,2,3,4-tetrahydroisoquinolinium (Figure S1a).

Atom Name	Atom Type	Partial Charge
C3	ca	-0.210812
H5	ha	0.173661
C2	ca	-0.102608
H4	ha	0.169559
C1	ca	-0.109233
H3	ha	0.171258
C6	ca	-0.258951
H6	ha	0.188969
C5	ca	0.099388
C4	ca	0.012968
C7	c3	0.033442
H7	hc	0.059688
H11	hc	0.059688
C8	c3	-0.106767
H8	hx	0.141482
H12	hx	0.141482
N1	n4	-0.296669
H1	hn	0.344286
H2	hn	0.344286
C9	c3	-0.192578
H9	hx	0.168730
H10	hx	0.168730

Table S 2: GAFF Atom types and partial charges for 1,2,3,4-tetrahydroisoquinolinium (Figure S1b).

Atom Name	Atom Type	Partial Charge	Atom Name	Atom Type	Partial Charge
O1	o	-0.799428	O5	oh	-0.715583
C4	c	0.757698	H14	ho	0.486549
O2	o	-0.799428	H13	h1	0.109616
C1	c3	0.268234	H11	h1	0.016452
N1	n3	-1.089399	H10	h1	0.033243
H1	hn	0.378206	O3	os	-0.587778
H2	hn	0.378206	C9	c3	0.414186
H3	h1	-0.026373	H15	h2	0.130389
C2	c3	-0.056728	N2	na	-0.382563
H4	hc	0.052668	C10	cc	0.423398
H5	hc	0.052668	H16	h5	0.082549
C3	c3	0.141526	N3	nd	-0.661217
H6	h1	0.034048	C11	ca	-0.018205
H7	h1	0.034048	C12	ca	0.798340
S1	ss	-0.392988	N4	nh	-0.968378
C5	c3	-0.285333	H17	hn	0.419257
H8	h1	0.169881	H18	hn	0.419257
H9	h1	0.169881	N5	nb	-0.829460
C6	c3	0.382666	C13	ca	0.626041
C7	c3	0.239395	H19	h5	0.049975
O4	oh	-0.713664	N6	nb	-0.768061
H12	ho	0.442695	C14	ca	0.490421
C8	c3	0.093094			

Table S 3: GAFF Atom types and partial charges for S-adenosyl-L-homocysteine (Figure S1c).

## 2 Input Preparation

Here, we describe the differences in topology preparation and input control settings between *sander* and *pmemd* for the model systems described in the main text. For each system, the topology was first prepared for *pmemd* and then atoms belonging to the other end state were stripped using the `parmed.py` module of AmberTools. This was done as the *pmemd* topology contains both end states, while *sander* requires two topologies, one corresponding to each end state.

### 2.1 Solvation Free Energy

For the solvation free energy system, the input topology for *pmemd* was the ligand in solution. The initial state was just the solvent, with the ligand decoupled from the rest of the system (effectively in the gas phase). The topology for the final state in the *sander* simulation was the same as the *pmemd* topology. The topology for the initial state in the *sander* simulation was created by using `parmed` to remove the atoms associated with the ligand

```
python parmed.py ti.prmtop
  loadRestrt ti.inpcrd
  setOverwrite True
  strip :1
  outparm ti_a.prmtop ti_a.inpcrd
  quit
```

```
cp ti.prmtop ti_b.prmtop
cp ti.inpcrd ti_b.inpcrd
```

where `ti.prmtop` and `ti.inpcrd` were the *pmemd* input files and `ti_a.*` and `ti_b.*` were the *sander* input files for the initial and final states respectively. The input control flags for *pmemd* and *sander* were

```
pmemd input control flags:
  icfe = 1, ifsc = 1,
  timask1='', timask2=':1', scmask1='', scmask2=':1',
```

```
sander input control flags:
  initial state:
    icfe = 1, ifsc = 1,
    scmask='',
  final state:
    icfe = 1, ifsc = 1,
    scmask=':1',
```

Note that *pmemd* uses only one input control file, while *sander* uses two files, one corresponding to the initial state and the other corresponding to the final state.

### 2.2 Relative Binding Free Energy

For the relative binding free energy calculation, the initial `pdb` was modified so that both ligands, 7-sulfamoyl-1,2,3,4-tetrahydroisoquinolinium and 1,2,3,4-tetrahydroisoquinolinium, were included. This was used to generate the *pmemd* topology files `ti.prmtop` and `ti.inpcrd`. Note that this was done with the ligands bound to PNMT and the ligands free in solution. The rest of this discussion will focus on the ligands bound to PNMT, but the only difference with the ligands free in solution is a change in the residue numbers. The *sander* topologies were created by removing the ligand corresponding to the other state using `parmed`

```
python parmed.py ti.prmtop
  loadRestrt ti.inpcrd
  setOverwrite True
  strip :272
  outparm ti_a.prmtop ti_a.inpcrd
```

```
quit
```

```
python parmed.py ti.prmtop
loadRestrt ti.inpcrd
setOverwrite True
strip :271
outparm ti_b.prmtop ti_b.inpcrd
quit
```

where residues :271 and :272 were the residue numbers for the ligands in the *pmemd* topology file. The corresponding input control flags were

```
pmemd input control flags:
icfe = 1, ifsc = 1,
timask1=':271', timask2=':272',
scmask1=':271@H3', scmask2=':272@S1,O1,O2,H12,H13,N2',
noshakemask=':271,272',
```

```
sander input control flags:
initial state:
icfe = 1, ifsc = 1,
scmask=':271@H3',
noshakemask=':271',
final state:
icfe = 1, ifsc = 1,
scmask=':271@S1,O1,O2,H12,H13,N2',
noshakemask=':271',
```

For the ligands free in solution, residue :271 became residue :1 and residue :272 became residue :2.

## 2.3 Mutation

The last model system studied was the R371A mutation of N1 neuraminidase (NMD). A multi-step transformation was used to calculate the free energies, which requires three separate simulations and therefore three sets of topologies. The construction of the topologies and the associated input flags for each step of the transformation is discussed below.

### 2.3.1 Charge Removal with Wild-Type NMD

The first step in this transformation was the removal of the partial charges on the residue to be mutated. In order to do this with *pmemd*, a special topology was created that had residue 371 duplicated. To create this topology, the entire NMD protein was duplicated in the pdb. Then LEaP was used to generate the topology files *ti1.prmtop* and *ti1.inpcrd*. Disulfide bonds were present in the protein, so these also had to be duplicated when using LEaP.

The duplication of the protein creates additional bonding terms which can decrease the performance of the calculation. The duplicate atoms and the additional bonding terms from the non-mutated residues were removed using *parmed*

```
python parmed.py ti1.prmtop
loadRestrt ti1.inpcrd
setOverwrite True
tiMerge :1-385 :386-770 :286 :671
outparm ti1_merged.prmtop ti1_merged.inpcrd
quit
```

where residues :1-385 and :386-770 are the first and second copies of the protein and residues :286 and :671 are the first and second copies of the residue that is being mutated. Note that these residue numbers do not match the original sequence in the pdb, as Amber will renumber the input residues sequentially. The new topology files, *ti1\_merged.prmtop* and *ti1\_merged.inpcrd*, have had all redundant atoms and bonding terms associated with the unperturbed part of the

system removed. The tiMerge command will also output the atom masks to use for the input flags. In this case these correspond to residue :286, which is the original residue 371, and residue :386, which is the duplicated residue. All other atoms from the second copy of the protein have been removed.

The *sander* topology was created by removing the atoms corresponding to the duplicated residue. The same topology was used for the initial and final states.

```
python parmed.py til_merged.prmtop
  loadRestrt til_merged.inpcrd
  setOverwrite True
  strip :386
  outparm til_a.prmtop til_a.inpcrd
  quit
```

```
cp til_a.prmtop til_b.prmtop
cp til_a.inpcrd til_b.inpcrd
```

The input control flags were

```
pmemd input control flags:
  icfe = 1,
  timask1=':286',
  timask2=':386',
  crgmask=':386',
```

```
sander input control flags:
  initial state:
    icfe = 1,
  final state:
    icfe = 1,
    crgmask=':286',
```

### 2.3.2 vdW Transformation

The second step in this transformation was the mutation of Arginine 371 to Alanine using a vdW-only transformation. Once again the entire protein was duplicated in the pdb. Then the residue corresponding to residue 371 in the second copy of the protein was mutated to an Alanine by removing the extra atoms in the pdb and changing the residue name to ALA. Then LEaP was used to prepare the prmtop and inpcrd files. The same tiMerge command that was used for the charge removal was used here to remove redundant atoms and bonding terms. This generated the *pmemd* topology files ti2\_merged.prmtop and ti2\_merged.inpcrd. Parmed was then used to generate the *sander* topology files ti2\_a.\* and ti2\_b.\*.

```
python parmed.py ti2_merged.prmtop
  loadRestrt ti2_merged.inpcrd
  setOverwrite True
  strip :386
  outparm ti2_a.prmtop ti2_a.inpcrd
  quit
```

```
python parmed.py ti2_merged.prmtop
  loadRestrt ti2_merged.inpcrd
  setOverwrite True
  strip :286
  outparm ti2_b.prmtop ti2_b.inpcrd
  quit
```

The input control flags were

```

pmemd input control flags:
  icfe = 1, ifsc = 1,
  timask1=':286',scmask1=':286',
  timask2=':386',scmask2=':386',
  crgmask=':286,386',

sander input control flags:
  initial state:
    icfe = 1, ifsc = 1,
    scmask=':286',crgmask=':286',
  final state:
    icfe = 1, ifsc = 1,
    scmask=':385',crgmask=':385',

```

### 2.3.3 Charge Removal with Mutant NMD

The final step of the transformation was the addition of charges back to the mutated residue. Once again the protein in the initial pdb was duplicated, but both copies of residue 371 were mutated to an Alanine. The rest of the procedure was exactly the same as for the charge removal with the wild-type NMD.

## 3 Speed of simulations

The speed of the simulations, in nanoseconds per day, is given in Table S4. This data was used to generate Figure 5 in the main text. The speed of the  $\lambda = 0.5$  window for the relative binding energy calculation used in the scaling tests is shown in Table S5. This data was used to generate Figure 6 in the main text.

	Sander	Pmemd	Sander Reference	Pmemd Reference
Model System	Speed (ns/day)	Speed (ns/day)	Speed (ns/day)	Speed (ns/day)
Solvation Free Energy	8.9	26.5	19.2	36.0
Relative Binding Energy (bound)	1.9	5.7	4.3	7.5
Relative Binding Energy (free)	10.4	32.6	23.2	44.0
Mutation (vdW)	1.2	4.5	2.7	4.8
Mutation Charge Removal (wt)	1.4	3.7	2.7	4.8
Mutation Charge Removal (mutant)	1.4	3.7	2.6	4.7

Table S 4: Speed of the production simulations in nanoseconds per day for each model system. The speed was averaged over the  $\lambda$  values for the alchemical transformations and over the two end states for the reference simulations. For the alchemical transformations, the results were then averaged over five independent simulations.

	Sander	Pmemd	Sander Reference	Pmemd Reference
Number of Cores	Speed (ns/day)	Speed (ns/day)	Speed (ns/day)	Speed (ns/day)
8	1.1	3.1	2.5	4.0
16	1.9	5.7	4.4	7.7
32	3.0	10.3	5.9	14.2
64	3.6	17.5	7.0	24.4
128	4.0	19.6	7.3	29.2

Table S 5: Speed of the  $\lambda = 0.5$  production simulation for the bound state of the relative binding energy model system as the number of cores is varied. Also shown is the speed for the corresponding reference simulation averaged over the two possible end states.

## 4 Free Energy Results

This section contains the free energies calculated for each of the model systems. The results calculated using MBAR and TI are summarized in Table S6 and Table S7 respectively. The deviation in the free energy was calculated using this data, which is represented in Figure 3 in the main text. The change in free energy when including the  $\lambda = 0.0$  and  $\lambda = 1.0$  end states for the solvation free energy model system is shown in Table S8.

MBAR	Sander	Pmemd
Model System	$\Delta F$ (kcal/mol)	$\Delta F$ (kcal/mol)
Solvation Free Energy	$-47.13 \pm 0.82$	$-47.15 \pm 1.39$
Relative Binding Energy (bound)	$-59.17 \pm 0.30$	$-59.06 \pm 0.51$
Relative Binding Energy (free)	$-55.42 \pm 0.01$	$-55.39 \pm 0.04$
Mutation (vdW)	$2.37 \pm 0.40$	$2.17 \pm 0.45$
Mutation Charge Removal (wt)	$211.46 \pm 0.57$	$211.70 \pm 0.79$
Mutation Charge Removal (mutant)	$27.94 \pm 0.26$	$27.85 \pm 0.19$

Table S 6: Calculated average free energies for the model systems simulated using *sander* or the new *pmemd* implementation. The free energies were calculated using MBAR, as implemented in the freely available pyMBAR script. Uncertainties represent standard deviation of results from five independent simulations.

TI	Sander	Pmemd
Model System	$\Delta F$ (kcal/mol)	$\Delta F$ (kcal/mol)
Solvation Free Energy	$-49.22 \pm 2.07$	$-49.33 \pm 0.87$
Relative Binding Energy (bound)	$-59.15 \pm 0.41$	$-58.78 \pm 0.39$
Relative Binding Energy (free)	$-55.62 \pm 0.02$	$-55.59 \pm 0.04$
Mutation (vdW)	$2.40 \pm 0.49$	$2.17 \pm 0.23$
Mutation Charge Removal (wt)	$211.47 \pm 0.59$	$211.64 \pm 0.69$
Mutation Charge Removal (mutant)	$28.10 \pm 0.21$	$27.90 \pm 0.20$

Table S 7: Calculated average free energies for the model systems simulated using *sander* or the new *pmemd* implementation. The free energies were calculated using TI with trapezoidal integration. Uncertainties represent standard deviation of results from five independent simulations.

Model System	Method	$\Delta F_{0.0 \rightarrow 1.0}$ (kcal/mol)	$\Delta F_{0.1 \rightarrow 0.9}$ (kcal/mol)	$\Delta \Delta F_{end}$ (kcal/mol)
Solvation Free Energy	TI	$-66.74 \pm 0.11$	$-49.33 \pm 0.87$	$-19.29 \pm 1.23$
	MBAR	$-65.62 \pm 1.39$	$-47.15 \pm 1.39$	$-18.47 \pm 1.96$

Table S 8: Comparison of the solvation free energy calculated using two different ranges of  $\lambda$  values, as indicated by subscripts. These calculations were run using the new *pmemd* implementation because the *sander* implementation is not able to run softcore simulations at the end states.  $\Delta \Delta F_{end}$  is the difference between these free energies and is an estimate of the error in the solvation free energy caused by neglecting the end states.