Molecular dynamics of class A β-lactamases – Effects of substrate binding

Supporting Material

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Abstract

The effects of substrate binding on class A β-lactamase dynamics were studied using molecular dynamics simulations of two model enzymes; forty 100-ns trajectories of the free and substrate-bound forms of TEM-1 (with benzylpenicillin) and PSE-4 (with carbenicillin) were recorded (totalling 4.0 μs). Substrates were parameterized with CGenFF. In both enzymes, the Ω loop exhibits a marked flexibility increase upon substrate binding, supporting the hypothesis of substrate-gating. However, specific interactions that are formed or broken in the Ω loop upon binding differ between the two enzymes: dynamics are conserved, but not specific interactions. Substrate binding also has a global structuring effect on TEM-1, but not on PSE-4. Changes in TEM-1's normal modes show long-range effects of substrate binding on enzyme dynamics. Hydrogen bonds observed in the active site are mostly preserved upon substrate binding, and new, transient interactions are also formed. Agreement between NMR relaxation parameters and our theoretical results highlights the dynamic duality of class A β-lactamases: enzymes that are highly structured on the ps-ns timescale, with important flexibility on the μs-ms timescale in regions such as the Ω loop.

This supporting material document contains the detailed parameterization protocol for penicillin and carbenicillin β-lactam antibiotics, supporting results and figures for β-lactamase backbone dynamics, and a list of supporting movies (essential dynamics normal modes). Tabular data and force field files are available in plain text format upon request.

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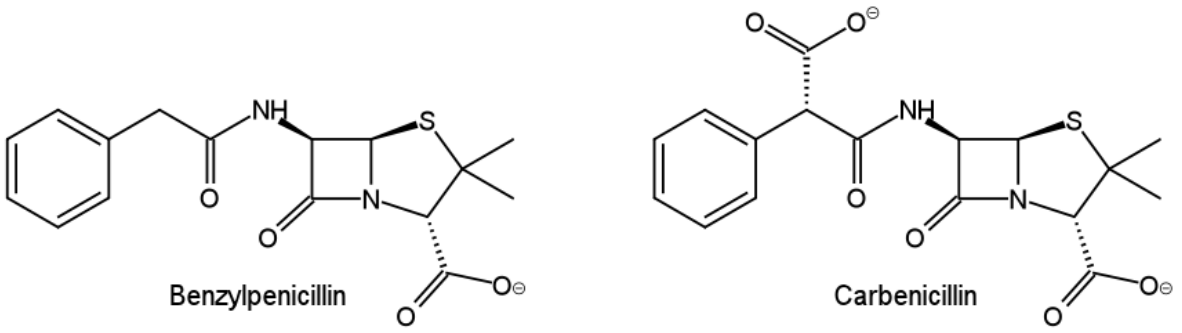

Figure S1: Structure of parameterization target compounds. Benzylpenicillin (BZP) is β-lactamase TEM-1's prefered substrate, while carbenicillin (CBC) is hydrolyzed efficiently by β-lactamase PSE-4.

β-lactam parameterization

Summary

Benzylpenicillin and carbenicillin (Figure S1) [1, 2] were parameterized within the context of the CGenFF forcefield [3], version 2b5. The four-member ring was the first and principal fragment considered. The substituted four member ring was then fused to the five-atom cycle to form the basic antibiotics scaffold. The C-S bond at the rings junction needed parameterization since it is currently missing from CGenFF. Afterwards, the other substituants were added to create benzylpenicillin. Finally, an additional carboxyl was added to form carbenicillin. At all steps, the suggested CGenFF parameterization protocol and philosophy were followed to obtain transferable parameters that could be used to construct a wide variety of penicillin-like β-lactams.

β-Lactam ring

Since β-lactam antibiotics are mimetic dipeptides, a first attempt was made at parameterizing a minimal β-lactam by analogy with existing amino acid parameters, chiefly proline (due to the disubstituted amide in the lactam cycle). However, MM geometry minimization showed that the cycle conformation differed from that suggested by quantum mechanics calculations (Figure S2): the cycle has a pronounced pucker, whilst the QM-geometry is nearly flat. All MM calculations in this work were carried out with CHARMM c35b1 [4]. All QM calculations in this work were carried out with Gaussian 03 [5].

MM-geometry being inadequate, the β-lactam ring was reparameterized. To avoid modifying existing CGenFF parameters, and considering that the chemical environment in a four-member ring is substantially different from anything else in CGenFF, we introduced new atom types for the four-member cycle. Initial parameters and partial charges were guessed by analogy with amino acids. Atom types are:

- CG315: Aliphatic C for CH in β-lactam ring, analog to CG311
- NG2S4: N,N-Disubstituted amide N in β-lactam ring, analog to NG2S0
- CG2O8: Amide carbonyl C in β-lactam ring, analog to CG2O1

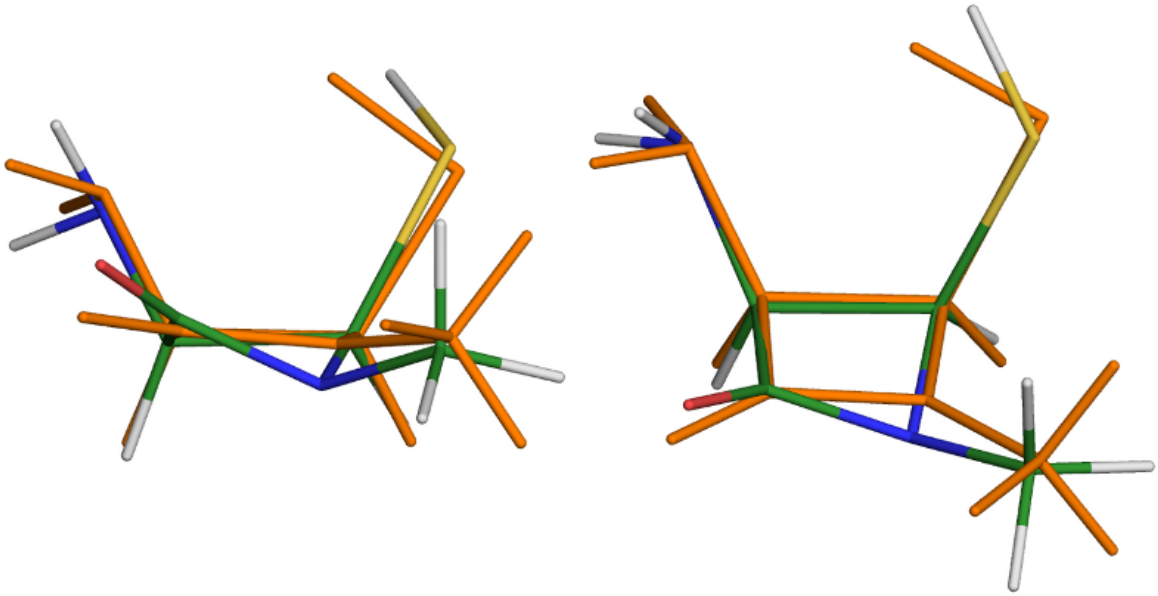

Figure S2: Comparison of QM and MM geometry optimization (two different orientations) for a minimal substituted β-lactam. QM-optimized structure in orange; MD-optimized structure is CPK-colored, with green carbons.

The previously shown β-lactam ring (Figure S2) was used for parameter optimization; atom numbering is shown in Figure S3.

Non-bonded terms

The non-bonded terms taken from existing atom types (CG311, NG2S0, CG2O1) were not reoptimized, in keeping with the recommended CGenFF strategy.

Geometry

Atom equilibrium distances, Urey-Bradley distances and angles involving the new atom types were set according to the QM-optimized geometry at the MP2 level of theory in the 6-31G(d) ensemble.

Partial charges

Merz-Kollman charges were computed at the MP2 level and used as initial guesses for partial atomic charges in the β-lactam ring, except for aliphatic protons, whose charge was set to 0.09 and not optimized. Substituent (CH3, SH and NH2) charges were set by analogy with existing CGenFF compounds and were not optimized. The equilibrium distances and energies of four water molecules were computed at the HF level (Figure S4).

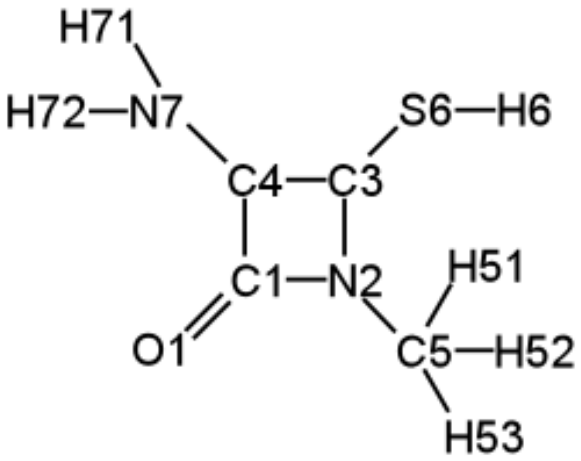

Figure S3: Substituted β-lactam used as parameterization starting point. Atom numbering is shown.

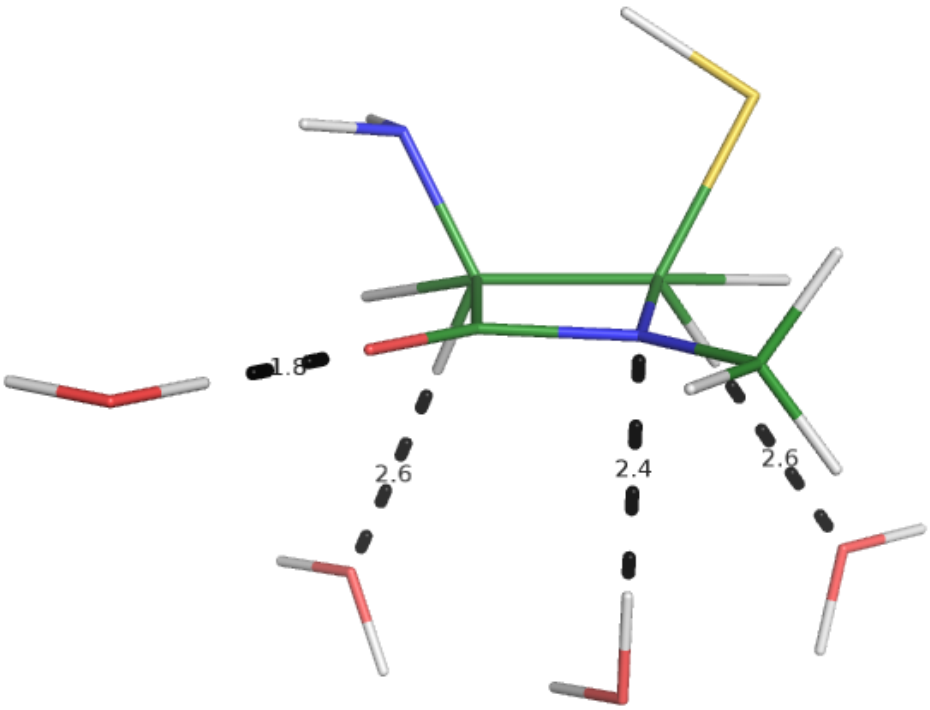

Figure S4: Solvation distances and orientations for β-lactam parameterization.

Table S1: Partial atomic charges on minimal substituted β-lactam

Atom	Type	Charge e	ΔE QM kcal/mol	ΔE MM kcal/mol	ΔΔE kcal/mol	d QM Å	d MM Å	Δd Å
C1	CG2O8	0.475						
O1	OG2D1	-0.475	-5.51	-5.52	0.01	2.06	1.79	0.27
N2	NG2S4	-0.410	0.21	0.29	-0.08	2.61	2.36	0.25
C3	CG315	0.195						
H3	HGA1	0.090	-2.52	-2.57	0.04	2.51	2.60	-0.09
C4	CG315	0.175						
H4	HGA1	0.090	-2.56	-2.63	0.07	2.54	2.60	-0.06
C5	CG331	-0.060						
H51	HGA3	0.090						
H52	HGA3	0.090						
S6	SG311	-0.230						
H6	HGP3	0.160						
N7	NG321	-0.960						
H71	HGPAM2	0.340						
H72	HGPAM2	0.340						

The same solvation was performed using the MM forcefield. Charges were adjusted manually to reproduce QM distances and interaction energies. Final partial charges are given in Table S1.

Bonded terms

Bond, angle and improper angle force constants involving new atom types were optimized by minimizing divergence between the QM and MM vibrational spectra. QM frequencies were computed at the MP2 level and compared to CHARMM-generated values (scaled by 94 %). The average difference between the two spectra was 8.5 % prior to optimization.

An automated optimization algorithm was used, which assumes a global minimum and slowly modifies all force constants to move towards that mimimum. Different starting points and algorithms were used, and all converged to the same point. After optimization, vibrational frequencies differed on average by 4.8 %. Optimized parameters are given in Tables S2 (bonds) and S3 (angles). Angles that contain only one new atom in a non central position were not reoptimized (Table S4). Improper angles were optimized (Table S5).

Optimizing dihedral angle and Urey-Bradley constants through vibrational spectra yeilded no measurable improvement compared to the values taken from CGenFF by analogy, so the initial values were kept unchanged. Since the β-lactam ring is very rigid, potential energy scans for dihedral constants (using *fit_ dihedral* [6], for instance) were deemed unnecessary. Dihedral and Urey-Bradley parameters are given in Tables S6 and S7, respectively.

Iteration

Partial charges were checked again since modifying the bonded terms could have an impact on solvation energies. However, no significant change was observed. A second round of optimization was therefore not necessary. (Solvation energies reported above are the final results after bonded

Table S2: Optimized bond constants for minimal substituted β-lactam

Bond		k	d
		kcal/mol	Å
CG2O8	OG2D1	725.0	1.22188
CG2O8	NG2S4	267.0	1.36713
CG2O8	CG315	297.5	1.53754
CG315	NG2S4	298.0	1.46504
CG315	HGA1	341.0	1.09676
CG315	CG315	270.0	1.57285
NG2S4	CG331	405.5	1.44255
CG315	SG311	283.5	1.80608
CG315	NG321	342.5	1.44688

Table S3: Optimized angle constants for minimal substituted β-lactam

Angle			k	θ
			kcal/mol	°
CG2O8	NG2S4	CG315	10.0	95.724
CG315	CG315	NG2S4	52.0	87.034
CG2O8	CG315	CG315	10.0	84.983
CG315	CG2O8	NG2S4	67.0	92.027
NG2S4	CG2O8	OG2D1	77.0	133.609
CG315	CG2O8	OG2D1	15.0	134.322
CG2O8	NG2S4	CG331	55.5	131.522
CG315	NG2S4	CG331	139.0	131.828
SG311	CG315	NG2S4	72.0	117.164
CG315	CG315	SG311	89.4	117.004
CG2O8	CG315	NG321	14.5	111.267
CG315	CG315	NG321	79.2	116.893
NG2S4	CG315	HGA1	45.5	112.900
CG315	CG315	HGA1	37.0	113.254
CG2O8	CG315	HGA1	40.0	113.556
SG311	CG315	HGA1	31.0	107.086
NG321	CG315	HGA1	13.0	115.059

Table S4: Angle constants not subjected to optimization for minimal substituted β-lactam

Angle			k	θ
			kcal/mol	°
NG2S4	CG331	HGA3	48.0	112.000
CG315	SG311	HGP3	38.8	95.000
CG315	NG321	HGPAM2	41.0	112.100

Table S5: Improper angle constants for minimal substituted β-lactam

Improper angle				k	m	p
				kcal/mol	-	°
CG2O8	X	X	OG2D1	161.5	0	0.00
NG2S4	CG2O8	CG315	CG331	0.0	0	0.00

Table S6: Dihedral angle constants for minimal substituted β-lactam

Dihedral angle				k	m	p
				kcal/mol	-	°
HGA1	CG315	NG2S4	CG2O8	0.8000	3	0.00
CG315	CG315	NG2S4	CG2O8	0.8000	3	0.00
SG311	CG315	NG2S4	CG2O8	0.8000	3	0.00
HGA3	CG331	NG2S4	CG2O8	0.0000	3	0.00
CG2O8	CG315	CG315	NG2S4	0.2000	3	0.00
CG2O8	CG315	CG315	HGA1	0.2000	3	0.00
CG2O8	CG315	CG315	SG311	0.2000	3	0.00
CG2O8	CG315	NG321	HGPAM2	0.3000	3	180.00
OG2D1	CG2O8	NG2S4	CG315	2.7500	2	180.00
OG2D1	CG2O8	NG2S4	CG315	0.3000	4	0.00
OG2D1	CG2O8	NG2S4	CG331	2.7500	2	180.00
OG2D1	CG2O8	NG2S4	CG331	0.3000	4	0.00
OG2D1	CG2O8	CG315	CG315	0.4000	1	180.00
OG2D1	CG2O8	CG315	CG315	0.6000	2	0.00
OG2D1	CG2O8	CG315	HGA1	0.4000	1	0.00
OG2D1	CG2O8	CG315	HGA1	0.6000	2	0.00
OG2D1	CG2O8	CG315	NG321	0.0000	1	0.00
NG2S4	CG2O8	CG315	CG315	0.4000	1	0.00
NG2S4	CG2O8	CG315	CG315	0.6000	2	0.00
NG2S4	CG2O8	CG315	HGA1	0.4000	1	180.00
NG2S4	CG2O8	CG315	HGA1	0.6000	2	0.00
NG2S4	CG2O8	CG315	NG321	0.3000	1	0.00
NG2S4	CG2O8	CG315	NG321	-0.3000	4	0.00
NG2S4	CG315	CG315	HGA1	0.2000	3	0.00
NG2S4	CG315	CG315	NG321	0.3000	3	180.00
NG2S4	CG315	SG311	HGP3	0.2000	3	0.00
CG315	CG2O8	NG2S4	CG315	2.7500	2	180.00
CG315	CG2O8	NG2S4	CG315	0.3000	4	0.00
CG315	NG2S4	CG331	HGA3	0.0000	3	0.00
CG315	CG315	NG321	HGPAM2	0.3000	3	180.00
HGA1	CG315	NG2S4	CG331	0.1000	3	0.00
HGA1	CG315	CG315	HGA1	0.1950	3	0.00
NG321	CG315	CG315	HGA1	0.1950	3	0.00
HGA1	CG315	SG311	HGP3	0.1950	3	0.00
CG315	CG2O8	NG2S4	CG331	2.7500	2	180.00
CG315	CG2O8	NG2S4	CG331	0.3000	4	0.00
CG315	CG315	NG2S4	CG331	0.1000	3	0.00
CG315	CG315	SG311	HGP3	0.1950	3	0.00
SG311	CG315	CG315	HGA1	0.1950	3	0.00
HGA1	CG315	NG321	HGPAM2	0.0100	3	0.00
SG311	CG315	NG2S4	CG331	0.1000	3	0.00
SG311	CG315	CG315	NG321	0.3000	3	180.00

Table S7: Urey-Bradley constants for minimal substituted β-lactam

Urey-Bradley			k	d
			kcal/mol	Å
CG315	CG315	HGA1	22.53	2.2445
NG321	CG315	HGA1	50.00	2.1554

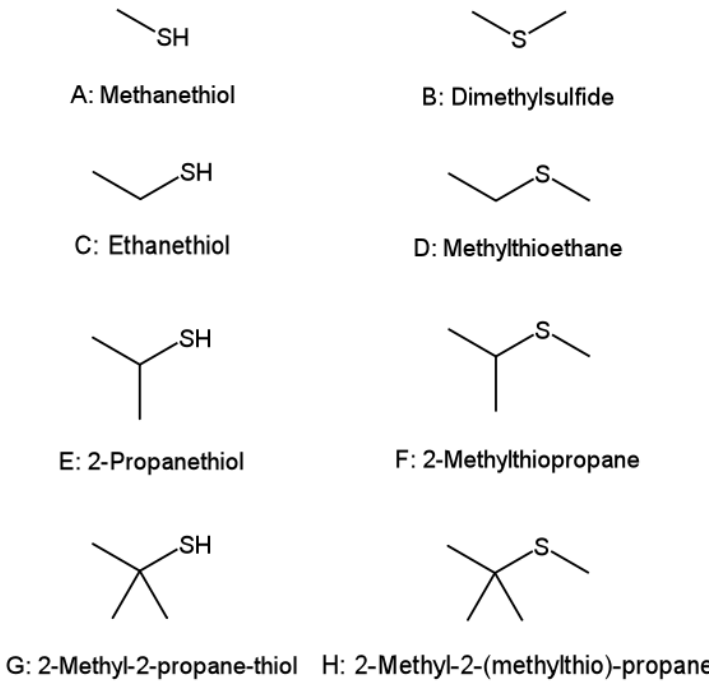

Figure S5: Sulfur-containing compounds used for SG311–GC301 bond parameterization.

parameters optimization.)

Sulfur-carbon bonds

Since the SG311–CG301 bond is not parameterized in CGenFF but is required to build the penicillin scaffold, it became the second parameterization step. Eight molecules were used to optimise carbon-sulfur bond and angle parameters (Figure S5).

Compounds A and B were used as controls for the CG331–SG311 bond already present in CGenFF. Compounds C and D were used as controls for the CG321–SG311 bond already present in CGenFF. Compounds E and F were used to parameterize the CG311–SG311 bond. Compounds G and H were used to parameterise the CG301–SG311 bond.

Partial charges for all atoms in these compounds were assigned using the systematic values seen in CGenFF: 0.09 for hydrogens, but 0.16 for sulfur-linked hydrogens. Sulfur atoms were given a charge of -0.23 if linked to only one carbon (compounds A, C, E, G) or -0.10 if linked to two carbons (compounds B, D, F and H). Carbons were given charges of -0.27, -0.18, -0.09 or 0.00 when linked to 3, 2, 1 or 0 hydrogen, respectively. Carbon charges were adjusted by +0.07 when linked to an –SH function, and

Table S8: Optimized bond constants for sulfur-containing compounds

Bond		k	d
		kcal/mol	Å
CG301	SG311	162.5	1.8387
CG311	SG311	197.0	1.8271

Table S9: Optimized angle constants for sulfur-containing compounds

Angle			k	θ
			kcal/mol	°
CG331	CG301	SG311	56.5	108.74
CG301	SG311	CG331	56.0	102.98
CG301	SG311	HGP3	54.5	96.22
CG331	CG311	SG311	51.5	109.77
SG311	CG311	HGA1	40.0	107.37
CG311	SG311	HGP3	51.0	96.59
CG311	SG311	CG331	93.5	100.48

by +0.05 when linked to an –S– function.

QM-geometry at MP2/6-31G(d) were used to determine equilibrium distances and angles. Values measured in compounds E and F were averaged to describe the CG311–SG311 bond and related angles. Values from compounds G and H were averaged to describe CG301–SG311.

Vibrational spectra were used to adjust bond and angle force constants as described previously. Final values are shown in Tables S8 (bonds) and S9 (angles). Values for existing parameters (controls, data not shown) were similar to CGenFF values (within 5 %). Dihedral angle optimization yielded no improvement over the values taken by analogy from existing CGenFF parameters (Table S10).

Penicillin scaffold

The β-lactam ring was then fused to the five-member cycle, and substituents added to form penicillin (Figure S6). Charges on removed hydrogen atoms were summed into the adjacent heavy atom. Charges on substituants were set according to the systematic values from existing CGenFF compounds. Missing dihedral angles were filled in by simple analogies with existing CGenFF parameters.

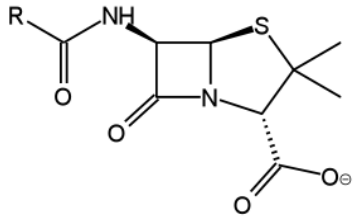

Figure S6: Penicillin scaffold allowing the generation of a variety of β-lactam antibiotics.

Table S10: Dihedral angle constants for sulfur-containing compounds

Dihedral angle				k	m	p
				kcal/mol	-	°
CG331	CG311	SG311	HGP3	1.3300	1	0.00
CG331	CG311	SG311	HPG3	0.1800	2	0.00
CG331	CG311	SG311	HGP3	0.3200	3	0.00
SG311	CG311	CG331	HGA3	0.1600	3	0.00
HGA1	CG311	SG311	HGP3	0.0000	3	0.00
CG331	CG311	SG311	CG331	0.4000	1	0.00
CG331	CG311	SG311	CG331	0.4900	3	0.00
HGA3	CG331	SG311	CG311	0.2840	3	0.00
HGA1	CG311	SG311	CG331	0.2840	3	0.00
CG331	CG301	SG311	CG331	0.4000	1	0.00
CG331	CG301	SG311	CG331	0.4900	3	0.00
SG311	CG301	CG331	HGA3	0.1600	3	0.00
HGA3	CG331	SG311	CG301	0.2840	3	0.00
CG331	CG301	SG311	HGP3	1.1300	1	0.00
CG331	CG301	SG311	HGP3	0.1400	2	0.00
CG331	CG301	SG311	HGP3	0.2400	3	0.00

Table S11: Bond lengths in final β-lactam compounds

Bond		Benzylpenicillin			Carbenicillin		
		QM	MM	Δ	QM	MM	Δ
		Å	Å	Å	Å	Å	Å
C1	N2	1.36	1.38	0.02	1.39	1.39	0.00
N2	C3	1.44	1.44	0.00	1.46	1.45	-0.01
C3	C4	1.55	1.57	0.02	1.57	1.57	0.00
C1	C4	1.54	1.55	0.01	1.55	1.55	0.00
C1	O1	1.19	1.22	0.03	1.22	1.22	0.00
N2	C5	1.45	1.45	0.00	1.46	1.45	-0.01
C3	S7	1.83	1.81	-0.02	1.84	1.81	-0.03
C4	N11	1.43	1.46	0.03	1.43	1.47	0.04

Final compounds

Toluene was then fused to penicillin to generate the classic benzylpenicillin antibiotics. Finally, a carboxyl function was fused to benzylpenicillin to generate carbenicillin. Charges on removed hydrogen atoms were summed into the adjacent heavy atom and charges on substituants were set according to the systematic values from existing CGenFF compounds. Missing dihedral angles were filled in by simple analogies with existing CGenFF parameters. Optimized geometry and dipole moment for the two compounds were compared to QM-derived values and shown to be similar (Figure S7 and Tables S11, S12 and S13).

Usage instructions

The atom nomenclature for benzylpenicillin (BZP) and carbenicillin (CBC) are given in Figure S8.

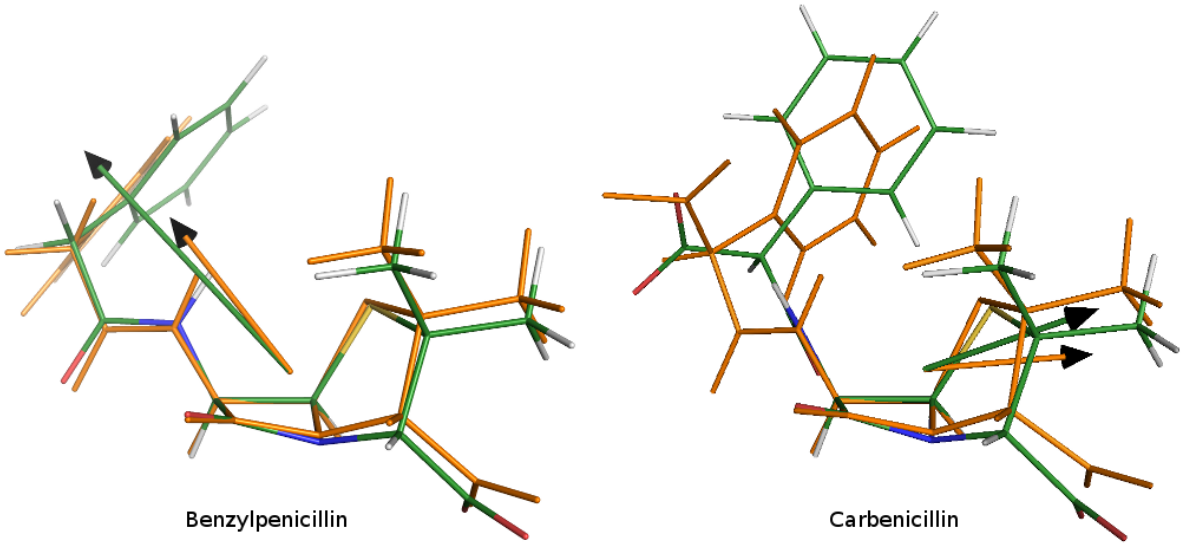

Figure S7: QM and MM Geometry and dipole comparison for benzylpenicillin and carbenicillin. QM-optimized structure in orange. MM-optimized structure is CPK-colored, with carbon in green. Arrows show dipole moment orientation (in orange and green for QM and MM, respectively).

Table S12: Angles in final β-lactam compounds

Angle			Benzylpenicillin			Carbenicillin		
			QM	MM	Δ	QM	MM	Δ
			°	°	°	°	°	°
C1	N2	C3	94.7	90.8	-3.9	93.8	90.1	-3.7
N2	C3	C4	88.0	90.6	2.6	88.3	90.5	2.2
C3	C4	C1	83.7	80.5	-3.2	83.9	79.9	-4.0
C4	C1	N2	91.5	93.6	2.1	91.1	93.8	2.7
O1	C1	N2	133.6	133.4	-0.2	133.4	132.6	-0.8
O1	C1	C4	134.9	132.9	-2.0	135.4	133.7	-1.7
C5	N2	C1	130.5	128.1	-2.4	130.5	127.4	-3.1
C5	N2	C3	117.1	121.8	4.7	115.3	122.1	5.8
S7	C3	N2	104.4	102.9	-1.5	104.3	102.7	-1.6
S7	C3	C4	117.9	121.2	3.3	115.9	122.8	6.9
N11	C4	C1	117.7	119.6	1.9	116.8	123.8	7.0
N11	C4	C3	119.5	115.5	-4.0	118.5	117.6	-0.9

Table S13: Dipoles in final β-lactam compounds

Dipole	BZP		CBC	
Debye	QM	MM	QM	MM
X	-8.4	-13.0	9.6	9.2
Y	5.2	8.4	1.4	-3.4
Z	8.6	11.8	1.4	4.8
T	13.1	19.5	9.8	11.0

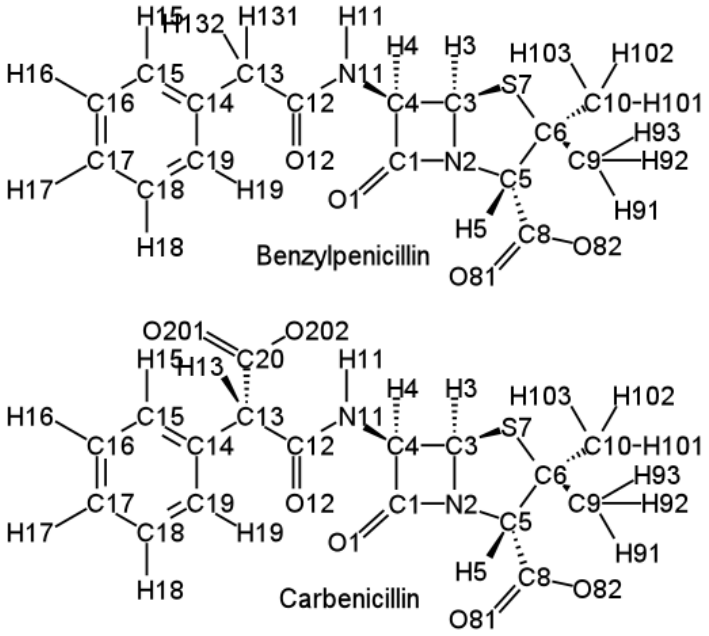

Figure S8: Atom names used in topology and parameter files for β-lactam antibiotics.

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* CHARMM Generalized Force Field beta-lactams
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36  1

RESI BZP  -1.0
ATOM      C1   CG2O8     0.475
ATOM      O1   OG2D1    -0.475
ATOM      N2   NG2S4    -0.410
ATOM      C3   CG315     0.195
ATOM      H3   HGA1      0.090
ATOM      C4   CG315     0.235
ATOM      H4   HGA1      0.090
ATOM      C5   CG3C51    0.030
ATOM      H5   HGA1      0.090
ATOM      C6   CG3C50    0.070
ATOM      S7   SG311    -0.230
ATOM      C8   CG2O3     0.340
ATOM      O81  OG2D2    -0.670
ATOM      O82  OG2D2    -0.670
ATOM      C9   CG331    -0.270
ATOM      H91  HGA3      0.090
ATOM      H92  HGA3      0.090
ATOM      H93  HGA3      0.090
ATOM      C10  CG331    -0.270
ATOM      H101 HGA3      0.090
ATOM      H102 HGA3      0.090
ATOM      H103 HGA3      0.090
ATOM      N11  NG2S1    -0.470
ATOM      H11  HGP1      0.310
ATOM      C12  CG2O1     0.510
ATOM      O12  OG2D1    -0.510
ATOM      C13  CG321    -0.180
ATOM      H131 HGA2      0.090
ATOM      H132 HGA2      0.090
ATOM      C14  CG2R61    0.000
ATOM      C15  CG2R61   -0.115
ATOM      H15  HGR61     0.115
ATOM      C16  CG2R61   -0.115
ATOM      H16  HGR61     0.115
ATOM      C17  CG2R61   -0.115
ATOM      H17  HGR61     0.115
ATOM      C18  CG2R61   -0.115
ATOM      H18  HGR61     0.115
ATOM      C19  CG2R61   -0.115
ATOM      H19  HGR61     0.115
```

```
BOND      C1   O1        C1   N2        C1   C4
BOND      N2   C3        N2   C5
BOND      C3   H3        C3   C4        C3   S7
BOND      C4   H4        C4   N11
BOND      C5   H5        C5   C6        C5   C8
BOND      C6   S7        C6   C9        C6   C10
BOND      C8   O81       C8   O82
BOND      C9   H91       C9   H92       C9   H93
BOND      C10  H101      C10  H102      C10  H103
BOND      N11  H11       N11  C12
BOND      C12  O12       C12  C13
BOND      C13  H131      C13  H132      C13  C14
BOND      C14  C15       C14  C19
BOND      C15  H15       C15  C16
BOND      C16  H16       C16  C17
BOND      C17  H17       C17  C18
BOND      C18  H18       C18  C19
BOND      C19  H19
IMPR      C1   N2   C4   O1
IMPR      N2   C1   C3   C5
IMPR      C8   C5   O81  O82
IMPR      N11  C4   C12  H11
IMPR      C12  N11  C13  O12
DONOR     H11  N11
ACCEPTOR  O1   C1
ACCEPTOR  O12  C12

IC  N2    C4   *C1    O1    1.3828   93.69  176.35  132.62  1.2226
IC  C4    C1    N2    C5    1.5486   93.69  149.24  128.45  1.4499
IC  C5    C1   *N2    C3    1.4499  128.45 -133.13   90.99  1.4415
IC  C4    N2   *C3    S7    1.5678   90.64  122.09  102.90  1.8104
IC  C4    N2   *C3    H3    1.5678   90.64 -120.21  114.47  1.0973
IC  C3    C1   *C4    N11   1.5678   80.55 -113.92  118.48  1.4619
IC  C3    C1   *C4    H4    1.5678   80.55  109.07  109.34  1.0958
IC  C1    N2    C5    C8    1.3828  128.45  156.55  109.47  1.5200
IC  C8    N2   *C5    C6    1.5200  109.47  118.72  103.65  1.5561
IC  C6    N    *C5    H5    1.5561  103.65  119.43  112.52  1.0988
IC  N2    C5    C8    O81   1.4499  109.47   39.51  117.97  1.2635
IC  O81   C5   *C8    O82   1.2635  117.97  179.38  115.31  1.2629
IC  S7    C5   *C6    C9    1.8894  107.10  115.59  111.14  1.5412
IC  S7    C5   *C6    C10   1.8894  107.10 -119.45  115.69  1.5401
IC  C5    C6    C9    H91   1.5561  111.14  -50.02  110.83  1.1091
IC  H91   C6   *C9    H92   1.1091  110.83  119.25  109.28  1.1103
IC  H91   C6   *C9    H93   1.1091  110.83 -121.21  110.67  1.1091
IC  C5    C6    C10   H101  1.5561  115.69 -174.81  110.87  1.1078
IC  H101  C6   *C10   H102  1.1078  110.87  120.70  109.69  1.1106
IC  H101  C6   *C10   H103  1.1078  110.87 -120.57  110.47  1.1116
IC  C1    C4    N11   C12   1.5486  118.48 -123.92  123.18  1.3318
IC  C12   C4   *N11   H11   1.3318  123.18  179.00  116.07  0.9946
IC  C4    N11   C12   C13   1.4619  123.18  178.84  117.69  1.5018
IC  C13   N11  *C12   O12   1.5018  117.69 -178.26  121.59  1.2230
```

```
IC  N11   C12   C13   C14   1.3318  117.69   32.50  126.90  1.5173
IC  C14   C12  *C13   H131  1.5173  126.90  123.38  105.57  1.1146
IC  H131  C12  *C13   H132  1.1146  105.57  112.32  105.23  1.1149
IC  C12   C13   C14   C19   1.5018  126.90 -169.20  117.43  1.4060
IC  C19   C13  *C14   C15   1.4060  117.43 -178.27  123.70  1.4095
IC  C13   C14   C15   C16   1.5173  123.70  179.82  120.44  1.4012
IC  C16   C14  *C15   H15   1.4012  120.44 -178.52  121.21  1.0788
IC  C14   C15   C16   C17   1.4095  120.44   -0.56  120.08  1.3998
IC  C17   C15  *C16   H16   1.3998  120.08 -179.84  119.80  1.0811
IC  C15   C16   C17   C18   1.4012  120.08   -0.45  119.98  1.4001
IC  C18   C16  *C17   H17   1.4001  119.98 -179.62  119.93  1.0804
IC  C19   C17  *C18   H18   1.4006  119.92  179.97  120.02  1.0803
IC  C18   C14  *C19   H19   1.4006  120.70 -179.26  119.58  1.0800

PATCH FIRST NONE LAST NONE

RESI CBC  -2.0
ATOM      C1   CG2O8     0.475
ATOM      O1   OG2D1    -0.475
ATOM      N2   NG2S4    -0.410
ATOM      C3   CG315     0.195
ATOM      H3   HGA1      0.090
ATOM      C4   CG315     0.235
ATOM      H4   HGA1      0.090
ATOM      C5   CG3C51    0.030
ATOM      H5   HGA1      0.090
ATOM      C6   CG3C50    0.070
ATOM      S7   SG311    -0.230
ATOM      C8   CG2O3     0.340
ATOM      O81  OG2D2    -0.670
ATOM      O82  OG2D2    -0.670
ATOM      C9   CG331    -0.270
ATOM      H91  HGA3      0.090
ATOM      H92  HGA3      0.090
ATOM      H93  HGA3      0.090
ATOM      C10  CG331    -0.270
ATOM      H101 HGA3      0.090
ATOM      H102 HGA3      0.090
ATOM      H103 HGA3      0.090
ATOM      N11  NG2S1    -0.470
ATOM      H11  HGP1      0.310
ATOM      C12  CG2O1     0.510
ATOM      O12  OG2D1    -0.510
ATOM      C13  CG311    -0.190
ATOM      H13  HGA1      0.090
ATOM      C14  CG2R61    0.000
ATOM      C15  CG2R61   -0.115
ATOM      H15  HGR61     0.115
ATOM      C16  CG2R61   -0.115
ATOM      H16  HGR61     0.115
ATOM      C17  CG2R61   -0.115
ATOM      H17  HGR61     0.115
```

```
ATOM      C18  CG2R61   -0.115
ATOM      H18  HGR61     0.115
ATOM      C19  CG2R61   -0.115
ATOM      H19  HGR61     0.115
ATOM      C20  CG2O3     0.620
ATOM      O201 OG2D2    -0.760
ATOM      O202 OG2D2    -0.760

BOND      C1   O1        C1   N2       C1   C4
BOND      N2   C3        N2   C5
BOND      C3   H3        C3   C4       C3   S7
BOND      C4   H4        C4   N11
BOND      C5   H5        C5   C6       C5   C8
BOND      C6   S7        C6   C9       C6   C10
BOND      C8   O81       C8   O82
BOND      C9   H91       C9   H92      C9   H93
BOND      C10  H101      C10  H102     C10  H103
BOND      N11  H11       N11  C12
BOND      C12  O12       C12  C13
BOND      C13  H13       C13  C14      C13  C20
BOND      C14  C15       C14  C19
BOND      C15  H15       C15  C16
BOND      C16  H16       C16  C17
BOND      C17  H17       C17  C18
BOND      C18  H18       C18  C19
BOND      C19  H19
BOND      C20  O201      C20  O202
IMPR      C1   N2   C4   O1
IMPR      N2   C1   C3   C5
IMPR      C8   C5   O81  O82
IMPR      N11  C4   C12  H11
IMPR      C12  N11  C13  O12
IMPR      C20  C13  O201 O202
DONOR     H11  N11
ACCEPTOR  O1   C1
ACCEPTOR  O12  C12

IC  N2   C4   *C1   O1    1.3873   93.79  178.23  133.62   1.2233
IC  C4   C1    N2   C5    1.5494   93.79  151.08  127.54   1.4521
IC  C5   C1   *N2   C3    1.4521  127.54 -132.20   90.11   1.4454
IC  C4   N2   *C3   S7    1.5743   90.53  123.81  102.68   1.8118
IC  C4   N2   *C3   H3    1.5743   90.53 -119.38  114.68   1.0969
IC  C3   C1   *C4   N11   1.5743   79.88 -116.51  123.77   1.4664
IC  C3   C1   *C4   H4    1.5743   79.88  108.93  107.73   1.0994
IC  C1   N2    C5   C8    1.3873  127.54  155.85  110.86   1.5215
IC  C8   N2   *C5   C6    1.5215  110.86  119.89  103.74   1.5571
IC  C6   N2   *C5   H5    1.5571  103.74  118.42  111.84   1.0990
IC  N2   C5    C8   O81   1.4521  110.86   38.99  118.26   1.2628
IC  O81  C5   *C8   O82   1.2628  118.26  179.22  115.30   1.2651
IC  S7   C5   *C6   C9    1.8882  107.32  115.45  111.15   1.5411
IC  S7   C5   *C6   C10   1.8882  107.32 -119.42  115.76   1.5405
IC  C5   C6    C9   H91   1.5571  111.15  -50.98  110.55   1.1099
```

```
IC  H91  C6   *C9   H92   1.1099  110.55  119.47  109.53   1.1095
IC  H91  C6   *C9   H93   1.1099  110.55 -120.52  110.60   1.1091
IC  C5   C6    C10  H101  1.5571  115.76 -175.73  110.69   1.1078
IC  H10  C6   *C10  H102  1.1078  110.69  120.75  109.70   1.1102
IC  H10  C6   *C10  H103  1.1078  110.69 -120.38  110.29   1.1115
IC  C1   C4    N11  C12   1.5494  123.77 -179.13  120.68   1.3383
IC  C12  C4   *N11  H11   1.3383  120.68  175.64  120.55   0.9995
IC  C4   N11   C12  C13   1.4664  120.68 -178.30  118.14   1.4903
IC  C13  N11  *C12  O12   1.4903  118.14  177.35  120.45   1.2280
IC  N11  C12   C13  C14   1.3383  118.14   73.23  112.86   1.5482
IC  C14  C12  *C13  C20   1.5482  112.86 -125.80  103.04   1.5281
IC  C14  C12  *C13  H13   1.5482  112.86  121.17  107.77   1.1058
IC  C12  C13   C14  C19   1.4903  112.86 -166.20  118.46   1.4108
IC  C19  C13  *C14  C15   1.4108  118.46 -173.62  124.04   1.4168
IC  C13  C14   C15  C16   1.5482  124.04  176.56  121.38   1.3997
IC  C16  C14  *C15  H15   1.3997  121.38  179.52  120.15   1.0795
IC  C14  C15   C16  C17   1.4168  121.38   -1.37  119.99   1.3986
IC  C17  C15  *C16  H16   1.3986  119.99  179.86  119.58   1.0801
IC  C15  C16   C17  C18   1.3997  119.99   -0.27  119.83   1.3988
IC  C18  C16  *C17  H17   1.3988  119.83  179.86  120.03   1.0793
IC  C19  C17  *C18  H18   1.3996  119.89  179.57  120.40   1.0793
IC  C18  C14  *C19  H19   1.3996  121.63 -177.67  118.65   1.0808
IC  C12  C13   C20  O201  1.4903  103.04  102.23  117.85   1.2582
IC  O20  C13  *C20  O202  1.2582  117.85 -179.13  115.41   1.2598

PATCH FIRST NONE LAST NONE

end

read param card flex append
* CHARMM Generalized Force Field beta-lactams
*

BONDS
CG2O8  OG2D1  725.0    1.22188
CG2O8  NG2S4  267.0    1.36713
CG2O8  CG315  297.5    1.53754
CG315  NG2S4  298.0    1.46504
CG315  HGA1   341.0    1.09676
CG315  CG315  270.0    1.57285
NG2S4  CG3C51 405.5    1.44255
CG315  SG311  283.5    1.80608
CG315  NG2S1  342.5    1.44688
CG3C50 SG311  162.5    1.8387
CG3C50 CG3C51 222.50   1.5000
CG3C50 CG331  222.50   1.5380

ANGLES
NG2S4  CG2O8  OG2D1  77.0    133.609
CG315  CG2O8  OG2D1  15.0    134.322
CG315  CG2O8  NG2S4  67.0     92.027
CG2O8  NG2S4  CG315  10.0     95.724
```

```
NG2S4  CG315  HGA1   45.5    112.900
CG315  CG315  NG2S4  52.0     87.034
CG315  CG315  HGA1   37.0   113.254  22.53   2.2445
CG2O8  CG315  CG315  10.0     84.983
CG2O8  CG315  HGA1   40.0    113.556
CG2O8  NG2S4  CG3C51  55.5  131.522
CG315  NG2S4  CG3C51 139.0  131.828
SG311  CG315  NG2S4  72.0  117.164
SG311  CG315  HGA1   31.0  107.086
CG315  CG315  SG311  89.4  117.004
CG2O8  CG315  NG2S1  14.5  111.267
CG315  CG315  NG2S1  79.2  116.893
NG2S1  CG315  HGA1   13.0  115.059   50.00   2.15540
NG2S4  CG3C51 HGA1     48.00    112.00
CG3C50 CG3C51 NG2S4    70.00    110.80
CG2O3  CG3C51 NG2S4    50.00    107.00
CG3C50 CG3C51 HGA1     34.60    110.10   22.53   2.179
CG2O3  CG3C51 CG3C50   52.00    108.00
CG3C51 CG3C50 SG311    56.5     108.74
CG3C51 CG3C50 CG331    58.35    113.50   11.16   2.561
CG331  CG3C50 SG311    56.5     108.74
CG331  CG3C50 CG331    58.35    113.50   11.16   2.561
CG315  SG311  CG3C50   93.5     100.48
CG3C50 CG331  HGA3     33.43    110.10   22.53   2.17900
CG315  NG2S1  HGP1     35.00    117.00
CG2O1  NG2S1  CG315    50.00    120.00
CG2O1  CG311  CG2R61   52.00    108.00
CG2O1  CG311  CG2O3    52.00    108.00
CG2O1  CG321  CG2R61   52.00    108.00

DIHEDRALS
HGA1   CG315  NG2S4  CG2O8     0.8000  3     0.00
CG315  CG315  NG2S4  CG2O8     0.8000  3     0.00
SG311  CG315  NG2S4  CG2O8     0.8000  3     0.00
CG2O8  CG315  CG315  NG2S4     0.2000  3     0.00
CG2O8  CG315  CG315  HGA1      0.2000  3     0.00
CG2O8  CG315  CG315  SG311     0.2000  3     0.00
OG2D1  CG2O8  NG2S4  CG315     2.7500  2   180.00
OG2D1  CG2O8  NG2S4  CG315     0.3000  4     0.00
OG2D1  CG2O8  CG315  CG315     0.4000  1   180.00
OG2D1  CG2O8  CG315  CG315     0.6000  2     0.00
OG2D1  CG2O8  CG315  HGA1      0.4000  1     0.00
OG2D1  CG2O8  CG315  HGA1      0.6000  2     0.00
NG2S4  CG2O8  CG315  CG315     0.4000  1     0.00
NG2S4  CG2O8  CG315  CG315     0.6000  2     0.00
NG2S4  CG2O8  CG315  HGA1      0.4000  1   180.00
NG2S4  CG2O8  CG315  HGA1      0.6000  2     0.00
NG2S4  CG315  CG315  HGA1      0.2000  3     0.00
CG315  CG2O8  NG2S4  CG315     2.7500  2   180.00
CG315  CG2O8  NG2S4  CG315     0.3000  4     0.00
HGA1   CG315  CG315  HGA1      0.1950  3     0.00
SG311  CG315  CG315  HGA1      0.1950  3     0.00
```

```
HGA1   CG3C51 NG2S4  CG2O8     0.8000  3     0.00
CG3C50 CG3C51 NG2S4  CG2O8     0.8000  3     0.00
CG2O3  CG3C51 NG2S4  CG2O8     0.8000  3     0.00
CG2O8  CG315  NG2S1  HGP1      0.0000  1     0.00
CG2O8  CG315  NG2S1  CG2O1     0.2000  1   180.00
OG2D1  CG2O8  NG2S4  CG3C51    2.7500  2   180.00
OG2D1  CG2O8  NG2S4  CG3C51    0.3000  4     0.00
OG2D1  CG2O8  CG315  NG2S1     0.0000  1     0.00
NG2S4  CG2O8  CG315  NG2S1     0.4000  1     0.00
NG2S4  CG315  CG315  NG2S1     0.2000  3     0.00
NG2S4  CG315  SG311  CG3C50    0.1400  3     0.00
NG2S4  CG3C51 CG3C50 SG311     0.0500  3     0.00
NG2S4  CG3C51 CG3C50 CG331     0.0500  3     0.00
OG2D2  CG2O3  CG3C51 NG2S4     0.0000  6   180.00
HGA1   CG3C51 NG2S4  CG315     0.1000  3     0.00
CG3C50 CG3C51 NG2S4  CG315     0.1000  3     0.00
CG2O3  CG3C51 NG2S4  CG315     0.1000  3     0.00
CG315  CG315  NG2S1  HGP1      0.0000  1     0.00
CG315  CG315  NG2S1  CG2O1     1.8000  1     0.00
CG3C51 CG3C50 SG311  CG315     0.1580  3     0.00
CG331  CG3C50 SG311  CG315     0.2000  3     0.00
HGA1   CG315  NG2S4  CG3C51    0.1000  3     0.00
NG2S1  CG315  CG315  HGA1      0.2000  3     0.00
HGA1   CG315  SG311  CG3C50    0.1950  3     0.00
CG311  CG2O1  NG2S0  CG3C51    2.7500  2   180.00
CG315  CG2O8  NG2S4  CG3C51    0.3000  4     0.00
CG315  CG315  NG2S4  CG3C51    0.1000  3     0.00
CG315  CG315  SG311  CG3C50    0.2000  3     0.00
OG2D1  CG2O1  NG2S1  CG315     2.5000  2   180.00
CG321  CG2O1  NG2S1  CG315     1.6000  1     0.00
CG321  CG2O1  NG2S1  CG315     2.5000  2   180.00
HGA1   CG315  NG2S1  HGP1      0.0000  1     0.00
HGA1   CG315  NG2S1  CG2O1     0.0000  1     0.00
SG311  CG315  NG2S4  CG3C51    0.1000  3     0.00
CG3C51 CG3C50 CG331  HGA3      0.1500  3   180.00
SG311  CG3C50 CG3C51 HGA1      0.0500  3     0.00
CG331  CG3C50 CG3C51 HGA1      0.0500  3     0.00
OG2D2  CG2O3  CG3C51 CG3C50    0.0500  6   180.00
SG311  CG315  CG315  NG2S1     0.2000  3     0.00
SG311  CG3C50 CG3C51 CG2O3     0.0500  3     0.00
SG311  CG3C50 CG331  HGA3      0.1600  3     0.00
CG331  CG3C50 CG3C51 CG2O3     0.1580  3     0.00
CG331  CG3C50 CG331  HGA3      0.1600  3     0.00
CG311  CG2O1  NG2S1  CG315     1.6000  1     0.00
CG311  CG2O1  NG2S1  CG315     2.5000  2   180.00
NG2S1  CG2O1  CG311  CG2R61    0.0000  1     0.00
NG2S1  CG2O1  CG311  CG2O3     0.0000  1     0.00
CG2R61 CG2R61 CG311  CG2O1     0.2300  2   180.00
OG2D2  CG2O3  CG311  CG2O1     0.0500  6   180.00
OG2D1  CG2O1  CG311  CG2R61    1.4000  1     0.00
OG2D1  CG2O1  CG311  CG2O3     1.4000  1     0.00
NG2S1  CG2O1  CG321  CG2R61    0.0000  1     0.00
```

```
CG2R61 CG2R61 CG321  CG2O1     0.2300  2   180.00
OG2D1  CG2O1  CG321  CG2R61    0.0500  6   180.00

IMPROPERS
CG2O8  X      X      OG2D1   161.5  0     0.00
NG2S4  CG2O8  CG315  CG3C51    0.0  0     0.00

end

return
```

To use this file, the CGenFF forcefield must be patched to add the new atom types. Here are the relevant lines which must be added to the appropriate sections in the forcefield files:

```
ATOMS
MASS   92  CG2O8  12.01100 ! Carboxyl C in beta-lactam ring
MASS   93  CG315  12.01100 ! Aliphatic C for CH in beta-lactam ring
MASS  126  NG2S4  14.00700 ! N,N-Disubstituted amide N in beta-lactam ring

NONBONDED
NG2S4  0.0  -0.2000  1.8500  0.0  -0.20  1.55
CG2O8  0.0  -0.1100  2.0000
CG315  0.0  -0.0320  2.0000  0.0  -0.01  1.90
```

Backbone dynamics

Simulation stability was assessed from global RMSD for main chain heavy atoms, as shown in Figure S9 and discussed in the main text. Example of correlation functions used to compute synthetic S^2 are given in Figure S10. These show that the vast majority of residues display converging functions; the averaging over ten trajectories further improve S^2 estimate, yielding parameters that are in agreement with NMR relaxation results, as discussed in the main text.

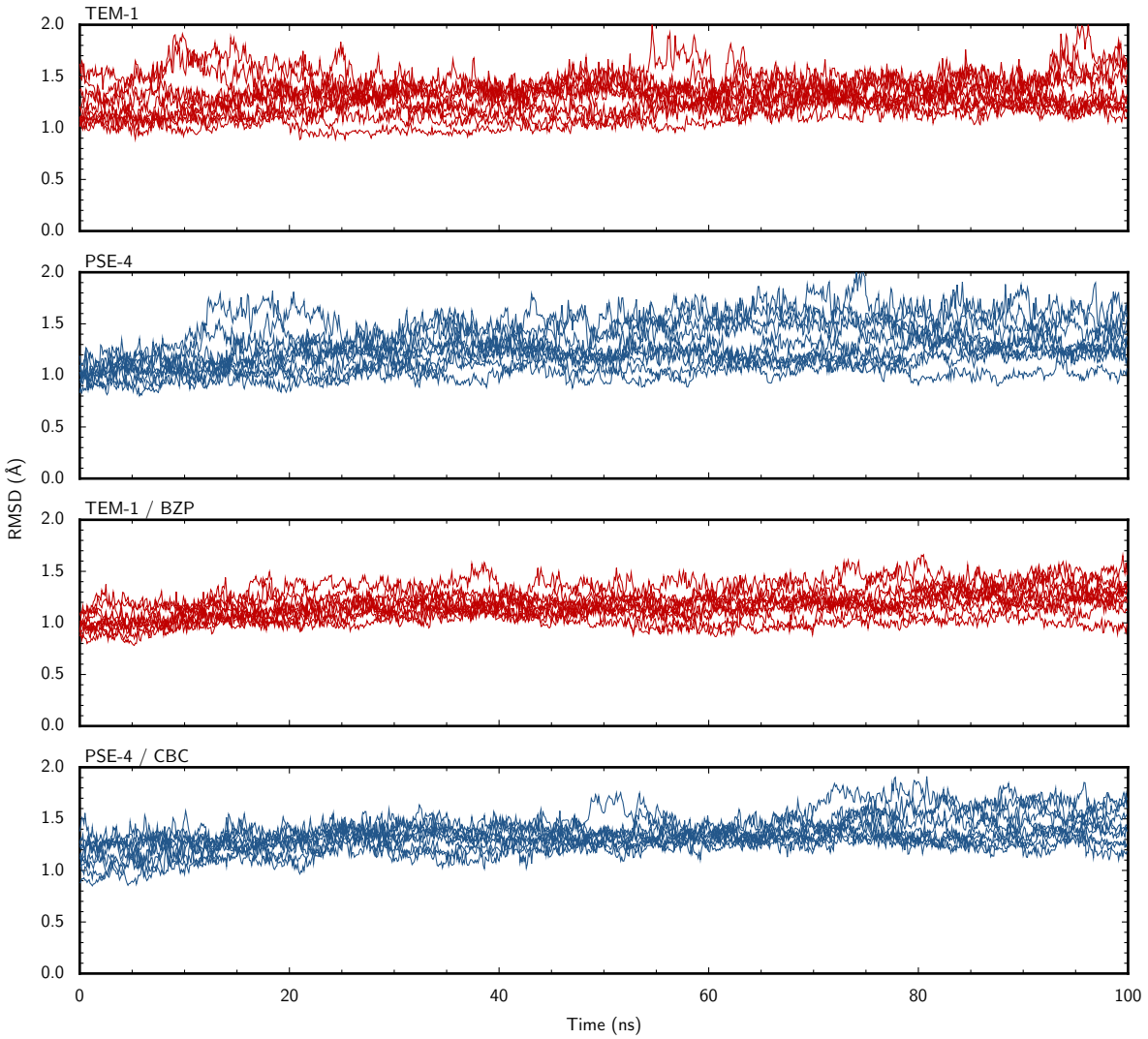

Figure S9: Global RMSD for all simulations. Only main chain heavy atoms were considered. For each of the four systems (TEM-1, PSE-4, TEM-1 / BZP and PSE-4 / CBC), ten 100 ns production trajectories were recorded and are shown together in the respective panels. Data points are 10 ps apart and averaged over that time period (10 values).

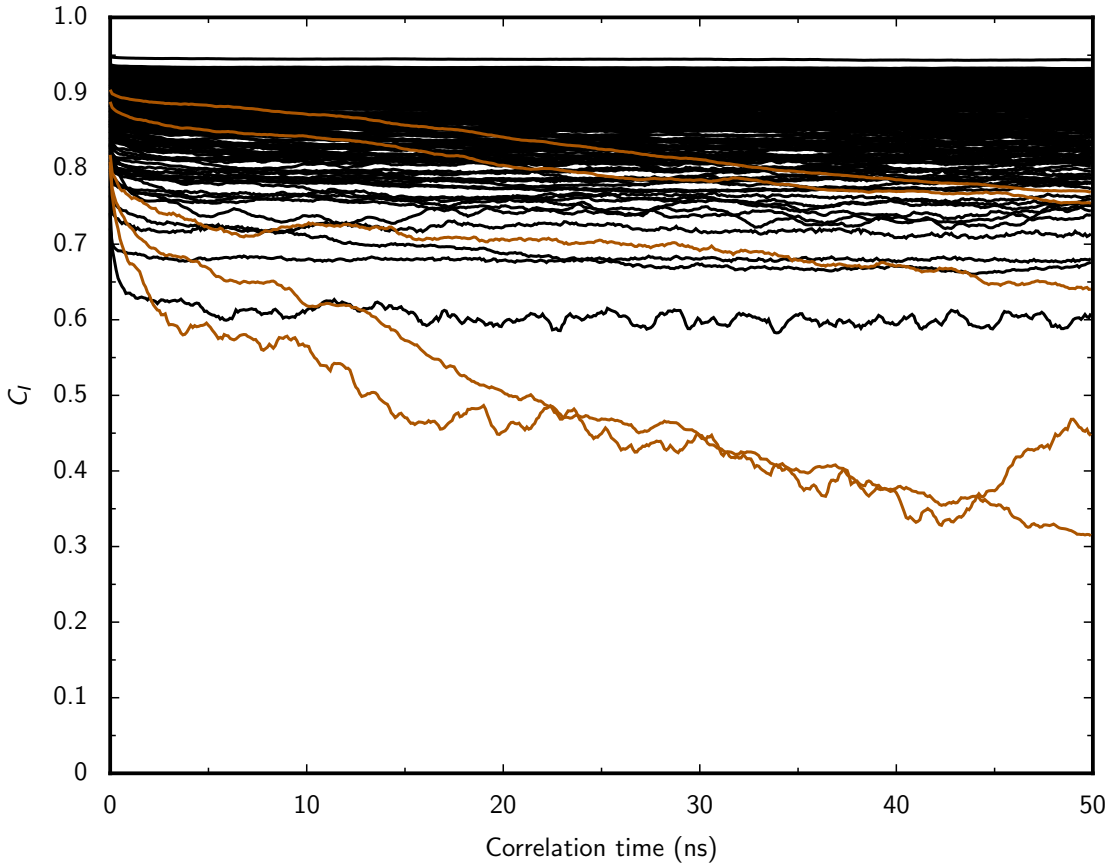

Figure S10: Example $C_I(t)$ autocorrelation functions for all residues in one free form TEM-1 trajectory. Functions that are unconverged at $t = 10\,\text{ns}$ are shown in orange. Convergence criteria is $\Delta C_I < 0.05$ between $t = 10\,\text{ns}$ and $t = 50\,\text{ns}$. There are five unconverged residues: R43, H158, K288, H289 and W290. Similar results are observed for the other trajectories, but unconverged residues vary.

Essential dynamics movie list

Movie S1: PCA for TEM-1 (free form) – Modes 1 to 5

Movie S2: PCA for PSE-4 (free form) – Modes 1 to 5

Movie S3: PCA for TEM-1 / BZP – Modes 1 to 5

Movie S4: PCA for PSE-4 / CBC – Modes 1 to 5

Eigenvectors are displayed consecutively in each movie. System and vector number are shown in upper-left corner. Residues are colored on a scale from blue to red according to their amplitude of motion within the eigenvector; blue means little amplitude; red high amplitude.

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