Can Molecular Dynamics and QM/MM NBO solve the Penicillin Binding Protein Protonation Puzzle?

Jacqueline C. Hargis, Justin K. White, Yu Chen[‡] & H. Lee Woodcock*

Department of Chemistry, University of South Florida, Tampa, Florida 33620, United States
[‡]*Department of Molecular Medicine, College of Molecular Medicine, University of South Florida, Tampa, Florida, 33612, United States *HLW email: hlw@usf.edu*

Supporting Information

NBO setup

Two NBO calculations were performed for each protonation state. For the remainder of the NBO methods, we will refer to them as NBO-1-X and NBO-2-X where X is the protonation state. NBO-1 is uniform for all link atoms and residues for all protonation states, however the inclusion of water molecules differs for each protonation state. This is also true for the NBO-2 calculations. All link atom cuts were chosen between carbon-carbon bonds to strategically include all atoms interacting with benzylpenicillin.

Table S1. DD-Peptidase NBO-1 QM region: This table includes the waters for each protonation state included in the QM region.

Protonation State	Xtal waters	Solvated Waters
I	451, 16, 160, 202, 291	6417, 6404, 6441, 8586
II	27, 16, 291	5837, 6386, 8038
III	16, 27, 110, 373	6990
IV	27, 16	8522
V	451, 16, 27	n/a
VI	451, 16, 27, 110	6401

Table S2: DD-Peptidase NBO-2 QM region: This table shows all atoms included in the QM region by type for each residue. Additionally, information about the link atoms and cuts are included. Not applicable was denoted by n/a.

Residue	QM Atoms	Link atom in QM	Link atom excluded
Benzylpenicillin	All	n/a	n/a
Ser62	N HN CA HA CB HB1 HB2 OG HG1	CA	C
Gly61	C O	C	CA
His298	N HN CA HA CB HB1 HB2 CD2 HD2 CG NE2 HE2 ND1 HD1 CE1 HE1	CA	C
Gly297	C O	C	CA
Tyr159	CB HB1 HB2 CG CD1 HD1 CE1 HE1 CZ OH HH CD2 HD2 CED2 HE2	CB	CA
Lys65	N HN CA HA CB HB1 HB2 CG HG1 HG2 CD HD1 HD2 CE HE1 HE2 NZ HZ1 HZ2 HZ3	CA	C
Thr64	C O	C	CA
Arg285	N HN CA HA CB HB1 HB2 CG HG1 HG2 CD HD1 HD2 NE HE	CA	C

	CZ NH1 HH11 HH12 NH2 HH21 HH22		
Leu284	C O	C	CA

Table S3: DD-Peptidase NBO-3 QM region: This table includes the waters for each protonation state included in the QM region.

Protonation State	Xtal waters	Solvated Waters
I	n/a	n/a
II	n/a	n/a
III	373	n/a
IV	n/a	n/a
V	372	8725
VI	103	8502, 9312, 6401, 6421, 6990

Table S4: DD-Peptidase NBO-4 QM region: The following table shows all atoms included in the QM region by type for each residue. Additionally, information about the link atoms and cuts are included.

Residue	QM Atoms	Link atom in QM	Link atom excluded
Benzylpenicillin	All	n/a	n/a
His298	C O	C	CA
Thr299	N HN CA HA CB HB OG1 HG1 CG2 HG21 HG22 HG23 C O (all)	n/a	n/a
Gly300	N HN CA HA1 HA2 C O (all)	n/a	n/a
Thr301	N HN CA HA CB HB OG1 HG1 CG2 HG21 HG22 HG23 C O (all)	n/a	n/a
Val302	N HN CA HA	CA (two cuts)	C, CB

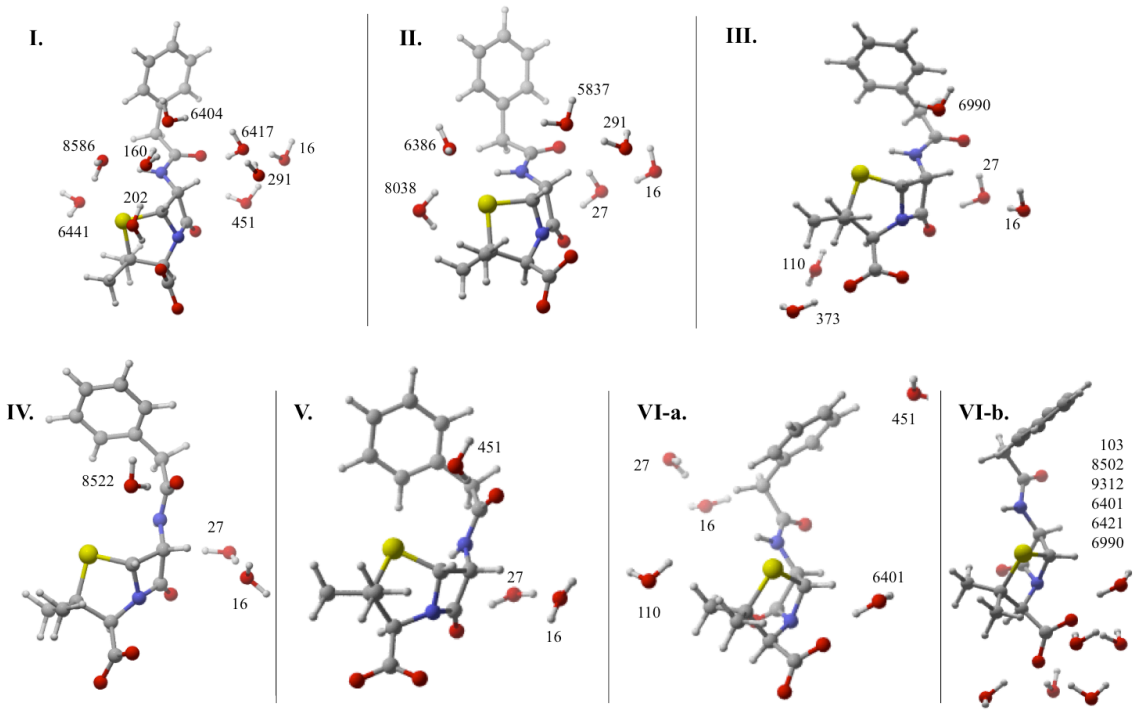

Figure S1: The figure shows the placement of waters in the NBO calculations.

Beta-Lactamase NBO Setup

The beta-lactamase calculations were broken up into four NBO calculations.

The first table includes the waters for each protonation state included in the QM region. The following tables show all atoms included in the QM region by type for each residue. Additionally, information about the link atoms and cuts are included. Not applicable was denoted by n/a.

Table S5. Beta-lactamase-NBO-1 QM region: This calculation included the CFXA ligand and the waters.

Protonation State	Xtal waters	Solvated Waters
A	146	1066, 7812, 570, 6635, 3588
B	274, 37, 53, 133	7874, 893, 964, 2416, 3413, 1736, 1399, 1726, 3526, 363, 6136, 1112, 5399, 1384, 3355, 1944, 3266, 6421, 3527

Table S6. Beta-lactamase-NBO-2 QM region: CFXA & Residues 70, 216, 233-236

Residue	QM Atoms	Link atom in QM	Link atom excluded
CFXA	All	n/a	n/a
Ser70	CB HB1 BH2 OG	CB	CA
Thr216	CB HB OG1 HG1 CG2 HG21 HG22 HG23	CB	CA
Asp233	C O	C	CA
Lys234	N HN CA HA CB HB1 HB2 CG HG1 HG2 CD HD1 HD2 CE HE1 HE2 NZ HZ1 HZ2 HZ3 C O (all)	n/a	n/a
Thr235	N HN CA HA CB HB OG1 HG1 CG2 HG21 HG22 HG23 C O (all)	n/a	n/a
Gly236	N HN CA HA1 HA2	CA	C

Table S7. Beta-lactamase-NBO-3 QM region: CFXA & Residues 104, 105, 130, 132

Residue	QM Atoms	Link atom in QM	Link atom excluded
CFXA	All	n/a	n/a
Asn104	H HN CA HA CB HB1 HB2 CG OD1 ND2 HD21 HD22	CA	C
Tyr105	CB HB1 HB2 CG CD1 HD1 CE1 HE1 CZ OH HH CD2 HD2 CE2 HE2	CB	CA
Ser130	N HN CA HA CB HB1 HB2 OG HG1	CA	C
Asn132	H HN CA HA CB HB1 HB2 CG OD1 ND2 HD21 HD22	CA	C

Table S8. Beta-lactamase-NBO-4 QM region: CFXA & Residues 73, 166, 167, 170, 236, 237, 218

Residue	QM Atoms	Link atom in QM	Link atom excluded
CFXA	All	n/a	n/a
Lys73	CB HB1 HB2 CG HG1 HG2 CD HD1 HD2 CE HE1 HE2 NZ HZ1 HZ2 HZ3	CB	CA
Glu166	CB HB1 HB2 CG HG1 HG2 CD OE1 OE2 HE2	CB	CA
Pro167	N CD HD1 HD2 CA HA CB HB1 HB2 CG HG1 HG2 C O	n/a	n/a
Asn170	N HN CA HA CB HB1 HB2 CG OD1 ND2 HD21 HD22	CA	C
Gly236	C O	C	CA
Ser237	N HN CA HA CB HB1 HB2 OG HG1 C O	n/a	n/a
Gly238	N HN CA HA1 HA1	CA	C