

Breaking Benzene Aromaticity – Computational Insights into the Mechanism of the Tungsten-Containing Benzoyl-CoA Reductase

Supporting Information

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S1 Supplementary figures

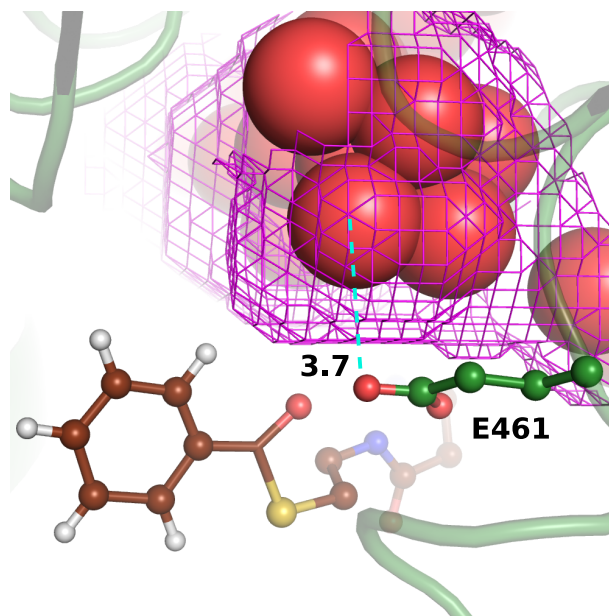


Figure S1: Water channel that leads to E461 and enables its protonation when the substrate is bound in the cavity. Magenta mesh represents the solvent accessible volumes, red spheres potential water molecules as placed by McVol. Distance between last water molecule and E461 is 3.7 Å.

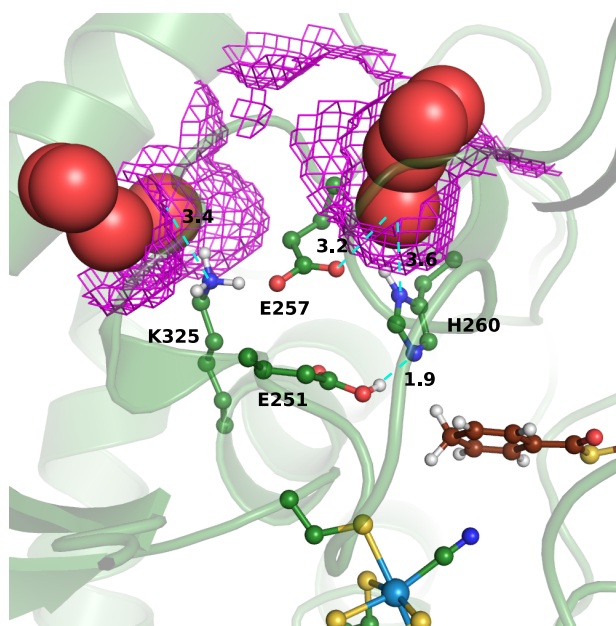


Figure S2: Proton transfer chain residues (K325, E257 and H260) are located in direct neighborhood of two water channels channels detected by McVol program. Magenta mesh represents the solvent accessible volumes, red spheres potential water molecules. Distances between last water molecule and the proton acceptors are in Å.

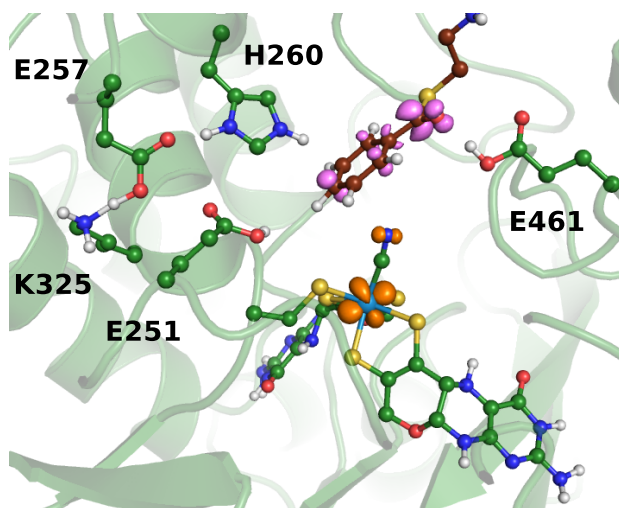


Figure S3: Unpaired spin density distribution with the substrate in the active site with $X = \text{CN}^-$ and total charge that corresponds to W(IV) oxidation state. α spin in orange, β spin in violet (isovalue $0.005 \text{ e}/\text{\AA}^3$).

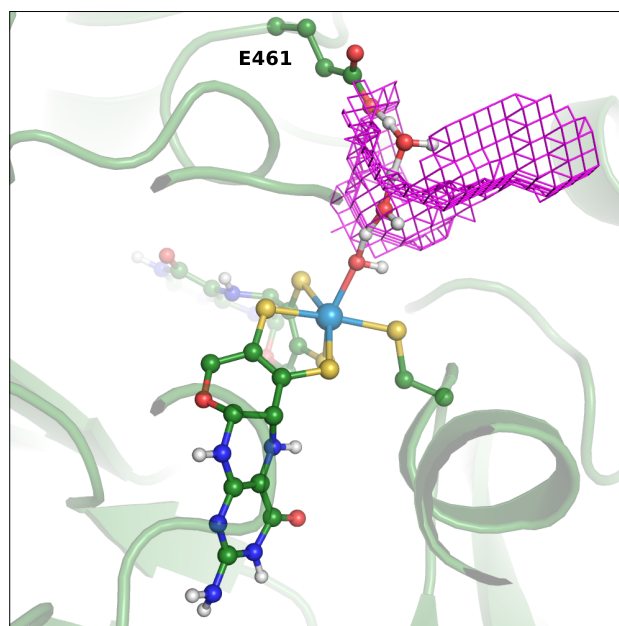


Figure S4: Transition state of the ligand X (OH here) reprotonation by a chain of two water molecules and E461 at W(IV) state without bound CoA ester. Magenta mesh represents the solvent accessible volume determined by McVol.

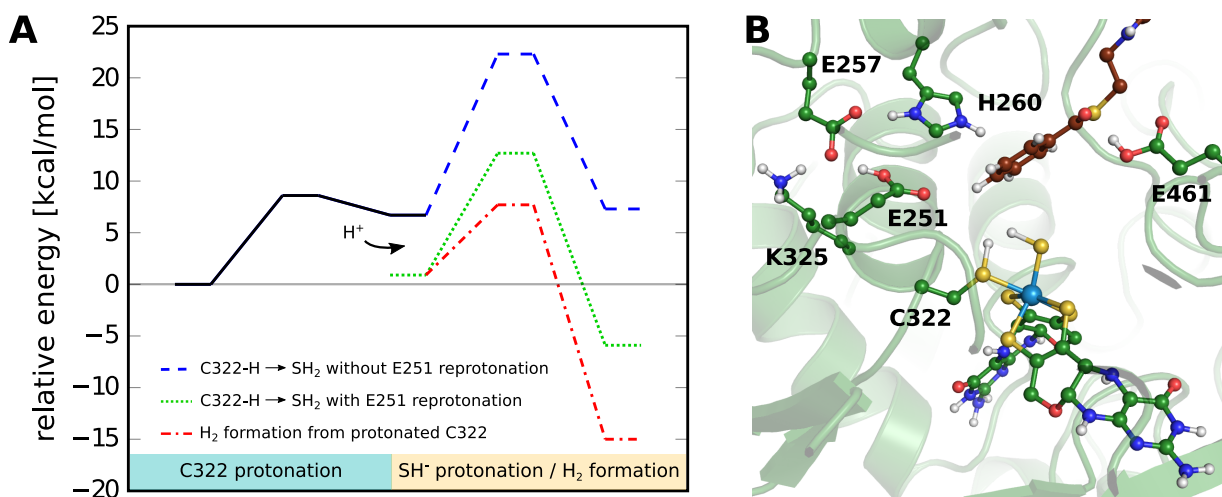


Figure S5: **A:** Energy profiles of the SH^- to SH_2 protonation with substrate bound in the cavity at W(IV) oxidation state. The reaction is unfeasible unless E251 gets reprotonated from the solvent immediately after it protonates C322. Even in this case, however, a competitive H_2 formation reaction has much lower energy barrier. **B:** Transition state structure for the H_2 formation reaction.

S2 Supplementary tables

Table S1: Differences in QM potential energies (kcal/mol) of the alternative orientations of the diatomic ligands X in an isolated BamB WPT-center model in gas phase at three tungsten oxidation states. For most cases, the orientation in which the carbon points towards the tungsten ion is preferred. The only exception is the CNH coordinated W(VI). However, this state is unlikely to occur because it is electrostatically disfavored, since the active site would have a charge of +1.

energy difference (kcal/mol)	W(IV)	W(V)	W(VI)
E(W-CN) - E(W-NC)	-9.4	-9.0	-3.9
E(W-CNH) - E(W-NCH)	-7.3	-0.9	2.7
E(W-CO) - E(W-OC)	-37.6	-24.8	-26.3

Table S2: Sums of Mulliken spin populations on different parts of the QM region for mechanism A & A': Ligand X donates the first proton, H260 donates the second proton.

X	Sub			In			Pro		
	W-S ₅ X	Pt ₂	bz.	W-S ₅ X	Pt ₂	bz.	W-S ₅ X	Pt ₂	bz.
SH ⁻	0.62	0.00	-0.61	0.00	0.00	0.00	0.00	0.00	0.00
SH ₂	0.12	0.02	-0.14	0.92	-0.42	-0.48	0.96	-0.96	0.00
OH ⁻	0.87	0.00	-0.75	0.00	0.00	0.00	0.00	0.00	0.00
OH ₂	0.10	0.03	-0.13	0.90	-0.35	-0.54	0.71	-0.70	0.00
CNH	0.00	0.13	-0.15	0.97	0.33	0.62	0.97	-0.98	0.02

W-S₅X Tungsten and its first and second sphere ligand atoms

Pt₂ Pterin parts of the pyranopterin cofactors

bz. QM-treated part of benzoyl-CoA

Table S3: Sums of Mulliken spin populations on different parts of the QM region for mechanism **B**: H260 donates both first and second proton.

X	Sub			In			In-H			Pro		
	W-S ₅ X	Pt ₂	bz.	W-S ₅ X	Pt ₂	bz.	W-S ₅ X	Pt ₂	bz.	W-S ₅ X	Pt ₂	bz.
SH ⁻	0.62	0.00	-0.61	1.00	-0.12	-0.86	0.86	-0.28	-0.56	0.80	-0.80	0.00
SH ₂	0.12	0.02	-0.14	0.26	0.74	-0.97	_a	_a	_a	_a	_a	_a
OH ⁻	0.87	0.00	-0.75	0.98	-0.21	-0.76	0.90	-0.40	-0.49	0.83	-0.83	0.00
OH ₂	0.10	0.03	-0.13	0.44	0.51	-0.92	_a	_a	_a	_a	_a	_a
CN ⁻	0.43	0.00	-0.42	0.99	-0.05	-0.94	0.87	-0.48	-0.38	0.94	-0.98	0.03
CNH	0.00	0.13	-0.15	-0.04	0.99	-0.92	_a	_a	_a	_a	_a	_a
CO	0.18	0.00	-0.18	0.00	0.97	-0.97	-0.01	0.85	-0.83	-0.01	0.12	-0.10

^a Not determined, since the first step is already energetically unfeasible.

W-S₅X Tungsten and its first and second sphere ligand atoms

Pt₂ Pterin parts of the pyranopterin cofactors

bz. QM-treated part of benzoyl-CoA

Table S4: Sums of Mulliken spin populations on different parts of the QM region for mechanism **C**: E251 donates the first proton, H260 donates the second proton.

X	Sub			In			Pro		
	W-S ₅ X	Pt ₂	bz.	W-S ₅ X	Pt ₂	bz.	W-S ₅ X	Pt ₂	bz.
SH ⁻	0.62	0.00	-0.61	0.83	-0.14	-0.66	0.90	-0.49	0.00
SH ₂	0.12	0.02	-0.14	0.22	0.70	-0.82	_a	_a	_a
OH ⁻	0.87	0.00	-0.75	0.86	-0.26	-0.57	0.87	-0.48	0.00
OH ₂	0.10	0.03	-0.13	0.45	0.53	-0.88	_a	_a	_a
CN ⁻	0.43	0.00	-0.42	0.96	-0.14	-0.79	0.98	-0.53	0.01
CNH	0.00	0.13	-0.15	0.03	0.96	-0.87	_a	_a	_a
CO	0.18	0.00	-0.18	0.03	0.93	-0.93	_a	_a	_a

^a Not determined, since the first step is already energetically unfeasible.

W-S₅X Tungsten and its first and second sphere ligand atoms

Pt₂ Pterin parts of the pyranopterin cofactors

bz. QM-treated part of benzoyl-CoA

Table S5: Sums of Mulliken spin populations on different parts of the QM region for mechanisms starting from W(V).

X	Sub			In (H260)			In (E251)			In (X)		
	W-S ₅ X	Pt ₂	bz.	W-S ₅ X	Pt ₂	bz.	W-S ₅ X	Pt ₂	bz.	W-S ₅ X	Pt ₂	bz.
SH ⁻	0.98	0.25	-0.23	0.91	-0.91	0.97	0.96	-0.89	0.94	0.04	0.63	0.32
OH ⁻	0.99	0.28	-0.30	0.91	-0.91	0.98	1.04	-0.91	0.85	0.02	0.60	0.36
CN ⁻	0.81	0.26	-0.07	1.02	-0.88	0.89	0.96	-0.87	0.93	-*	-*	-*

* Ligand X carries no proton to donate.

W-S₅X Tungsten and its first and second sphere ligand atoms

Pt₂ Pterin parts of the pyranopterin cofactors

bz. QM-treated part of benzoyl-CoA

Table S6: First 15 most probable protonation states of the active site residues at pH 7 in W(IV)-S²⁻ state with benzoyl-CoA in the cavity.

state	ΔE (kcal/mol)	total charge	E251	E257	H260	K325	E461
1	0.0	0	0	(-)	δ	(+)	0
2	0.1	1	0	(-)	δ, ε (+)	(+)	0
3	0.3	0	0	0	ε	0	0
4	0.8	1	0	0	ε	(+)	0
5	2.2	0	0	(-)	ε	(+)	0
6	2.6	0	0	0	δ	0	0
7	4.2	1	0	0	δ	(+)	0
8	5.7	0	0	(-)	δ, ε (+)	(+)	(-)
9	6.8	1	0	0	δ, ε (+)	0	0
10	7.3	-1	0	0	ε	0	(-)
11	7.3	0	0	0	ε	(+)	(-)
12	7.9	-1	0	(-)	δ	(+)	(-)
13	9.4	-1	0	(-)	ε	(+)	(-)
14	9.5	0	0	(-)	δ, ε (+)	0	0
15	10.2	-1	0	0	δ	0	(-)

Table S7: First 15 most probable protonation states of the active site residues at pH 7 in W(V)-S²⁻ state with benzoyl-CoA in the cavity.

state	ΔE (kcal/mol)	total charge	E251	E257	H260	K325	E461
1	0.0	0	0	(-)	δ	(+)	0
2	0.9	0	0	0	ε	0	0
3	2.2	1	0	(-)	$\delta, \varepsilon (+)$	(+)	0
4	2.3	1	0	0	ε	(+)	0
5	2.7	0	0	(-)	ε	(+)	0
6	2.7	0	0	0	δ	0	0
7	5.3	1	0	0	δ	(+)	0
8	5.9	0	0	(-)	$\delta, \varepsilon (+)$	(+)	(-)
9	6.0	-1	0	0	ε	0	(-)
10	6.1	-1	0	(-)	δ	(+)	(-)
11	6.9	0	0	0	ε	(+)	(-)
12	8.0	-1	0	(-)	ε	(+)	(-)
13	8.5	-1	0	0	δ	0	(-)
14	8.9	1	0	0	$\delta, \varepsilon (+)$	0	0
15	10.6	0	0	0	δ	(+)	(-)

Table S8: First 15 most probable protonation states of the active site residues at pH 7 in W(VI)-S²⁻ state with benzoyl-CoA in the cavity.

state	ΔE (kcal/mol)	total charge	E251	E257	H260	K325	E461
1	0.0	0	0	(-)	δ	(+)	0
2	1.8	0	0	0	ε	0	0
3	2.9	0	0	0	δ	0	0
4	3.3	0	0	(-)	ε	(+)	0
5	3.6	-1	0	(-)	δ	(+)	(-)
6	4.3	1	0	0	ε	(+)	0
7	4.4	-1	0	0	ε	0	(-)
8	5.0	1	0	(-)	$\delta, \varepsilon (+)$	(+)	0
9	6.2	-1	0	(-)	ε	(+)	(-)
10	6.3	-1	0	0	δ	0	(-)
11	6.3	0	0	(-)	$\delta, \varepsilon (+)$	(+)	(-)
12	6.5	0	0	0	ε	(+)	(-)
13	6.6	1	0	0	δ	(+)	0
14	9.5	0	0	0	δ	(+)	(-)
15	10.1	-1	0	(-)	δ	0	0

Table S9: First 15 most probable protonation states of the active site residues at pH 7 in W(IV)-SH⁻ state with benzoyl-CoA in the cavity.

state	ΔE (kcal/mol)	total charge	E251	E257	H260	K325	E461
1	0.0	0	0	(-)	δ	(+)	0
2	0.8	0	0	0	ε	0	0
3	2.0	1	0	(-)	δ, ε (+)	(+)	0
4	2.1	1	0	0	ε	(+)	0
5	2.6	0	0	(-)	ε	(+)	0
6	2.7	0	0	0	δ	0	0
7	5.2	1	0	0	δ	(+)	0
8	5.6	0	0	(-)	δ, ε (+)	(+)	(-)
9	5.8	-1	0	0	ε	0	(-)
10	6.0	-1	0	(-)	δ	(+)	(-)
11	6.6	0	0	0	ε	(+)	(-)
12	7.8	-1	0	(-)	ε	(+)	(-)
13	8.4	-1	0	0	δ	0	(-)
14	8.7	1	0	0	δ, ε (+)	0	0
15	10.4	0	0	0	δ	(+)	(-)

Table S10: First 15 most probable protonation states of the active site residues at pH 7 in W(V)-SH⁻ state with benzoyl-CoA in the cavity.

state	ΔE (kcal/mol)	total charge	E251	E257	H260	K325	E461
1	0.0	0	0	(-)	δ	(+)	0
2	1.9	0	0	0	ε	0	0
3	3.0	0	0	0	δ	0	0
4	3.5	0	0	(-)	ε	(+)	0
5	3.5	-1	0	(-)	δ	(+)	(-)
6	4.5	-1	0	0	ε	0	(-)
7	4.6	1	0	0	ε	(+)	0
8	5.4	1	0	(-)	δ, ε (+)	(+)	0
9	6.2	-1	0	0	δ	0	(-)
10	6.3	-1	0	(-)	ε	(+)	(-)
11	6.6	0	0	(-)	δ, ε (+)	(+)	(-)
12	6.7	0	0	0	ε	(+)	(-)
13	6.8	1	0	0	δ	(+)	0
14	9.6	0	0	0	δ	(+)	(-)
15	9.9	0	(-)	0	ε	(+)	0

Table S11: First 15 most probable protonation states of the active site residues at pH 7 in W(VI)-SH⁻ state with benzoyl-CoA in the cavity.

state	ΔE (kcal/mol)	total charge	E251	E257	H260	K325	E461
1	0.0	0	0	(-)	δ	(+)	0
2	1.0	-1	0	(-)	δ	(+)	(-)
3	2.8	0	0	0	ε	0	0
4	2.9	-1	0	0	ε	0	(-)
5	3.2	0	0	0	δ	0	0
6	4.0	-1	0	0	δ	0	(-)
7	4.1	0	0	(-)	ε	(+)	0
8	4.4	-1	0	(-)	ε	(+)	(-)
9	6.0	0	0	0	ε	(+)	(-)
10	6.5	1	0	0	ε	(+)	0
11	6.8	0	0	(-)	δ, ε (+)	(+)	(-)
12	7.8	0	(-)	0	δ	(+)	0
13	8.0	1	0	0	δ	(+)	0
14	8.1	1	0	(-)	δ, ε (+)	(+)	0
15	8.3	0	0	0	δ	(+)	(-)

Table S12: First 15 most probable protonation states of the active site residues at pH 7 in W(IV)-SH₂ state with benzoyl-CoA in the cavity.

state	ΔE (kcal/mol)	total charge	E251	E257	H260	K325	E461
1	0.0	0	0	(-)	δ	(+)	0
2	2.2	0	0	0	ε	0	0
3	2.8	-1	0	(-)	δ	(+)	(-)
4	3.0	0	0	0	δ	0	0
5	3.6	0	0	(-)	ε	(+)	0
6	4.0	-1	0	0	ε	0	(-)
7	4.7	1	0	0	ε	(+)	0
8	5.5	-1	0	0	δ	0	(-)
9	5.6	1	0	(-)	δ, ε (+)	(+)	0
10	5.7	-1	0	(-)	ε	(+)	(-)
11	6.0	0	0	0	ε	(+)	(-)
12	6.1	0	0	(-)	δ, ε (+)	(+)	(-)
13	6.7	1	0	0	δ	(+)	0
14	8.8	0	0	0	δ	(+)	(-)
15	10.0	-1	0	(-)	δ	0	0

Table S13: First 15 most probable protonation states of the active site residues at pH 7 in W(V)-SH₂ state with benzoyl-CoA in the cavity.

state	ΔE (kcal/mol)	total charge	E251	E257	H260	K325	E461
1	0.0	0	0	(-)	δ	(+)	0
2	0.3	-1	0	(-)	δ	(+)	(-)
3	2.5	-1	0	0	ε	0	(-)
4	3.2	0	0	0	ε	0	0
5	3.3	0	0	0	δ	0	0
6	3.3	-1	0	0	δ	0	(-)
7	4.0	-1	0	(-)	ε	(+)	(-)
8	4.4	0	0	(-)	ε	(+)	0
9	5.7	0	0	0	ε	(+)	(-)
10	6.7	0	0	(-)	$\delta, \varepsilon (+)$	(+)	(-)
11	6.9	1	0	0	ε	(+)	0
12	7.4	0	(-)	0	δ	(+)	0
13	7.7	0	0	0	δ	(+)	(-)
14	8.2	1	0	0	δ	(+)	0
15	8.2	0	(-)	0	ε	(+)	0

Table S14: First 15 most probable protonation states of the active site residues at pH 7 in W(VI)-SH₂ state with benzoyl-CoA in the cavity.

state	ΔE (kcal/mol)	total charge	E251	E257	H260	K325	E461
1	0.0	-1	0	(-)	δ	(+)	(-)
2	1.3	0	0	(-)	δ	(+)	0
3	2.6	-1	0	0	ε	0	(-)
4	3.1	-1	0	0	δ	0	(-)
5	4.0	-1	0	(-)	ε	(+)	(-)
6	4.7	0	0	0	δ	0	0
7	4.9	0	0	0	ε	0	0
8	6.1	0	0	(-)	ε	(+)	0
9	6.3	-1	(-)	0	δ	(+)	(-)
10	6.7	-1	(-)	0	ε	(+)	(-)
11	6.8	0	0	0	ε	(+)	(-)
12	6.8	0	(-)	0	δ	(+)	0
13	7.4	-1	(-)	(-)	$\delta, \varepsilon (+)$	(+)	(-)
14	7.9	-2	(-)	0	δ	0	(-)
15	8.0	-1	(-)	0	δ	0	0

Table S15: First 15 most probable protonation states of the active site residues at pH 7 in W(IV)-O²⁻ state with benzoyl-CoA in the cavity.

state	ΔE (kcal/mol)	total charge	E251	E257	H260	K325	E461
1	0.0	0	0	(-)	δ	(+)	0
2	0.4	1	0	(-)	δ, ε (+)	(+)	0
3	0.5	0	0	0	ε	0	0
4	1.0	1	0	0	ε	(+)	0
5	2.4	0	0	(-)	ε	(+)	0
6	2.6	0	0	0	δ	0	0
7	4.3	1	0	0	δ	(+)	0
8	5.6	0	0	(-)	δ, ε (+)	(+)	(-)
9	7.0	0	0	0	ε	(+)	(-)
10	7.0	-1	0	0	ε	0	(-)
11	7.1	1	0	0	δ, ε (+)	0	0
12	7.5	-1	0	(-)	δ	(+)	(-)
13	9.1	-1	0	(-)	ε	(+)	(-)
14	9.8	-1	0	0	δ	0	(-)
15	9.8	0	0	(-)	δ, ε (+)	0	0

Table S16: First 15 most probable protonation states of the active site residues at pH 7 in W(V)-O²⁻ state with benzoyl-CoA in the cavity.

state	ΔE (kcal/mol)	total charge	E251	E257	H260	K325	E461
1	0.0	0	0	(-)	δ	(+)	0
2	1.0	0	0	0	ε	0	0
3	2.4	1	0	(-)	δ, ε (+)	(+)	0
4	2.5	1	0	0	ε	(+)	0
5	2.7	0	0	0	δ	0	0
6	2.8	0	0	(-)	ε	(+)	0
7	5.3	1	0	0	δ	(+)	0
8	5.6	-1	0	(-)	δ	(+)	(-)
9	5.7	-1	0	0	ε	0	(-)
10	5.8	0	0	(-)	δ, ε (+)	(+)	(-)
11	6.7	0	0	0	ε	(+)	(-)
12	7.7	-1	0	(-)	ε	(+)	(-)
13	8.1	-1	0	0	δ	0	(-)
14	9.2	1	0	0	δ, ε (+)	0	0
15	10.2	0	0	0	δ	(+)	(-)

Table S17: First 15 most probable protonation states of the active site residues at pH 7 in W(VI)-O²⁻ state with benzoyl-CoA in the cavity.

state	ΔE (kcal/mol)	total charge	E251	E257	H260	K325	E461
1	0.0	0	0	(-)	δ	(+)	0
2	1.8	0	0	0	ε	0	0
3	2.9	0	0	0	δ	0	0
4	3.4	0	0	(-)	ε	(+)	0
5	3.5	-1	0	(-)	δ	(+)	(-)
6	4.4	-1	0	0	ε	0	(-)
7	4.5	1	0	0	ε	(+)	0
8	5.2	1	0	(-)	δ, ε (+)	(+)	0
9	6.2	-1	0	(-)	ε	(+)	(-)
10	6.2	-1	0	0	δ	0	(-)
11	6.5	0	0	(-)	δ, ε (+)	(+)	(-)
12	6.6	0	0	0	ε	(+)	(-)
13	6.8	1	0	0	δ	(+)	0
14	9.6	0	0	0	δ	(+)	(-)
15	9.8	0	(-)	(-)	δ, ε (+)	(+)	0

Table S18: First 15 most probable protonation states of the active site residues at pH 7 in W(IV)-OH⁻ state with benzoyl-CoA in the cavity.

state	ΔE (kcal/mol)	total charge	E251	E257	H260	K325	E461
1	0.0	0	0	(-)	δ	(+)	0
2	1.1	0	0	0	ε	0	0
3	2.5	1	0	(-)	δ, ε (+)	(+)	0
4	2.5	1	0	0	ε	(+)	0
5	2.7	0	0	0	δ	0	0
6	2.8	0	0	(-)	ε	(+)	0
7	5.3	-1	0	(-)	δ	(+)	(-)
8	5.3	1	0	0	δ	(+)	0
9	5.5	-1	0	0	ε	0	(-)
10	5.5	0	0	(-)	δ, ε (+)	(+)	(-)
11	6.4	0	0	0	ε	(+)	(-)
12	7.4	-1	0	(-)	ε	(+)	(-)
13	7.8	-1	0	0	δ	0	(-)
14	9.3	1	0	0	δ, ε (+)	0	0
15	9.9	0	0	0	δ	(+)	(-)

Table S19: First 15 most probable protonation states of the active site residues at pH 7 in W(V)-OH⁻ state with benzoyl-CoA in the cavity.

state	ΔE (kcal/mol)	total charge	E251	E257	H260	K325	E461
1	0.0	0	0	(-)	δ	(+)	0
2	1.9	0	0	0	ε	0	0
3	2.9	0	0	0	δ	0	0
4	3.0	-1	0	(-)	δ	(+)	(-)
5	3.5	0	0	(-)	ε	(+)	0
6	4.0	-1	0	0	ε	0	(-)
7	4.5	1	0	0	ε	(+)	0
8	5.3	1	0	(-)	δ, ε (+)	(+)	0
9	5.7	-1	0	0	δ	0	(-)
10	5.8	-1	0	(-)	ε	(+)	(-)
11	6.0	0	0	(-)	δ, ε (+)	(+)	(-)
12	6.1	0	0	0	ε	(+)	(-)
13	6.7	1	0	0	δ	(+)	0
14	9.0	0	0	0	δ	(+)	(-)
15	10.0	-1	0	(-)	δ	0	0

Table S20: First 15 most probable protonation states of the active site residues at pH 7 in W(VI)-OH⁻ state with benzoyl-CoA in the cavity.

state	ΔE (kcal/mol)	total charge	E251	E257	H260	K325	E461
1	0.0	0	0	(-)	δ	(+)	0
2	0.8	-1	0	(-)	δ	(+)	(-)
3	2.6	-1	0	0	ε	0	(-)
4	2.7	0	0	0	ε	0	0
5	3.2	0	0	0	δ	0	0
6	3.8	-1	0	0	δ	0	(-)
7	4.1	0	0	(-)	ε	(+)	0
8	4.2	-1	0	(-)	ε	(+)	(-)
9	5.9	0	0	0	ε	(+)	(-)
10	6.5	1	0	0	ε	(+)	0
11	6.6	0	0	(-)	δ, ε (+)	(+)	(-)
12	7.4	0	(-)	0	δ	(+)	0
13	7.8	0	(-)	0	ε	(+)	0
14	8.0	1	0	(-)	δ, ε (+)	(+)	0
15	8.1	1	0	0	δ	(+)	0

Table S21: First 15 most probable protonation states of the active site residues at pH 7 in W(IV)-OH₂ state with benzoyl-CoA in the cavity.

state	ΔE (kcal/mol)	total charge	E251	E257	H260	K325	E461
1	0.0	0	0	(-)	δ	(+)	0
2	2.3	0	0	0	ε	0	0
3	2.4	-1	0	(-)	δ	(+)	(-)
4	3.0	0	0	0	δ	0	0
5	3.7	-1	0	0	ε	0	(-)
6	3.8	0	0	(-)	ε	(+)	0
7	4.9	1	0	0	ε	(+)	0
8	5.2	-1	0	0	δ	0	(-)
9	5.4	-1	0	(-)	ε	(+)	(-)
10	5.8	0	0	0	ε	(+)	(-)
11	5.9	1	0	(-)	δ, ε (+)	(+)	0
12	6.0	0	0	(-)	δ, ε (+)	(+)	(-)
13	6.8	1	0	0	δ	(+)	0
14	8.4	0	0	0	δ	(+)	(-)
15	10.0	-1	0	(-)	δ	0	0

Table S22: First 15 most probable protonation states of the active site residues at pH 7 in W(V)-OH₂ state with benzoyl-CoA in the cavity.

state	ΔE (kcal/mol)	total charge	E251	E257	H260	K325	E461
1	0.0	0	0	(-)	δ	(+)	0
2	0.3	-1	0	(-)	δ	(+)	(-)
3	2.4	-1	0	0	ε	0	(-)
4	3.1	0	0	0	ε	0	0
5	3.2	0	0	0	δ	0	0
6	3.3	-1	0	0	δ	0	(-)
7	3.9	-1	0	(-)	ε	(+)	(-)
8	4.4	0	0	(-)	ε	(+)	0
9	5.6	0	0	0	ε	(+)	(-)
10	6.6	0	0	(-)	δ, ε (+)	(+)	(-)
11	6.8	1	0	0	ε	(+)	0
12	7.5	0	(-)	0	δ	(+)	0
13	7.7	0	0	0	δ	(+)	(-)
14	8.1	1	0	0	δ	(+)	0
15	8.3	0	(-)	0	ε	(+)	0

Table S23: First 15 most probable protonation states of the active site residues at pH 7 in W(VI)-OH₂ state with benzoyl-CoA in the cavity.

state	ΔE (kcal/mol)	total charge	E251	E257	H260	K325	E461
1	0.0	-1	0	(-)	δ	(+)	(-)
2	1.4	0	0	(-)	δ	(+)	0
3	2.5	-1	0	0	ε	0	(-)
4	3.1	-1	0	0	δ	0	(-)
5	3.9	-1	0	(-)	ε	(+)	(-)
6	4.7	0	0	0	δ	0	0
7	4.9	0	0	0	ε	0	0
8	6.1	0	0	(-)	ε	(+)	0
9	6.6	0	0	0	ε	(+)	(-)
10	6.7	-1	(-)	0	δ	(+)	(-)
11	7.1	-1	(-)	0	ε	(+)	(-)
12	7.3	0	(-)	0	δ	(+)	0
13	7.7	-1	(-)	(-)	δ, ε (+)	(+)	(-)
14	7.9	0	0	(-)	δ, ε (+)	(+)	(-)
15	8.3	0	0	0	δ	(+)	(-)

Table S24: First 15 most probable protonation states of the active site residues at pH 7 in W(IV)-CN⁻ state with benzoyl-CoA in the cavity.

state	ΔE (kcal/mol)	total charge	E251	E257	H260	K325	E461
1	0.0	0	0	(-)	δ	(+)	0
2	0.6	0	0	0	ε	0	0
3	1.8	1	0	(-)	δ, ε (+)	(+)	0
4	2.1	1	0	0	ε	(+)	0
5	2.5	0	0	(-)	ε	(+)	0
6	2.7	0	0	0	δ	0	0
7	5.3	1	0	0	δ	(+)	0
8	6.1	0	0	(-)	δ, ε (+)	(+)	(-)
9	6.2	-1	0	0	ε	0	(-)
10	6.6	-1	0	(-)	δ	(+)	(-)
11	7.2	0	0	0	ε	(+)	(-)
12	8.3	-1	0	(-)	ε	(+)	(-)
13	8.6	1	0	0	δ, ε (+)	0	0
14	9.0	-1	0	0	δ	0	(-)
15	10.3	0	0	(-)	δ, ε (+)	0	0

Table S25: First 15 most probable protonation states of the active site residues at pH 7 in W(V)-CN⁻ state with benzoyl-CoA in the cavity.

state	ΔE (kcal/mol)	total charge	E251	E257	H260	K325	E461
1	0.0	0	0	(-)	δ	(+)	0
2	1.6	0	0	0	ε	0	0
3	2.9	0	0	0	δ	0	0
4	3.1	0	0	(-)	ε	(+)	0
5	4.1	-1	0	(-)	δ	(+)	(-)
6	4.2	1	0	0	ε	(+)	0
7	4.7	-1	0	0	ε	0	(-)
8	4.7	1	0	(-)	δ, ε (+)	(+)	0
9	6.5	-1	0	(-)	ε	(+)	(-)
10	6.6	0	0	(-)	δ, ε (+)	(+)	(-)
11	6.7	1	0	0	δ	(+)	0
12	6.8	-1	0	0	δ	0	(-)
13	6.8	0	0	0	ε	(+)	(-)
14	9.5	0	(-)	(-)	δ, ε (+)	(+)	0
15	9.7	0	(-)	0	ε	(+)	0

Table S26: First 15 most probable protonation states of the active site residues at pH 7 in W(VI)-CN⁻ state with benzoyl-CoA in the cavity.

state	ΔE (kcal/mol)	total charge	E251	E257	H260	K325	E461
1	0.0	0	0	(-)	δ	(+)	0
2	1.8	-1	0	(-)	δ	(+)	(-)
3	2.4	0	0	0	ε	0	0
4	3.1	0	0	0	δ	0	0
5	3.2	-1	0	0	ε	0	(-)
6	3.8	0	0	(-)	ε	(+)	0
7	4.6	-1	0	0	δ	0	(-)
8	4.8	-1	0	(-)	ε	(+)	(-)
9	6.3	1	0	0	ε	(+)	0
10	6.6	0	0	0	ε	(+)	(-)
11	7.1	0	(-)	0	δ	(+)	0
12	7.1	0	0	(-)	δ, ε (+)	(+)	(-)
13	7.3	0	(-)	0	ε	(+)	0
14	7.6	1	0	(-)	δ, ε (+)	(+)	0
15	7.9	0	(-)	(-)	δ, ε (+)	(+)	0

Table S27: First 15 most probable protonation states of the active site residues at pH 7 in W(IV)-CNH state with benzoyl-CoA in the cavity.

state	ΔE (kcal/mol)	total charge	E251	E257	H260	K325	E461
1	0.0	0	0	(-)	δ	(+)	0
2	2.1	0	0	0	ε	0	0
3	2.7	-1	0	(-)	δ	(+)	(-)
4	3.0	0	0	0	δ	0	0
5	3.6	0	0	(-)	ε	(+)	0
6	3.8	-1	0	0	ε	0	(-)
7	4.6	1	0	0	ε	(+)	0
8	5.4	-1	0	0	δ	0	(-)
9	5.5	1	0	(-)	δ, ε (+)	(+)	0
10	5.5	-1	0	(-)	ε	(+)	(-)
11	5.8	0	0	0	ε	(+)	(-)
12	5.9	0	0	(-)	δ, ε (+)	(+)	(-)
13	6.7	1	0	0	δ	(+)	0
14	8.6	0	0	0	δ	(+)	(-)
15	10.1	-1	0	(-)	δ	0	0

Table S28: First 15 most probable protonation states of the active site residues at pH 7 in W(V)-CNH state with benzoyl-CoA in the cavity.

state	ΔE (kcal/mol)	total charge	E251	E257	H260	K325	E461
1	0.0	-1	0	(-)	δ	(+)	(-)
2	0.3	0	0	(-)	δ	(+)	0
3	2.2	-1	0	0	ε	0	(-)
4	3.0	-1	0	0	δ	0	(-)
5	3.5	0	0	0	ε	0	0
6	3.6	0	0	0	δ	0	0
7	3.7	-1	0	(-)	ε	(+)	(-)
8	4.8	0	0	(-)	ε	(+)	0
9	5.4	0	0	0	ε	(+)	(-)
10	6.4	0	0	(-)	δ, ε (+)	(+)	(-)
11	7.3	1	0	0	ε	(+)	0
12	7.4	0	0	0	δ	(+)	(-)
13	7.9	0	(-)	0	δ	(+)	0
14	8.3	-1	(-)	0	δ	(+)	(-)
15	8.4	-1	(-)	0	ε	(+)	(-)

Table S29: First 15 most probable protonation states of the active site residues at pH 7 in W(VI)-CNH state with benzoyl-CoA in the cavity.

state	ΔE (kcal/mol)	total charge	E251	E257	H260	K325	E461
1	0.0	-1	0	(-)	δ	(+)	(-)
2	2.2	0	0	(-)	δ	(+)	0
3	2.7	-1	0	0	ε	0	(-)
4	3.1	-1	0	0	δ	0	(-)
5	4.1	-1	0	(-)	ε	(+)	(-)
6	5.5	0	0	0	δ	0	0
7	5.8	0	0	0	ε	0	0
8	6.6	-1	(-)	0	δ	(+)	(-)
9	6.8	0	0	0	ε	(+)	(-)
10	7.0	0	0	(-)	ε	(+)	0
11	7.0	-1	(-)	0	ε	(+)	(-)
12	7.8	-1	(-)	(-)	δ, ε (+)	(+)	(-)
13	8.0	0	(-)	0	δ	(+)	0
14	8.3	-2	(-)	0	δ	0	(-)
15	8.3	0	0	(-)	δ, ε (+)	(+)	(-)

Table S30: First 15 most probable protonation states of the active site residues at pH 7 in W(IV)-CO state with benzoyl-CoA in the cavity.

state	ΔE (kcal/mol)	total charge	E251	E257	H260	K325	E461
1	0.0	0	0	(-)	δ	(+)	0
2	1.9	0	0	0	ε	0	0
3	2.9	0	0	0	δ	0	0
4	3.3	-1	0	(-)	δ	(+)	(-)
5	3.4	0	0	(-)	ε	(+)	0
6	4.2	-1	0	0	ε	0	(-)
7	4.5	1	0	0	ε	(+)	0
8	5.3	1	0	(-)	δ, ε (+)	(+)	0
9	6.0	-1	0	(-)	ε	(+)	(-)
10	6.0	-1	0	0	δ	0	(-)
11	6.3	0	0	(-)	δ, ε (+)	(+)	(-)
12	6.3	0	0	0	ε	(+)	(-)
13	6.7	1	0	0	δ	(+)	0
14	9.3	0	0	0	δ	(+)	(-)
15	10.0	-1	0	(-)	δ	0	0

Table S31: First 15 most probable protonation states of the active site residues at pH 7 in W(V)-CO state with benzoyl-CoA in the cavity.

state	ΔE (kcal/mol)	total charge	E251	E257	H260	K325	E461
1	0.0	0	0	(-)	δ	(+)	0
2	0.8	-1	0	(-)	δ	(+)	(-)
3	2.6	-1	0	0	ε	0	(-)
4	2.8	0	0	0	ε	0	0
5	3.2	0	0	0	δ	0	0
6	3.7	-1	0	0	δ	0	(-)
7	4.1	0	0	(-)	ε	(+)	0
8	4.1	-1	0	(-)	ε	(+)	(-)
9	5.8	0	0	0	ε	(+)	(-)
10	6.5	1	0	0	ε	(+)	0
11	6.6	0	0	(-)	δ, ε (+)	(+)	(-)
12	7.5	0	(-)	0	δ	(+)	0
13	7.9	0	(-)	0	ε	(+)	0
14	8.1	1	0	0	δ	(+)	0
15	8.1	0	0	0	δ	(+)	(-)

Table S32: First 15 most probable protonation states of the active site residues at pH 7 in W(VI)-CO state with benzoyl-CoA in the cavity.

state	ΔE (kcal/mol)	total charge	E251	E257	H260	K325	E461
1	0.0	-1	0	(-)	δ	(+)	(-)
2	0.9	0	0	(-)	δ	(+)	0
3	2.1	-1	0	0	ε	0	(-)
4	3.0	-1	0	0	δ	0	(-)
5	3.6	-1	0	(-)	ε	(+)	(-)
6	4.0	0	0	0	ε	0	0
7	4.1	0	0	0	δ	0	0
8	5.2	0	0	(-)	ε	(+)	0
9	6.2	0	0	0	ε	(+)	(-)
10	6.9	-1	(-)	0	δ	(+)	(-)
11	6.9	-1	(-)	0	ε	(+)	(-)
12	7.0	0	(-)	0	δ	(+)	0
13	7.3	0	0	(-)	δ, ε (+)	(+)	(-)
14	7.3	-1	(-)	(-)	δ, ε (+)	(+)	(-)
15	7.7	0	(-)	0	ε	(+)	0

S3 Sequence Alignment

The sequence alignment to identify conserved residues E178, D352 and D529 was performed with 133 sequences that share a sequence identity of 50% and more (query cover over 90%) with BamB of *G. metallireducens* (WP_004514579.1). Standard protein BLAST (NCBI; against non-redundant protein sequences) was performed (reference date August 20, 2017) and the 133 resulting complete FASTA files aligned with MUSCLE [1] and default settings. The obtained alignment is provided in an extra file (ja7b07012_si_003.txt).

D352 and D529 are conserved 100% (0 of 133 sequences differed). At position 178 nine out of 133 sequences are different from glutamate. In seven cases glutamate is replaced by aspartate which can perform the same function to potentially stabilize a positively charged pyranopterin centered radical (accessions: OGO38769.1, OGQ49165.1, OGA55611.1, OGA33951.1, WP_054692113.1, WP_092238595.1, WP_031453124.1; all sequences shared an identity of 53-57% to BamB of *G. metallireducens*). In two cases glutamate is replaced by glutamine (accessions: WP_006523462.1 and KPK26351.1; sequence identity of 65% and 67% to BamB of *G. metallireducens*). It is known that most obligately anaerobic bacteria that degrade aromatic compounds possess at least two *bamB* paralogues per genome [2]. In *G. metallireducens* the second BamB paralogue (BamB2), whose function is unknown, shares a sequence identity of 66% with the investigated BamB1. For both organisms (*Desulfotomaculum gibsoniae* and *Nitrospira bacterium* SG8_3) that contain each one BamB homologue with Q178 at least one other BamB exists with E178 which is more similar to BamB1 of *G. metallireducens* than the Q178-containing one [3]. Therefore, a potential loss of function could be compensated by another, more similar and E178-containing BamB homologue or these more remote BamB homologues perform another function.

[1] Edgar, R. C. *Nucleic Acids Res.*, **2004**, 32 (5), 1792-1797

[2] Löffler, C.; Kuntze, K.; Vazquez, J. R.; Rugor, A.; Kung, J. W.; Böttcher, A.; Boll, B. *Environ. Microbiol.*, **2011**, 13 (3), 696-709

[3] Kuever, J., et al., *Stand. Genomic Sci.*, **2014**, 9 (3), 821-839