Asymmetric structure of the dimerization domain of PhoR, a sensor kinase important for the virulence of *Mycobacterium tuberculosis*

Daniel Xing[†§], *Michelle B. Ryndak*[‡¶], *Liqin Wang*[†], *Irina Kolesnikova*[‡], *Issar Smith*[‡], *and Shuishu Wang*[†*]

[†]Department of Biochemistry and Molecular Biology, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, Maryland 20814; [‡]Public Health Research Institute Center, New Jersey Medical School, Rutgers, The State University of New Jersey, 225 Warren Street, Newark, New Jersey 07103, USA

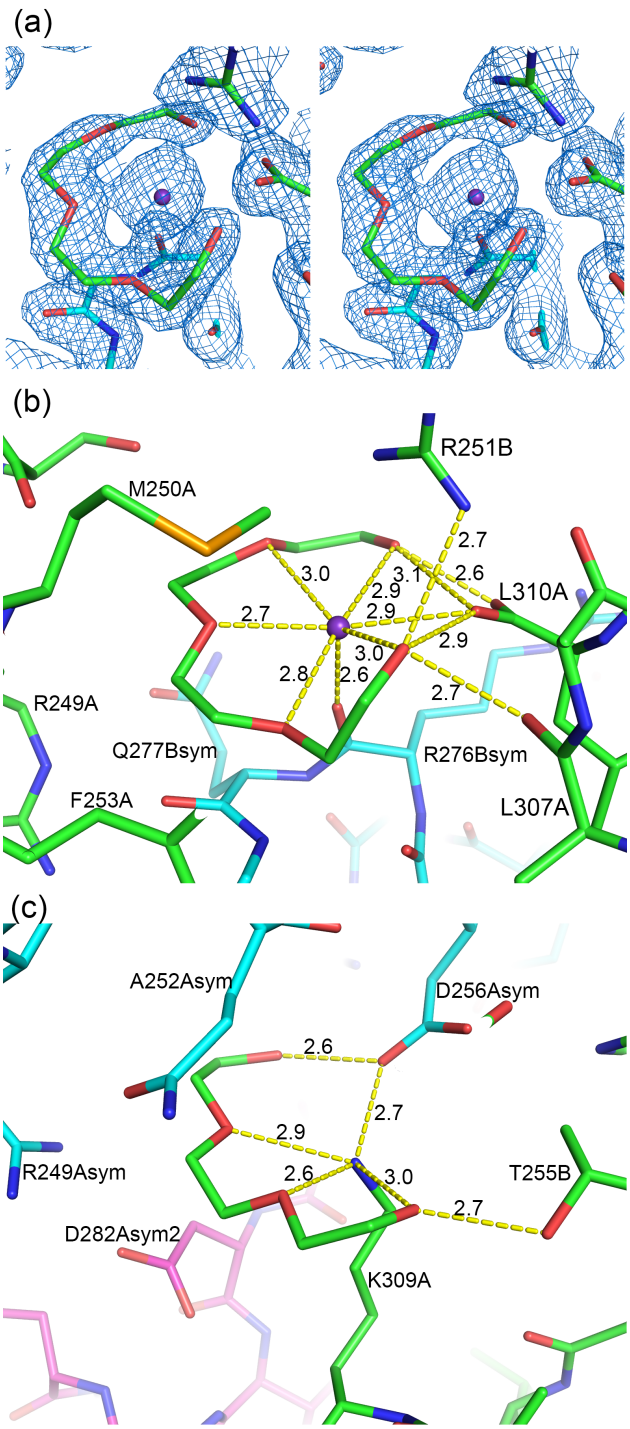

Figure S1. Fragments of PEG identified in the crystal structure. (a) Stereo view of the electron density of a tetraethylene glycol encircling a K^+ ion. The electron density was contoured at 1 σ. Crystallographic-symmetry related PhoRD molecules are shown with different colors (same for other panels). (b) Binding interactions of the tetraethylene glycol molecule. Distances of the

glycol molecule to the K^+ and hydrogen bonds to the protein are marked with dashed lines. The ethylene groups had hydrophobic interactions with the protein, while the oxygen atoms interacted with the cation in the center. Terminal oxygen atoms of the tetraethylene glycol also have hydrogen bonds to protein side chains. (c) Binding interactions of a bound triethylene glycol with the protein. This figure was prepared from the structural coordinates derived from the SeMet-labeled crystal. The native structure has similar binding of PEG fragments.

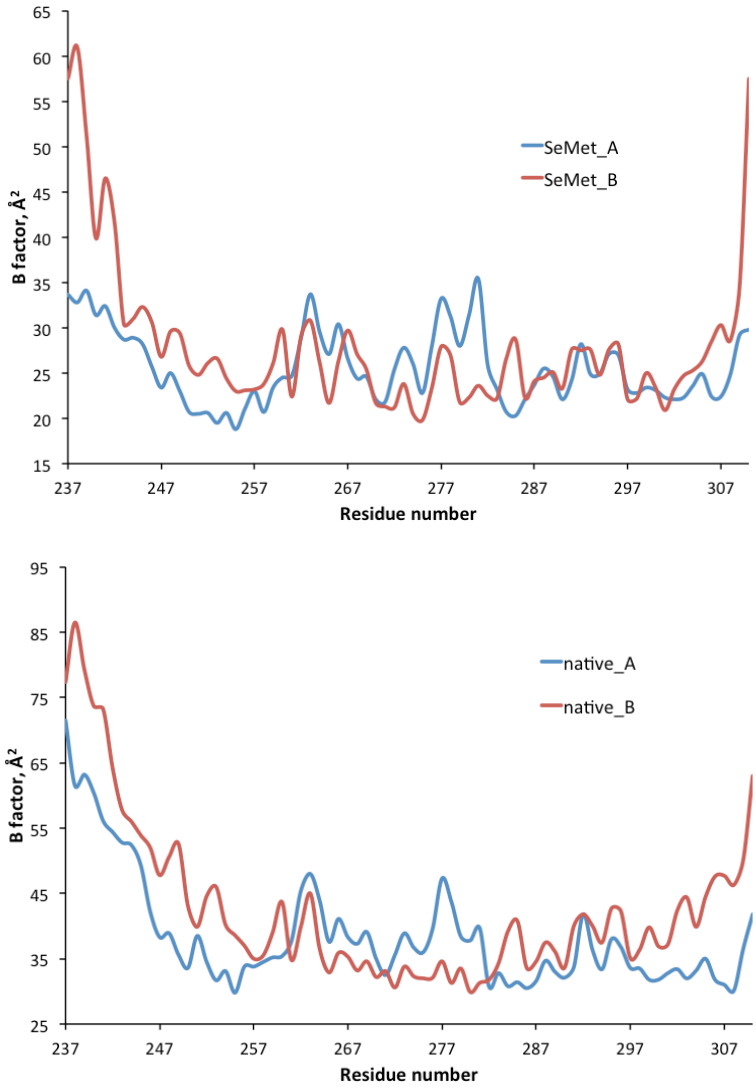

Figure S2. Plots of average B factors of main chain atoms of the structural models from the SeMet (top) and the native data (bottom). The curves for chain A of both structures are shown in blue, and those for chain B are in red. For each structure, the two chains have slightly different profile, with chain B having a higher B factors near the termini and a higher overall average B factor. Despite the higher overall B factor in the native structure, however, the B factor profiles for chain A for both structures are similar, and so are those for chain B.

Table S1. Analysis of interactions at the PhoRD dimer interface. The PhoRD dimer structure was analyzed with PISA (ref: E.B. Krissinel and K. Henrick Inference of macromolecular assemblies from crystalline state, J.Mol.Biol. 2007, 372, 774-797). The output of the PISA analysis for the dimer interface includes three hydrogen bonds with distances that are longer than 3.7 Å. These hydrogen bonds are not included in the discussion of interface interactions in the paper. H represents hydrogen bonds; S represents salt bridges; ASA, accessible surface area; BSA, buried surface area; SE, solvation energy.

1. Interface Summary

	Structure 1	Structure 2
Selection range	subunit B	subunit A
Atoms in the interface	164 (28.1%)	159 (27.3%)
Atoms on the surface	444 (76.2%)	447 (76.7%)
Total atoms	583 (100.0%)	583 (100.0%)
Residues in the interface	41 (55.4%)	41 (55.4%)
Residues on the surface	74 (100.0%)	74 (100.0%)
Total residues	74 (100.0%)	74 (100.0%)
Buried ASA, Å^2	1630.1 (25.9%)	1566.5 (24.3%)
Total ASA, Å^2	6285.4 (100.0%)	6445.8 (100.0%)
Solvation energy, kcal/mol	-43.4	-43.9
SE gain, kcal/mol	-13.6	-12.0

2. Hydrogen Bonds

##	Structure 1	Dist. Å	Structure 2
1	B:SER 246 [OG]	2.8	A:GLU 247 [OE1]
2	B:ARG 276 [NH1]	3.0	A:GLY 284 [O]
3	B:ARG 251 [NH1]	2.9	A:ALA 308 [O]
4	B:ARG 251 [NH1]	2.7	A:LEU 310 [OXT]
5	B:SER 246 [OG]	3.8	A:ARG 251 [NH2]
6	B:GLU 247 [OE2]	3.7	A:GLU 247 [N]

7	B:GLU 247 [OE2]	2.5	A:SER 246 [OG]
8	B:LEU 307 [O]	3.8	A:ARG 251 [NH1]
9	B:ALA 308 [O]	2.9	A:ARG 251 [NH1]
10	B:LEU 310 [O]	2.8	A:ARG 251 [NH1]

3. Salt Bridges

##	Structure 1	Distance, Å	Structure 2
1	B:ARG 276 [NE]	2.8	A:GLU 291 [OE1]
2	B:ARG 276 [NH1]	3.0	A:GLU 291 [OE1]
3	B:ARG 262 [NE]	2.8	A:ASP 302 [OD1]
4	B:ARG 262 [NH1]	2.9	A:ASP 302 [OD1]
5	B:ARG 262 [NH1]	3.7	A:ASP 302 [OD2]
6	B:GLU 291 [OE1]	3.1	A:ARG 276 [NE]
7	B:GLU 291 [OE1]	3.4	A:ARG 276 [NH2]
8	B:GLU 291 [OE2]	2.9	A:ARG 276 [NE]
9	B:ASP 302 [OD1]	3.1	A:ARG 262 [NH1]
10	B:ASP 302 [OD1]	3.5	A:ARG 262 [NE]

4. Interfacing Residues: Structure 1

Residues	HS	ASA, Å^2	BSA, Å^2	ΔG, kcal/mol
B:GLY 237		98.51	12.10	-0.14
B:MSE 239		162.75	25.48	0.39
B:ALA 240		66.68	49.47	0.65
B:ALA 243		65.19	59.60	0.83
B:ARG 244		133.51	5.41	0.07
B:SER 246	H	49.98	20.55	-0.05
B:GLU 247	H	75.95	52.55	0.02
B:MSE 250		79.21	73.27	1.76
B:ARG 251	H	147.60	61.47	-0.77
B:ILE 254		93.09	89.05	1.42
B:THR 255		54.62	31.91	0.17
B:SER 258		56.26	55.35	0.32
B:HIS 259		117.15	22.09	0.05
B:LEU 261		33.46	33.13	0.53
B:ARG 262	S	163.26	55.70	-0.59
B:LEU 265		97.33	85.16	1.27
B:ILE 268		41.59	36.11	0.58
B:ARG 269		181.29	6.13	0.07
B:ALA 272		33.17	31.12	0.49
B:GLU 273		106.36	3.13	0.01
B:TYR 275		38.23	1.69	0.03
B:ARG 276	HS	211.38	82.58	-1.31
B:VAL 283		94.34	24.46	0.39

B:GLY 284		48.83	20.82	0.23
B:LEU 286		17.31	12.11	0.19
B:LEU 287		96.39	92.89	1.43
B:SER 288		57.89	10.26	0.07
B:ILE 290		47.64	46.81	0.75
B:GLU 291	S	127.40	80.57	-0.25
B:ALA 294		48.73	48.73	0.59
B:SER 295		70.64	27.22	0.33
B:MSE 297		32.61	32.61	0.75
B:GLY 298		35.57	22.35	0.34
B:VAL 301		65.43	64.93	1.04
B:ASP 302	S	85.80	19.87	-0.15
B:LEU 304		39.16	34.64	0.48
B:LEU 305		113.31	96.37	1.54
B:LEU 307	H	49.15	5.26	0.08
B:ALA 308	H	70.09	52.40	0.35
B:LYS 309		162.02	18.49	-0.09
B:LEU 310	H	182.48	26.24	-0.22

5. Interfacing Residues: Structure 2

Residues	HS	ASA, Å^2	BSA, Å^2	ΔG, kcal/mol
A:MSE 239		152.88	28.16	0.44
A:ALA 240		50.50	27.52	0.42
A:ALA 243		60.98	43.65	0.56
A:ARG 244		147.68	11.00	0.14
A:SER 246	H	69.58	23.33	-0.12
A:GLU 247	H	77.11	61.58	0.29
A:MSE 250		106.61	58.55	1.14
A:ARG 251	H	148.45	98.41	-1.22
A:PHE 253		74.45	5.88	0.09
A:ILE 254		94.38	94.38	1.51
A:THR 255		69.63	33.98	0.13
A:ALA 257		7.93	3.35	0.05
A:SER 258		54.20	54.08	0.31
A:HIS 259		104.66	12.82	-0.09
A:LEU 261		34.86	34.53	0.55
A:ARG 262	S	151.07	50.74	-0.52
A:LEU 265		73.04	69.51	1.11
A:ILE 268		33.62	33.62	0.54
A:ARG 269		175.73	58.78	-0.51
A:ALA 272		52.00	51.51	0.75
A:GLU 273		77.86	5.01	-0.01
A:TYR 275		126.30	65.20	0.41
A:ARG 276	S	192.12	44.08	-0.56

A:VAL 283		62.85	23.69	0.38
A:GLY 284	H	50.41	18.13	0.14
A:LEU 286		19.63	4.19	0.07
A:LEU 287		92.33	70.96	1.14
A:SER 288		60.56	6.36	0.08
A:ILE 290		34.50	34.50	0.55
A:GLU 291	S	103.52	52.13	-0.22
A:ALA 294		49.32	35.29	0.44
A:MSE 297		36.76	36.76	0.77
A:GLY 298		31.04	13.07	0.20
A:VAL 301		59.90	59.90	0.96
A:ASP 302	S	83.09	33.63	-0.28
A:LEU 304		43.96	33.04	0.50
A:LEU 305		113.84	91.33	1.45
A:LEU 307		68.21	0.27	-0.00
A:ALA 308	H	73.44	54.61	0.40
A:LYS 309		168.54	17.81	0.04
A:LEU 310	H	181.70	11.12	-0.07