Supplemental Information

Assembly of the preactivation complex for urease maturation in *Helicobacter pylori*: Crystal Structure of the UreF/UreH complex

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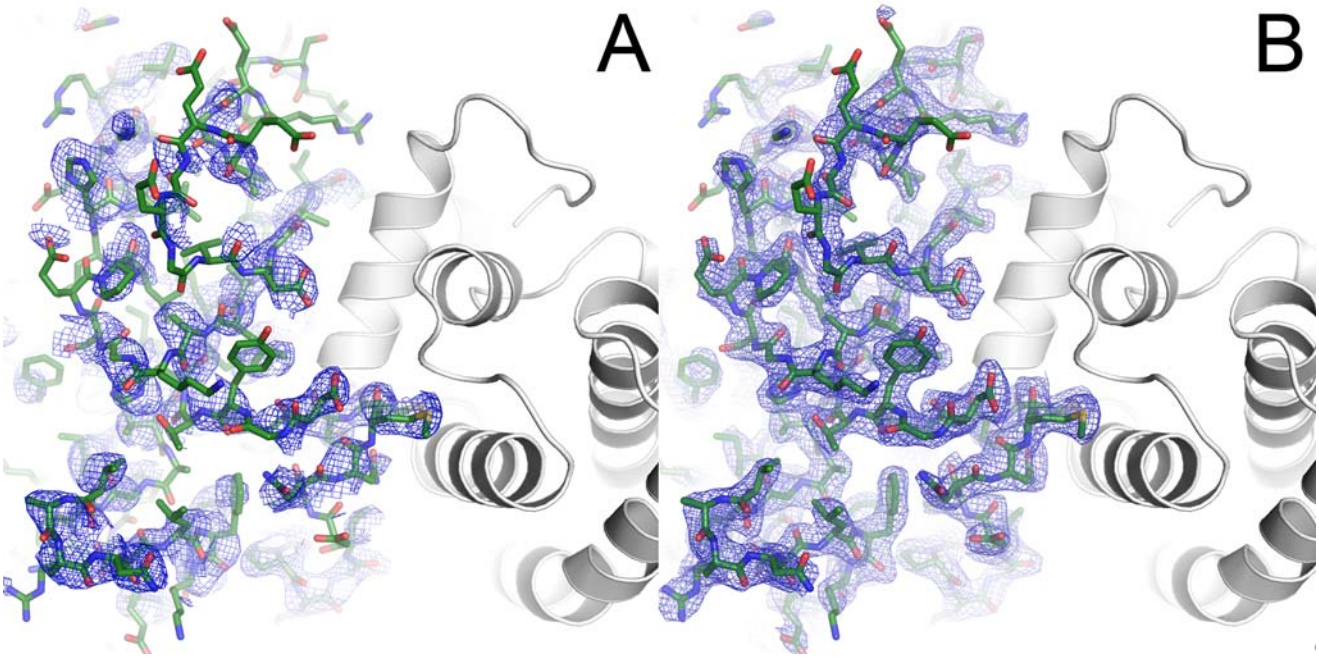

Figure S1. The 2Fo-Fc electron density map of UreH after density modification and model building. (A) After an initial molecular replacement solution was found using UreF (white) as search model, the electron density was improved by density modification. The resulting 2Fo-Fc electron density map is shown contoured at 1.0 σ. Clear electron density of the UreH (green) chain is observed. (B) 2Fo-Fc electron density map of UreH after model building is completed is shown contoured at 1.0 σ.

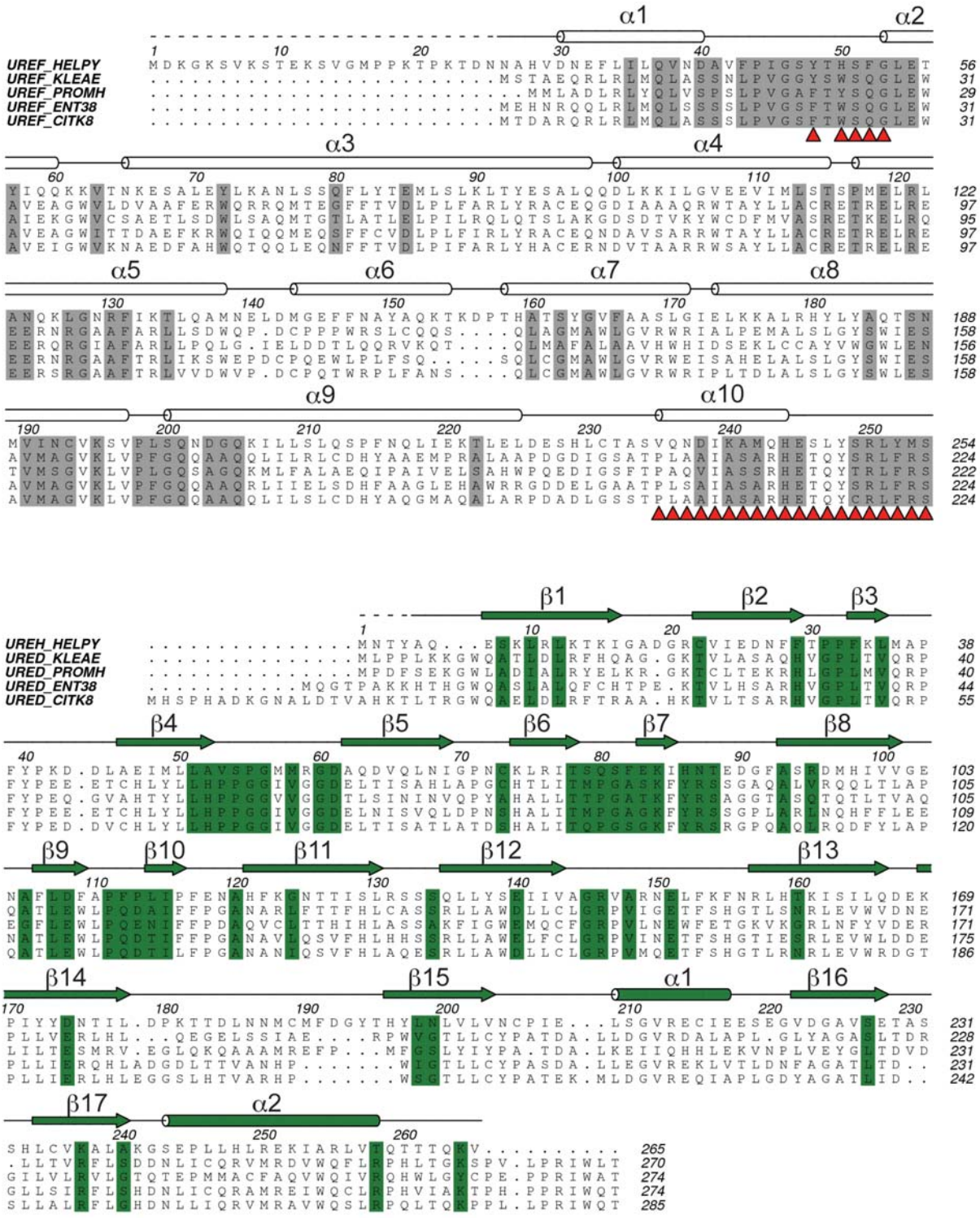

Figure S2. The sequence alignment of UreF and UreH(D). Sequences of UreF and UreH(D) from Swiss-Prot database were aligned using the program MUSCLE (1). The multiple sequence alignment obtained was used to calculate the conservation scores using the CONSURF server (2). Protein sequences from 5 selected species are shown (*Helicobacter pylori* – HELPY; *Klebsiella aerogenes* – KLEAE; *Proteus mirabilis* – PROMH; *Enterobacter sp.* strain 638 – ENT38; *Citrobacter koseri* – CITK8). α-helices and β-strands of *H. pylori* UreF and UreH are indicated as cylinders and arrows, respectively. Residues with conservation scores 8-9 are highlighted. Residues of UreF involved in conformational changes are marked with red triangles.

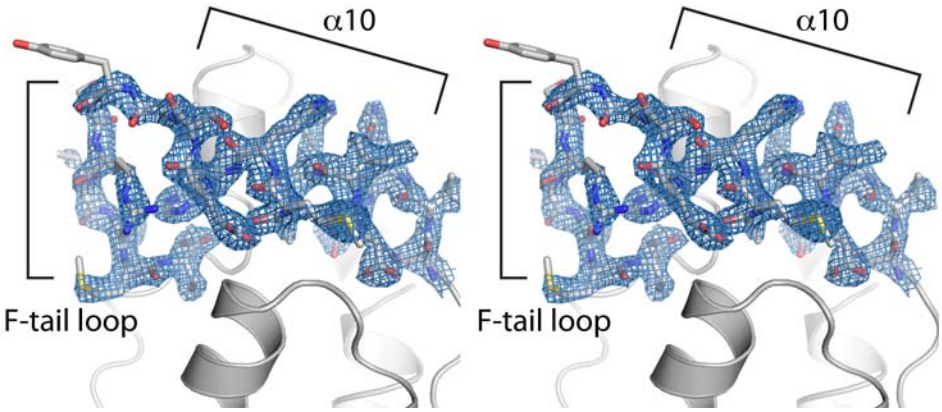

Figure S3. The C-terminal residues of UreF become ordered upon complex with UreH. An sigma-weighted 2Fo-Fc electron density map complex is contoured at 1.5σ for residues $S234^{UreF}$-$S254^{UreF}$ of the UreF/UreH complex. These residues form an extra helix α10 (236-243) and the F-tail loop structure (244-254). Note that these C-terminal residues are missing in the crystal structure of the free form of UreF.

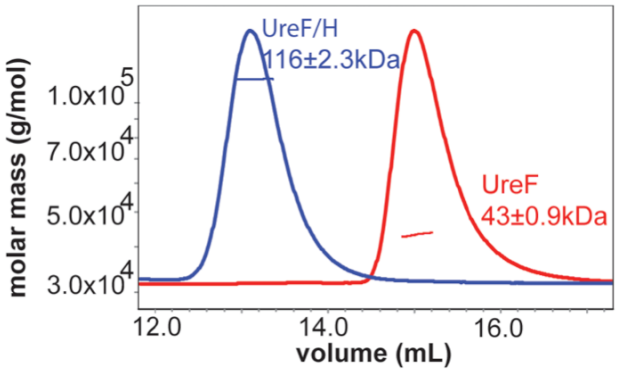

Figure S4. Determination of solution oligomerization state of UreF and UreF/UreH complex. Purified samples of UreF and UreF/UreH complex were loaded to a Superdex 200 analytical gel filtration column pre-equilibrated with phosphate buffer saline, and analyzed by static light scattering. Both UreF and UreF/UreH were eluted as single peaks. The molecular weights of UreF and the UreF/UreH complex were 43 ± 0.9 kDa and 116 ± 2.3 kDa, respectively.

Table S1. Data collection, phasing and refinement statistics for UreF [a]

	Native (PDB Code: 3O1Q)	SeMet		
		Peak	Inflection	Remote
Space Group	C2	C2		
Unit Cell Parameters (a, b, c, β)	135.3 Å, 90.0 Å, 67.0 Å, 94.6°	134.7 Å, 89.0 Å, 65.7 Å, 93.9°		
Wavelength (Å)	1.54187	0.98080	0.98100	0.97040
Resolution (Å)	50.95-1.85 (1.95-1.85)	40.11-2.28 (2.55-2.28)	40.11-2.41 (2.55-2.41)	40.11-2.41 (2.55-2.41)
No. of unique reflections	68238 (9897)	33836 (4605)	26725 (1604)	26707 (1629)
Redundancy	4.6 (4.5)	10.3 (9.7)	3.9 (3.6)	3.8 (3.8)
Completeness (%)	100 (100)	96.1 (89.8)	99.4 (98.3)	99.3 (99.5)
Mosaicity (°)	0.35	1.85	1.31	1.35
Average I/s	9.9 (3.9)	22.7 (8.0)	10.2 (3.4)	9.3 (3.6)
R_{merge}	0.103 (0.334)	0.072 (0.249)	0.088 (0.309)	0.088 (0.289)
Figure of Merit	—	0.496		
R_{work}	0.169 (0.183)			
R_{free}	0.208 (0.218)			
Average B-factor (Å^2)	24.5			
R.m.s. deviation bond:				
bond lengths (Å)	0.006			
angles (°)	0.926			
Ramachandran analysis:				
preferred regions (%)	98.6			
allowed regions (%)	100			
Diffraction Precision Index (Å^2)	0.1109			

[a] Values in parentheses correspond to the highest resolution shell.

Table S2. Summary of hydrogen bonds and salt bridges between UreF and UreH generated using PISA(3)

Hydrogen Bonds

	UreF	UreH	Distance [Å]
1	ALA 233[N]	ASN 175[O]	2.82
2	TYR 57[OH]	MET 188[O]	2.8
3	VAL 235[N]	CYS 189[O]	3.3
4	ASN 237[N]	CYS 189[O]	3.03
5	GLN 236[N]	CYS 189[O]	3.17
6	ASN 237[ND2]	CYS 189[SG]	3.63
7	GLN 236[N]	MET 190[O]	2.8
8	GLN 236[NE2]	ASP 192[OD2]	2.71
9	LYS 62[NZ]	ASP 192[OD2]	3.05
10	GLN 243[NE2]	ASP 223[OD1]	2.92
11	GLN 236[NE2]	ASP 223[OD2]	2.99
12	LYS 240[NZ]	ASP 223[OD2]	2.81
13	GLN 243[NE2]	GLY 224[O]	2.85
14	MET 118[N]	SER 227[OG]	2.97
15	ARG 121[NH2]	GLU 228[O]	2.53
16	LEU 113[O]	TYR 173[N]	3.19
17	SER 228[O]	ASN 175[ND2]	3.04
18	LEU 230[O]	ASN 175[ND2]	3.22
19	GLU 245[OE1]	ARG 213[NH2]	3.45
20	SER 116[O]	SER 227[OG]	3.48
21	GLU 245[OE2]	SER 227[OG]	2.78
22	SER 234[O]	LYS 237[NZ]	3.15

Salt Bridges

	UreF	UreH	Distance [Å]
1	LYS 62[NZ]	ASP 192[OD2]	3.05
2	LYS 240[NZ]	ASP 223[OD1]	3.31
3	LYS 240[NZ]	ASP 223[OD2]	2.81
4	GLU 245[OE1]	ARG 213[NH2]	3.45
5	ASP 238[OD2]	LYS 237[NZ]	3.11

REFERENCES

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2. Landau, M., Mayrose, I., Rosenberg, Y., Glaser, F., Martz, E., Pupko, T., and Ben-Tal, N. (2005) *Nucleic Acids Res.* **33**, W299-302
3. Krissinel, E., and Henrick, K. (2007) *J. Mol. Biol.* **372**, 774-797