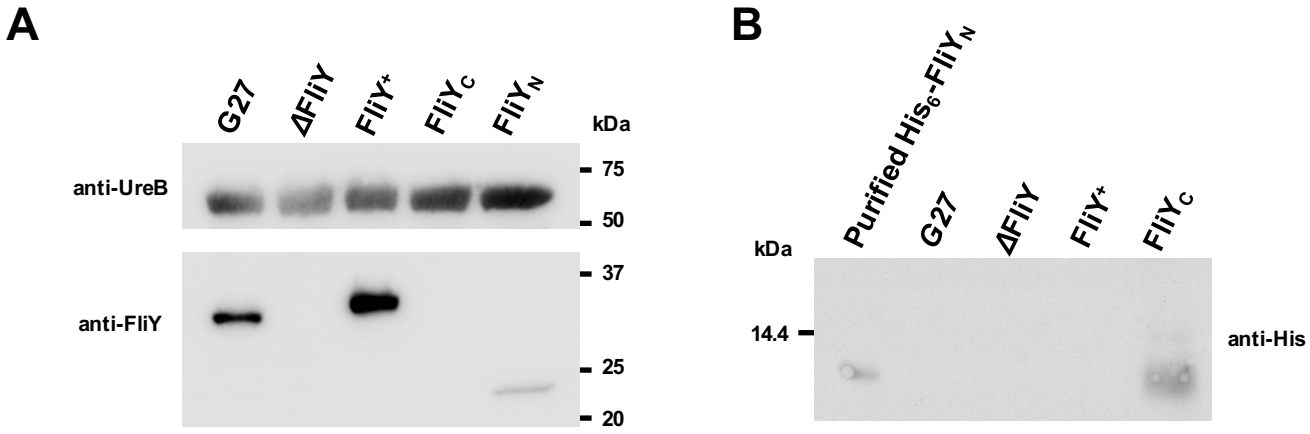
Supplemental Table 1. Data collection and refinement statistics.

	$FliN_C/FliY_C$
Data collection	
Space group	P 1
Cell dimensions	
a, *b*, *c* (Å)	48.21, 49.21, 52.37
α, β, γ (°)	69.34, 82.98, 76.01
Resolution (Å)	29.42 - 2.50 (2.60 - 2.50)
R-meas	0.057 (0.117)
CC1/2	0.994 (0.980)
I/σ(I)	6.0 (3.0)
Completeness (%)	87.3 (89.6)
Redundancy	1.9 (1.9)
Refinement	
Resolution (Å)	25.37 – 2.5
No. reflections	13190
R_{work}/R_{free}	0.2026/0.2286
No. atoms	2486
Protein	2408
Ligand/ion	-
Water	78
B-factors	43.2
Protein	43.0
Ligand/ion	-
Water	47.5
R.m.s. deviations	
Bond lengths (Å)	0.009
Bond angles (°)	1.220

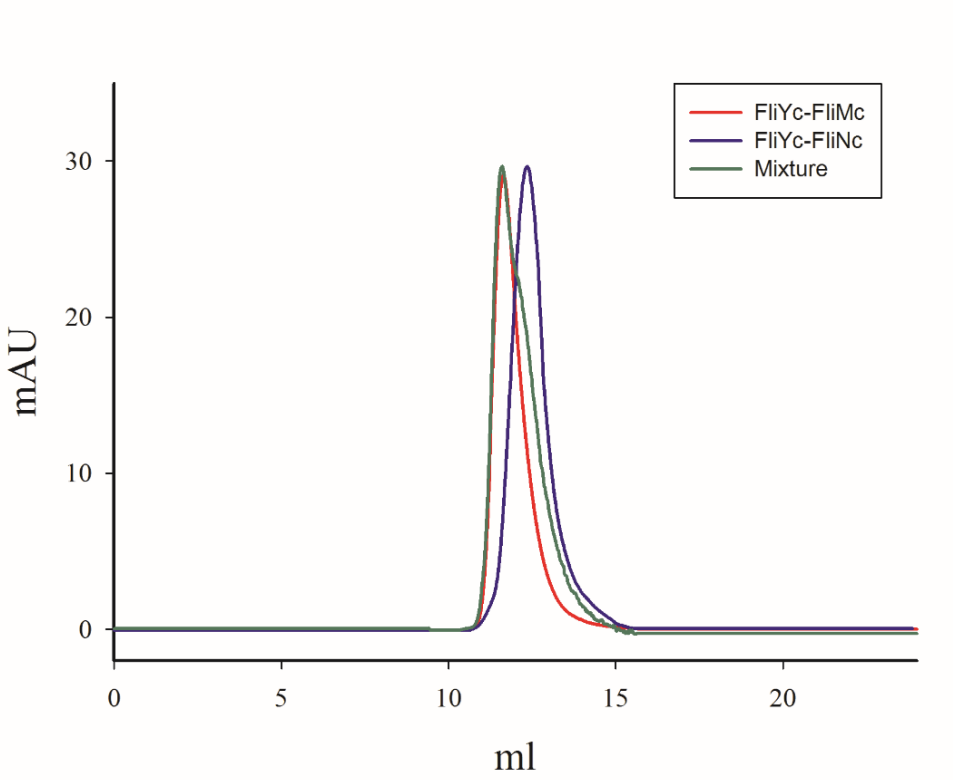
Statistics for the highest-resolution shell are shown in parentheses.

Supplemental Figure 1



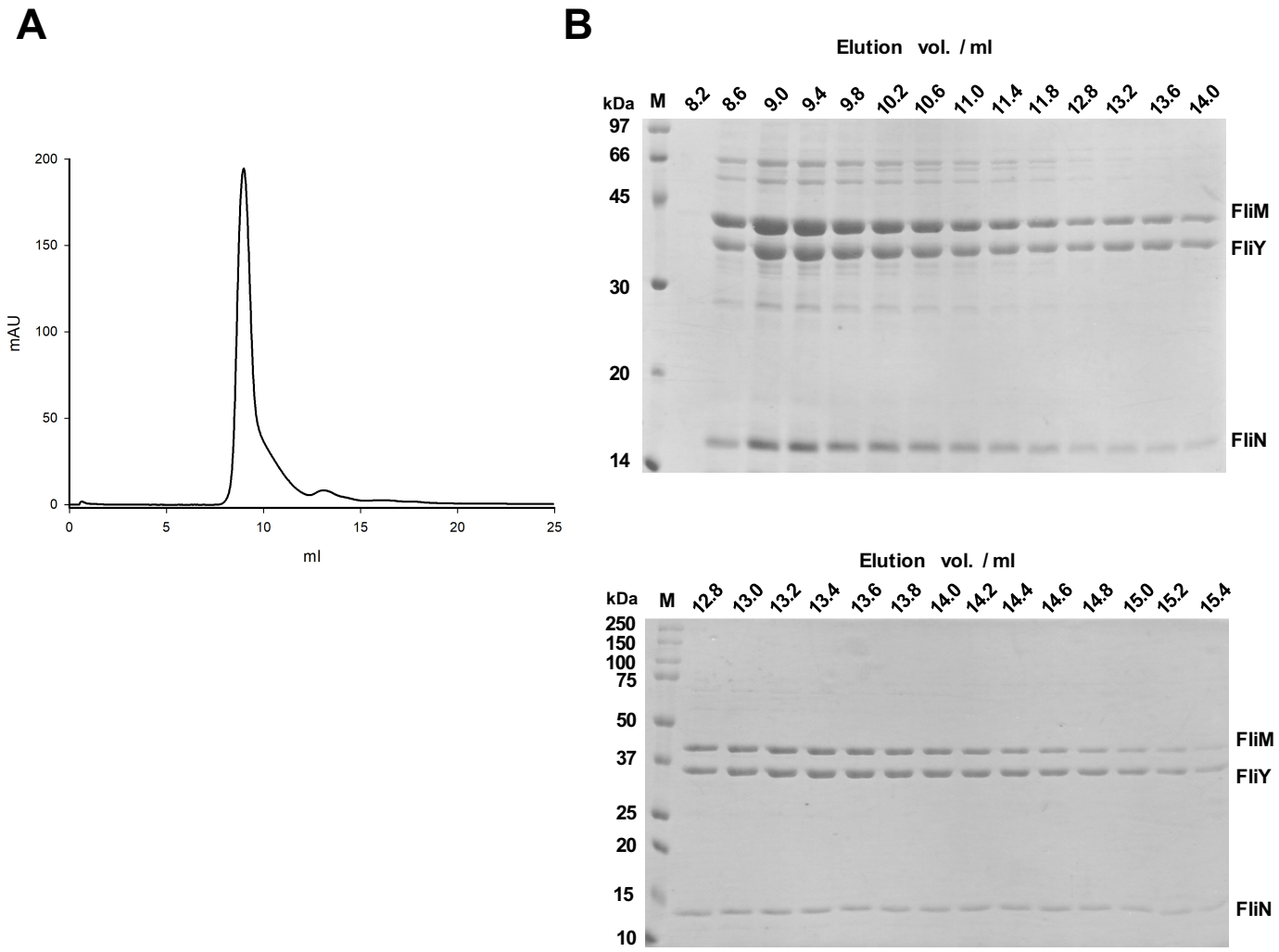
Western analysis of FliY, $FliY_N$ and $FliY_C$ expression in complemented strains. (A). Expression of FliY and $FliY_N$ was detected using anti-FliY antibody which was raised by using purified recombinant $FliY_N$ protein. Loading control using urease B was included. (B). Expression of $FliY_C$ was detected using anti-His antibody. We are not able to detect the his_6-FliY in $FliY^+$ complemented strain using anti-His antibody, possibly due to the N-terminal his_6-tag in FliY is degraded. *H. pylori* cells were cultured in BB10 medium with a starting OD_{600}=0.02. After an overnight incubation, the cells were collected and washed with PBS. OD_{600} was measured for each strain and same amount of cells were used for the western experiments.

Supplemental Figure 2



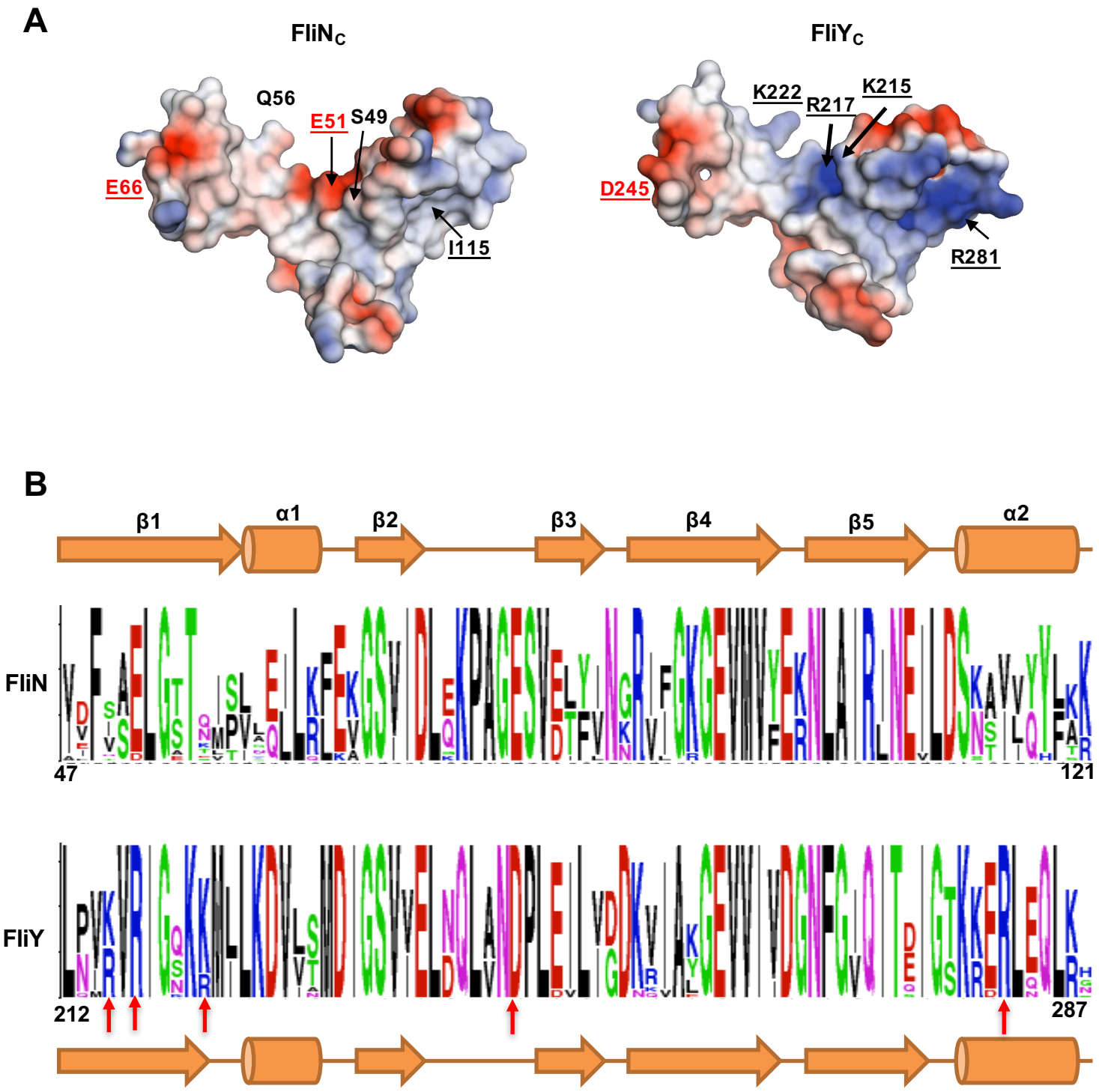
$FliY_C$–$FliM_C$ complex does not interact with $FliY_C$–$FliN_C$ complex. Purified $FliY_C$–$FliN_C$ and $FliY_C$–$FliM_C$ complexes were mixed at equimolar ratio at 4°C for overnight and were separated by Superdex S75. The elution profiles of $FliY_C$–$FliN_C$ and $FliY_C$–$FliM_C$ were overlaid for comparison.

Supplemental Figure 3



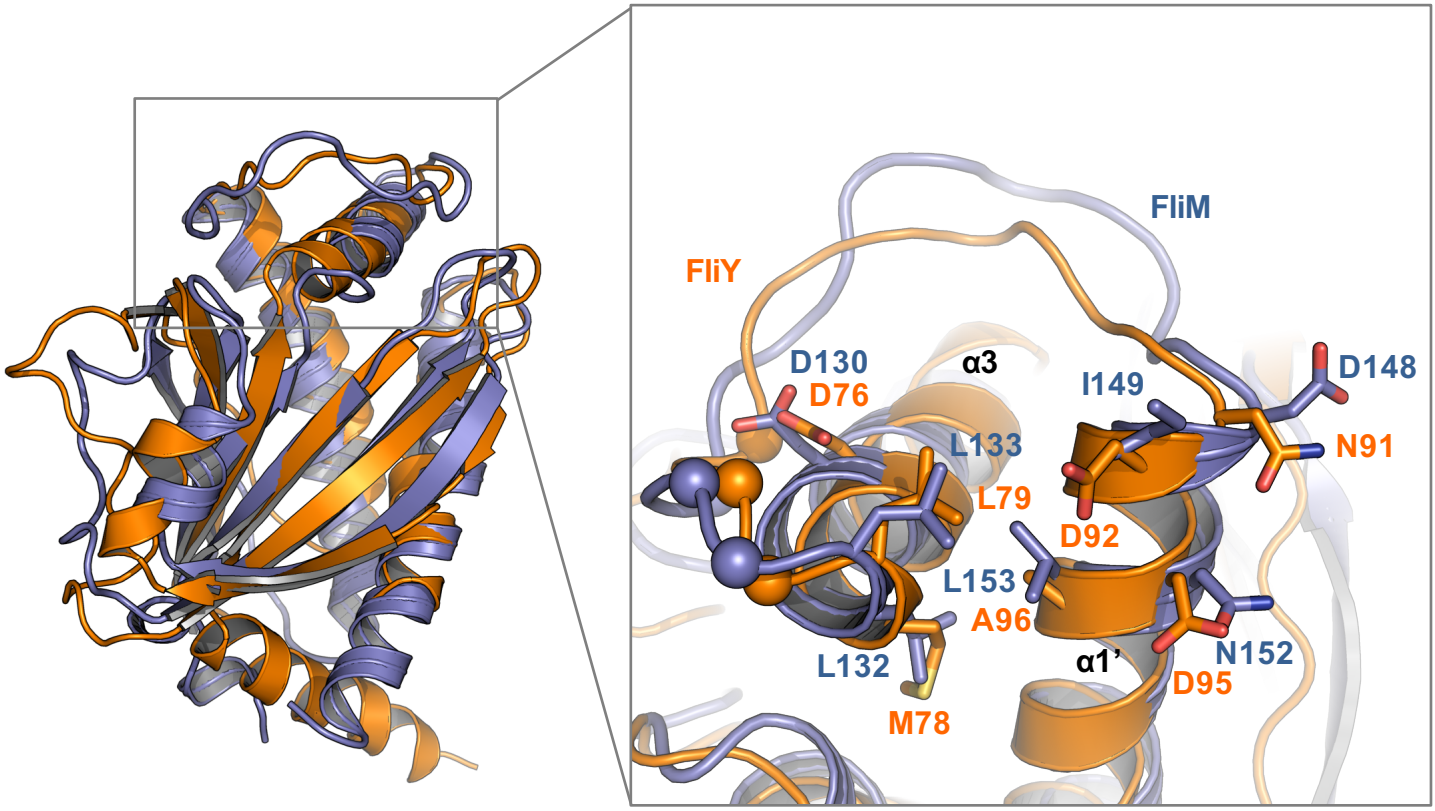
Co-purification of FliY–FliN–FliM complex. GST-FliM was co-expressed with His_6-FliN and FliY. After nickel and GST affinity chromatography, the partially purified FliY–FliN–FliM complex was subjected to Superdex S200 size exclusion analysis. (A). The elution profile from Superdex S200. (B). Eluted fractions were analyzed by SDS-PAGE. (Upper panel) Fractions eluted from 8.2–14.0 ml using 15% SDS-PAGE. (Lower panel) Fractions eluted from 12.8–15.4 ml using 10-20% gradient SDS-PAGE.

Supplemental Figure 4



Unique residues at the interface of FliY$_C$ and FliN$_C$ may favor the formation of FliY–FliN heterodimeric complex. (A) The binding interface of FliY$_C$–FliN$_C$ shown as electrostatic surface. Note that the FliY$_C$ is characterized by electropositive surface while FliN$_C$ is more electronegative. (B) Amino acid composition of FliN$_C$ and FliY$_C$ from ε-proteobacteria that contains both FliY and FliN (in *H. pylori* numbering) (Lowenthal et al., 2009). The sequences were aligned by T-coffee and depicted as sequence logo. Secondary structure of FliN$_C$ and FliY$_C$ were shown above and below the sequences, respectively. FliY and FliN-specific charged interacting residues at the interface are marked and indicated by arrows.

Supplemental Figure 5



Superimposition of $FliM_M$ (PDB-ID: 4FQ0) with $FliY_N$ model highlighting the sequence variations at the FliG interacting surface between these two proteins. The conserved GGXG motif is represented as sphere. The residues at α3 and α1' of $FliM_M$ mediating FliG–FliM interaction as well as the corresponding FliY residues are shown as stick. Note that I149 is replaced by aspartic acid in $FliY_N$, and $FliM_M$ I149D mutation abolished FliG–FliM interaction as demonstrated from our previous study (Lam et al., 2013). The model of $FliY_N$ was generated by Modeller using $FliY_M$ from *T. maritima* (PDB-ID: 4HYN) as template (Eswar et al., 2007).