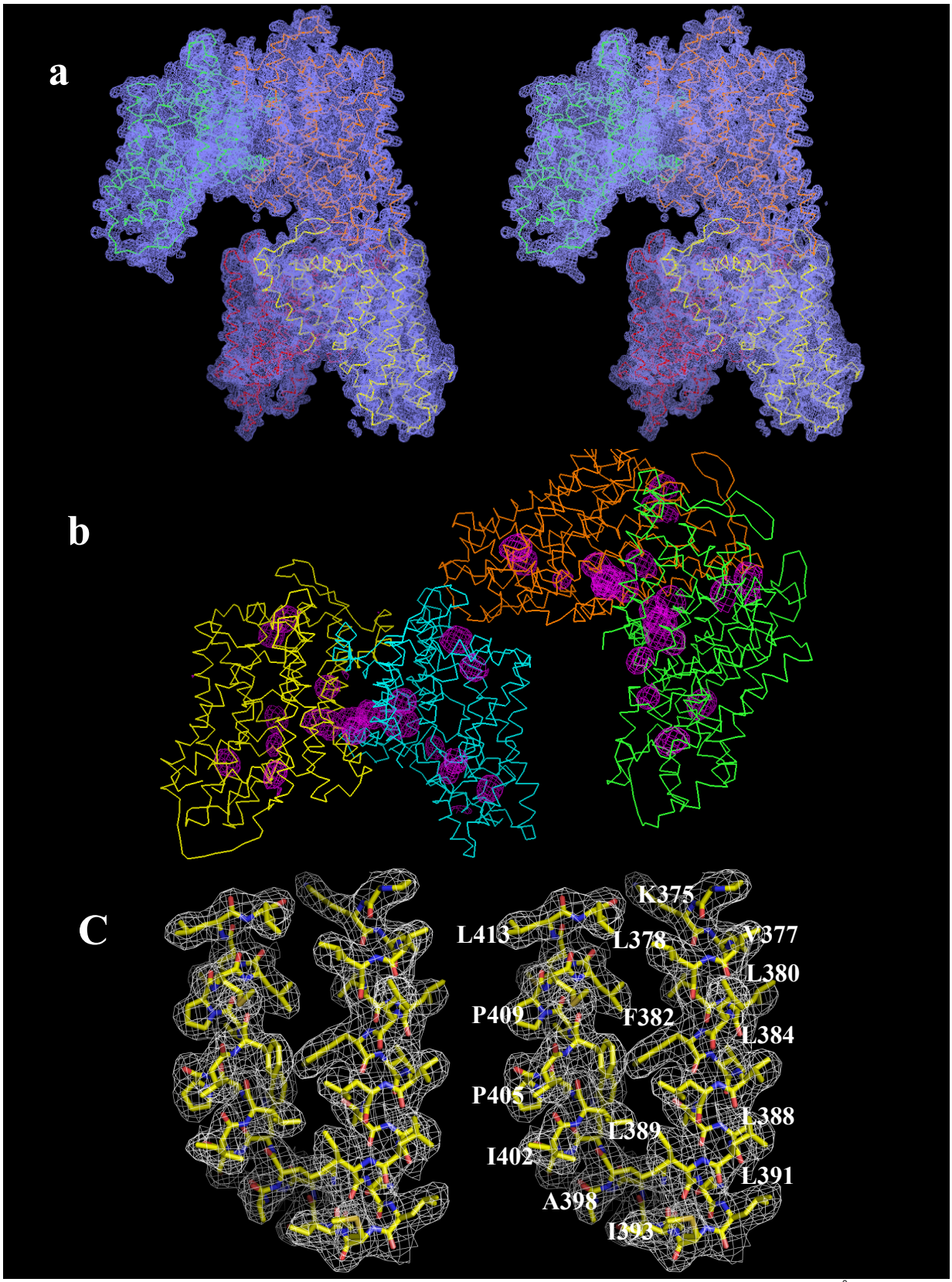
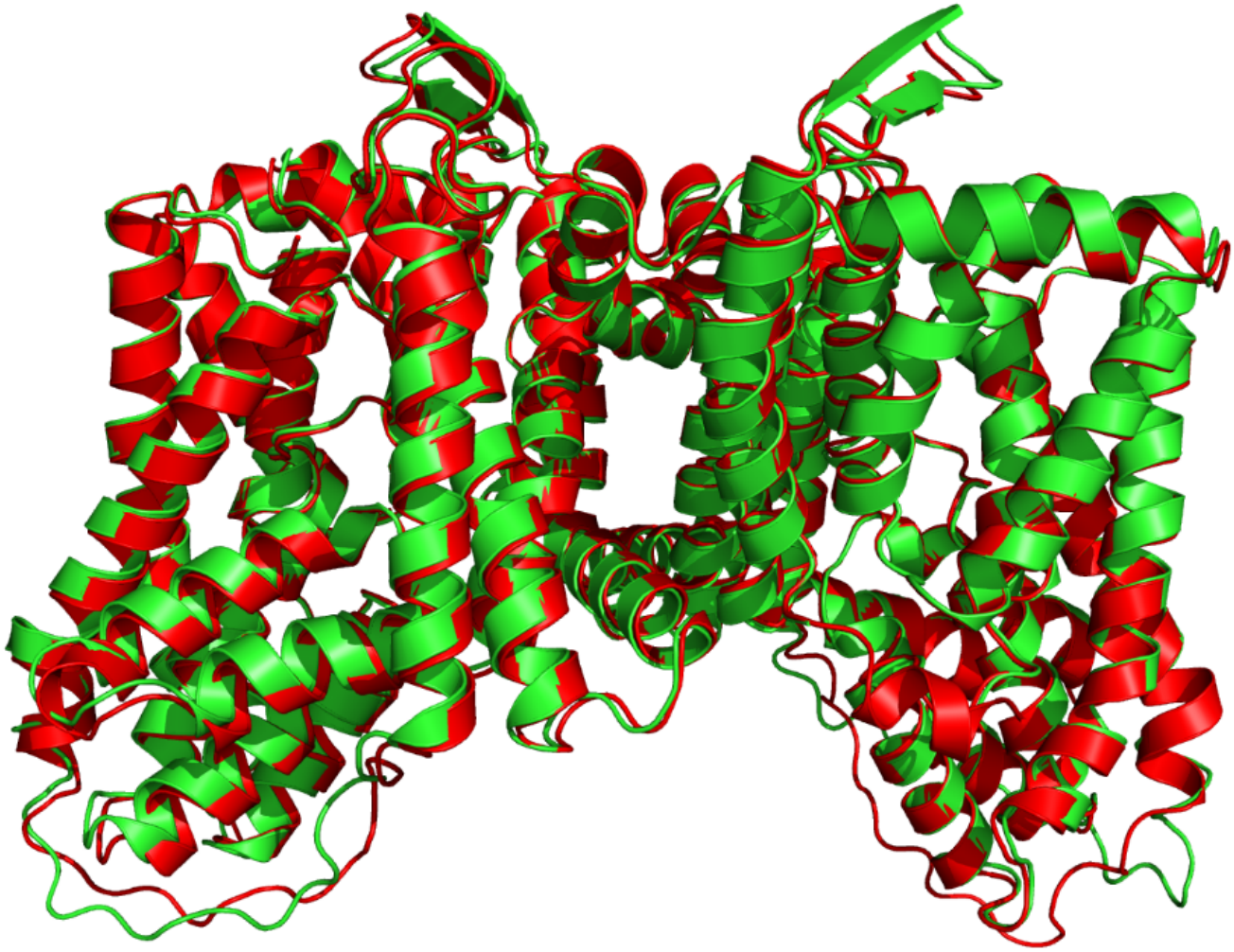
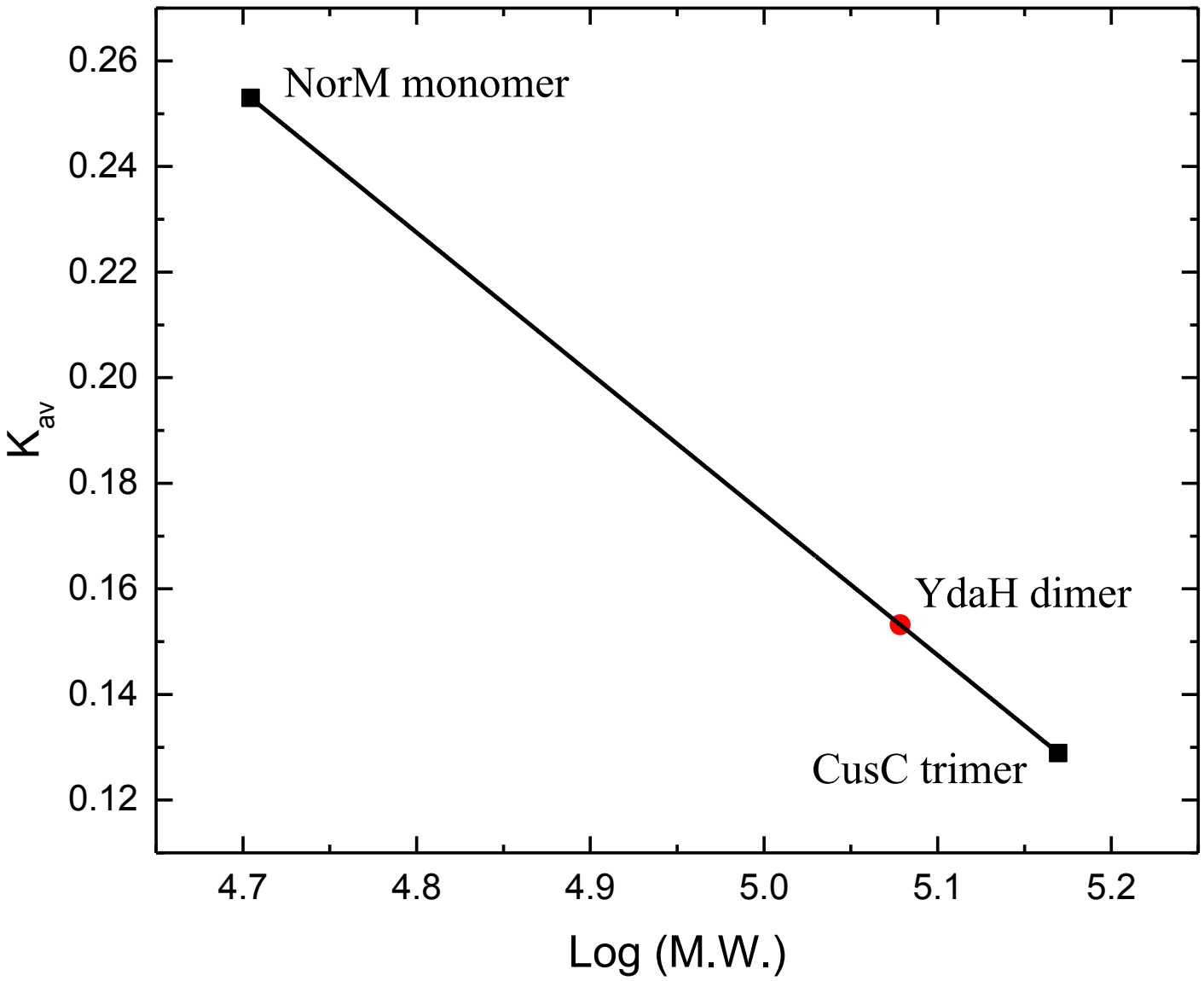
Supplementary Figure 1. Sequence and topology of *A. borkumensis* YdaH. Alignment of the amino acid sequences of the AbgT family of transporters were done using CLUSTAL W. *, identical residues; :, >60% homologous residues. Secondary structural elements are indicated: TM, transmembrane helix; β, strand. The sequence and topology of *A. borkumensis* YdaH are shown at the top. Conserved residues involved in lining the channel of the inner core of the protein are highlight with green bars. Residues coordinating with the bound Na^+ are indicated with red arrows.



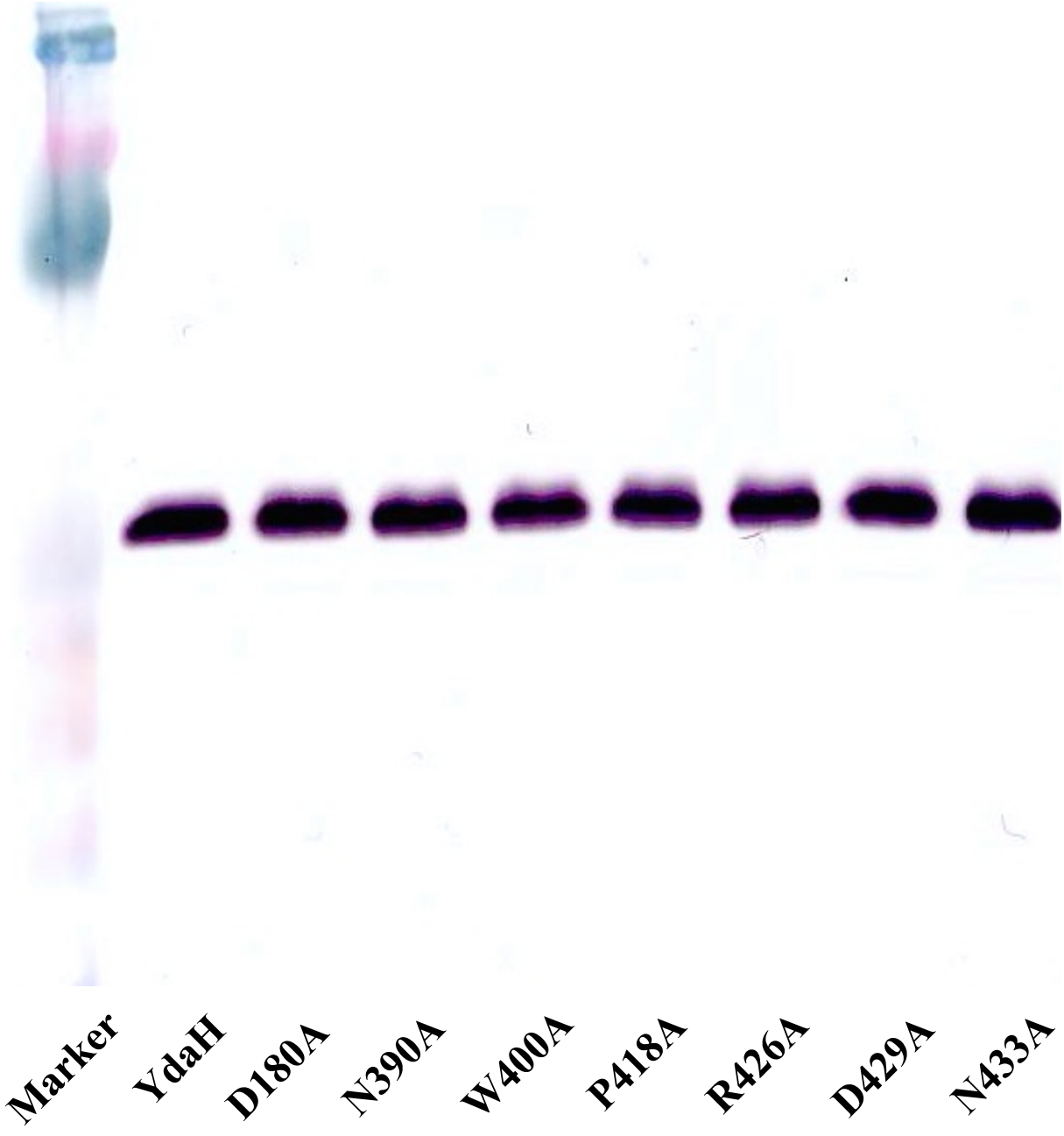
Supplementary Figure 2. Stereo view of the electron density maps of YdaH at a resolution of 2.96 Å. (a) The electron density maps are contoured at 1.2 σ. The Cα traces of the two YdaH dimers in the asymmetric unit are included. (b) Anomalous maps of the 44 selenium sites (contoured at 4 σ). Four protomers forming two dimers of YdaH are found in the asymmetric unit. Each protomer contributes 11 selenium sites corresponding to the 11 methionines (magenta). The Cα traces of the four YdaH monomers are colored green, orange, cyan and yellow. (c) Representative section of the electron density within HP2 of YdaH. The electron density (colored white) is contoured at the 1.2 σ level and superimposed with the final refined model (yellow, carbon; red, oxygen; blue, nitrogen).



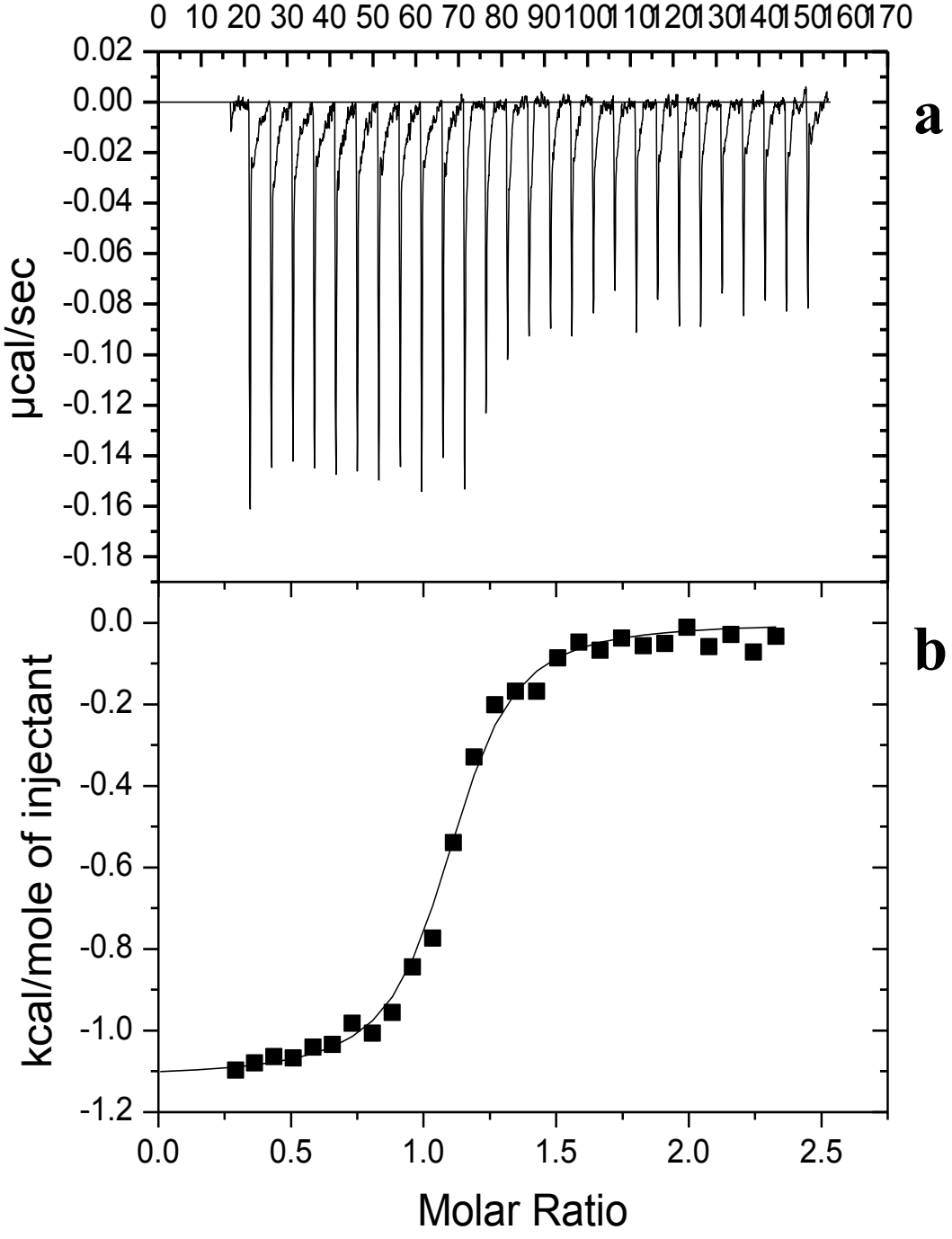
Supplementary Figure 3. Comparison of the two YdaH dimers within the asymmetric unit. Superimposition of the two YdaH dimers (green and red) results in an RMSD of 1.0 Å for 942 Cα atoms.



Supplementary Figure 4. Representative gel filtration experiment. The experiment demonstrated that YdaH exists as a dimer in solution. The *y*-axis values were defined as: $K_{av} = (V_e - V_0)/(V_T - V_0)$, where V_T, V_e, and V_0 are the total column volume, elution volume, and void volume of the column, respectively. Standards used were the trimeric *E. coli* CusC channel (M_r 12,400) and monomeric *N. gonorrhoeae* NorM efflux pump (M_r 29,000). The void volume was measured using blue dextran (M_r 2,000,000).



Supplementary Figure 5. Expression level of the YdaH pumps. An immunoblot against YdaH of crude extracts from 50 μg dry cells of strain BL21(DE3)Δ*abgT*Δ*pabA* expressing the YdaH wild-type and mutant (D180A, N390, W400A, P418A, R426A, D429A and N433A) pumps are shown.



Supplementary Figure 6. Representative isothermal titration calorimetry for the binding of sulfamethazine to YdaH. (a) Each peak corresponds to the injection of 10 µl of 20 µM monomeric YdaH in buffer containing 20 mM Tris-HCl pH 7.5, 50 mM NaCl and 0.03% DDM into the reaction containing 0.4 mM sulfamethazine in the same buffer. (b) Cumulative heat of reaction is displayed as a function of the injection number. The solid line is the least-square fit to the experimental data, giving a K_D of 0.41 ± 0.03 µM.

Supplementary Table 1. Primers for site-directed mutagenesis.

Primer	Sequence
D180A-forward	5′-CCCGGTGGCTGCAACCCTGGCTGGTCTGTCAACGGA-3′
D180A-reverse	5′-CAGGGTTGCAGCCACCGGGCCGACCAGCAGATTCGCC-3′
N390A-forward	5′-GTGCTGACGGCCCTGATTGCCCTGATGATCGGTAGTGCG-3′
N390A-reverse	5′-CGCACTACCGATCATCAGGGCAATCAGGGCCGTCAGCAC-3′
W400A-forward	5′-CGCCAAGGCGAGTATTCTGGCCCCGGTGTTCATCCCG-3′
W400A-reverse	5′-CAGAATACTCGCCTTGGCGGACGCACTACCGATCATCAG-3′
P418A-forward	5′-CATCAGTGCGGAAGCATCCCAGGCAGCTTATCGCGT-3′
P418A-reverse	5′-GATGCTTCCGCACTGATGCCCAGCAGCATCAGCATC-3′
R426A-forward	5′-GCATCCCAGGCAGCTTATGCCGTTGGTGATTCATCGACC-3′
R426A-reverse	5′-GGTCGATGAATCACCAACGGCATAAGCTGCCTGGGATGC-3′
D429A-forward	5′-GCAGCTTATCGCGTTGGTGCTTCATCGACCAATATTATC-3′
D429A-reverse	5′-GATAATATTGGTCGATGAAGCACCAACGCGATAAGCTGC-3′
N433A-forward	5′-GTTGGTGATTCATCGACCGCTATTATCACGCCGCTGATG-3′
N433A-reverse	5′-CATCAGCGGCGTGATAATAGCGGTCGATGAATCACCAAC-3′

Supplementary Table 2. MICs of sulfamethazine, Sulfadiazine, Sulfathiazole and Sulfanilamide for different YdaH variants expressed in *E. coli* BL21(DE3)Δ*abgT*Δ*pabA*.

Gene in BL21(DE3)Δ*abgT*Δ*pabA*	Sulfamethazine (μg/mL)	Sulfadiazine (μg/mL)	Sulfathiazole (μg/mL)	Sulfanilamide (μg/mL)
Empty vector	62.5	31.25	62.5	500
ydaH (wild-type)	2000	>250	>500	4000
ydaH (D180A)	1000	31.25	62.5	2000
ydaH (N390A)	62.5	31.25	62.5	2000
ydaH (W400A)	62.5	31.25	62.5	2000
ydaH (P418A)	250	31.25	62.5	2000
ydaH (R426A)	125	>250	>500	2000
ydaH (D429A)	62.5	31.25	62.5	2000
ydaH (N433A)	62.5	31.25	62.5	2000

Supplementary Table 3. Binding of sulfamethazine, sulfadiazine, sulfathiazole and sulfanilamide by YdaH.

	K_D (μM)	ΔH (kcal•mol^{-1})	ΔS (cal•mol•deg^{-1})
Sulfamethazine	0.41 ± 0.03	-1114.0 ± 16.2	25.5
Sulfadiazine	7.04 ± 0.54	-403.1 ± 17.9	22.2
Sulfathiazole	0.60 ± 0.02	-2540.0 ± 91.9	19.9
Sulfanilamide	4.97 ± 0.26	-467.0 ± 22.6	22.7