Flavin-induced oligomerization in *Escherichia coli* adaptive response protein AidB†

Supporting Information

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Table S1. Primer pairs used to generate the triple mutant of AidB (*mt*AidB)

Mutation	**Primer pair used**
T185V-S191R	forward: 5′- GGCATGGGAATGGTGGAAAAGCAGGGCGGTAGGGATGTTATGAGC- 3′ reverse: 5′-GCTCATAACATCCCTACCGCCCTGCTTTTCCACCATTCCCATGCC- 3′
R324D	forward: 5′-TATCATGCACATCAAGACCATGTTTTTGGTAAT-3´ reverse: 5′-ATTACCAAAAACATGGTCTTGATGTGCATGATA-3´

Table S2. Data collection statistics

	holo *wt*AidB
Beamline	APS 24ID-C
Wavelength (Å)	1.0000
Detector	Q315 ADSC
Space group	$P3_2$
Unit cell parameters (Å)	a = b = 179.29
	c = 204.24
Resolution (Å)[a]	50.0 – 2.80 (2.90 – 2.80)
No. of unique reflections[a]	174735 (17687)
R_{sym}(%)[a,b]	9.5 (45.7)
Completeness(%)[a]	96.2 (97.4)
Multiplicity[a]	6.1 (5.1)
$\langle I \rangle / \langle \sigma(I) \rangle$[a]	14.1 (3.3)
Wilson *B*-Factor (Å^2)	45.3

[a]Numbers for the highest resolution shell are shown in parentheses.

[b]$R_{sym} = (\sum_i \sum_{hkl} I_i(hkl) - \langle I(hkl) \rangle) / \sum_{hkl} \langle I(hkl) \rangle$, where $I_i(hkl)$ is the intensity of the i^{th} measured reflection, and $\langle I(hkl) \rangle$ is the mean intensity for the reflection with the Miller index (*hkl*).

Table S3. Crystallographic refinement statistics

	holo *wt*AidB
Resolution range (Å)	46.2 – 2.80
R_{cryst}[a]	0.204
R_{free}[a]	0.229
No. of non-hydrogen atoms	
protein	49649
FAD	636
chloride	12
water	297
Average isotropic *B*-Factors (Å^2)	
protein main chain / side chain	47.3/48.6
FAD	45.6
chloride	50.9
water	28.6
r.m.s.d.[b] for bond lengths (Å)	0.005
r.m.s.d.[b] for bond angles (°)	0.828
Ramachandran statistics (% residues)	
favored	98.1
allowed	1.8
disallowed	0.1

[a] $R_{cryst} = (\sum_{hkl} \| F_{obs}(hkl) \| - \| F_{calc}(hkl) \|) / \sum_{hkl} \| F_{obs}(hkl) \|$; R_{free} is calculated identically, using 5% of reflections omitted from refinement.

[b] r.m.s.d. = root-mean-square deviation.

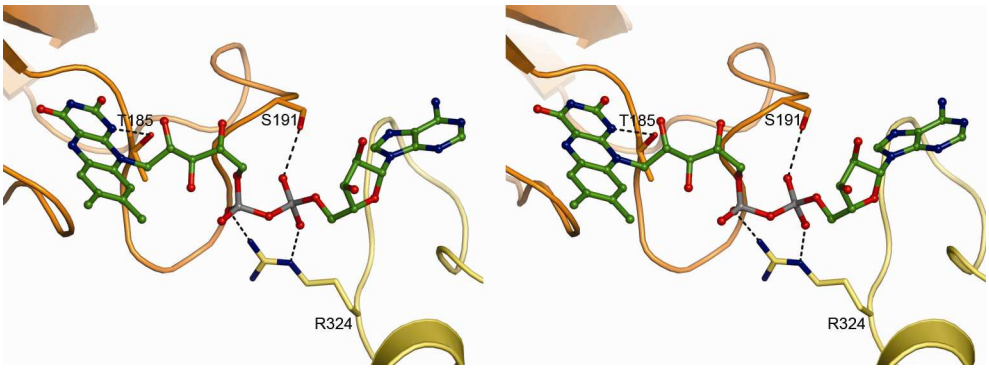

Figure S1. Wall-eyed stereo view of the flavin-binding site of holo *wt*AidB and the residues mutated to generate the flavin-binding deficient *mt*AidB. The two protomers contributing to flavin binding are shown in orange and yellow ribbons. Mutated residues are shown in sticks, with carbon atoms colored according to their protomer. Interactions between these residues and the FAD cofactor are indicated by dashed black lines.

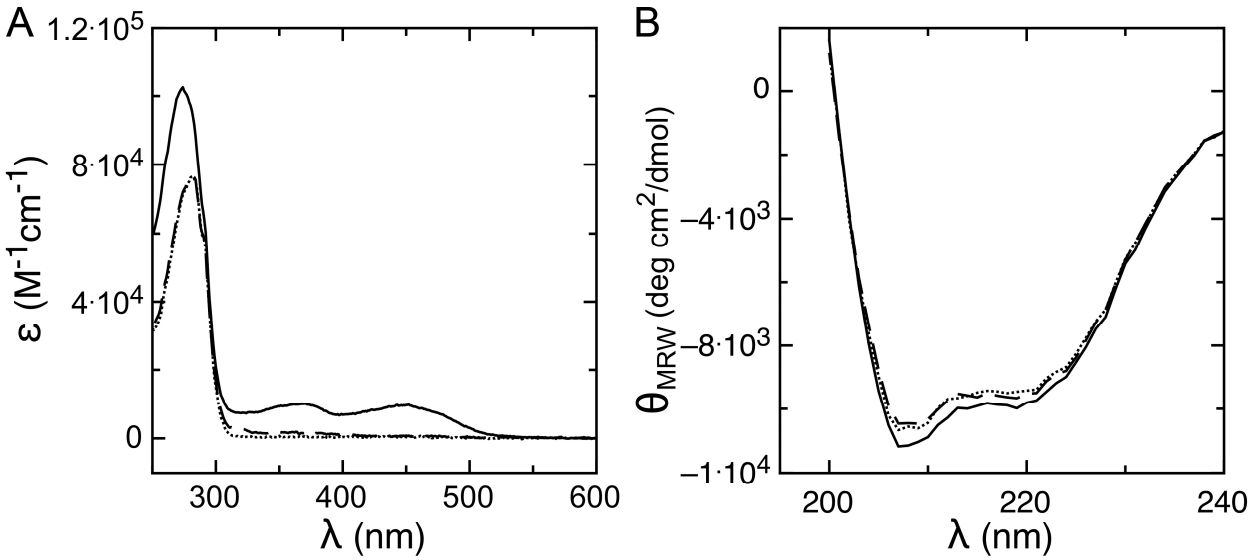

Figure S2. Spectroscopic properties of holo *wt*AidB, apo *wt*AidB, and apo *mt*AidB. (A) UV/Vis spectra of holo *wt*AidB (solid line), apo *wt*AidB (dotted line), and apo *mt*AidB (dashed line). (B) CD spectra of holo *wt*AidB (solid line), apo *wt*AidB (dotted line) and apo *mt*AidB (dashed line).

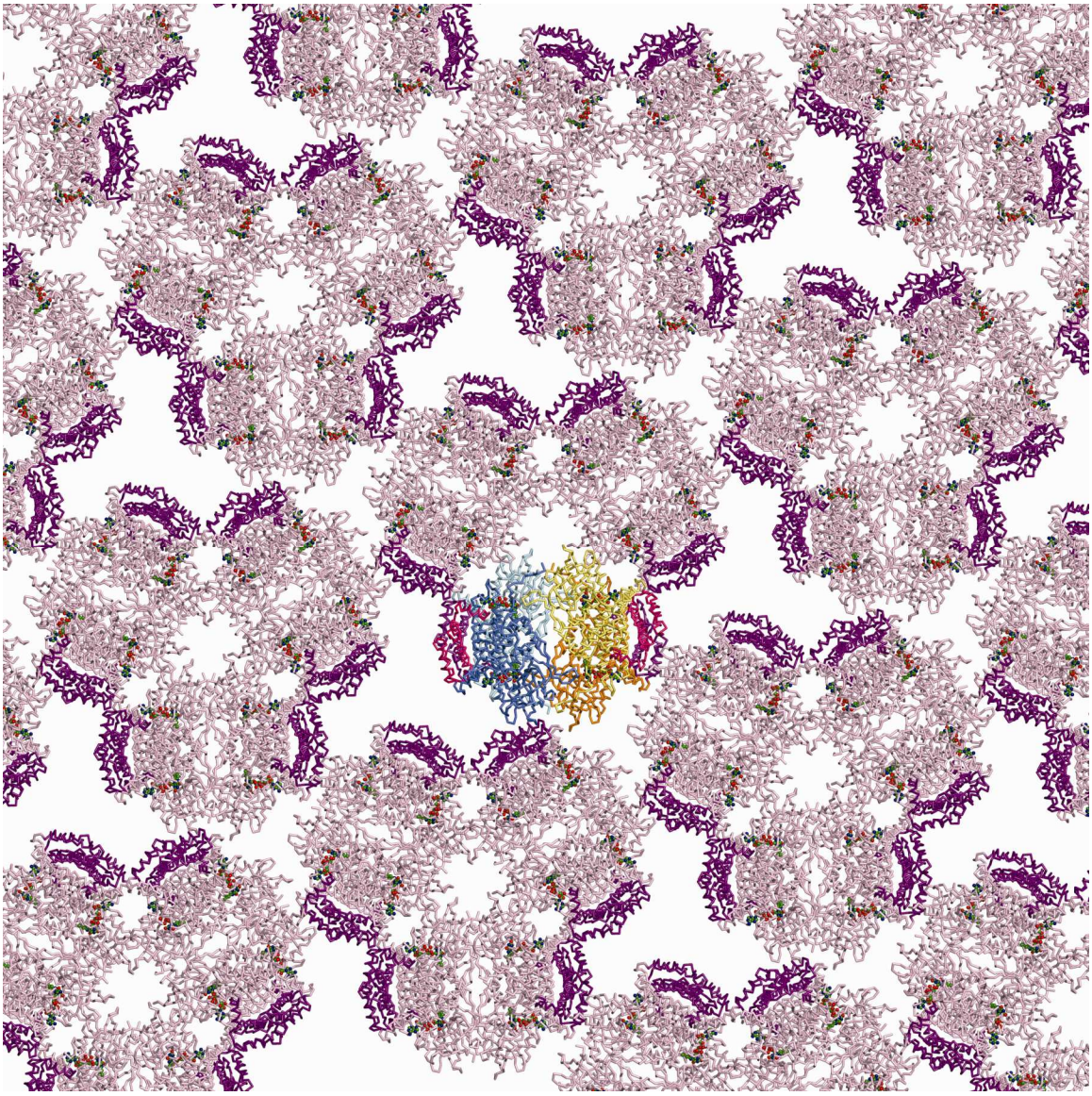

Figure S3. C_α-traces of holo *wt*AidB molecules in the $P3_2$ crystal lattice. One tetramer of AidB is colored by protomer in yellow, orange, cyan, and blue. The other tetramers are shown in pink. The putative DNA-binding regions are highlighted in purple and magenta. Bound FAD molecules are shown in the ball-and-stick representation with carbon atoms in green.

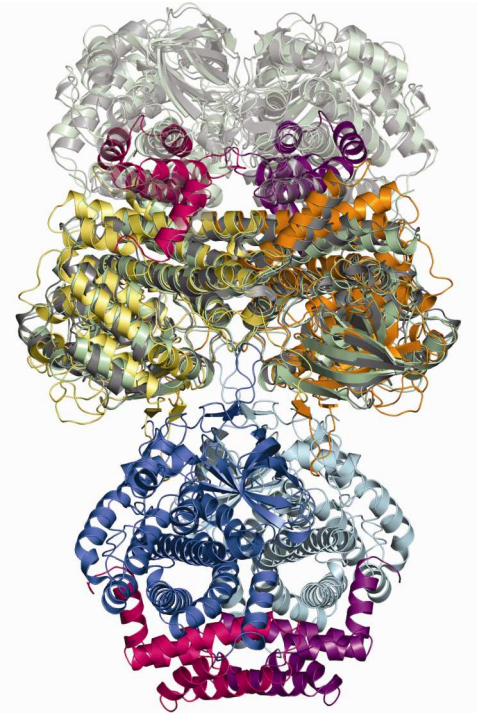

Figure S4. Superimposition of holo *wt*AidB with IVD (PDB code 1IVH) and MCAD (PDB code 3MDD). The AB dimer of AidB, shown in yellow and orange, superposes well with dimers of IVD (r.m.s.d. 1.68 Å over 314 C_{α}-atoms) and MCAD (r.m.s.d. 1.70 Å over 303 C_{α}-atoms), shown as solid gray and solid green ribbons, respectively. The tetramer of AidB exhibits a different architecture than the tetramers of IVD and MCAD. In AidB, the putative DNA-binding domain, colored in magenta and purple, prevents the formation of the tetramer observed in IVD and MCAD. The position of the second dimer of AidB, shown in blue and cyan, does not correlate with the position of the second dimer for IVD (transparent gray) and MCAD (transparent green).