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Supporting information for article:

Structural studies of a novel auxiliary domain-containing phenylalanine hydroxylase from *Bacillus cereus* ATCC 14579

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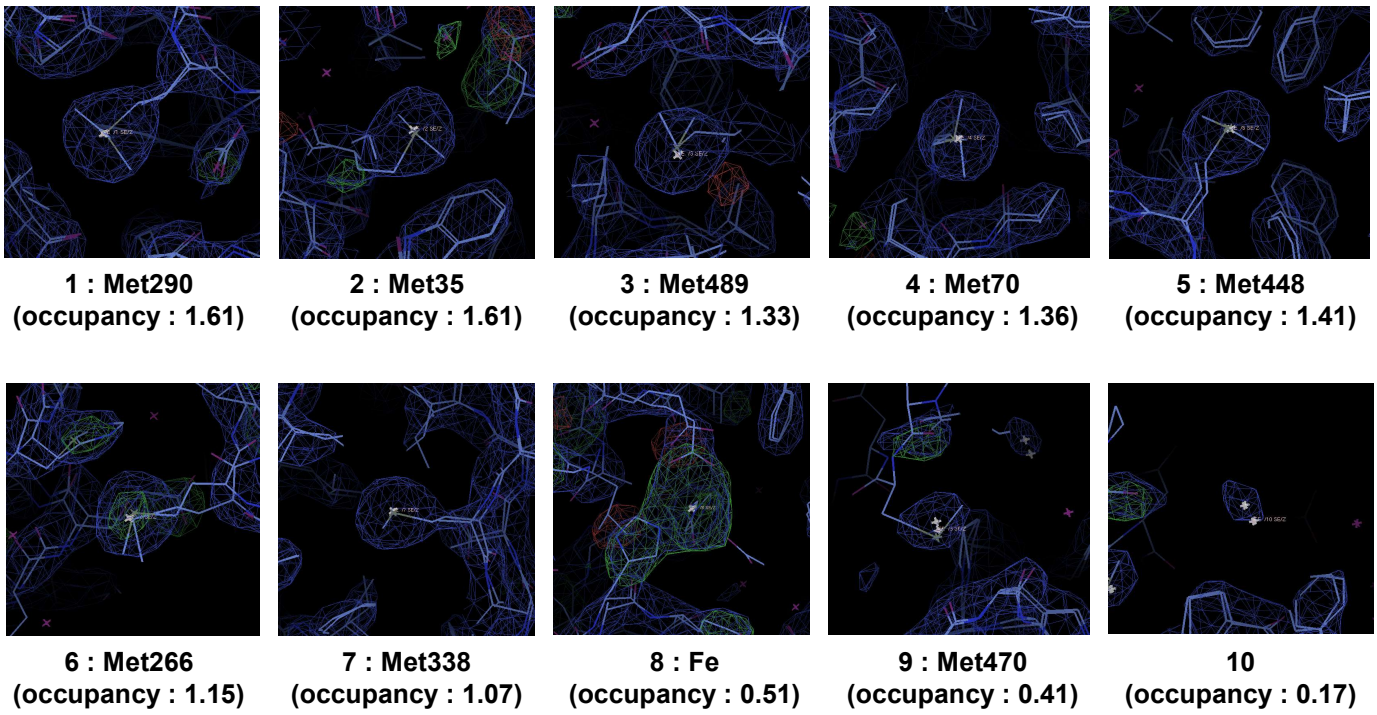

Figure S1. Initial SAD map of *Bc*PAH. The initial SAD map is shown as a blue-colored mesh with 1.50 rmsd contour. The initial and final refined structure of *Bc*PAH were indicated as a stick model.

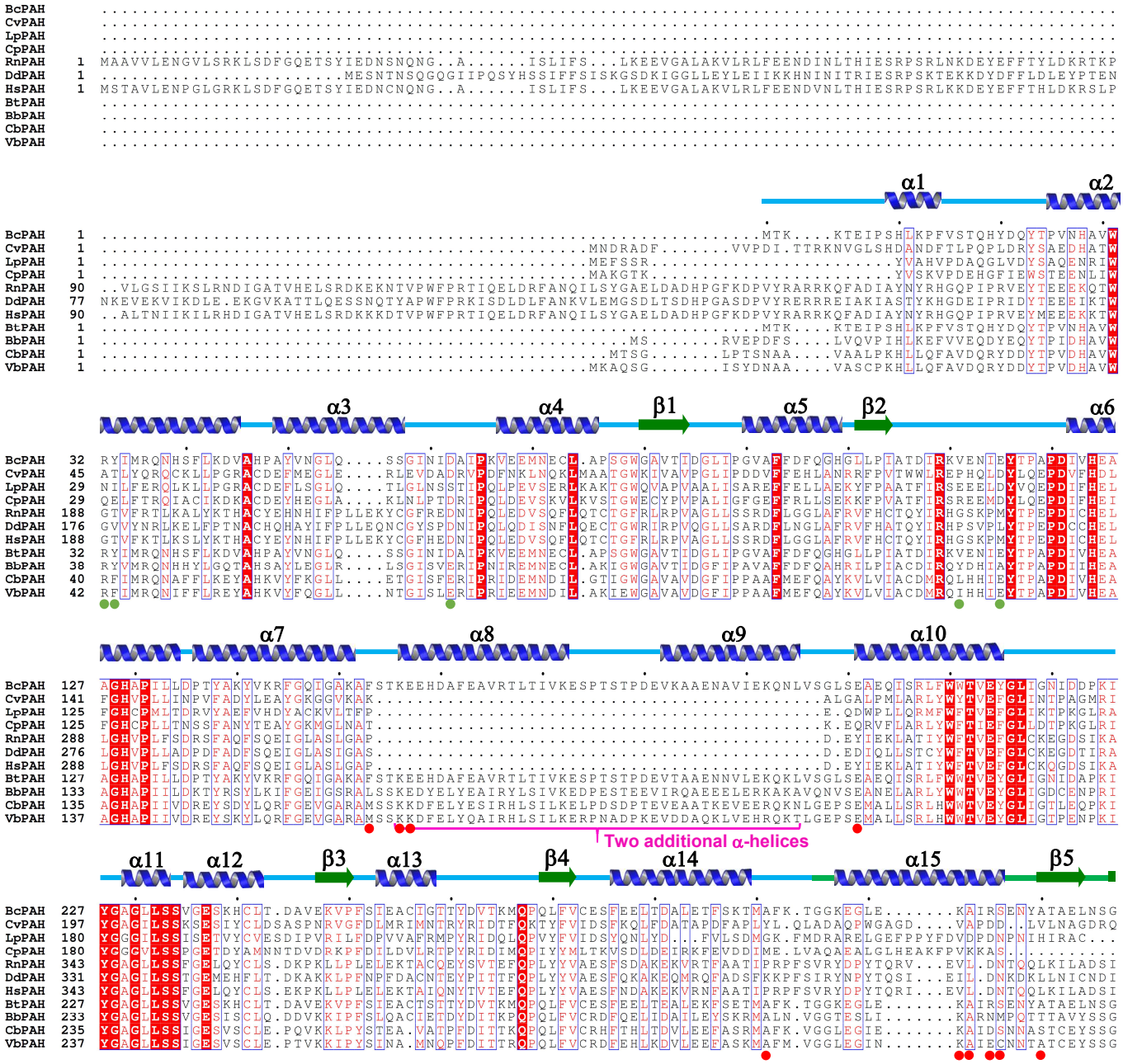

Figure S2. Full amino acid sequence alignment of PAHs. The secondary structures are drawn based on the *Bc*PAH structure. The cyan-, green-, pink-, yellow-colored lines under the secondary structure indicate *Bc*PAH-CD, *Bc*PAH-AD1, AD1-AD2 linker, and *Bc*PAH-AD2, respectively. The residues involved in the interactions between *Bc*PAH-CD and *Bc*PAH-AD1, and between *Bc*PAH-CD and *Bc*PAH-AD2 are indicated by red-, and green-colored circles, respectively.

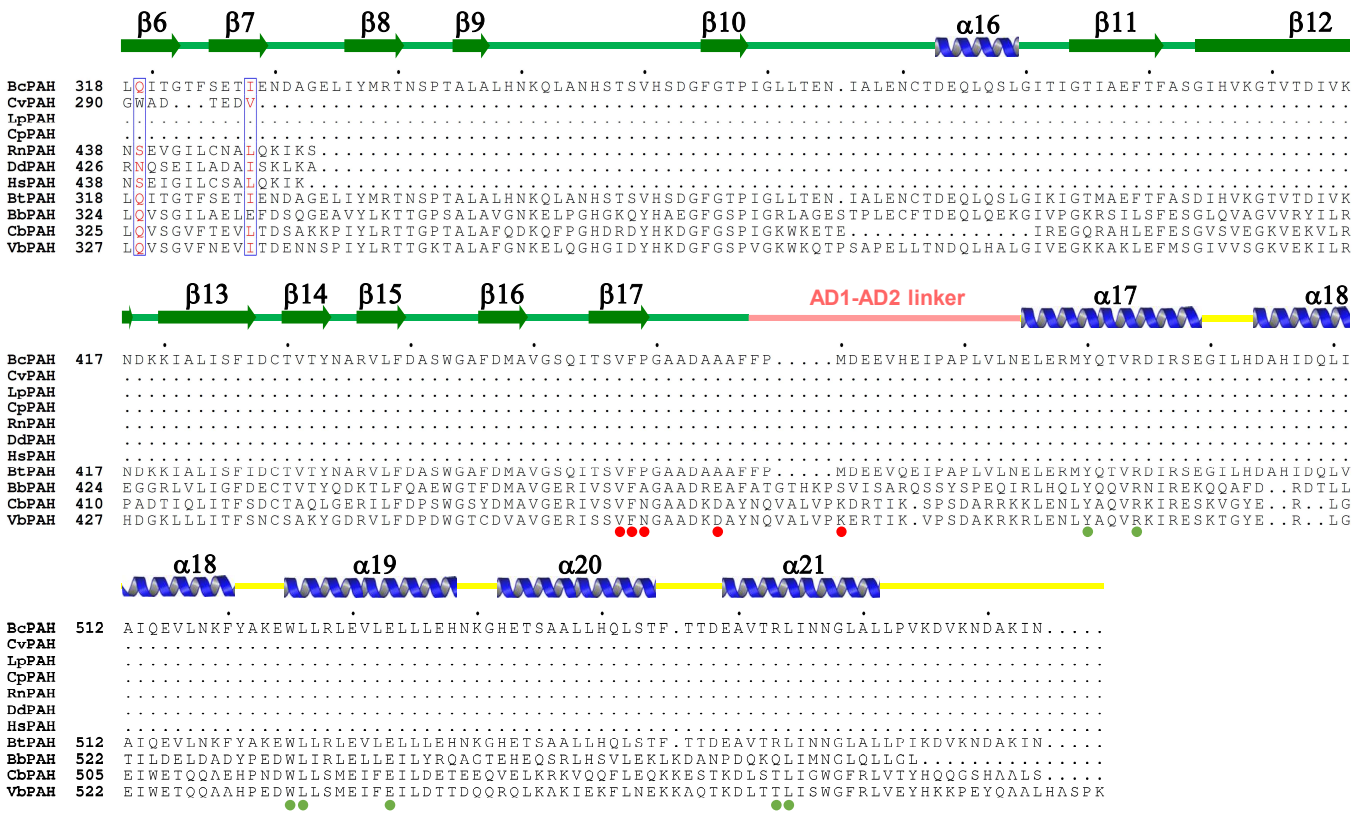

Figure S2. Full amino acid sequence alignment of PAHs (continued).

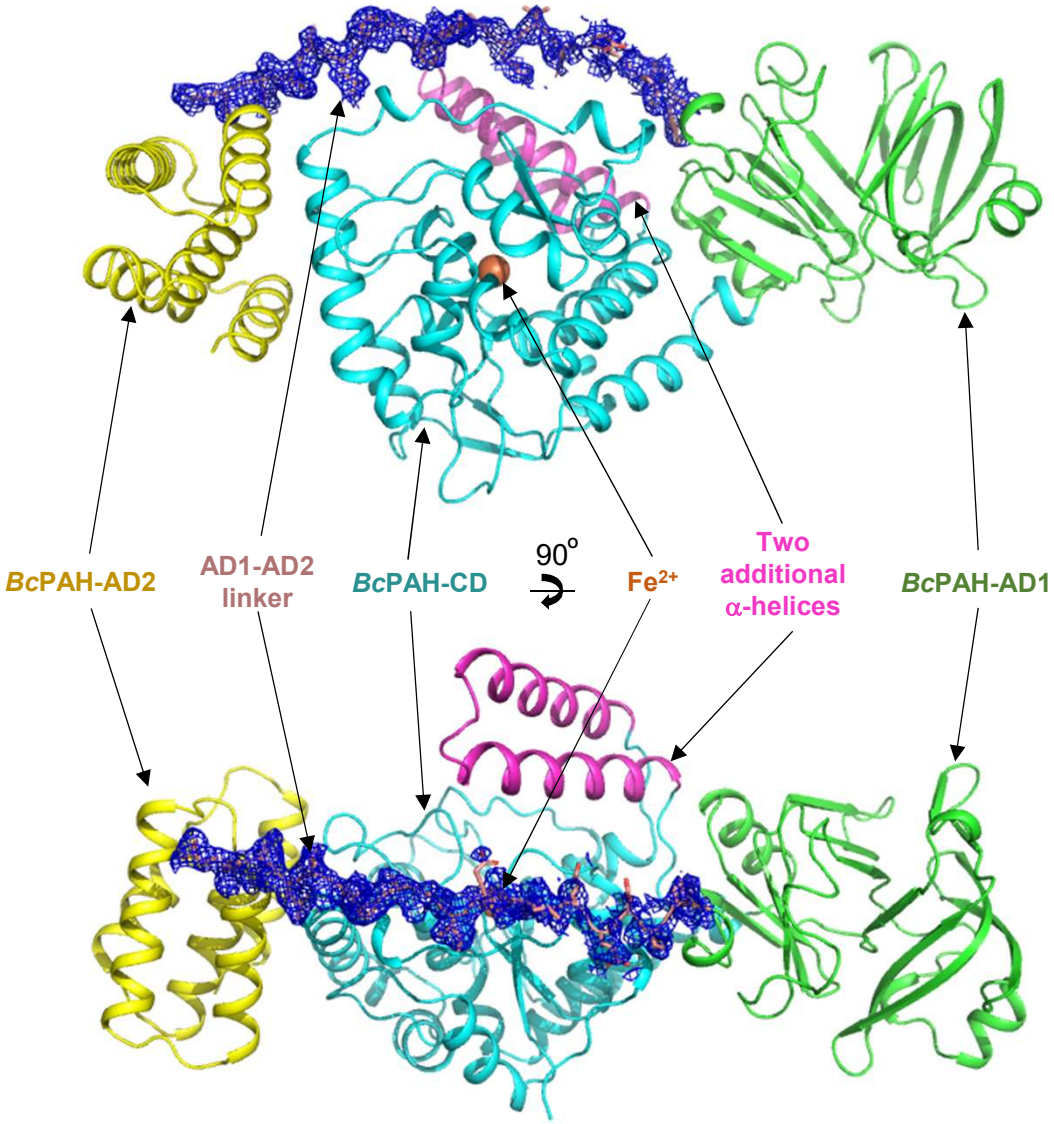

Figure S3. Electron density map of AD1-AD2 linker of *Bc*PAH. The Fo-Fc electron density map is shown as a blue-colored mesh with 1.0 σ contour. The structure of *Bc*PAH is shown as a cartoon diagram, and each domain is distinguished by different colors, and labeled. The residues constituting AD1-AD2 linker of *Bc*PAH are shown as a stick model.

Analysis of the protein interfaces has not revealed any specific interactions that could result in the formation of stable quaternary structures. Most probably, the structures do not form a complex in solution.

Complex Summary

Multimeric state	1	Surface area, Å^2	26902.1	ΔG^{int}, kcal/mol	−12.8	$T\Delta S^{diss}$, kcal/mol	0.1
Copies in unit cell	N/A	Buried area, Å^2	141.2	ΔG^{diss}, kcal/mol	12.7	Symmetry number	1
Formula	Aa						
Composition	A[FE]						
Dissociation pattern	A + [FE]						

Figure S4. The PISA results of *Bc*PAH. The PISA analysis was conducted using the structure of *Bc*PAH in which chemicals other than iron ion were deleted.

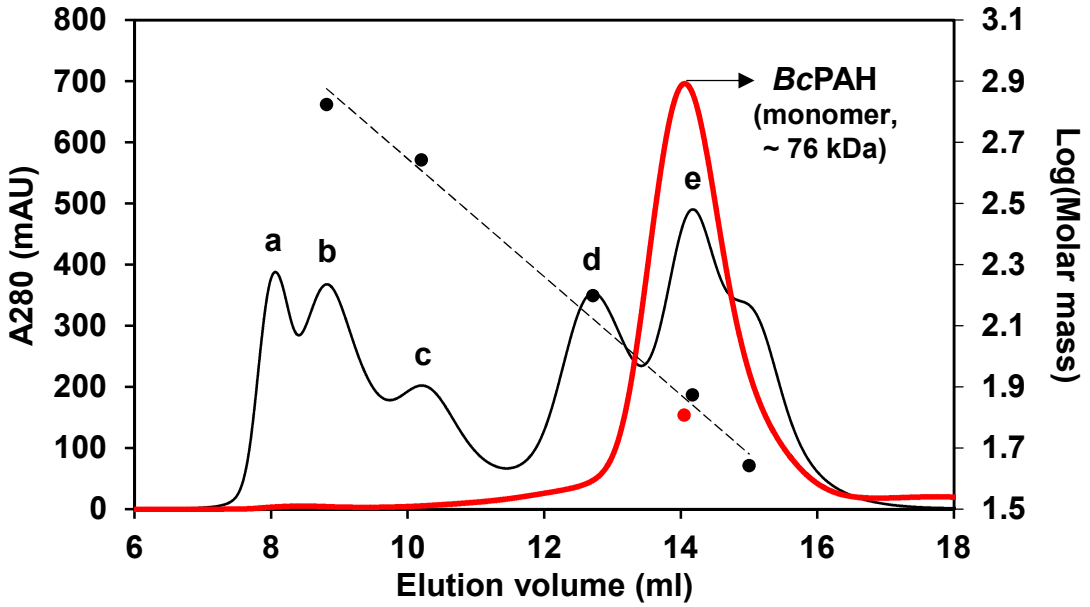

Figure S5. Size-exclusion chromatography of *Bc*PAH. The red- and black-colored lines indicate *Bc*PAH and the standard samples, respectively. a-e indicate standard samples of thyroglobulin (669 kDa), ferritin (440 kDa), aldolase (158 kDa), conalbumin (75 kDa), and ovalbumin (44 kDa), respectively. The dashed-line shows calibration curve of elution volume versus log(molar mass). Each black dot corresponds to each standard sample (a–e), respectively, and the red dot indicates *Bc*PAH.

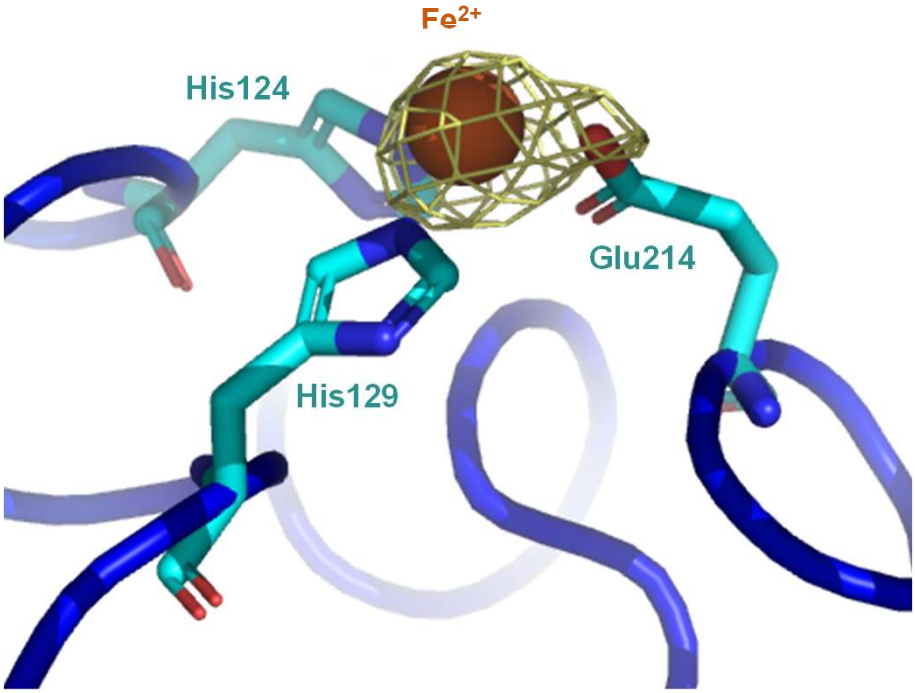

Figure S6. Electron density map of the iron ion bound at the active site and B-factor putty model of *Bc*PAH. The Fo-Fc electron density map is shown as a yellow-colored mesh with 4.0 σ contour. The structure of BcPAH is represented as a B-factor putty model. The bound iron ion in *Bc*PAH is presented with an orange-colored sphere and the residues involved in the coordination of the iron ion are shown as a cyan-colored stick model.

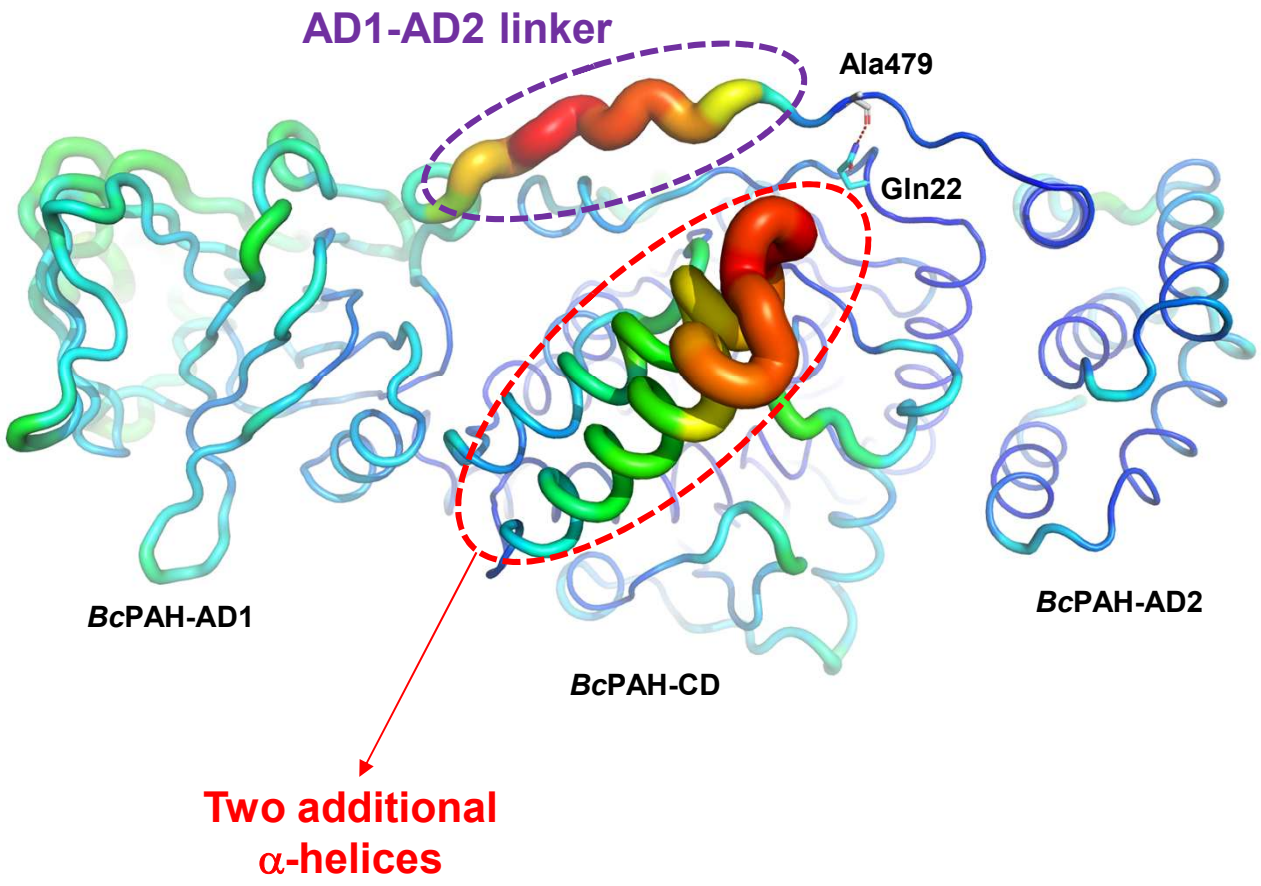

Figure S7. B-factor presentation of *Bc*PAH. The thick and red regions indicate high B-factor and the thin and blue regions indicate low B-factor. The residues forming a hydrogen bond between AD1-AD2 linker and BcPAH-CD are indicated by a stick model.

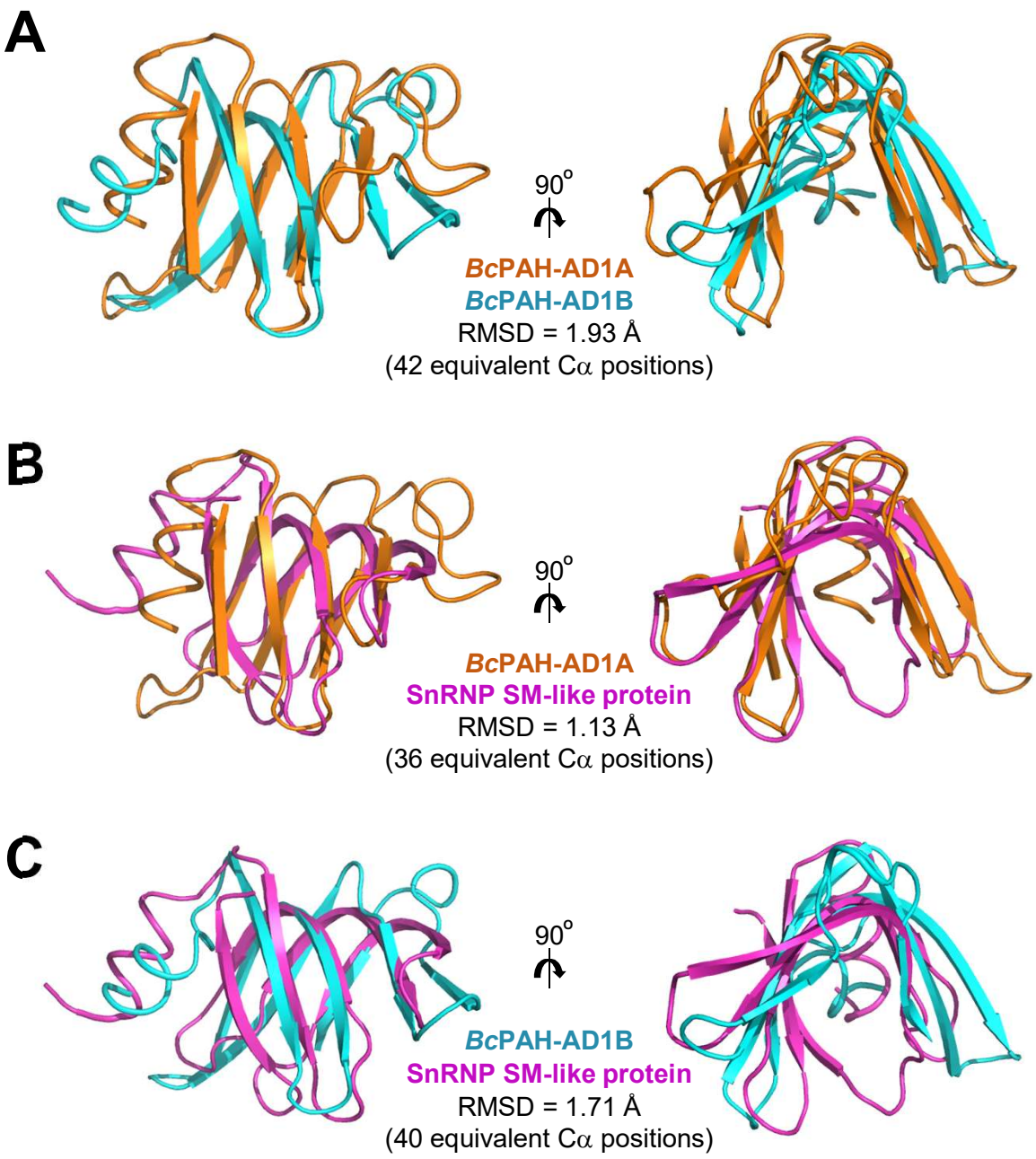

Figure S8. Superposition of the subdomains of *Bc*PAH-AD1. (A) Superposition of *Bc*PAH-AD1A and *Bc*PAH-AD1B. (B) Superposition of *Bc*PAH-AD1A and the SnRNP SM-like protein. (C) Superposition of *Bc*PAH-AD1B and the SnRNP SM-like protein.

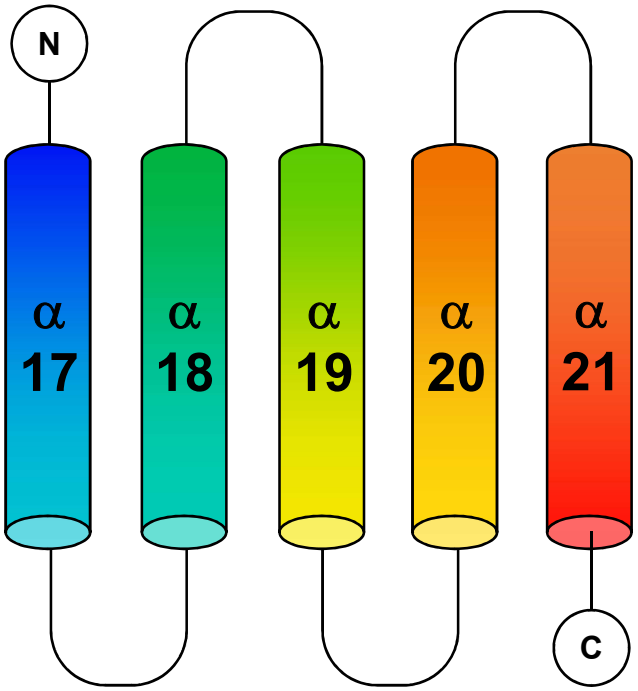

Figure S9. Schematic diagram of *Bc*PAH-AD2. The number of α-helices are labeled.

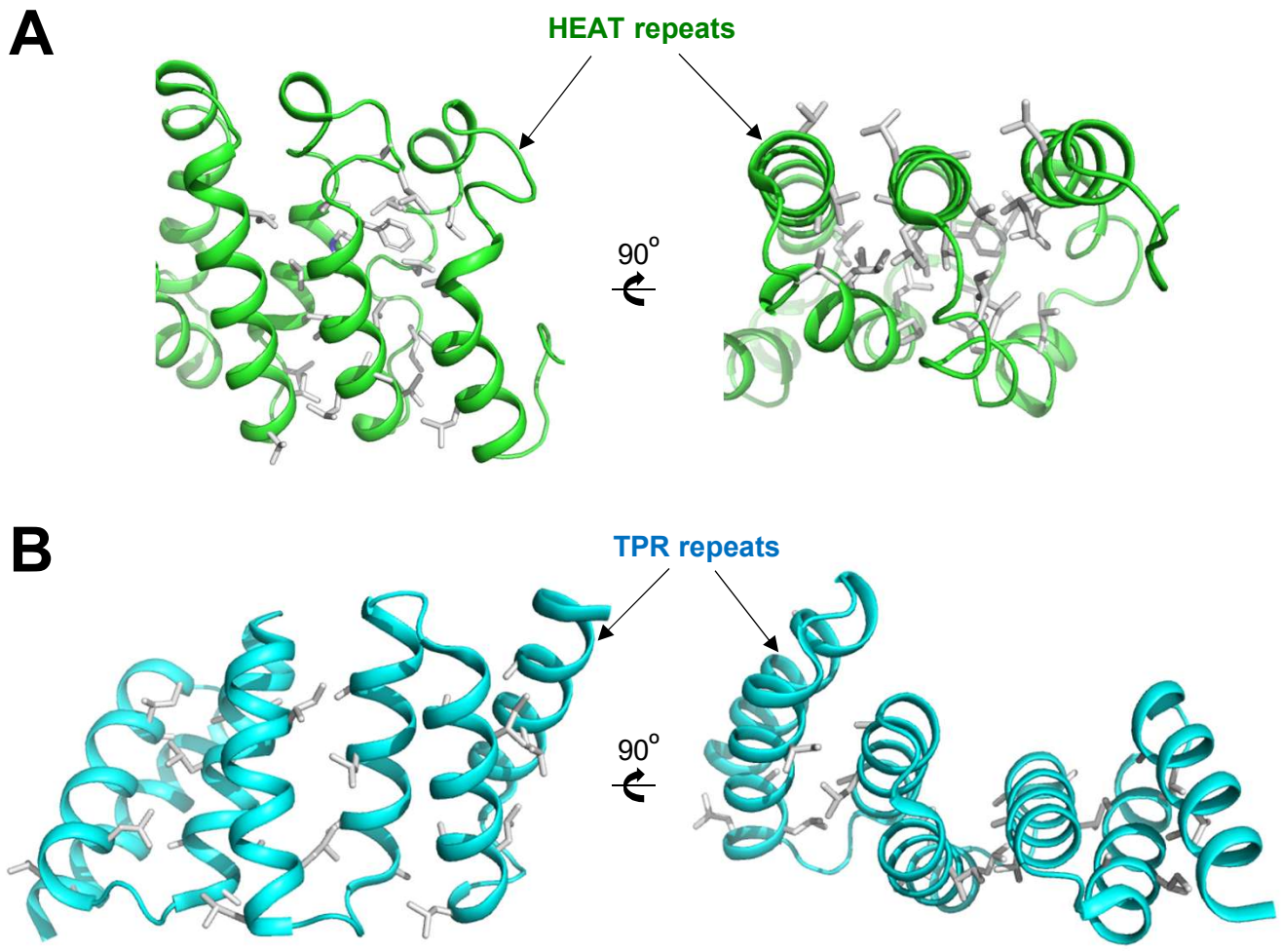

Figure S10. Other α-helical structures and hydrophobic contacts. The structures of HEAT repeats (PDB code 2IAE) and TPR repeats (PDB code 1NA0) are shown as a cartoon model with colors of green and cyan. The hydrophobic residues involved in the formation of the helical bundle are shown with gray-colored sticks.