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Supporting Information

B-factor Guided Proline Substitutions in *Chromobacterium violaceum* Amine Transaminase: Evaluation of the Proline Rule as a Method for Enzyme Stabilization

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Author Contributions

H.L. Methodology: Equal; Writing – original draft: Equal; Writing – review & editing: Equal
J.C. Methodology: Equal; Writing – original draft: Equal; Writing – review & editing: Equal.

Supporting information

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Table S1. All α-helices and β-turns of *Cv*-ATA and the residues that belong to them.

Residues[a]	Secondary structure element	Residues (Cont.)	Secondary structure element (Cont.)
6-16	α-Helix 1	240-252	α-Helix 9
24-31	α-Helix 2	266-269	β-Turn 11
40-43	β-Turn 1	273-278	α-Helix 10
46-49	β-Turn 2	287-290	β-Turn 12
62-65	β-Turn 3	289-292	β-Turn 13
68-80	α-Helix 3	291-294	β-Turn 14
93-103	α-Helix 4	303-313	α-Helix 11
108-111	β-Turn 4	320-323	β-Turn 15
119-138	α-Helix 5	325-342	α-Helix 12
140-143	β-Turn 5	346-350	α-Helix 13
149-152	β-Turn 6	352-363	α-Helix 14
158-163	α-Helix 6	367-370	β-Turn 16
166-171	α-Helix 7	376-379	β-Turn 17
187-190	β-Turn 7	388-391	β-Turn 18
192-195	β-Turn 8	398-410	α-Helix 15
196-216	α-Helix 8	418-421	β-Turn 19
217-220	β-Turn 9	431-457	α-Helix 16
230-233	β-Turn 10		

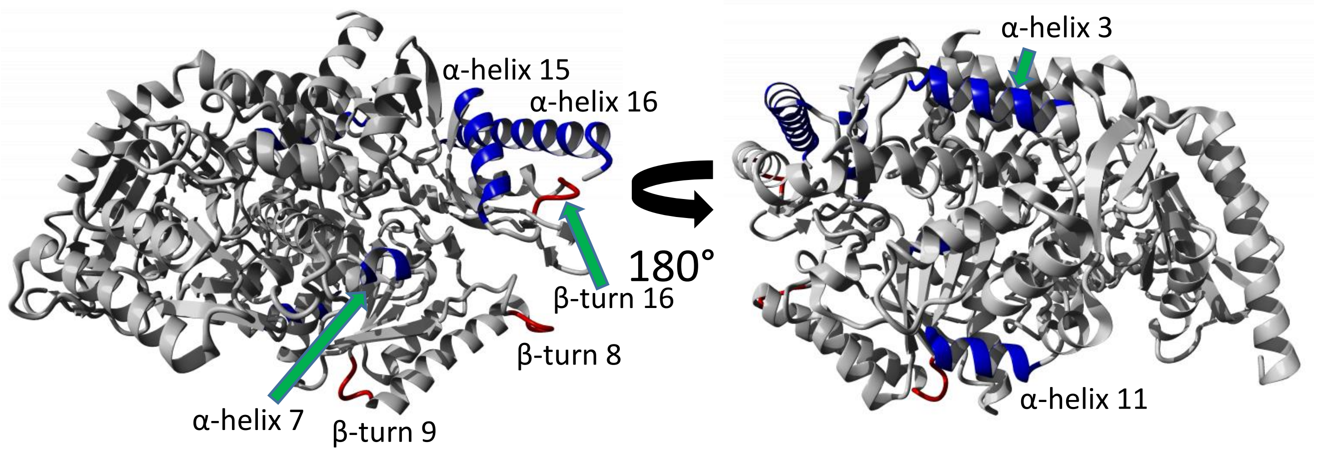

Figure S1. X-Ray crystal structure of *Cv*-ATA (PDB ID: 4A6T). Secondary structure elements selected for proline substitutions are highlighted by color. Blue: α-helix. Red: β-turn.

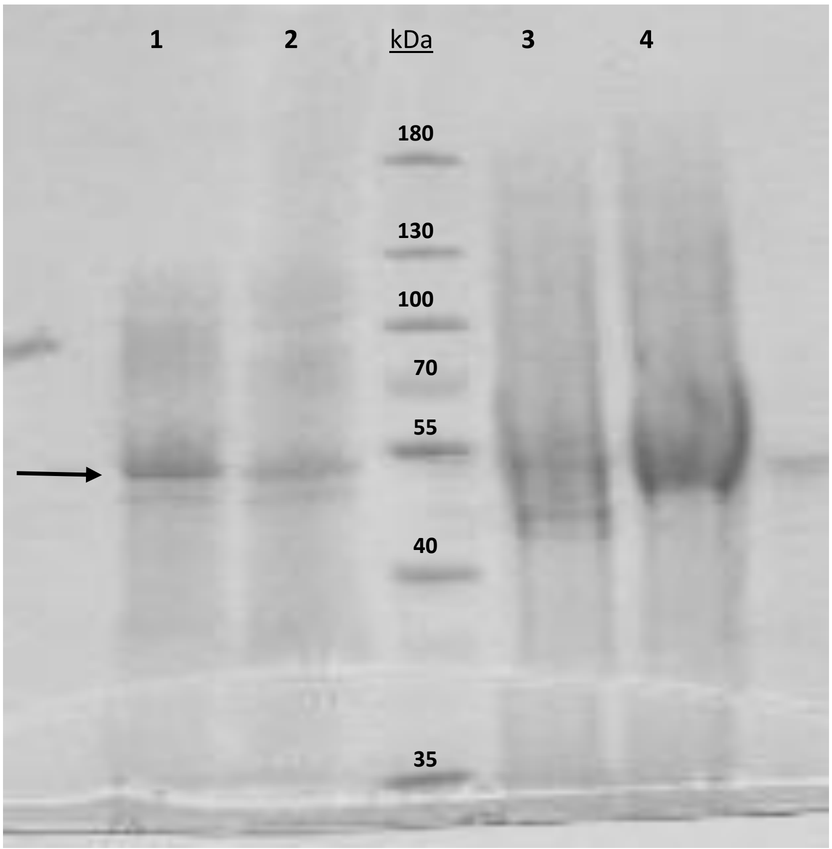

Figure S2. SDS-PAGE gel of *Cv*-ATA WT soluble fraction (1) and insoluble fraction (3) as well as Pro8 soluble fraction (2) and insoluble fraction (4).

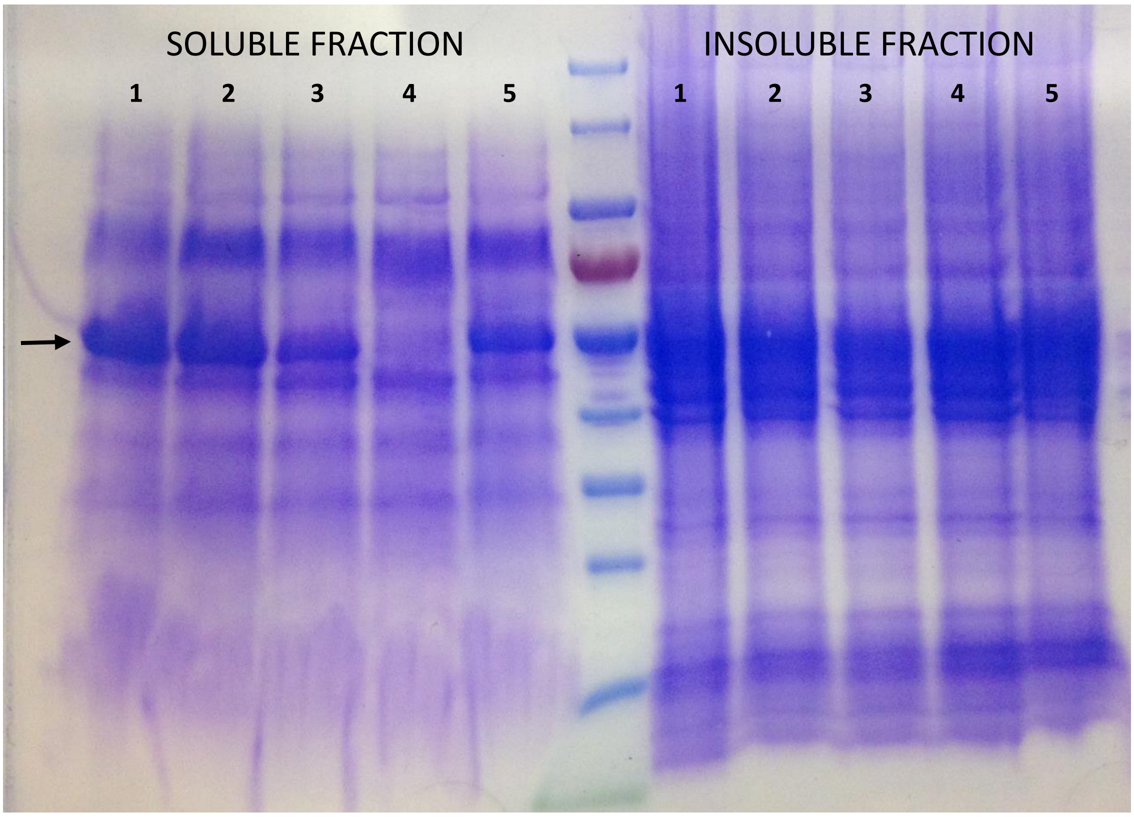

Figure S3. SDS-PAGE gel image of soluble and insoluble fractions of *Cv*-ATA WT (1), K69P/K304P (2), K167P/E368P (3), K193P/G398P (4) and D218P/R432P (5).

Table S2. Conservation of amino acid positions chosen for proline substitutions based on alignment with 500 most similar sequences in the IMG database.

Residue	Conservation in 500 most similar proteins[a]
K69	37% (K 37%)
K167	38% (G 49%)
K193	8% (G37%)
D218	12% (E 79%)
K304	3% (D 67%)
E368	6% (P 90%)
G398	97% (G 97%)
R432	37% (R 37%)

[a] Residue in parenthesis is most conserved residue in that position.

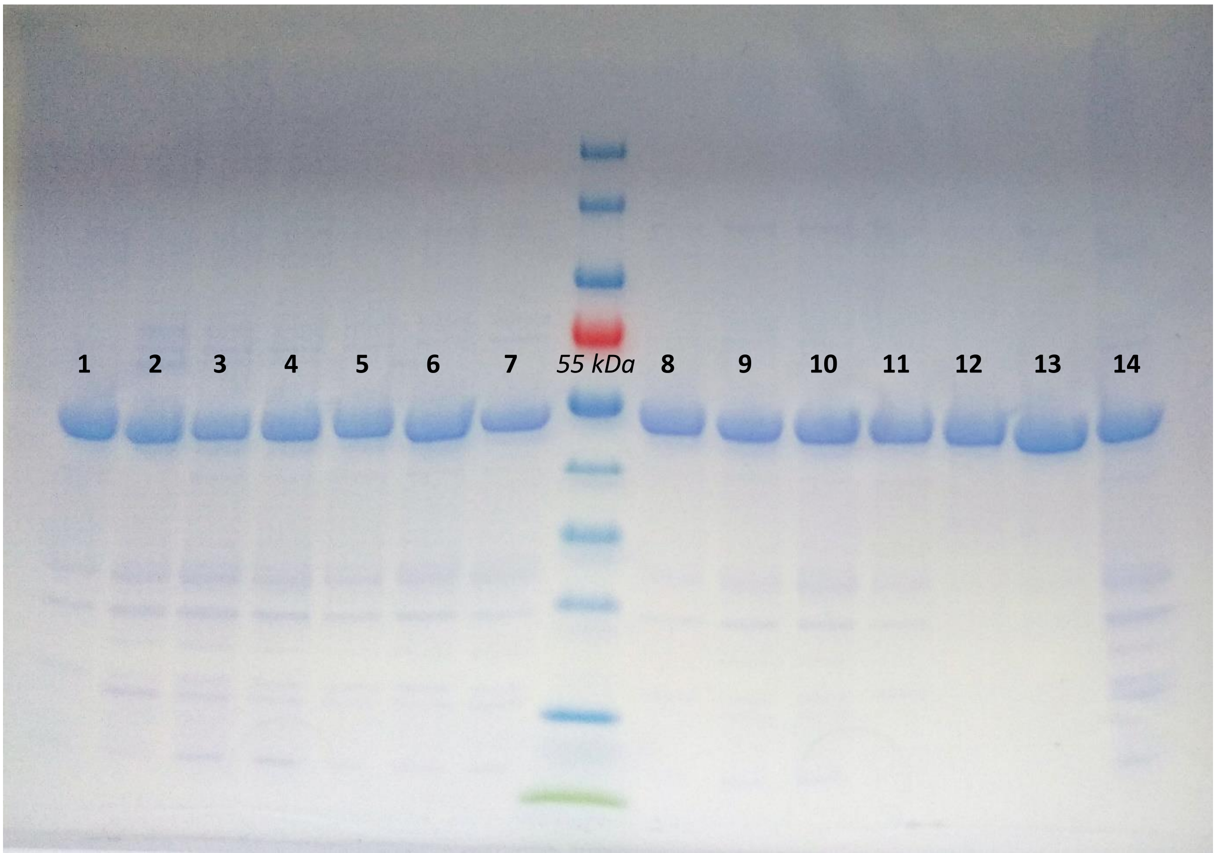

Figure S4. SDS-PAGE gel image of purified (5 µg) *Cv*-ATA WT and mutants. (1): WT, (2): Pro7, (3): Pro5, (4): Pro4, (5): K69P/K304P, (6): K167P/E368P, (7): D218P/R432P, (8): K69P, (9): K167P, (10): K193P, (11): D218P, (12): K304P, (13): E368P and (14): R432P.

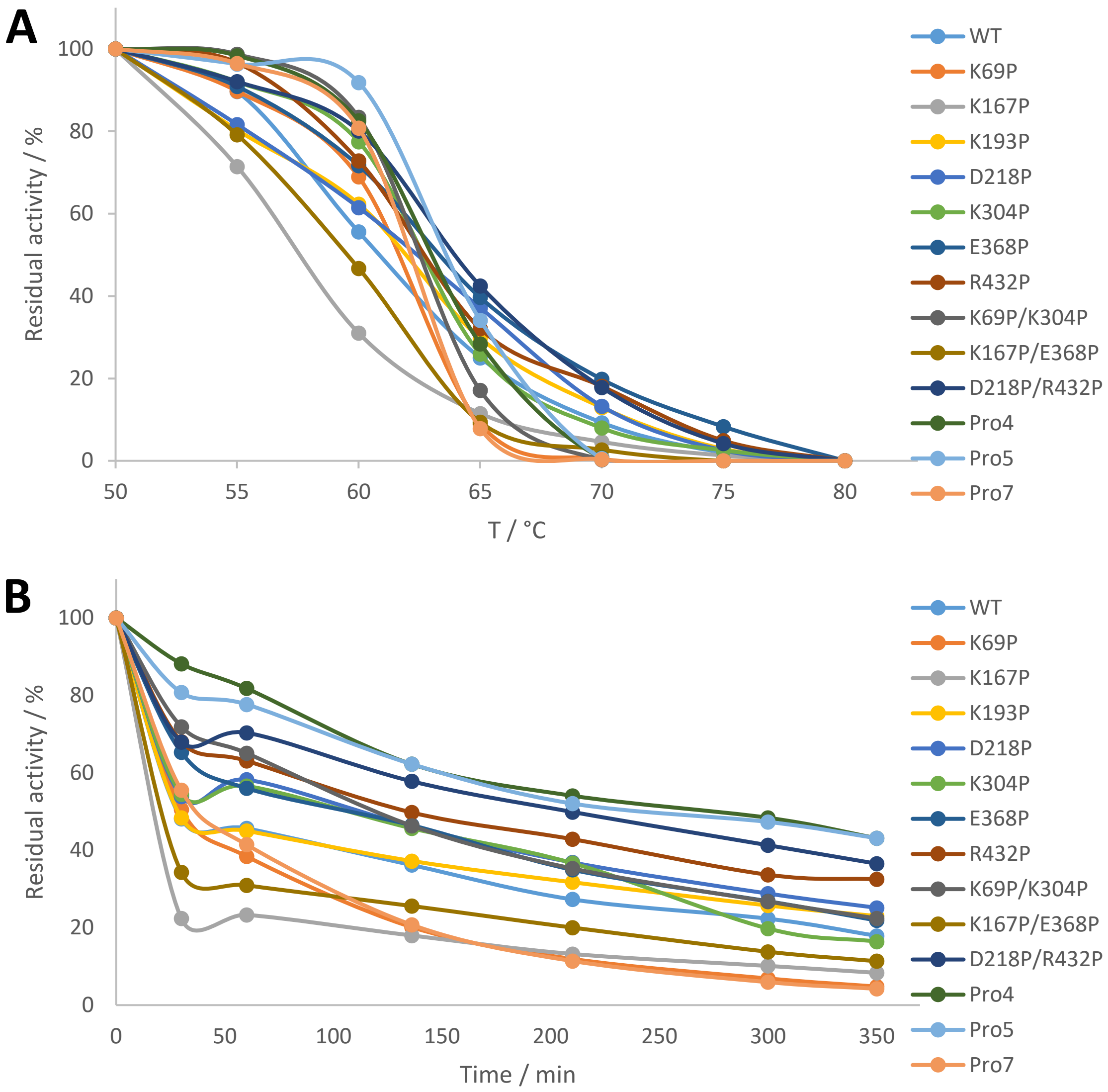

Figure S5. Experimental data that was used to calculate *T_{50}* (A) and $t_{1/2}$ (B).

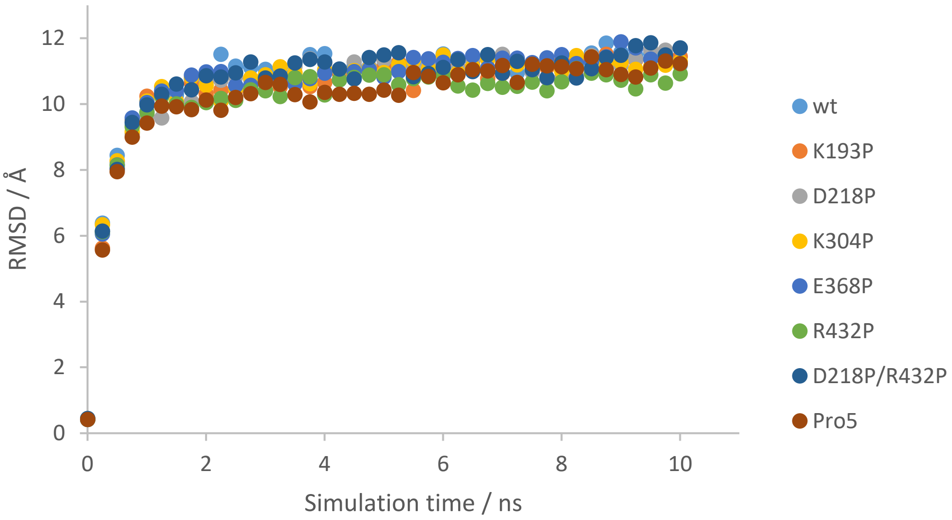

Figure S6. Total RMSD for all atoms over time for *Cv*-ATA WT and variants obtained from MD-simulations.