



Supplementary Information for an Article Titled:

Structural Characterization of an ACP from Thermotoga maritima: Insights into Hyperthermal Adaptation

Yeongjoon Lee ¹, Ahjin Jang ¹, Min-Cheol Jeong ¹, Nuri Park ¹, Jungwoo Park ¹, Woo Cheol Lee ¹, Chaejoon Cheong ² and Yangmee Kim ^{1,*}

- ¹ Department of Bioscience and Biotechnology, Konkuk University, Seoul 05029, Korea; lyj7956@konkuk.ac.kr (Y.L.); ajin931017@konkuk.ac.kr (A.J.); boby8520@konkuk.ac.kr (M.-C.J.); snfl235@konkuk.ac.kr (N.P.); jhopark123@konkuk.ac.kr (J.P.); wclee3@konkuk.ac.kr (W.C.L.)
- ² Magnetic Resonance Team, Korea Basic Science Institute, Ochang 28199, Chungcheongbuk-do, Korea; cheong@kbsi.re.kr
- * Correspondence: ymkim@konkuk.ac.kr; Tel.: +82-2-450-3421

Received: 12 March 2020; Accepted: 7 April 2020; Published: 9 April 2020



© 2020 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).

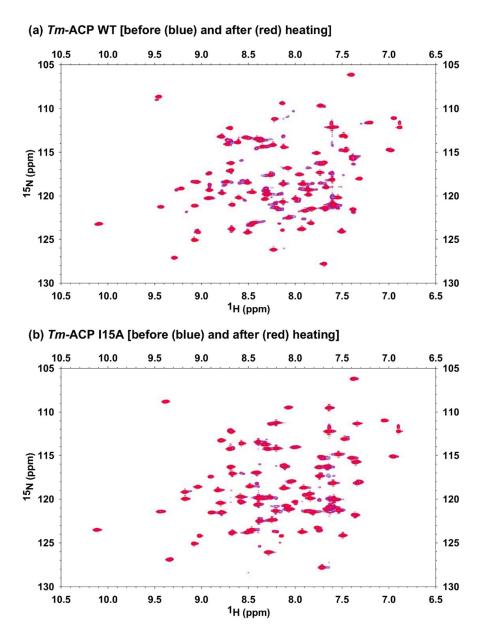


Figure S1. Reversibility of the thermal denaturation of (a) the wild-type and (b) the I15A mutant holo *Tm*-ACPs was confirmed by comparing the ¹H-¹⁵N HSQC spectra of each protein before (blue) and after (red) heat-treatments. The annealing was performed by heating the native sample in a boiling water bath for 15 min and cooling down to room temperature.

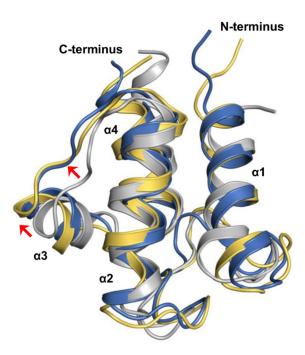


Figure S2. Superimposition of three structures: the solution structure of holo *Tm*-ACP (blue, PDB ID: 6LVT) with the lowest energy, the crystal structure of apo *Tm*-ACP (yellow, PDB ID: 6LVU) and the solution structure of holo *Ec*-ACP (grey, PDB ID: 2k93) [38]. The red arrows indicate the protrusion of helix III of *Tm*-ACP compared to that of *Ec*-ACP.

Tm-ACP WT (black) and I15A (red) 6.5 + 105 10.5 105 | 10.0 9.5 9.0 8.5 8.0 7.5 7.0 110 110 120 (bbm) 120 115 120 125 125 130 130 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 1_H (ppm)

Figure S3. ¹H-¹⁵N HSQC spectral overlay of the wild-type (black) and the I15A mutant (red) holo *Tm*-ACPs. The chemical shift perturbation of the eight residues, V11, L19, V26, L32, L36, L46, F50, and V69, whose sidechains contact closely with that of I15, were indicated as blue arrows.