Molecular Motions and Free Energy Landscape of Protease Proteinase K in relation to Its Cold-Adaptation: A Comparative Molecular Dynamics Simulation Study and the underlying Mechanisms

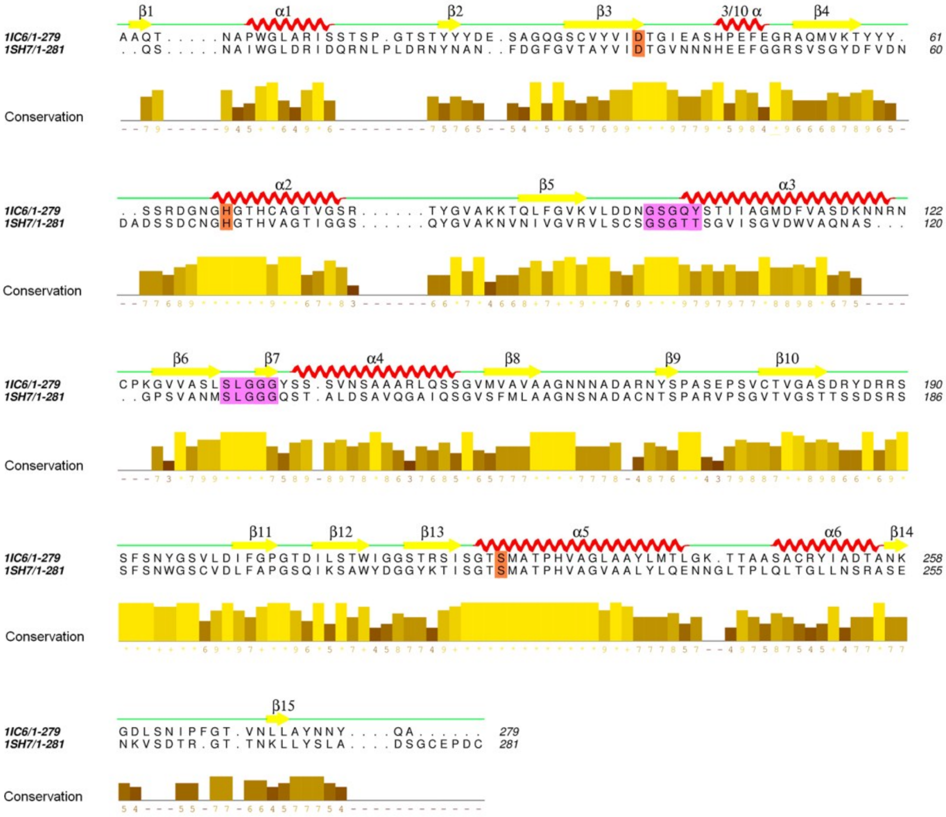

Figure S1. Structural alignment by Dali server. Secondary structures are illustrated above the corresponding amino acid sequence (red helix: α-helix, yellow arrow: β-sheet). The sequence positions corresponding to the catalytic triad residues and to the substrate binding residues are highlighted in orange and magenta, respectively. The conservation profile was generated by JarView. The degree of conservation in the corresponding alignment position was represented by yellow color (the lighter the yellow color is, the higher the corresponding residues are conserved).

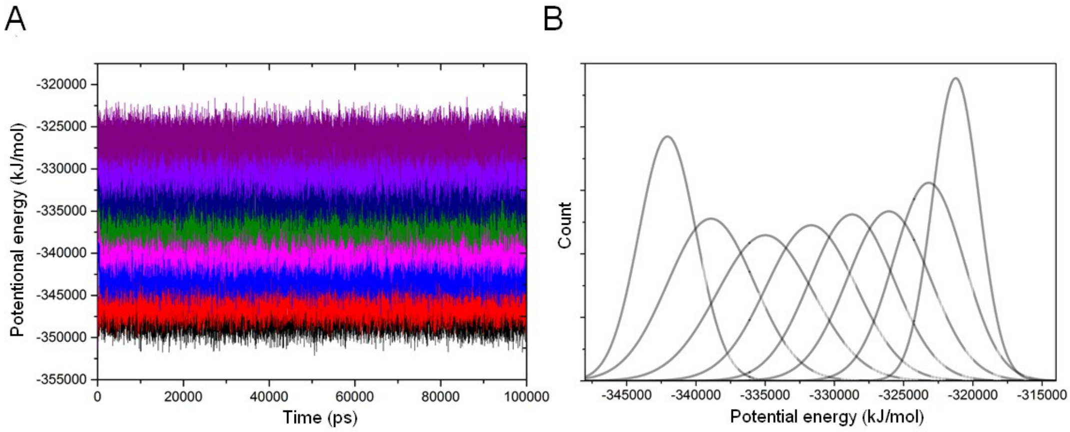

Figure S2. Potential energy and its distribution during PTMetaD-WTE simulation. (A) Time series of potential energy at different temperatures of the PTMetaD-WTE simulation. The eight replicas are represented by curves of different colors. (B) Potential energy distribution of the eight replicas. In this study, the temperatures of the eight replicas are 300K, 312K, 325K, 332K, 337K, 342K, 352K and 360K, in each of which 100ns well-tempered MetaD simulation was performed. The average exchange probability between each replica was evaluated to be 60%.

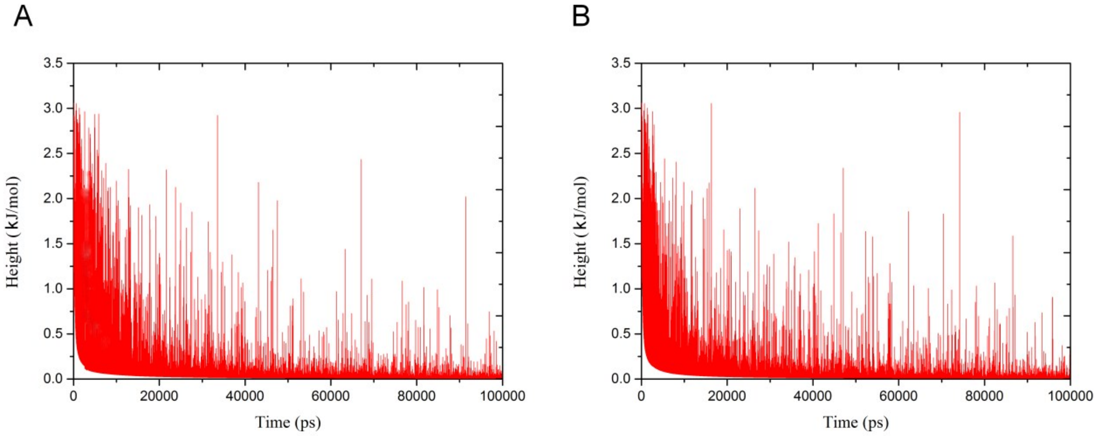

Figure S3. Plot of the Gaussian Height added to the system during the well-tempered metadynamics simulation in replica 300K. The added Gaussian decrease gradually during simulation and becomes very small since 40ns time point. This event leads to the convergence in the estimation of the FEL.

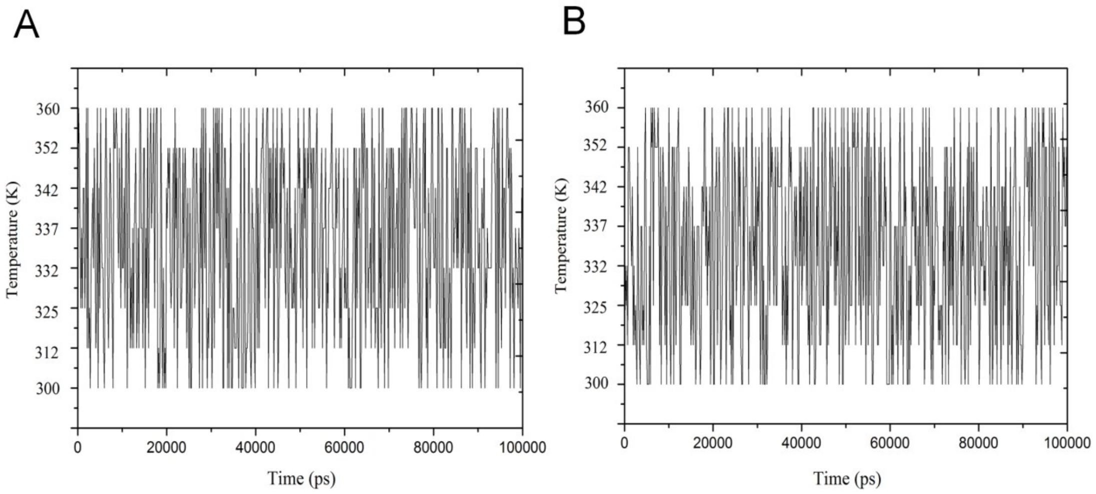

Figure S4. The temperature space of replica 300K as a function of simulation time. (A) 1IC6. (B) 1SH7. From this analysis, we can conclude that all the eight replicas are diffusing effectively in the temperature space, indicating that the structural coordinate and conformation space of the other seven replicas have been exchanged to the 300K replica.