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Supporting information for article:

**Conformational dynamics of the essential sensor histidine kinase
Walk**

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Kong, Xingqiang Chen, Chen Wang, Jianwei Shuai and Aidong Han**

A *lpWalk*

380 390 400 410

lpWalkD VTEQQKIDND.....RKQFVSNVSHHE.....LRTPLTSLRSYIEALSDG
smVicKDATEQKEERE.....RRLFVSNVSHHE.....LRTPLTSVKSYLEALDDG
tmHK853 NVTESKELERLKRIDRM.....KTEFIANISHHE.....LRTPLTAIKAYAETIYNS
tmThkADDVTELYKYEEERKRRLSILGEMTARVAHE.....IRNPITIIIGGFIMRMKKH
bsDesKNERIAELVKLEE.....RQRITARDLHDHT.....LGQKLSLIGLKSDLARK
bsSpo0BS LQKYQDRV.....FEMIEEMVIDAKHESKLSN LKTPHLAFDFTLFWKTH
ecCpxA GASFNQMVTALERMMTS.....QQRLLSDSLISHE.....LRTPLTLRLQLGTALLRRR

lpWalk 420 430 440 450 460

lpWalk A.WK DPEVAPGFLKVTQEETDRMIRMINELL.SLSRMDSGTTRVDMELVNIINEMFNIVLD
smVicK A.LT E.SVAPSFIKVSLDETNRMMRMITDLL.SLSRIDNQTSHLDVDELINFTAFMNIYILD
tmHK853 LGELDLSTLKEFLLEVIDQS NHLLENLLELL.DFSRLERKSLQINREKVDLCDLVESAVN
tmThkALDPEPETLKKYIINIITNELSRLLETIVKEIIL.EYSKERQVLE.....FTEFNLNELLIREVYV
bsDesK LIYK DPEQAAARELKS VQQTARTSLNEVRKIVSSMKGI.....RLKDELINIKQILEAAD
bsSpo0B Y.....M.....TLEYEVLGEIK.DLSAYDQKL.....AKLMRKLFFH
ecCpxA SGE.....SKE LERIETEAQRLDSMINDLL.VMSRNQKKNALVS.ETIKANQLWSEVLD

lpWalk 470 480 490 500 510 520 TT--

lpWalk RFDMI LKKDDNPAKYITIKREFTKRD L WVEIDTDK...FTQVLDNIMNNNAIKYSPDGG..
smVicK RFDQIQSQQ.STNKVYEIIRDYPDKSVWIEIDTDK...MTQVIDNINLNNAIKYSPDGG..
tmHK853 AIKEFASSH.NVNVLFESE...NVPCPVEAYIDPTR...IRQVLLNLLNNGVKYSKKDAPD
tmThkA LFEEKIRK...MNIDF...CFETDNEDLRVEADRTTR...IKQVLLNLVQNAIEATGGENG..
bsDesKIMFIYEEEKWPENISLLNENILSMCLKEAVTNVVKHSSQAK...
bsSpo0B LFDQA VSRSESNHLTVSLQTDHPDRQLI LYLDFHGAFADPSAFDDIRQNGYEDV.....
ecCpxA NAAFEAEQ...MG...KSLTVNFPPGPWP LYGPNNA...LESALENINVRNALRYSHTKI..

lpWalk 530 540 550 560 570 580

lpWalk .VVT CRLLETHNQ VIISISDQGLGIPRADLGH VFFDRFFRVDKARSRAQG GTGLGLAI SKE
smVicK .KVT ITMQTTDTQ LIILSISDQGLGIPKKDLPL IFDRFVRVDKARSRAQG GTGLGLAI AKE
tmHK853 KYVK VILDEKDGGLI IIVEDNGI GIPDHAKDR IFEQFYRVDSSTLYEVP GTGLGLAI TKE
tmThkA .KIK ITSDMYTK VRVSVWNSGP PIPEELKEK IFSPFFTTK...TQGTGLGLSICRK
bsDesK .TCRVIDIQQLWKE VVITVSDGTGFKGEEN...SFSKGHGLLMGRE
bsSpo0B .DIMRFEITSHECLIEI GLD...SFGHGLLMGRE
ecCpxA ...EVGFVAVDKDG IITITVD DGP GVS PEDREQ IFRPFYRTDEARDRESG GTGLGLAI VET

lpWalk 590 600 610 620

lpWalk VVQ.MLGGR I W VDS VEGK GSTFY ISLP YEPYE.EEDLWDDDSQA.
smVicK IVK.QHKGR I W VDS VEGK GSTFT I VLP YENDNDAIDEWEDEDES
tmHK853 IVE.LHGGR I W VDS VEGK GSRRFVWIP KDRAGEDN.RQDN...
tmThkA I I EDEHGGK I W TEN REN.GVVFI FEFIPKTPEKR...
bsDesK RLE.FANGSL H I D TEN..GTKL TMAIPNNSK...
bsSpo0B
ecCpxA AIQ.QHRGW V K AED SPLG GLRLV I W LP LYKRS...

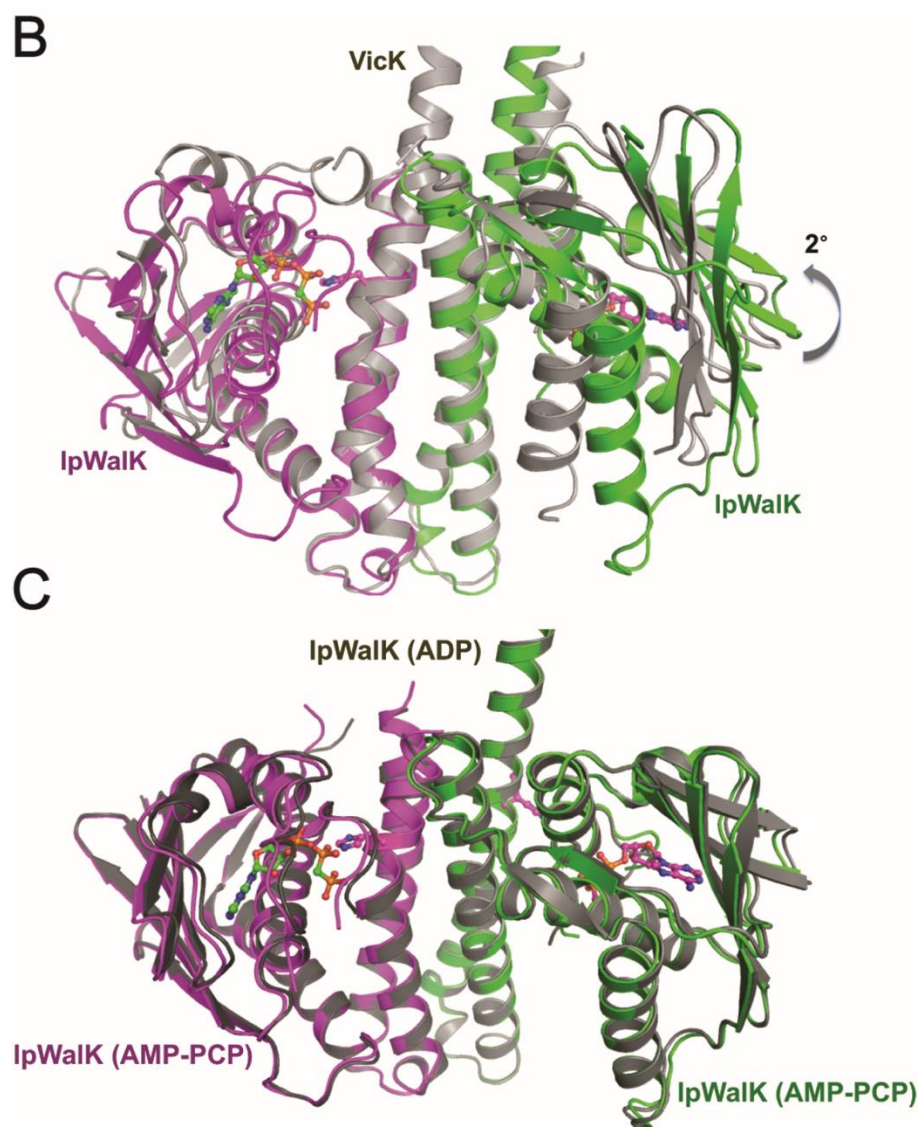


Figure S1 Global alignments of the IpWalK. (A) Sequence alignment of IpWalK with other SKs. The secondary structure elements are shown above the alignments as spiral lines and arrows for α helices and β strands. Highly conserved residues are shown in white letters over red background. Partially conserved residues are shown in red letters in blue boxes. These bacteria are *Lactobacillus plantarum*, *Streptococcus mutans*, *Thermotoga maritima*, *Bacillus subtilis*, *Escherichia coli*. (B) IpWalK aligned with the corresponding region of *S. mutans* VicK (smVicK). IpWalK is colored in magenta and green, and VicK in grey. The CA domain of the open chain of smVicK (rmsd of 1.1 Å for 186 C α atoms) rotates 2° relative to the DHp, while a closed chain is well aligned for both domains (rmsd of 1.5 Å for 163 C α atoms). The rotation angle of the open chains is labeled with a grey arrow on the right. (C) An alignment of IpWalK bound with AMP-PCP (colored in magenta and green) with IpWalK bound with ADP (ADP is

not shown) at rmsd of 0.69 Å for 410 C α atoms. The AMPPCP ligands bound in IpWalK are shown in sticks.

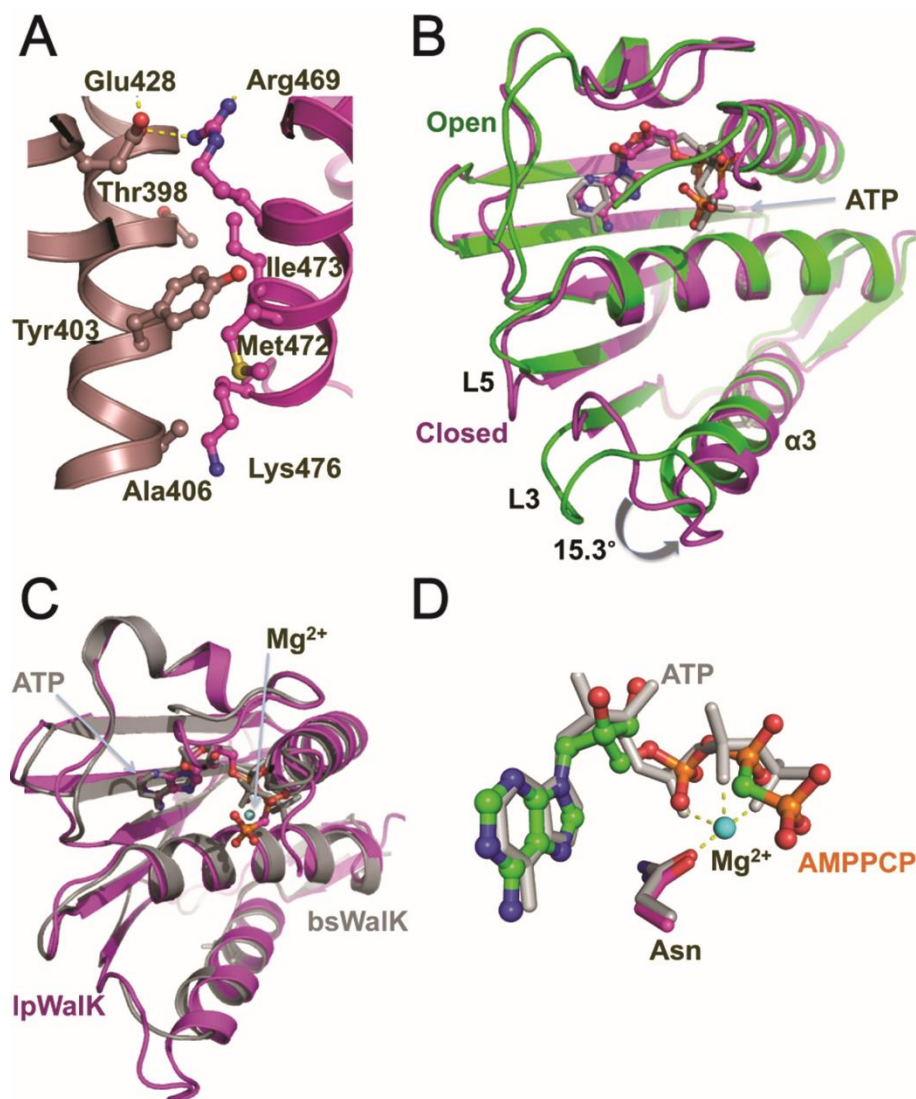
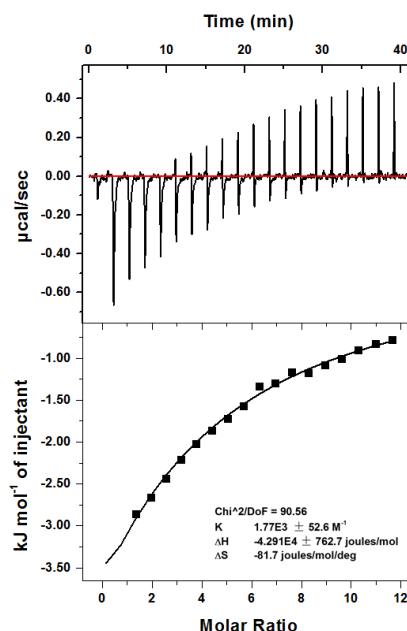
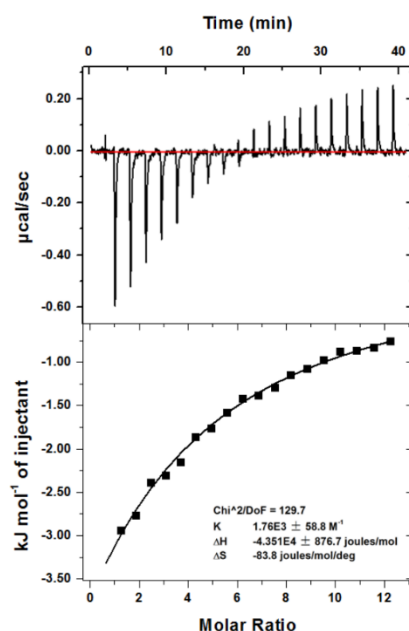


Figure S2 Detailed conformational analyses of the closed IpWalK state in presence of AMPPCP.

(A) The hydrophobic patch between the CA and DHp domains in the chain with the closed conformation. The key residues are shown in sticks. A hydrogen bond is shown in yellow dashed line. (B) Structural rigidity of the CA domain of WalK. Alignment of the CA domains of IpWalK in the closed conformation with that in the open conformation. The CA domain of the closed conformation is colored with magenta, and the open conformation in green. A rotation angle of the helix $\alpha 3$ is indicated with a grey arrow. (C) Alignment of the IpWalK CA domain in the closed conformation with bsWalK. IpWalK is colored in

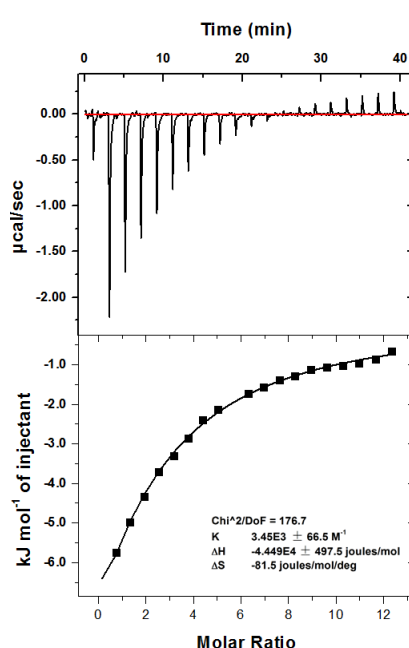
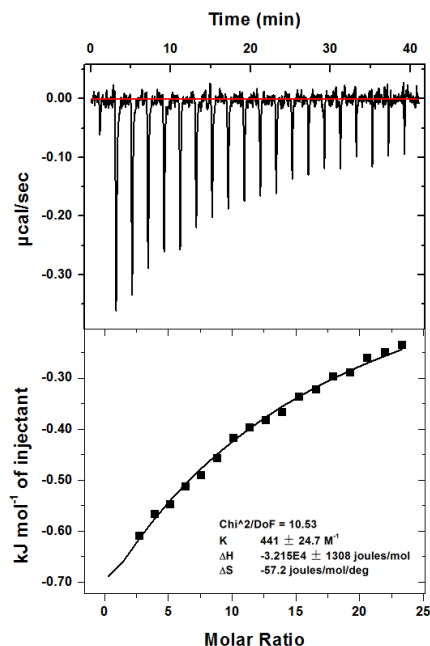
magenta, and bsWalK in grey. ATP in the closed chain of lpWalK is shown in orange sticks. (D) The AMPPCP conformation in lpWalK loses two coordinates with Mg^{2+} . ATP in bsWalK is aligned to AMPPCP, showing coordinates of Mg^{2+} . Asparagines from both structures are shown in sticks. ATP in bsWalK is shown in grey sticks. The Mg^{2+} associated with ATP is shown in a cyan ball.

A. smVick 196-450 and AMPPNP B. smVick 196-450 and ADP

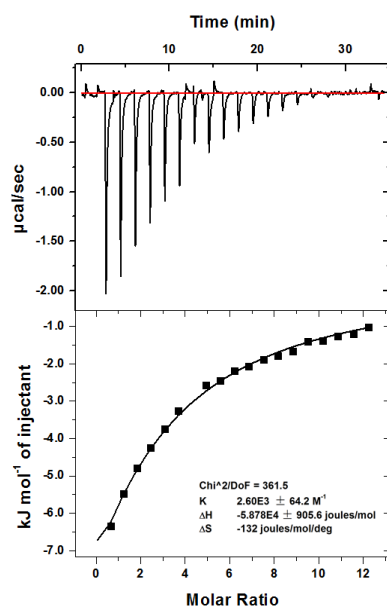


C. smVick 196-450 and

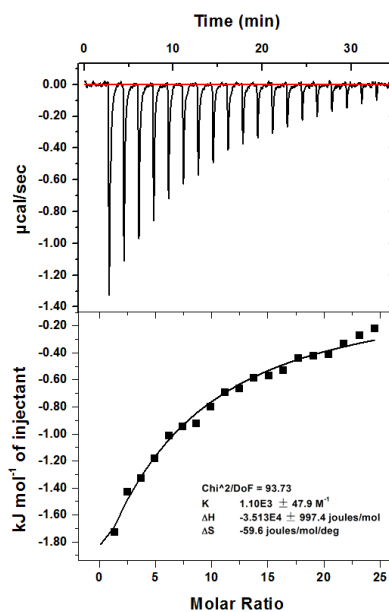
D. smVick 271-450 and AMPPNP



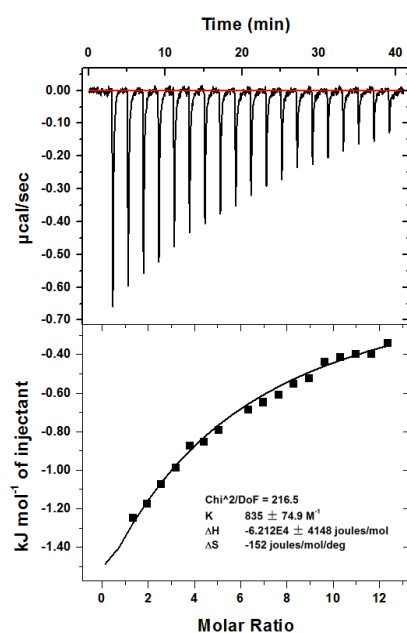
E. smVick 271-450 and ADP



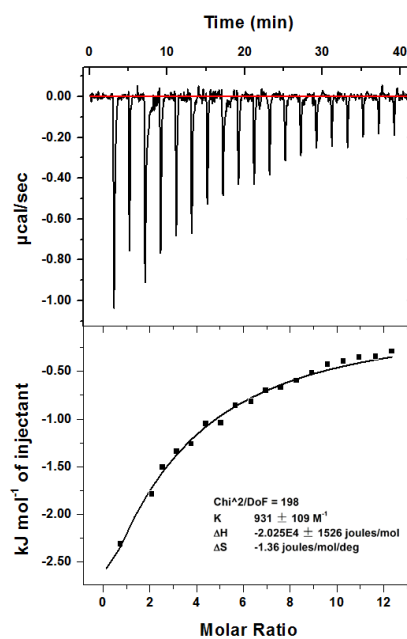
F. smVick 271-450 and AMP



G. IpWaIK and AMPPNP



H. IpWaIK and ADP



I. IpWalk and AMP

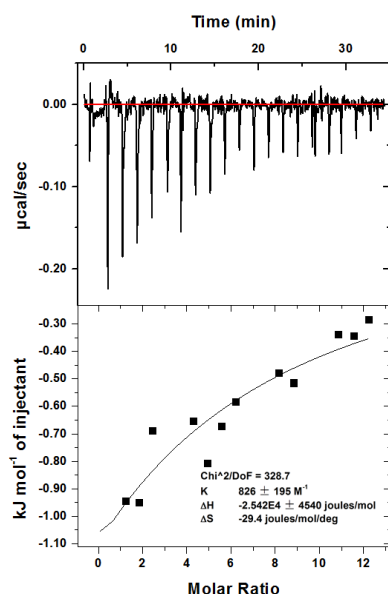


Figure S3 Quantifications for interactions of smVicK and nucleotides by Isothermal titration calorimetry. (A-C) The catalytic region of smVicK (aa 196-450) that comprises both DHp and CA domains was titrated with 3 mM AMPPNP, 3 mM ADP and 6 mM AMP, respectively. (D-F) The CA domain of smVicK (aa 271-450) was titrated with 3 mM AMPPNP, 3 mM ADP and 6 mM AMP, respectively. Nucleotides as injectants were simply diluted into the same buffer of 20 mM Tris-HCl, pH 8.0 and 5 mM MgCl₂. The titrations were performed at room temperature. (G-I) Titration of IpWalk (aa 370-624) with 3 mM AMPPNP, 3 mM ADP and 6 mM AMP, respectively. Nucleotides as injectants were diluted into the same buffer. The titrations were performed at room temperature.

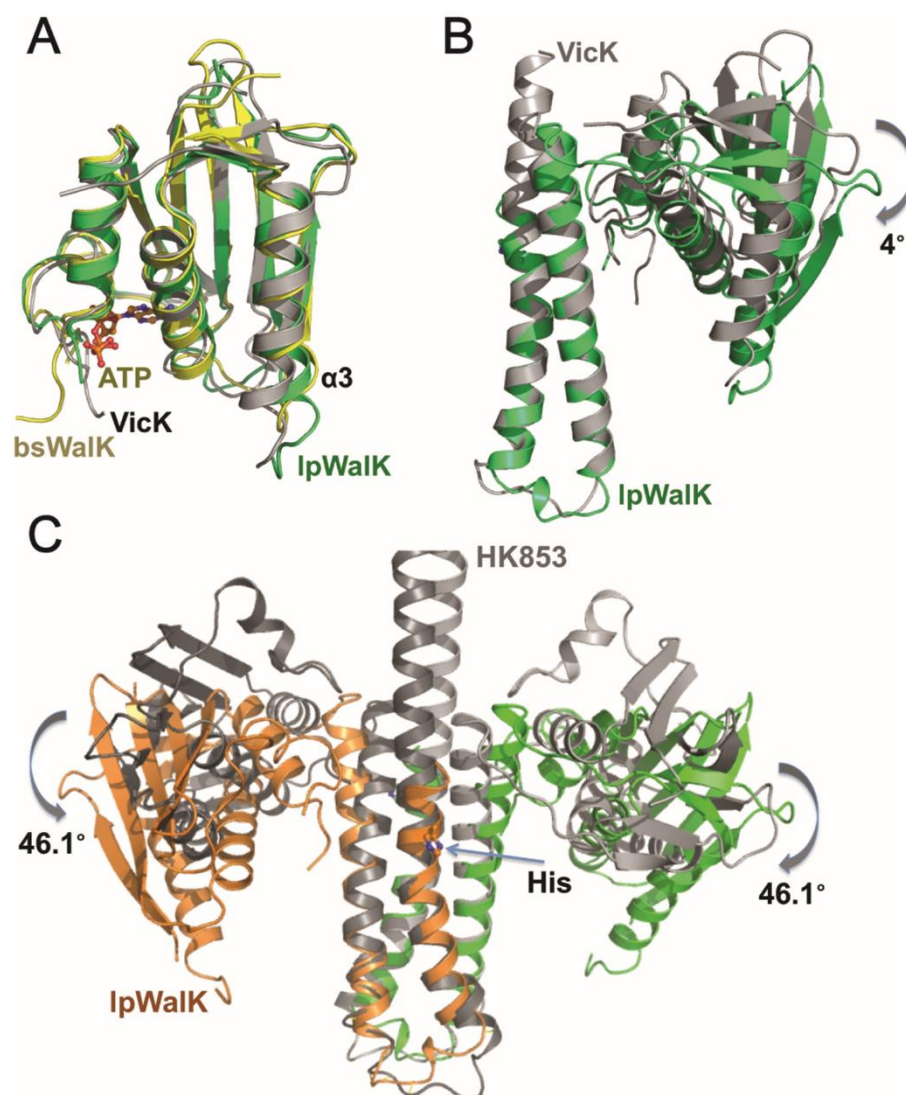


Figure S4 Comparisons of the open conformation of WalK. (A) The CA domain of IpWalK aligned with its homologs. The CA domain of *S. mutans* VicK is colored in grey, and IpWalK in green, and bsWalK in yellow. ATP from the bsWalK structure is shown in sticks. (B) The monomer of IpWalK aligned with the inactive chain of smVicK. (C) Global alignment of IpWalK with *T. maritima* HK853. IpWalK is colored in gold and green, and HK853 in grey. The phosphorylatable histidine is shown in sticks.

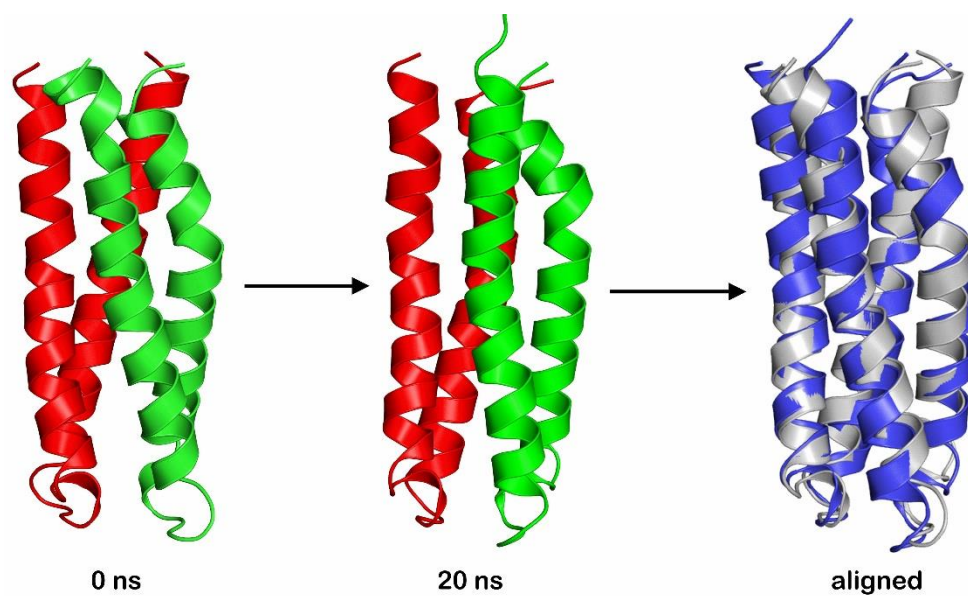


Figure S5 Relative stability of the DHp domain with the equal length of the helix $\alpha 1$ in molecular dynamic simulation for 20 ns. The simulated DHp domain (20 ns) in blue was aligned to its original conformation (0 ns) in grey on the right. No bending switch was observed.

Supplementary video S1. Conformational transition of IpWalk from the open state to the closed state. The homodimer is colored in magenta and green. The alignment based on the DHp domain is performed consistent to the Fig. 4.

Supplementary video S2. The molecular dynamic simulation of the DHp domain from the open state of IpWalk. The homodimer is colored in yellow and green. Pro396 as a bending point is highlighted with a red ball.

Supplementary video S3. The molecular dynamic simulation of the DHp domain from the closed state of IpWalk. The homodimer is colored in magenta and green. Pro396 as a bending point is highlighted with a white ball.

Supplementary video S4. The molecular dynamic simulation of the engineered DHp domain from the closed state. The color scheme is the same as video 3. A short green helix on the top of magenta monomer is a helical extension switched from its green monomer.

Supplementary video S5. The molecular dynamic simulation of the engineered DHp domain from the closed state. The color scheme is the same as video 3. The extended helix of the green monomer was manually rebuilt to be a random coil.