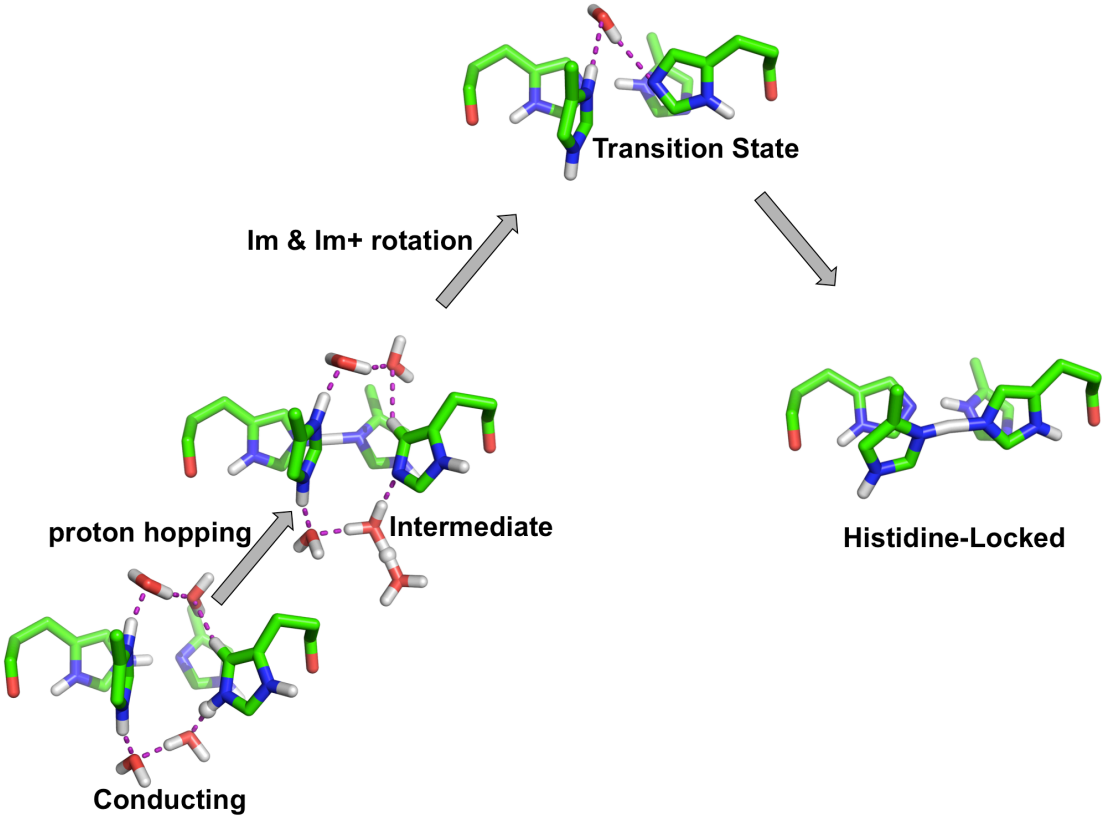

Fig. S1 Energy barrier encountered in the transition from the conducting state to the histidine-locked state. To obtain these results, the His37-Trp41 quartet was scanned along various paths connecting the two end states. Starting from **D4** (representing the conducting state), the proton on Nε2 of chain C was transferred to water molecules underneath, with or without restraining the χ_2 angles of His37 residues on chains C and D toward the values found in **D2** (i.e., the histidine-locked state). A moderate energy increase of about 2 kcal/mol was encountered without restraints on the χ_2 angles whereas a much greater barrier (about 10 kcal/mol) occurred while the imidazole rings were forced to rotate toward **D2**. Charge-charge repulsion between the protonated His37 on chain D and the leaving proton is likely a significant contributing factor to the latter higher energy, which suggests that the proton may hop to and then move along the water chain underneath before the rings undergo substantial rotation. The resulting intermediate is displayed, with the leaving proton highlighted as a sphere. Thereafter, the χ_2 angles of His37 residues on chains C and D (the former now deprotonated) were scanned along different paths. Along the minimum energy path, the barrier was about 8 kcal/mol. In the transition state, both Nε2 of chain C and Nδ1 of chain D can form only weak hydrogen bonds with a water molecule above (N-O distances about 3.4 Å). Interestingly, the total energy barrier is again about 10 kcal/mol.

Table S1. Comparison of Models Calculated from the 3LBW and 2L0J Backbone Structures

	3LBW			2L0J		
	D2	**D3**	**D4**	**D2**	**D3**	**D4**
Cβ-Cβ distance (Å)[a]	7.0/6.9 6.8/6.7	7.0/6.8 6.8/6.8	6.9/6.8 6.8/6.8	7.1/7.2 7.0/7.2	7.1/7.2 7.1/7.2	7.1/7.2 7.1/7.2
Nδ-Nε distance (Å)[b]	2.6/2.6	2.7/-	2.7/-	2.7/2.8	2.9/-	2.9/-
Inter-ring angle (°)[c]	23/19	22/64	20/55	30/27	30/70	17/64
χ_1 (°)[d]	-173/-170 -164/-165	-165/-178 -163/179	-166/-179 -164/-177	-170/-159 -177/-169	-175/-172 -173/-166	-176/-166 -176/-162
χ_2 (°)[d]	-7/-8 -100/-93	-11/64 -91/-135	-9/56 -88/-158	2/-11 -111/-96	-3/31 -103/-109	15/11 -107/-136

[a]In each entry, the first two numbers refer to the distances between chains A and B and between chains C and D, and the last two refer to the distances between chains B and C and between chains A and D.

[b]In each entry, the numbers refer to the distances between chains A and B and between chains C and D.

[c]In each entry, the numbers refer to the inter-ring angles between His37 sidechains on chains A and B and between those on chains C and D.

[d]In each entry, the first two numbers refer to the those for chains A and C, and the last two refer to those for chains B and D.