**Supplemental Data text. -** Trajtenberg *et al.*

*Structure solution and refinement methods.* The structure was determined at 1.7 Å resolution and was phased using **S**ingle wavelength **A**nomalous **D**iffraction (SAD) data collected with our home X ray source (Table 1), after quick-soaking the crystals with NaI (1). Five $I^-$ atoms were found using direct methods (2). Using this initial anomalous scatterer substructure, iodide occupancy, iso/anisotropic atomic displacement factors and 3D atomic coordinates were refined (3), with iterative identification of seven extra iodide atoms. After substructure refinement convergence, solvent flipping and histogram matching algorithms were applied, allowing to break the single wavelength phase ambiguities. The resulting electron density maps proved to be of excellent quality, allowing for automatic interpretation of >95% of the atomic model using Arp/wArp (4). Final corrections and loop modeling were done with Coot (5). Restrained refinement was straightforward using Refmac5 (6), including a TLS model.

*Sequence-based analyses* - Homologous sequences were retrieved from bacterial genomes stored at the IMG system (7) with a Blastp search (8), default parameters, keeping sequences with E-values $\leq 10^{-12}$. After redundancy removal, 406 unique sequences were retained and further aligned with Muscle (9) with default settings. For the comparative analysis with known histidine kinases, whole sequences corresponding to the 'HisKA_3' family were retrieved from the Pfam database (10). Removing identical sequences left us with 762 unique proteins, which were in turn aligned with Muscle. Next, homolmapper (11) was used to map multiple sequence alignments (MSAs) onto the molecular surfaces of DesKABD and the DHp domain (3GIE, same model used for the docking calculations). The alignments displayed in figure 3 are a subset of the original MSA of Pfam sequences, obtained by trimming the MSA into a non-redundant set of proteins at a cutoff of 88% pair-wise identity, using Jalview (12). Some long proteins were also manually removed from this set and new MSAs were computed with Mafft (13) for the reduced sets. All MSAs featuring secondary structure information were rendered with ESPript (14). The comparative analysis of '(NarQ/DesK)-like' proteins (Fig. S1) was done as follows. The Pfam team has split the 'HisKA_3' into 77 domain organizations (architectures); we were focused on a single architecture, namely, DHp-ABD. Thus, the full-length protein sequences sharing this domain organization (808 in total) were retrieved and formatted into a blastp-searchable database (using the 'formatdb' command from the BLAST suite of programs). Next, NarQ and DesK were used as queries against this smaller in-house database. Sequences with E-values $< 10^{-6}$ were kept from both searches, comprising 227 and 203 unique sequences for DesK and NarQ, respectively. Identity levels among pair-wise alignments reported by Blastp, went from 27% to 100%. The two data sets had 74 sequences in common, which we finally took to build an MSA using T-Coffee (15) with default settings. On visual inspection, 17 sequences with large insertions were removed, and the remaining proteins were realigned with T-Coffee.

*Structural comparisons*. In order to determine if there are differences between the ATP-bound DesKABD and the same domain but complexed with ADP (as observed in the structure of the point mutant DesKCH188V (16)) we used the program Escet (17) to quantitate the conformational variability between the two models of the ATP binding domain using error-scaled difference distance matrices. The ABD at high resolution (this report) was compared to the two domains present in the point mutant of the histidine kinase H188V (3EHH) using a 2.5σ cutoff level (where σ is the experimental uncertainty in the measurement of the distance between two atoms). The Cα's of the statistically invariant residues were then used for least-squares superposition. Fold searches and comparisons were done with DALI (18). Electrostatic calculations were performed with APBS (19), and figures prepared with PyMOL (http:// pymol.sourceforge.net). We identified a structurally invariant region at a conservative 2.5σ level, using error-scaled difference distance matrices (Fig. S3A). Helix α5 shows a significant movement compared to the other two helices (Fig. S3B). ATP γP is H-bonded to the nitrogen of G336 and the imidazole Nδ1 of H335. This network of interactions seems to be accounting for the stabilization of the ATP lid, and also for the movements of helix α5, comparing the ATP- vs ADP-bound structures. The same changes were confirmed using a second ABD in complex with ADP (Fig. S3C, S3D), independently solved and refined (DesKCH188E (16), the single point mutation affecting the DHp domain). DesKCH188E crystals belong to a different space group and display a completely different crystal packing arrangement. Although the significance of the movements is smaller for DesKCH188E (a 1.5σ cutoff was used due to its higher coordinate uncertainties at 2.65Å resolution), they are clearly discernable over the background noise and, more importantly, in full agreement with the ones previously identified when comparing to DesKCH188V-ADP. Overall, these comparisons strongly suggest that the nucleotide-linked changes affecting helix α5, are neither due to crystal packing constraints, nor to alternative interaction configurations with the central DHp domain.

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