Supplementary Materials

Supplementary Information

Figure S1  (a) Time evolution of RMSD for C\(_{\alpha}\) atoms from the NMR structure of Af1503\(^1\) in the MD simulation of model 1. Blue: the entire HAMP domain, superimposed upon the helical region of the NMR structure; Red: helical regions only; Gray: connector loop region only. (b) The average RMSF for C\(_{\alpha}\) atoms averaged for the last 50 ns in the simulation of model 1. Coloring scheme is the same as that in Fig. 4.

Figure S2  Time evolution of the contacts involved in the connector regions. The dots are plotted every 1 ns. Each panel consists of two datasets, chain B (left) and chain A (right). (a) Polar contacts in Af1503, and (b) hydrophobic contacts in Af1503. (c) Polar contacts in HtrII, and (d) hydrophobic contacts in HtrII.
Figure S3 Inter-chain contacts. (a) Tube model of the Af1503 HAMP domain (taken from a snapshot of the trajectory at 100 ns). The color scheme of the chain is the same as that in Fig. 4. (b) Schematic representation of the contacts in Af1503. Magenta and gray lines represent polar and hydrophobic contacts, respectively. Solid lines indicate the contacts appearing in the corresponding pairs of residues in Af1503 and HtrII. Broken lines represent the contacts appearing in either of the two molecules. (c) Tube model of the HtrII HAMP domain (taken from a snapshot of the trajectory at 160 ns). (d) Schematic representation of the contacts in HtrII. (e) Time evolution of the inter-chain contacts in Af1503 and (f) in HtrII. The scheme used here is the same as that in Fig. S2.
Figure S4  Ramachandran map of residues in the connector loops.  (a) 297–299 (Af1503) and (b) 103–105 (HtrII).  (c) Histogram of $\phi + \psi$ for G297 in Af1503, and (d) for G103 in HtrII.  (e) Time evolution of backbone hydrogen bond distances; K293–G297 (gray) and I294–G297 (magenta) in Af1503, and (f) R99–G103 (gray) and M100–G103 (magenta) in HtrII.  (g) Backbone hydrogen bonds in Af1503, and (h) in HtrII.
Figure S5  Side-chain crick angles of GCN4 (PDBid: 1 gcl), a typical canonical parallel coiled-coil four-helix bundle. (a) $a$ (blue) and $d$ (red) sites in the heptad repeat pattern, and (b) $e$ (red) and $g$ (blue) sites.

### Table S1  Average side-chain crick angles (in degrees)

| NMR structures | Af1503     | HtrII      |
|----------------|------------|------------|
| x-position    |            |            |
| Layer 1 (I284/L90) | 5.4±1.2   | 13.9±3.2   | 16.3±4.2   |
| Layer 2 (L315/L120) | −5.1±0.7  | −7.9±2.9   | −11.1±2.1  |
| Layer 3 (A291/A97) | 10.5±0.5  | 6.0±2.6    | 15.7±3.2   |
| Layer 4 (L322/M127) | −26.6±0.6 | −22.9±1.7  | −15.3±3.8  |
| Average difference$^a$ |          | +0.8       | +2.7       |
| Average difference from a typical knobs-into-holes$^e$ |          | −7.5       | −5.6       |
| da-position (intra-chain)$^b$ |            |            |            |
| Layer 1 (I312/I117) | 39.4±1.0  | 43.4±4.5   | 36.2±3.3   |
| Layer 2 (L287/L93)  | −47.8±0.6 | −47.9±3.5  | −30.2±3.0  |
| Layer 3 (I319/F124) | 36.5±0.7  | 27.2±2.9   | 23.6±2.8   |
| Layer 4 (I294/M100) | −42.1±0.6 | −50.9±2.4  | −33.5±3.7  |
| Average difference$^b$ |          | −0.9       | +10.6      |
| Average difference from a typical knobs-into-holes$^e$ |          | −22.1      | −10.7      |
| da-position (inter-chain)$^c$ |            |            |            |
| Layer 1 (E311/E116) | −55.2±11.6| −62.6±6.8  | −75.9±6.0  |
| Layer 2 (S288/A94)  | 74.7±1.3  | 68.2±3.0   | 73.7±3.8   |
| Layer 3 (S318/A123) | −49.6±0.7 | −78.3±3.2  | −69.7±3.3  |
| Layer 4 (A295/G101) | 59.2±0.8  | 50.3±3.0   | 58.8±5.0   |
| Average difference$^c$ |          | +5.2       | +9.9       |
| Average difference from a typical knobs-into-holes$^e$ |          | −19.0      | −14.3      |

Table shows the averages and standard deviations for 22 NMR structures from 2asz$^1$ and for the last 50 ns trajectories of the simulations of Af1503 and HtrII.

$^a$ Corresponding to the top panel of Fig. 7.

$^b$ Corresponding to the middle panel of Fig. 7.

$^c$ Corresponding to the bottom panel of Fig. 7.

$^d$ Average differences are the values from those of the average of the NMR structures. A positive value signifies a shift from knobs-to-knobs to knobs-into-holes, and a negative value signifies a shift to the opposite direction.

$^e$ Average difference from a typical knobs-into-hole is difference from the values calculated from the result of Fig. S5, $a$ at 19° and $d$ at −21°; bottom: $e$ at 88° and $g$ at −79°. A negative value signifies a shift from knobs-into-holes to knobs-to-knobs.
Supplementary Discussion

Stability of model 1

The results of the MD simulation for 100 ns of model 1 of the HAMP domain of HtrII (Fig. 1) are shown in Fig. S1. Even though the RMSD values (Fig. S1a) are comparable to those calculated for model 2, the RMSF values (Fig. S1b) in a chain exceeded 2 Å. The large fluctuation in the connector loop was caused by the absence of the side-chain contacts formed in model 2, R112-D125/E126 and V107-R128.

Polar and hydrophobic contacts

To demonstrate the stability of the polar and hydrophobic contacts shown in Fig. 5, we presented time evolutions of these contacts in Fig. S2. Data indicate that all of the contacts are stably formed during the simulations.

Inter-chain contacts are shown in Fig. S3. In Af1503, there are five polar inter-chain contacts, while in HtrII two of them (T281-E331 and S288-S318) were replaced by two hydrophobic contacts (A87-E116 and A94-A123), respectively. The ionic contacts, D292-R321 and E296-R321 in Af1503 were absent in HtrII because the corresponding pairs of the amino acids, S98-E126 and D120-E126, do not have attractive charges on their side-chains.

Peculiar interactions in the C-terminal part of AS1 are illustrated in Fig. S4. When focusing on the backbone conformation in the connector loops, we noticed that the corresponding residues, 297–299 in Af1503 and 103–105 in HtrII, showed almost the same $\phi - \psi$ distributions (Figs. S4a and b). Moreover, both distributions have unoccupied regions dividing the distribution into two parts. It was found that the sum $\phi + \psi$ correlated positively with the distance of a hydrogen bond K293-G297 (R99-G103) and I294-G297 (M100-G103). This implies that the two peaks in the histogram of $\phi + \psi$ correspond to the hydrogen bonds of K293-G297 (R99-G103) and I294-G297 (M100-G103), respectively (Figs. S5c, d, e, and f). The homology model of HtrII succeeded in reproducing the dynamic exchange of the hydrogen bonds.

Side-chain crick angles

We calculated side-chain crick angles for GCN4, a leucine zipper core, as a typical parallel coiled-coil four-helix bundle forming knobs-into-holes (Fig. S5). We used the assignment of the heptad positions a-g of GCN4 described in ref. 2. The averages values, 19°, −21°, 88°, and −79° for a, d, e, and g, respectively, may deviate from the ideal value of ±26° and ±78°, partly due to fluctuations of the side-chain orientations and skewed packing of the helices.

Table S1 presents the average values of the side-chain crick angles for the 22 NMR structures (PDBid: 2asx), and the simulation results of Af1503 and HtrII for the last 50 ns. Data in the table clearly show that layer 4 of the NMR structure is in knobs-into-holes packing, and that layer 3 is in an intermediate state, and layers 1 and 2 are definitely in the knobs-to-knobs. The change during the simulation of Af1503 is small and shows that the packing state was stably maintained. In addition, the trend of a shift to knobs-into-holes in HtrII is clearly seen here.

References

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2. Harbury, P. B., Zhang, T., Kim, P. S. & Alber, T. A switch between two-, three-, and four-stranded coiled coils in GCN4 leucine zipper mutants. Science 262, 1401–1407 (1993).