Communicating helices: molecular simulation of allosteric receptor proteins
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HAMP domains are vital signal conversion modules of bacterial chemotaxis receptors. So far it is unknown how this homodimeric four helical bundle transmits signal motions from one domain to another. Among the existing mechanistic models of HAMP signaling, the dynamic bundle model, derived from large scale mutational studies of the HAMP domains in the *E. coli* serine receptor Tsr, proposes that packing stability of the HAMP domains plays important roles in the communication between the input and output domains of HAMP\[27, 28\]. Experimentally no structural evidence is yet available to support the dynamic bundle model. This work studies the structure and dynamics of the Tsr HAMP via all-atom molecular dynamics simulations. We show that Tsr-HAMP is unstable when modeled on the crystal structure of HAMP2 from the tri-HAMP chain of soluble receptor Aer2 (PDB entry 3LNR)\[1\]. Instead Tsr-HAMP is relatively stable when modeled on the Af1503 structure (PDB entry 2L7H), but more flexible than the wild-type Af1503 HAMP with different geometry of its hydrophobic layers and with less helical characters at its terminal parts. Two point Tsr mutants L256A and I229A, modeled on Af1503, are more flexible than wild type Tsr. Both mutants have larger sidechain flexibility. L256A has similar degree of helicity as wild type Tsr-HAMP while I229A is less helical. These results support the extended dynamic bundle model\[28\].
5.1 Introduction

Signaling processes of prokaryotes involve many two-component signal transduction pathways[23]. Many of the proteins in these signal pathways contain HAMP (Histidine kinases, Adenylate cyclases, Methyl accepting proteins and Phosphatases) domains that function as signal relay modules coupling the motions of sensor domains to the activity of a downstream cytoplasmic output domain. The mechanism by which HAMP domains propagate the input signal towards their output domains is essential in understanding the signaling process in prokaryotes.

Transmembrane (TM) chemotaxis receptors are among the best studied examples of HAMP-containing TM proteins[29, 26]. Typically, HAMP domains occur as a single unit in chemotaxis receptors, connecting the second transmembrane helix (TM2) to the cytoplasmic kinase-module [9]. HAMP domains that occur as a single unit contain a conserved P...[DExG] motif and are classified as canonical HAMPs[5]. The structure of a HAMP domain Af1503 from A. fulgidus, resolved via solution NMR spectroscopy, currently serves as the prototype for all canonical HAMPs[25, 2, 20]. This structure (PDB entry 2L7H) is a dimeric coiled-coil complex with four helices in parallel orientation. Containing 58 residues, one monomer (M1 or M2) consists of two helices (N-terminal and C-terminal) linked by a ∼14 residue flexible connector. The four helices (two N-terminal helices N1 and N2, two C-terminal helices C1 and C2) contain two heptad repeat (a-g) in which the positions a and d are occupied mainly by hydrophobic residues which form an interface, see FIG. 5.1. These hydrophobic residues form 5 hydrophobic layers (L1-L5), colored in the sequence alignment in FIG. 5.2 (L1-pink, L2-blue, L3-red, L4-green, L5-orange). All layer residues of Af1503 and their locations (from the N-terminal and C-terminal helices or the connector) are listed in TAB. 5.1.

Through the HAMP domain, chemoreceptors relay and propagate the environmental signals to a network of molecules and regulate the rotational pattern of the flagellar on the cell surface of the bacteria. The default counter-clock wise (CCW) rotation of flagellar make the bacteria to swim while the clock-wise (CW) rotation, initially activated by an attractant binding to the chemoreceptor, make the bacteria to tumble in space. For details of this regulating process, the reader is referred to SEC. 1.1.1.

The signaling mechanism of canonical HAMPs has been extensively studied experimentally and resulted in two classes of models: the static model[8, 24, 12, 17] and the dynamic bundle model[27]. The static model assumes that HAMP can adopt at least two different conformational states during signaling. Several types of motions have been proposed to link these separate states: (I) rotation of the helices in the gearbox model[12], (II) piston shifts of helices in the piston model[8, 17], (III) tilting of the helices in the scissor model[24]. In Chapter 2, we have shown via Molecular Dynamics and Well-Tempered Metadynamics simulations that for an isolated Af1503 HAMP, the piston-like and scissor-like motions of the helices are coupled while helical rotation is a less coupled mode of motion. Two extra states than the NMR structure (P10/P01, P11) were also identified for Af1503 HAMP in terms of the piston mode. P10 corresponds to a piston shift of +1Å for N1 and -1Å for C2 with respect to the NMR structure (PDB entry 2L7H); alternatively, due to the symmetry of homodimer HAMP, P01 corresponds to the same shift for the other pair of helices +1Å for N2 and -1Å for C1; P11 corresponds to the piston shifts of both pairs of helices.

The dynamic bundle model, on the other hand, focuses on the dynamic properties of
Figure 5.1: Geometry of hydrophobic layers of Af1503 and Tsr-HAMP. Color code indicates residues from different layers: *white* monomer 1, *black* monomer 2, *pink* Layer 1 $L_1$, *blue* Layer 2 $L_2$, *red* Layer 3 $L_3$, *green* Layer 4 $L_4$, *orange* Layer 5 $L_5$. 
HAMP. Here we give a brief overview of the dynamic bundle model. A detailed description of the model is given in the Appendix SEC. 5.6. By varying the connector residues of the E. coli Tsr-HAMP[2], Zhou et al identified three critical connector residues for which most replacements destroyed Tsr function[2], consistent with the structure of the prototype Af1503 HAMP[12]. The consistency of Tsr-HAMP with the Af1503 structure was further strengthened by a subsequent mutational study on all helical residues[27]. Among the total ∼370 mutations, Zhou et al obtained 131 mutations with loss of function, of which 51 are located on the N-terminal helices and 80 on the C-terminal helices. In each helix, six residues were identified as functionally critical due to complete loss of function when they were varied. When aligning the Tsr-HAMP sequence on the structure of Af1503 HAMP (see FIG. 5.2 and TAB. 5.1), these critical residues are all located at the interface of the HAMP bundle. Furthermore, the mutations revealed that replacing residues on the C-terminal end of C1 and C2 (residues 256-264) with proline resulted in altered functionality of Tsr. In contrast, changing residues in other regions of HAMP to proline had little effect.

Subsequent studies focused on the nature of 118 mutations, grouping them in 6 categories according to phenotype[2, 27, 28]. Linking the location of a mutation in the helical bundle to a specific phenotype resulted in the postulation of the (extended) biphasic dynamic bundle model. In this model, signal transduction occurs by changing the degree of packing in the helical bundle: binding of attractant induces tighter packing of the HAMP bundle whereas binding of repellent results in more loosely packed bundle. However, the response of the flagellar rotation for such change of HAMP depends on the initial packing degree: (I) very tight and very loose initial packing of HAMP both induce CCW behavior; (II) if the initial packing is tighter than a threshold degree, HAMP works in the normal regime - tighter packing enhances CW behavior; (III) if the initial packing is looser than the threshold degree, HAMP works in the reversed regime - looser packing of HAMP enhances CW behavior (see Figure 7 of Ref[28]). According to this model, the wild-type Tsr HAMP falls in the normal regime while a number of mutants, such as I229A, work in the reversed regime. Mutants that lock the flagellar rotation CW, e.g. L256A, were proposed to hold the threshold packing degree[28].

<table>
<thead>
<tr>
<th></th>
<th>N(N2)</th>
<th>Connector</th>
<th>C(C2)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Af1503</strong></td>
<td>L1</td>
<td>Pro283, Ile284</td>
<td>Ile312</td>
</tr>
<tr>
<td></td>
<td>L2</td>
<td>Leu287</td>
<td>Leu315, Ala316</td>
</tr>
<tr>
<td></td>
<td>L3</td>
<td>Ala291</td>
<td>Ile319</td>
</tr>
<tr>
<td></td>
<td>L4</td>
<td>Ile294, Ala295</td>
<td>Leu322</td>
</tr>
<tr>
<td></td>
<td>L5</td>
<td></td>
<td>Leu298, Leu326</td>
</tr>
<tr>
<td><strong>EcTsr</strong></td>
<td>L1</td>
<td>Pro221, Met222</td>
<td>Met249</td>
</tr>
<tr>
<td></td>
<td>L2</td>
<td>Leu225, Ile226</td>
<td>Leu252, Ala253</td>
</tr>
<tr>
<td></td>
<td>L3</td>
<td>Ile229</td>
<td>Leu256</td>
</tr>
<tr>
<td></td>
<td>L4</td>
<td>Ile232, Ala233</td>
<td>Met259</td>
</tr>
<tr>
<td></td>
<td>L5</td>
<td></td>
<td>Leu237, Leu263</td>
</tr>
</tbody>
</table>

Table 5.1: Residues in the layers of Af1503 (PDB entry 2L7H) and HAMP2 (PDB entry 3LNR). Residues are distinguished by their locations (from N-terminal helix, connector or C-terminal helix). Bold residues form the hydrophobic core of each layer.
5.1 Introduction

In addition to the canonical HAMP domains in chemoreceptors, HAMP domains also occur in proteins as multiple consecutive copies\[1, 5\]. Such HAMPs are typically missing the P...DExG motif and classified as divergent HAMPs\[5\]. Crane et al obtained the first crystal structure of the N-terminal tri-HAMP chain from the Aer2 receptor from \textit{P. aeruginosa} (PDB entry 3NLR)\[1\]. Although all three HAMPs from this chain are divergent HAMPs, Crane et al found that HAMP1 and HAMP3 are structurally similar to the prototype canonical HAMP Af1503 while the HAMP2 crystal has a different conformation. HAMP2 has an offset of approximately one helical turn between the N-terminal helix N1 and the C-terminal helix C1, as illustrated in the sequence alignment of FIG. 5.2. Based on the layer shift of HAMP2 and a bioinformatic prediction\[20\], HAMP2 has been suggested as an accessible packing conformation during signal transduction by the canonical Tsr-HAMP to support the dynamic bundle model\[1, 20, 14\].

The dynamic bundle model is inspiring in the sense of its focus on a change in the dynamical properties of HAMP rather than a conformational change of HAMP during signaling. However it involves considerable speculations. So far, no structural evidence is available to support the idea of weakened or strengthened packing interactions of Tsr-HAMP during signaling. The terms of "loosely" and "tightly" packed HAMP bundle were derived purely from speculations on the destabilizing effect of the mutations might have on the bundle and are not defined. In fact, one point mutation could change the sidechain volume, charge distribution and helical property of that residue at the same time. It has been suggested that Molecular Dynamics simulations could shed light on the dynamics of Tsr-HAMP\[16\]. To date, only one all-atom Molecular Dynamics study was performed on the sensor module and the HAMP domain of the Tar receptor, a chemoreceptor closely related to Tsr\[19\]. This work proposed that signal ligand binding to the sensor module makes the N-terminal helices of Tar-HAMP less dynamic and the C-terminal helices more dynamic. However the authors did not characterize the HAMP domain in details that enable direct comparison with the mutational studies that led to the dynamic bundle model.

In this chapter, we model the sequence of Tsr-HAMP on two template structures Af1503\[12\] and HAMP2\[1\] and characterize the dynamical properties of these two Tsr-HAMP systems via all-atom Molecular Dynamics simulations. We address the following questions about these two systems: (I) can Tsr-HAMP fluctuate around the NMR Af1503 conforma-
tion or the crystal HAMP2 conformation? (II) is Tsr-HAMP helical throughout the bundle? (III) What is the difference between the Tsr-HAMP conformations? Our results indicate that the Af1503 HAMP is a reasonable template for Tsr-HAMP while the HAMP2 crystal with shifted layers is not. We also show that Tsr-HAMP is highly dynamic, with less helical character than Af1503 on its two terminal ends. To gain further insights into the destabilizing effects of mutations, we model two mutant sequences of Tsr-HAMP L256A and I229A on the Af1503 NMR structure. MD simulations of the two mutants show that (I) L256A is more flexible than wild-type Tsr-HAMP with larger side-chain flexibility of the layer residues (II) I229A is even more flexible than L256A with less helical character. The results of our 6µs MD simulations seem consistent with the biphasic diagram of the HAMP stability in the extended dynamic bundle model[28].

5.2 Methods
5.2.1 Simulation Systems
We used the Modeller version 9.10[22] to model the sequence of wild-type and mutant Tsr-HAMP on two template structures, using the sequence alignment in FIG. 5.2. The Tsr-HAMP model based on the Af1503 structure (PDB entry 2L7H) is referred to as EcTsr_WT, the mutant model L256A as EcTsr_L256A and I229A as EcTsr_I229A. The Tsr-HAMP model based on the crystal structure of HAMP2 (residues 59-116 of pdb structure 3LNR) is referred to as EcTsr_H2.

5.2.2 Molecular Dynamics
With GROMACS v.4.5.4[10] as the Molecular Dynamics (MD) engine, we employed the OPLS force field[13] to describe the interactions between atoms for both systems, in combination with the TIP4P water models[18]. Na+ and Cl− ions were added to both systems to meet physiological conditions [NaCl]=0.15M and to maintain electrostatic neutrality of the systems. Periodic boundary condition were applied to the simulation boxes. ∼9000 water molecules were added to a cubic box of dimension of 67Å. Both systems were energy minimized using the conjugate gradient method. To equilibrate the hydrogen atoms and water molecules, we ran a 10 ps molecular dynamics at a temperature of 298 K and a pressure of 1 bar for each system after energy-minimization, in which the heavy atoms in the protein were position-restrained.

The production MD runs were performed in the isothermal-isobaric ensemble at room temperature 298K with the Nose-Hoover thermostat[11] and at ambient pressure 1 bar with Parrinello-Rahman barostat[21]. Long-range electrostatic interactions were treated via the Particle Mesh Ewald method[4, 7] with a short-range electrostatic cutoff distance at 11Å. Van der Waals interactions were cut off at 11Å. The timestep is set at 2fs. Bonds were constrained using the LINCS algorithm. Frames were recorded every 1ps. 8 independent runs of 60ns were performed EcTsr_H2. 16 independent 150ns trajectories were obtained for EcTsr_WT. 8 independent 200ns runs were performed for EcTsr_L256A and EcTsr_I229A respectively. Independent runs started with different velocities drawn randomly from the Maxwell-Boltzmann distribution.
5.2.3 Analysis of MD simulations

As HAMP is a homodimer, we distinguished residues from different monomers by the presence or absence of the prime label. For example, Val65 is from Monomer 1 (white in FIG. 5.1) whereas Val65' is located in Monomer 2 (black in FIG. 5.1); N1 denotes the N-terminal helix from Monomer 1, whereas N2 denotes the N-terminal helix from Monomer 2.

To capture the essential structural features of Tsr-HAMP, we computed the RMSD of Tsr-HAMP with respect to the two template structures, using positions of the $C_\alpha$ atoms of the helical residues span the hydrophobic layers ($L_1$ to $L_4$). For $rmsd_{\_af}$, the RMSD referenced on the NMR Af1503 (PDB entry 2L7H), this range includes residues 221-233 of the N-terminal helices and residues 248-260 of the C-terminal helices. For $rmsd_{\_h2}$, the RMSD referenced on the HAMP2 crystal (PDB entry 3LNR), this range is larger in the N-terminal helices (residues 218-233) because the crystal HAMP2 structure has an extra layer $L_x$ (see SEC. 4.1 and FIG. 5.2).

Since the dynamic bundle model predicts that helicity of Tsr-HAMP could be lost at the C-terminal residues, we need a measure of helical character per residue. We define a helical hydrogen bond as formed when the distance between the Oxygen atom of residue $n-4$ and the Hydrogen atom of residue $n$ is smaller than 2.5Å. Then the helicity of a specific residue pair $n-4$ and $n$ is measured via the probability $P_{n}^{hhb}$ of whether this helical hydrogen bond is formed:

$$P_{n}^{hhb} = \langle h(r_{cut} - |r_{O_{n-4}} - r_{H_{n}}|) \rangle$$

(5.1)

(5.2)

Here $r$ denotes the configurational position of an atom, $n$ the residue number, $O$ and $H$ the Oxygen and Hydrogen atom respectively with $\langle \ldots \rangle$ indicating the ensemble average assuming the sampling of our MD simulations is sufficient, $r_{cut} = 2.5\text{Å}$ is the cutoff distance, $h(x)$ is the Heaviside step function:

$$h(x) = \begin{cases} 
0, & \text{if } x < 0 \\
1, & \text{if } x \geq 0
\end{cases}$$

(5.3)

The Tsr-HAMP conformations obtained in our simulations often have distorted helices, disabling structural analysis via the program samCC specialized in four helical bundle analysis[6]. We characterize the geometry of Tsr-HAMP via the side-chain distances between the residues that form its hydrophobic layers. The distances are computed using the center of mass of the heavy atoms of the side-chains $D_{sc}$ of the residues within each layer. For example, $D_{sc}$-Leu252 denotes the side-chain distance between the residue Leu252 and Leu252'.

5.3 Results

5.3.1 Wild type Tsr-HAMP modeled on HAMP2

Among the four available HAMP structures, the NMR structure of canonical Af1503, the crystal of HAMP1 and HAMP3 from the polyHAMP chain of Aer2 receptor are very similar, whereas the HAMP2 domain adopts a different conformation[1], see SEC. 4.1. In the
Predicting the solution structure of the HAMP domain from the serine receptor Tsr

Figure 5.3: (A) Time traces of \( \text{rmsd}_{h2} \) (residues 218-233 and 249-260) of the \( EcTsr\_H2 \) model. RMSD computed with respect to the HAMP2 crystal structure. (B) Probability of helical hydrogen bond \( P_{hhb} \) computed using all 240ns data.

Bioinformatics analysis by Crane et al that threads different HAMP sequences on these four known structures, HAMP2 has a low threading score of 459 (in Rosetta energy units), indicating that HAMP2 might be an accessible conformation for Tsr-HAMP\[^1\]. HAMP2 was also proposed as a possible state for Tsr-HAMP during signaling\[^20\]. Therefore we projected the Tsr-HAMP sequence on the HAMP2 crystal structure using the alignment in FIG. 5.2 and obtained the model \( EcTsr\_H2 \) rendered on the left of FIG. 5.3A.

FIG. 5.3A plots the time traces of \( \text{rmsd}_{h2} \) of the 8 60ns MD trajectories of \( EcTsr\_H2 \), referenced on the HAMP2 crystal structure. Here \( \text{rmsd}_{h2} \) is computed from C\( \alpha \) atoms of residues 218-233 and 249-260 corresponding to L1-L4 of HAMP2 (including Lx). The majority of trajectories are at least 2Å from the crystal structure: four trajectories relaxed to \( \text{rmsd}_{h2} \approx 2.2\text{Å} \) (blue, gray, green and brown), two to 3Å (red and purple) and one to 3.5Å (yellow). In only one trajectory (black), \( EcTsr\_H2 \) remains close to the HAMP2 crystal structure with \( \text{rmsd}_{h2} = 1.5\text{Å} \) within 60ns, but appears drifting away in a relaxation process. The snapshots extracted at 60ns of the red, purple and yellow trajectories further reveal that \( EcTsr\_H2 \) is very unstable with considerable loss of helical characters. We thus computed the helical hydrogen bond probability \( P_{hhb} \) (see SEC. 5.2.3) using all 240ns data. As shown in FIG. 5.3B, \( P_{hhb} \) is less than 0.7 for the majority of residue pairs. These results indicate strongly that the HAMP2 crystal is not a suitable solution structure of Tsr-HAMP and should fall beyond the operational range of Tsr-HAMP during signaling.

5.3.2 Tsr-HAMP modeled on Af1503

5.3.2.1 Tsr-HAMP fluctuates around a Af1503-like structure

For the \( EcTsr\_WT \) system, we performed 16 150ns MD simulations for a direct comparison with our previous work on wild type Af1503 (see Chapter 3). To check the relaxation in each trajectory(FIG. 5.4), we computed the \( \text{rmsd}_{af} \) of Tsr-HAMP, the C\( \alpha \) RMSD of the residues spanning the hydrophobic layers L1-L4 with respect to the template Af1503 NMR structure. For all 8 reference trajectories of wild type Af1503, \( \text{rmsd}_{af} \) is 0.6Å. For the majority of \( EcTsr\_WT \) trajectories, \( \text{rmsd}_{af} \) fluctuates between 0.9Å and 1.7Å. A representative conformation extracted from these trajectories is shown in FIG. 5.4. In only
5.3 Results

Figure 5.4: Time traces of Root Mean Square Deviation $\text{rmsd}_\text{af}$ of wild-type Tsr-HAMP $\text{EcTsr}_\text{WT}$. Solid lines are $\text{EcTsr}_\text{WT}$ trajectories. Dashed lines are $\text{Af1503}$ trajectories. Inset figure presents the $\text{EcTsr}_\text{WT}$ conformation that deviates most from the template structure $\text{Af1503}$ (PDB code 2L7H).

One $\text{EcTsr}_\text{WT}$ trajectory (yellow), $\text{rmsd}_\text{af}$ is able to deviate beyond 2Å at 30ns that corresponds to a conformation with strongly distorted C1 helix. However by the end of this trajectory, Tsr-HAMP returns from this distorted conformation to a conformation with $\text{rmsd}_\text{af}=1.5$Å and normal helicity. These results indicate that $\text{EcTsr}_\text{WT}$ remain close to the template NMR structure $\text{Af1503}$, yet with mild structural differences and different dynamic properties as described in SEC. 5.3.2.2 and SEC. 5.3.2.3.

5.3.2.2 The Middle layers of Tsr-HAMP are more helical

To characterize the structural variety of the $\text{EcTsr}_\text{WT}$ conformations, we first measured the helicity per residue of the layered region via computing the probability of helical hydrogen bonds $P_n^{\text{hhb}}$, as described in the methods section. In FIG. 5.5, we compare the probability of helical hydrogen bond $P_n^{\text{hhb}}$ computed using all 400ns data of $\text{Af1503}$ and all 2.4$\mu$s data of $\text{EcTsr}_\text{WT}$.

In the $\text{Af1503}$ simulations (black curves in FIG. 5.5A,B), the $P_n^{\text{hhb}}$ is larger than 70% from L1 to L4. This is consistent with the high stability of $\text{Af1503}$. The low helicity of the last 3 bonds of the N-terminal helices (right of FIG. 5.5A) and the first 2 bonds of C-terminal helices (left of FIG. 5.5B) are due to the fact that the connector residues are unable to form hydrogen bonds with the C-terminal region of the helix N1/N2 or the N-terminal region of C1/C2, see FIG. 5.2. The low helicity of the first 4 bonds of the N-terminal helices (left of FIG. 5.5A) is because of our system setup: the HAMP domains are isolated in solution without constraints from the input domains, the first few N-terminal residues...
Predicting the solution structure of the HAMP domain from the serine receptor Tsr

Figure 5.5: Probability of helical hydrogen bonds $P_{hhb}^N$ (A) the N-terminal helices. (B) the C-terminal helices. Solid lines are the average values of $P_{hhb}^N$ over the two monomers. Error bars indicate the average deviation of the two monomers from the average values. In the x-axis, each one letter notation labels the residue $n$ from whose backbone hydrogen atom contributes to the corresponding helical hydrogen bond. The four rows are for Af1503, wild type Tsr-HAMP EcTsr _WT, two mutants of Tsr-HAMP EcTsr _L256A and EcTsr _I229A respectively. Residues that form the hydrophobic layers are highlighted. Color codes: black Af1503, red EcTsr _WT, green EcTsr _L256A and blue EcTsr _I229A.

are solvent-exposed and become very flexible. Among the hydrogen bonds in which both contributing residues are within $L_1 - L_4$ (residues 283-295 and 311-323) of the bundle, a few bonds occur with $P_{hhb}^N$ values of 70-80%, slightly weaker than the majority of 80%: Ile285-Asn289, Asn289-His293 of N1/N2, Ile312-Ala316, Ile314-Ser318 of C1/C2. Moreover, two bonds between $L_4$ and $L_5$ (Met259-Leu263, Gln260-Met264) in C1/C2 are also weaker (75%), which reflects the flexibility of $L_5$ in which two contributing residues are located at the connector.

For EcTsr _WT (red curves in FIG. 5.5A,B), $P_{hhb}^N$ is lower than Af1503, indicating considerable loss of helicity in Tsr-HAMP than in Af1503. The hydrogen bonds in EcTsr _WT corresponding to hydrogen bonds in Af1503 become weaker with $P_{hhb}^N = 30-70%$. Especially, the hydrogen bonds between $L_4$ and $L_5$ in the C-terminal helices are all destabilized, hinting at much larger flexibility of $L_5$ than in Af1503. Meanwhile, although the hydrogen bonds in the region $L_1 - L_4$ are less stable, at least one hydrogen bond with $P_{hhb}^N > 80%$ occurs between two neighbor layers: Met222-Ile226, Leu225-Ile229, Ile226-Arg230 and Ile229-Ala233 in N1/N2, Gly250-Glu254, Gln251-Ser255, Leu252-Leu256 and Leu256-Gln260 in C1/C2. These results indicate that EcTsr _AF has helical character, but less than Af1503.
Since the helicity of Af1503 is larger than 70% and Af1503 is a very stable bundle, we use $P_{hhb} = 70\%$ as the criterion to define a stable helical hydrogen bond for EcTsr\_WT. By this criterion, EcTsr\_WT has stable helical structures only between L1 and L3 in the N-terminal helices and between L2 and L3 in the C-terminal helices, i.e. only the middle layers (L2 – 3) of EcTsr\_WT are constantly helical.

5.3.2.3 Hydrophobic Layers
The loss of helicity in EcTsr\_WT forbids one to use the samCC program (a structural biology program for four-helical-bundle analysis\[6\]) or our previous methodology of rigid body motion analysis (see Chapter 3, 4) to characterize the conformations of Tsr-HAMP. However, one gains important insights by comparing the side-chain distances $D_{sc}$ between the layer residues.

In FIG. 5.6, we plot the averages and standard deviations of these distances computed using all data for Af1503 and EcTsr\_WT. Both the averages and the standard deviations of the distances are different between Af1503 and EcTsr\_WT. The difference in the averages indicate that the side-chain packing of EcTsr\_WT is distinguishable from AF1503. In general, the standard deviations of EcTsr\_WT are larger than those of Af1503, indicating larger sidechain flexibility of EcTsr\_WT.

In L1, the fluctuations of $D_{sc}$-Met222 and $D_{sc}$-Met249 in EcTsr\_WT are both around 1Å, 100% larger than the value of 0.5Å for $D_{sc}$-Ile284 and $D_{sc}$-Ile312 in Af1503. We attribute this increase of sidechain flexibility to the change in sidechain volume. The “original” space of L1 available (see FIG. 5.1) in the template Af1503 structure can not accommodate all 4 Methionine side-chains. This appears correlated with the loss of helicity between L1 and L2 of C1 and C2 (FIG. 5.5B) because full accommodation of the Methionine side-chains in L1 has to enlarge the space of L1 and disrupt the helicity of the relevant residues. In L2, Leu225 and Leu225’ of EcTsr\_WT ($D_{sc}$-Leu225=8.5Å) seems closer to each other than Leu287 and Leu287’ in Af1503 ($D_{sc}$-L287=10.7Å) while Leu252 and Leu252’ of EcTsr\_WT ($D_{sc}$-Leu252=6.5Å) are more distant from each other than Leu315 and Leu315’ in Af1503 ($D_{sc}$-L315=5.3Å). In L3, the most significant difference between EcTsr\_WT and Af1503 is found between the two connector residues Val303 and Ile241. $D_{sc}$-I241=14.5Å is 2.5Å smaller than that $D_{sc}$-V303=17Å, indicating that in EcTsr\_WT, the two Val303 residues are much more closely packed to the helical residues and could play more essential roles during signaling than the role of Ile241 in Af1503. In L4, $D_{sc}$-Ile232=14Å and $D_{sc}$-Ala232=15.5Å from EcTsr\_WT are both larger and more broadly distributed than $D_{sc}$-Ile294=12.7Å and $D_{sc}$-Ala295=13.2Å. Such increase in the distance between the N-terminal helices and the flexibility of the side-chains appears due to the fact that the more bulky Met259 in EcTsr\_WT separate the residues from the N-terminal helices more than Leu322 do in Af1503.

5.3.2.4 Destabilizing effects of mutations - L256A & I229A
To further explore the extended dynamical bundle model and characterize the destabilizing effects of the mutations on wild-type Tsr-HAMP, we made two mutant models EcTsr\_L256A and EcTsr\_I229A using the Af1503 NMR structure as the template structure. We selected these two mutants because of the following considerations: (I) L256A has...
the CW-locked phenotype that is located on the interface between the normal and reversed regime in the proposed extended dynamic bundle model [28]; (II) I229A exhibits the inverted phenotype and falls in the reversed regime; (III) the two point mutations both occur in the middle layer L3 rather than the layers on the terminal ends that are already very flexible in our setup of isolated wild-type Tsr-HAMP. The destabilizing effects of them could be more obvious than mutations in other layers; (IV) both mutations are hydrophobic replacements involving minimal change of the side-chain property - only changing the side-chain volume but not charge distribution or helicity. In contrast, replacement into proline residues change the side-chain volume and helicity; replacement into charged or polar residues change the side-chain volume and charge at the same time.

For each mutant system, we obtained 8 200ns trajectories. FIG. 5.7 plots the time-traces of \( \text{rmsd}_{af} \) for the L256A mutant. Among the 8 trajectories of EcTsr\_L256A, \( \text{rmsd}_{af} \) remains smaller than 1.5Å in 4 trajectories, i.e. close to the template Af1503 structure. In the other 4 trajectories, EcTsr\_L256A relaxes to 4 different conformations. The one with the largest \( \text{rmsd}_{af} = 2.7\text{Å} \) (green trajectory) correspond to a tilted conformation in which L1 is disassembled and L2 is strongly distorted. In the conformation that has \( \text{rmsd}_{af} = 2.5\text{Å} \) (red trajectory), one observes a clear upper-shift of Monomer 1 with respect to Monomer 2, disturbing all the hydrophobic layers. The conformations at around \( \text{rmsd}_{af} = 1.75\text{Å} \) (blue
5.3 Results

Figure 5.7: Time traces of Root Mean Square Deviation \(rmsd_{af}\) of the L256A mutant of Tsr-HAMP \(EcTsr_{L256A}\). Solid lines are \(EcTsr_{L256A}\) trajectories. Dashed lines are Af1503 trajectories. Inset figures present the \(EcTsr_{L256A}\) conformations obtained at the end of the corresponding trajectories.

Line) exhibit disturbed L4 and L5 but intact L1-L3. In the \(rmsd_{af}=1.5\)Å conformation (brown trajectory), the two monomers are almost in parallel. These distorted conformations clearly illustrate the destabilization by L256A mutation.

Surprisingly, \(P_{hhb}^n\) of \(EcTsr_{L256A}\) (FIG. 5.5) remains very similar to that of \(EcTsr_{WT}\). This means the L256A mutation causes structural perturbation without reducing helicity of Tsr-HAMP. In FIG. 5.6 we observe that the standard deviations of the side-chain distances are generally larger than that of \(EcTsr_{WT}\), confirming the increased side-chain flexibility of \(EcTsr_{L256A}\) over \(EcTsr_{WT}\).

In FIG. 5.8 we show \(rmsd_{af}\) traces of \(EcTsr_{I229A}\). In 6 out of the 8 trajectories, \(rmsd_{af}\) fluctuates between 1.0Å and 1.8Å. In these trajectories, L4 typically has a different geometry from the one shown in FIG. 5.1: the two M259 sidechains point to the N-terminal helix rather than the C-terminal helix of the other monomer. In the conformation with \(rmsd_{af}=2.3\)Å (red trajectory), the N1 helix is strongly unfolded dissembling L3. The conformation with the largest value of \(rmsd_{af}=3\)Å exhibits a distorted L4 and partially unfolded N-terminus of the N1 helix, such that two L4 sidechains are in contact with L3 sidechains. The standard deviations of the side-chain distances of \(EcTsr_{I229A}\) (FIG. 5.6) are comparable with those of \(EcTsr_{L256A}\) and thus also larger than those of \(EcTsr_{WT}\). Importantly, the \(P_{hhb}^n\) of \(EcTsr_{I229A}\) (FIG. 5.5) reveals that I229A has less helical character in the N-terminal part of the bundle with respect to both \(EcTsr_{WT}\) and \(EcTsr_{L256A}\).

When comparing side chain distances in (FIG. 5.6) between \(EcTsr_{WT}\) and
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Figure 5.8: Time traces of Root Mean Square Deviation $rmsd_{af}$ of the I229A mutant of Tsr-HAMP $EcTsr_{I229A}$. Solid lines are $EcTsr_{I229A}$ trajectories. Dashed lines are Af1503 trajectories. Inset figures present the $EcTsr_{I229A}$ conformations obtained at the end of the corresponding trajectories.

EcTsr_{I229A}, we find that the largest differences occur in $L1$ (M249) and $L3$ (I241). The latter can be explained as it is in the direct vicinity of the mutation. The former includes increase in both the average values and fluctuations of $D_{sc}$-M249 in EcTsr _I229A over EcTsr _WT. This indicates that the bulky Methionine sidechains in L1 of Tsr-I229A are allowed to form multiple packing modes than in wild-type Tsr-HAMP, possibly due to the loss of helicity in EcTsr _I229A.

5.4 Discussion

5.4.1 Af1503 - the only functional conformation?

This work presents the first dynamical study of the Tsr-HAMP domain at atomic resolutions. We find that the NMR Af1503 HAMP\cite{12,5} is a reasonable structural template for Tsr-HAMP while the crystal structure of HAMP2 from Aer2 receptor is not. Moreover, Tsr-HAMP is indeed dynamic. Both of these findings are consistent with the following (I) HAMP structures are particularly difficult to obtain because of its flexibility; (II) Previous mutational evidence underlying the dynamic bundle model already indicated that Af1503 might be the only functionally relevant conformation for Tsr-HAMP. Thus these findings support two elements of the dynamic bundle model (I) high (tunable) flexibility of Tsr-HAMP; (II) Tsr-HAMP fluctuates around a Af1503-like structure\cite{27}. 

5.4.2 The biphasic diagram of the extended dynamic bundle model

Our results of the L256A and I229A mutation confirm the destabilizing effects of mutations on the Tsr-HAMP bundle. Moreover, the I229A mutation appears more destabilizing than the L256A mutation, since the former disrupts helicity while the latter does not. Interestingly, in the extended dynamic bundle model, L256A is predicted to exhibit an intermediate degree of flexibility and to sit on the interface between the normal signaling regime and the reversed signaling regime, while I229A is predicted to be much more flexible than L256A and sits in the reversed signaling regime. If one assumes that the dynamics of the isolated Tsr-HAMP in our simulation setup properly represents the dynamics of the Tsr-HAMP during signaling, our results appear consistent with the prediction of the biphasic diagram of the extended dynamic bundle model.

5.4.3 Predicting other mutational effects on Tsr-HAMP

Our simulations on the wild type Tsr-HAMP (EcTsr_AF) provide important insights how mutations[2, 27, 28] might change the dynamical properties of HAMP. Tsr-HAMP with the wild type sequence is likely to represent the functional motions exhibited during Tsr signaling. Although Tsr-HAMP is very flexible and largely non-helical in other regions, it does remain relatively helical in the region between L1 and L3. Proline mutations replacing residues between L1 and L3 will disrupt the helicity in this region, indicating that helical conformation of L1-L3 is essential for activation of the kinase. Indeed, mutants showing the CCW-locked phenotype mostly occur on the N-terminal residues of the bundle[28].

The fact that Tsr-HAMP is helical between L3 and L5 in C1/C2 seem consistent with the prediction by Zhou et al that the helicity of the AS2c residues (256-364) might be a property adjustable by the output module during Tsr signaling[27]. In wild-type Tsr-HAMP, there are two stable helical hydrogen bonds ($P_{hhb}^n > 0.7$, see FIG. 5.5C) and several moderately stable bonds (0.3 $< P_{hhb}^n < 0.7$) between L3 and L4. Proline mutations on these residues modify the helicity pattern of this region, and could bias the kinase activity.

The layer geometry of L4 in Tsr-HAMP is different from Af1503. Given the special geometrical arrangement of the Met259 residues, one speculates that two larger (longer) bulky Phenylalanine sidechains at this position (see FIG. 5.4) could have more restraints on each other and thus enhance the flexibility of the side-chains of Ile232 and Ala233. Oppositely, if one mutates Gln260 into Phenylalanine, one adds bulky hydrophobic sidechains surrounding the existing hydrophobic sidechains in L4 and should form a larger hydrophobic core and reduce the flexibility of L4. Indeed, the M259F mutant exhibit CCW-biased phenotype while the Q260F mutant has the CW-locked phenotype[28].

5.5 Conclusion

Using all-atom molecular dynamics simulations, we have characterized the possible solution conformations of the Tsr-HAMP. We found that Tsr-HAMP is dynamic, fluctuating among conformations very similar to the Af1503 NMR structure. Tsr-HAMP has less helical character on the two terminal regions and mild changes in the packing of the hydrophobic residues. The HAMP2 crystal from Aer2 receptor is not a stable solution conformation for Tsr-HAMP. In addition, two mutations L256A and I229A both destabilize the HAMP bundle. The I229A
mutation is more destabilizing than the L256A mutation. These results support the extended dynamic bundle model[25].

5.6 Appendix - the Dynamic Bundle Model

The model was based on results from a large-scale mutagenesis study of the HAMP domain of the chemotaxis receptor Tsr in *E. coli*[27]. Obtaining an atomic-resolution HAMP structure of Tsr has been unsuccessful due to the high intrinsic flexibility of HAMP domains in chemoreceptors, including the Tsr receptor. The prototype structure Af1503 [12] is a unusually stable HAMP structure.

Mutation of the connector residues of the *E. coli* Tsr-HAMP[2], Zhou et al. identified three critical connector residues Gly235, Leu237 and Ile241, for which most replacements destroyed Tsr function. The region around Gly245 tolerated deletion of 1 residue and insertions of up to 10 glycines, suggesting that this region is a non-specific flexible linker. These results are consistent with the structure of the prototype Af1503 HAMP[12]: Gly235 is part of a critical turn at the C-terminal end of the N-terminal helices; Leu237 and Ile241 are residues located in L5 and L3 respectively, see FIG. 5.2.

The consistency of Tsr-HAMP with the Af1503 structure was further strengthened by a subsequent mutational study on all residues of the helices[27]. Among the total ∼370 mutations, Zhou et al obtained 131 null mutations (loss of function) of Tsr-HAMP, 51 were made on the N-terminal helices (N1 and N2) and 80 were made on the C-terminal helices (C1 and C2). This study had two major findings. Firstly, 6 residues in each helix were identified as functionally critical, because the majority of mutations on these residues produced a complete loss-of-function phenotype. These critical residues are Leu218, Met222, Leu225, Ile229, Ile232, Ala232 in N1 and Glu248, Met249, Leu252, Leu256, Met259, Leu263 in C1 and C2. Except for Leu218 and Glu248, these residues all correspond to the layer residues at the interface of the HAMP bundle, when modeling the Tsr-HAMP sequence on that of the AF1503 HAMP (see FIG. 5.2 and TAB. 5.1). Secondly, Zhou et al found that all residues on the C-terminus of the C-terminal helices (AS2c) (residues 256-264) are very sensitive to proline replacements. In contrast, no non-critical residues position in other parts of HAMP are sensitive to proline replacements. This sensitivity to proline replacements seems to indicate the importance of maintaining a helical conformation at AS2c. Also this AS2c region between the C-terminal helices and the output kinase control module has a discontinuity in the heptad repeat, equivalent to an insertion of four residues into (or removal of three residues from) a regular heptad repeat. This discontinuity, referred to as a “stutter” [3, 15], is known to destabilize the coiled-coil pairing interactions[3]. These observations led the authors to propose a dependence of signal output control on the packing stability of HAMP-AS2c.

Since the mutational evidence points at only one functionally important conformation, Zhou et al argued that the mechanism of HAMP signaling is based on the packing stability of the bundle, rather than adopting different stable conformational states. The most stable HAMP conformation could be the Af1503 NMR conformation. The least stable would be a fully denatured bundle. HAMP domains operate within some region of this dynamic range, oscillating between bundle conformations of different stabilities. Later, another study that performed mutagenesis on the control cable between the transmembrane helices and Tsr-HAMP revealed that helicity of the control cable is not a requirement for proper Tsr
functioning\cite{14}. This work added credibility to the dynamic bundle model, because it sug-
ests that the input motions from the sensor domain are unlikely to induce simple rigid body
motions in the N-terminal helix of Tsr-HAMP.

In subsequent works\cite{28}, Zhou et al further analyzed the functional defects of 118 null
mutations of Tsr-HAMP obtained in Ref\cite{2,27} and extended the dynamic bundle model.
The mutants were grouped into 6 categories according to their phenotypes: (I) attractant-
mimic (unbiased), the mutants have minor functional defects; (II) CCW-locked, the mutated
chemoreceptors are irresponsible to environmental signals, the flagellar keep the default CCW
rotation and can not be switched into CW rotation; (III) CCW-biased, the mutants have
reduced ability to switch the flagellar into CW rotation; (IV) CW-biased, the mutants have
enhanced ability to switch the flagellar rotation; (V) CW-locked, the flagellar always rotates
CW; (VI) inverted, the mutated chemoreceptors behave oppositely to its normal behavior:
gradient of environmental signal molecules turn the flagellar from CW to CCW rotation.
Phenotype (I) only appear for mutations on two residues Pro221 and Glu248, which are
residues around $L_1$. Phenotype (II) and (III) were exhibited by any mutations on residues
at $L_3$ of N-terminal helices (Ile229) and $L_1$, $L_2$ (Met222, Met249, Leu225, Leu252, Leu253).
Phenotype (IV) and (V) were shown by any replacements of residues at $L_4$ of N-terminal
helices (Ala233), $L_3−L_5$ of C-terminal helices (Leu256, Met259, Glu262) and the connector
residue in $L_3$ (Ile241). The authors then attempted to relate these phenotypes to speculated
degrees of destabilization of the HAMP bundle by the mutations\cite{28} and proposed a biphasic
stability diagram of the HAMP bundle consisting of two regimes: the normal regime and
the reversed regime. The wild-type Tsr-HAMP lies in the normal regime, in which enhanced
packing stability of HAMP enhances CCW flagellar rotation while destabilizing the packing
of HAMP up to a certain extend encourages CW flagellar rotation. Once Tsr-HAMP is
over-destabilized, it enters the reversed regime, in which a very unstable HAMP promotes
CCW flagellar rotation while a less unstable HAMP enhances CW rotation (see Figure 7 of
Ref\cite{28}).
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Bibliography


