Heme-based Oxygen Sensor

FixL: Heme-bound PAS domain
Heme-based gas sensor protein

Signal (O₂, CO, NO)

Heme

Sensor domain (Heme)

Functional domain

Protein structural change

Ec DOS, FixL, HemAT, sGC, CooA

Heme

Sensor domain (Gas-bound heme)

Functional domain

Regulation of catalysis and transcription
Heme-bound domain

- PAS domain

-(GAF domain)

-Globin domain
Figure 3: Typical protein structures of the heme-bound PAS (A), globin (B) and GAF (C) domains. The heme iron complex is bound to these domains, forming the gas-sensing site. FixL, EcDOS, AxPDEA1 and Aer2 are heme-based oxygen sensors containing a heme-bound PAS domain.
FIGURE 1. Alignment of heme-bound oxygen-sensing and functional domains of the heme-based oxygen sensors. Left, the C-terminal HK domain in the two-component system containing N-terminal heme-bound globin, PAS, or GAF domains. Right, the C-terminal functional domains associated with MCP, c-diGMP homeostasis (DGC, synthesis; and PDE, degradation), and unknown functions (transmembrane (TM)) containing the N-terminal heme-bound globin or PAS domain. GCSs are defined as chimeric proteins composed of heme-bound globin and functional domains (14–18). Note that EcDOS and FixL have two tandem PAS domains; the heme iron complex is bound to the first PAS domain in EcDOSand to the second PAS domain in FixL. Similarly, DevS and DosT have two tandem GAF domains; in both cases, the heme iron complex is bound to the first GAF domain. Note that globin folds are not always localized at the N terminus but are inserted into diverse regions, as predicted from the amino acid sequences of sensor globins that are yet to be characterized (14–18). AxpPDEA1, A. xylinum PDEA1.

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Mechanisms of ligand discrimination by heme proteins

Fig. 3 A Structure of the heme domain of BjFixL. The FG loop is shown in green. B Comparison of the structure of FG loop and conformation of Arg220 in the unliganded “on” (blue) and liganded “off” (tan) state [32, 33]

PAS Structure
Fruit basket structure
Hand structure
FIGURE 3. A, heme environmental structures of GCSs and sperm whale Mb. Left, the heme Fe(III)-CN complex of HemAT-Bs (Protein Data Bank code 1OR4) (22). Center, the heme Fe(III) complex of GsGCS (code 2W31) (19). Right, the heme Fe(II)-O₂ complex of sperm whale (SW)Mb (code 1A6M) (98). Note that HemAT-Bs and GsGCS are GCSs, whereas sperm whale Mb is a vertebrate globin. The color of the helix corresponds to helix notification in Fig. 3B. Distal B- and E- and proximal F-helices are shown in orange, green, and light blue, respectively. B, alignment of amino acid sequences of selected GCSs and sperm whale Mb with helix notification. Helix notification was based on sperm whale Mb and HemAT (14–16, 38, 98). The distal Tyr residue (B10), which binds to O₂ in the heme Fe(II)-O₂ complex, and the proximal His residue (F8) as a heme-binding site of GCS are shown in blue and red, respectively. The distal His residue (E7) interacting with O₂ in the heme Fe(II)-O₂ complex of sperm whale Mb is shown in green.
Fe (II) + O2 → Fe (II)$_{Globin-coupled Ec DOS}$

Fe (II) + O2 → Fe (II)$_{AxPDEA1 FixL DevS, DosT}$

Inactive Forms

Active Forms
Two-component signal transduction system: FixL

1. Signal
   - Fe (II)
   - O₂ dissociation

2. Autophosphorylation
   - Histidine kinase (HK)
   - Asp - P

3. Phosphotransfer
   - ATP → ADP

4. Output
   - Regulatory output
   - FixJ
   - Response regulator (RR)

Regulation of N₂ fixation
Figure 1. Domain organization and signaling in TCSs. (a) Basic system consisting of an RR composed of REC and effector domains, and a membrane-bound HK composed of a sensor domain, two transmembrane helices (TM1 and TM2) and the DHp and CA domains, showing the bound ATP and the phosphorylatable His (H) and Asp (D). The path of the phosphoryl group (symbolized by a \(~P\)) between ATP and His, and between the His and Asp in the phosphotransfer reaction is symbolized by red arrows. (b) A more complex phosphorelay system. The complexity arises from the incorporation of a cytosolic sensor domain (S_c) following the transmembrane helices, the addition of covalently bound REC and histidine phosphotransfer domain (HPt) following the CA domain, and the representation of potential ancillary connector proteins (triangles) docked at their target domains. When a domain is variable, the most common variants (either fold variants or functional variants) are listed together with the frequency of occurrence when such a frequency is known [3, 4] and [37].

Figure 4. Signal transduction model. The signal, acting on the sensor domain, induces subtle piston-like or rotation changes in a helix that connects this domain to the transmembrane helix 2 (TM2). Concomitant movements of TM2 transmit the signal to the cytoplasm, through TM2 coupling to DHp helix 1, either directly or by way of an interposed cytoplasmic sensor domain such as the HAMP domain. This results in rotation of the cytoplasmic helix, causing changes in DHp dimer packing, with coordinated displacement of the CA domains, which sit on these helices. In this way, the different relative disposition of CA and DHp are responsible of the three functional states (kinase, phosphotransferase, and phosphatase) involved in the signaling mechanism of TCSs.
Figure 2. Structural bases of partner recognition and specificity among TCSs. Crystal structures of the complexes (a) Spo0B–Spo0F (PDB: 2FTK), (b) ThkA–TrrA (PDB: 3A0R) and (c,d) HK853–RR468 (PDB: 3DGE). RRs are shown in cartoon representation, in different hues of green, whereas the HK or analog dimer is shown in Connolly surface representation, with one subunit colored salmon and the other blue, with CA domains in darker hues. The phosphorylatable His and Asp are shown, with C atoms in yellow, N atoms in blue, and O atoms in red. ThkA has an extra domain of the PAS type (white) in each subunit. In (d) the HK dimer and the RR that is closer to the reader are shown in cartoon representation, whereas the rear RR is in semi-transparent surface representation. (e–g) Detailed views of regions of interaction in the HK853–RR468 complex, showing amino acid side-chains and labeling structural elements that play important roles in the interactions. Colors are as for (d). (h) Scheme to exemplify the protein engineering studies of Laub and co-workers [14••] in which chimeric molecules were built from two different HKs to rewire specificity for the partner RR. The interaction was monitored as phosphotransfer to the RR.
Figure 3. Structural insight into the kinase, phosphotransfer and phosphatase reactions of TCSs. (a) CA-binding domain of DesK (PDB: 3EHG) with ATP bound. The ATP lid is colored magenta. (b) HK domain conformations considered to be competent for the kinase, phosphotransferase and phosphatase reactions in TCSs. One subunit is blue and the other white. DHp and CA domains are in cartoon and surface representations, respectively, DHp-CA linkers are shown as red strings, and the acceptor His and the nucleotide are illustrated and labeled. DesK structures correspond to PDB files 3GIE (top left), 3GIG (top right) and 3EHH (bottom right). HK853 is taken from the HK853-RR468 complex (PDB file 3GDE). (c) Models for trans- and cis-autophosphorylation occurring in DesK (atomic coordinates kindly supplied by A. Buschiazzo, I. Pasteur, Montevideo) [26] and HK853 [8*]. When both subunits are shown, one is colored salmon and the other blue. (d) Trans and cis autophosphorylation involve different dispositions of the CA domain, as illustrated with DesK (PDB file 3GIG) and HK853. These HKs are viewed from the cell membrane with helix 1 of the DHp domain placed in identical position. For clarity, only one CA domain is represented. Dotted arrows connect the nucleotide to its target His. (e) The different handedness of the connection between helices α1 and α2 in EnvZ, relative to HK853 and DesK, is revealed by the superimposition of the DHp domains of these HKs. Different hues of the same color are used for each subunit in each dimer (blue, HK853; green, DesK, PDB file 3EHH; violet, EnvZ, PDB file 1JOY). The view is along the twofold axis. The phosphoacceptor His residues are shown and labeled in HK853. (f, g) Active center for the phosphotransfer reaction in Spo0B/Spo0F (f) and YPD1/SLN1 (g) (PDB file 2R25). Each complex, with its two proteins in different colors, has one bound BeF$_3^-$ molecule. Black broken lines represent apical bonds that break or form as the reaction proceeds. (h) Active center for the phosphatase reaction in the HK853:RR468 complex. One sulfate found at this site and the BeF$_3^-$ observed in a RR468 crystal structure (PDB file 3GL9) are superimposed. One water molecule (W) sits over the O atom of the sulfate that is closest to the active His residue. The Nɛ atom of the His activates this water for attacking the phosphate (black broken lines are used to represent this activation as well as the apical bonds in the pentavalent phosphorus transition state). (i) The superimposition of the complex of the CheX phosphatase (blue) with CheY3 (pink) (PDB file 3HZH) with the reported HK853:RR468 complex supports the phosphatase nature of this latter complex. N99 of CheX resembles His260 of HK853, since it polarizes a water molecule (W) for attack on the phosphate group of CheY3 (mimicked by BeF$_3^-$). The broken lines in magenta in panels (f-i) correspond to Mg$^{2+}$ coordination bonds.

View high quality image (1970K)
Table 1 Influence of oxidation state and ligands on the activities of S. meliloti and B. japonicum FixLs at 23 C and pH 8.0

(A) Autophosphorylation (% h⁻¹)

<table>
<thead>
<tr>
<th></th>
<th>FeII</th>
<th>FeIII</th>
<th>Ratio</th>
</tr>
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<tbody>
<tr>
<td>RmFixLT</td>
<td>33</td>
<td>33</td>
<td>1.0</td>
</tr>
<tr>
<td>BjFixL</td>
<td>33</td>
<td>33</td>
<td>1.0</td>
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Turnover (h⁻¹)

<table>
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<tr>
<th></th>
<th>RmFixLT</th>
<th>BjFixL</th>
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<tbody>
<tr>
<td></td>
<td>12</td>
<td>0.12</td>
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<tr>
<td></td>
<td>26</td>
<td>24</td>
<td>1.1</td>
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(B) Inhibition factors for turnover

<table>
<thead>
<tr>
<th></th>
<th>RmFixLT</th>
<th>BjFixL</th>
<th>R220A BjFixL</th>
</tr>
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<tbody>
<tr>
<td>Oxygen</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>–</td>
</tr>
<tr>
<td>Imidazole</td>
<td>&gt;260</td>
<td>4.7</td>
<td>10</td>
</tr>
<tr>
<td>Cyanide</td>
<td>2</td>
<td>47</td>
<td>1.7</td>
</tr>
<tr>
<td>Carbon monoxide</td>
<td>2.7</td>
<td>3.2</td>
<td>1.1</td>
</tr>
<tr>
<td>Nitric oxide</td>
<td>2.3</td>
<td>1.9</td>
<td>0.90</td>
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</table>

(A) The effect of oxidation state on the autophosphorylation and turnover activities.
(B) The inhibition of turnover caused by binding of heme ligands [7,40].

The turnover number represents the molecules of FixJ phosphorylated by one molecule of FixL in 1 h. The inhibition factors are computed by dividing the activity of the unliganded form (met or deoxy) by the activity of the same oxidation state bound to ligand. The activities of liganded forms were measured for protein more than 99% saturated with ligand, except for O₂. Since it is not feasible to saturate RmFixLT and BjFixL completely with O₂, for these FixLs the O₂ inhibition factors are conservative estimates based on subtracting the contribution of the deoxy fraction; actual inhibition factors may be much higher. For R220A BjFixL, the O₂ inhibition factor at standard temperature and pressure was not measurable because of the extraordinarily low affinity of this protein.
Fig. 7. Conformational switching in the BjFixL heme-binding PAS domain. In deoxy-BjFixLH (a) the Gb-2 arginine (Arg 220) on the distal side of the heme forms a hydrogen bond to the heme propionate 7, and the Fa9 arginine (Arg 206) on the proximal side interacts with the FG loop [41]. In oxy-BjFixLH (b), the Gb-2 and Fa9 arginines switch their hydrogen-bonding interactions to the bound O2 and the heme propionate 6, respectively [41]. The structures of the met and cyanomet forms closely resemble the deoxy and oxy forms, respectively [26,41].
Figure 2 Ribbons and ball-and-stick model diagrams of the BjFixLH heme-binding pocket for (A) met-BjFixLH, (B) deoxy-BjFixLH, (C) CO-BjFixLH, (D) NO-BjFixLH, (E) oxy-BjFixLH, (F) the revised cocrystallized cyanomet-BjFixLH, (G) imidazole-BjFixLH, and (H) MeIm-BjFixLH. W1 represents water. The dash lines show the hydrogen bonding or the salt-bridge interactions. Biochemistry 41, 12952 (2002) Structure-based mechanism of O2 sensing and ligand discrimination by the FixL heme domain
O2 affinities of FixL, *Ec* DOS, YddV, AfGcHK and Sperm Whale Myoglobin

<table>
<thead>
<tr>
<th>O2 sensor</th>
<th>$K_d$ (µM)</th>
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<tr>
<td>FixL</td>
<td>29 - 140</td>
</tr>
<tr>
<td><em>Ec</em> DOS</td>
<td>74 - 340</td>
</tr>
<tr>
<td>YddV</td>
<td>14</td>
</tr>
<tr>
<td>AfGcHK</td>
<td>0.077, 0.67</td>
</tr>
<tr>
<td>Sperm Whale Myoglobin</td>
<td>0.88</td>
</tr>
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Therefore, O2 affinities of some heme-based O2 sensors are lower than myoglobin.