Metal-containing PAS/GAF domains in bacterial sensors

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Highlights

- PAS/GAF domains bind disparate metal cofactors
- Metal can be the signal sensed by the PAS/GAF sensor
- O_2 and redox potential are the favorite signaling stimuli of metal-binding PAS/GAF
- Metal-binding induces conformational changes that modulate the effector activity

Abstract

PAS and related GAF are ubiquitous and highly versatile sensing domains that associate a range of signaling effectors. Each year, new bacterial PAS and GAF domains are characterized but genomes analysis suggests this list is far from complete.

This review aims to synthesize the current state of knowledge on the structure and activity of metal-binding PAS/GAF domain proteins. PAS/GAF domains can bind disparate metal cofactors with very precise coordination and high specificity concomitant with their extensive sequence diversity. Bound-metal can directly be the signal or can mediate signal detection such as gases or redox variation. Metal-containing PAS/GAF input modules are mainly associated to histidine kinases of two-component signaling systems and σ^{54}-dependent transcription factors. In both cases, metal-binding induces conformational changes that modulate the effector activity.

It appears that metal-containing PAS/GAF domains, as other PAS/GAF domains, offer great possibilities for coupling a broad range of ligand binding to various cellular responses and it is very likely that they have not yet revealed all their secrets.

Contents

1. Introduction

To survive, living organisms must adapt to new environments or to changes in their current environment. Bacteria actively sense and adapt to environmental cues through sensory and signal transduction systems. Per-ARNT-Sim (PAS) and cGMP-specific phosphodiesterases, adenylyl cyclases and Fhla (GAF) domains are common components of signal transduction systems serving as universal signal sensors and interaction hubs [1-4]. Both PAS and GAF domains are composed of ~ 110 amino acids with limited primary sequence homology but share a remarkably conserved core structure [3-6]. Although PAS and GAF domains are distinguished in the sequence-based annotations in the Pfam database [5], they are linked by evolution and are structurally and functionally related [1,3,6]. Hence, they are considered together in this review. PAS/GAF occur in all living organisms but are most highly represented among prokaryotic genomes, with 90% of PAS domains identified in Eubacteria [2,7]. For example, the deltaproteobacteria *Desulfovibrio* contains the largest number of PAS domain proteins (141) found in a genome [7]. PAS/GAF-containing proteins can contain both PAS and GAF domains or several PAS/GAF domains but are commonly associated with a wide range of enzymatic and non-enzymatic functions [2,4]. PAS/GAF proteins play roles as receptors, signal transduction mediators and transcription factors [4]. In bacteria and archaea, PAS/GAF clusters are mostly involved in two-component systems such as histidine kinases, but they have been identified in other proteins, such as serine/threonine kinases, guanylate cyclases, phosphodiesterases, ion channels, chemotaxis proteins and transcription factors [3-5].

PAS/GAF domains usually serve as versatile sensors and interaction modules in signal transduction proteins. These domains mainly exert their physiological roles without cofactors but some of them have been shown to bind cofactors to regulate the activity of certain effector domains [2,3,8]. Hence, cofactors such as hemes, flavins, di-and tricarboxylic acids, amino acids, divalent metal cations, coumaric acid, fatty acids, non-heme iron and Fe-S clusters can be found in PAS/GAF domains [2]. These cofactors, either covalently or not covalently bound to the sensory module, can emit a signal to which the proteins directly respond or mediate signal detection. In all cases, the signal is propagated to the effector domain by mechanisms and strategies that diverge according to the PAS/GAF module [2,3,8].

Predictions of the functions of PAS/GAF domain proteins based on sequence and signal transduction information remain challenging. Comparison of structurally and functionally characterized PAS and GAF domain-containing proteins known to bind ligands will help to

identify the functions/activities of PAS/GAF proteins that have not yet been characterized. Henry and Crosson in 2011 [2] and Unden et al in 2013 [8] provided overviews of the PAS/GAF signaling protein diversity in prokaryotes. This review aims to synthesize the current state of knowledge on the structures and activities of these proteins, focusing on metal-binding PAS/GAF domains associated with two-component signal transduction systems (TCS) and σ^{54}-dependent transcriptional regulators proteins (Figure 1).

2. Structural basis of metal-binding in the PAS and GAF domain family proteins

The PAS domain is composed of five β-strands and four α-helices organized in four main structural elements: the PAS core, the β-scaffold, the helical connector and the N-terminal cap (Figure 2A) [4]. Despite the possibility of an additional antiparallel β-sheet in the GAF domain, the GAF core itself is small and folds similar to the PAS core [3]. By way of example, Photoactive Yellow Protein, which entirely consists of a single PAS domain, is closely superimposable with the GAF domain of the YGK9 protein over a region that comprises the beta-sheet and the irregular layer (Figure 2B) [2].

PAS/GAF domains can bind a large variety of metallic ligands, including metal-containing cofactors such as heme, Fe-S clusters, and divalent metal cations, which contribute to the determination of the input signal [2].

2.1 Divalent metal cation-binding PAS/GAF domain

Non-heme iron is found in the GAF domain of NorR, a bacterial enhancer-binding protein (bEBP) of the AAA+ class of proteins that activates the σ^{54}-dependent transcription of *norVW* in response to NO [9]. EPR spectroscopy has shown that the N-terminal GAF domain of NorR contains a non-heme iron center that binds NO to form a high-spin $\{Fe(NO)\}^7$ (S=3/2) complex [10]. A model with the hexa-coordination of Fe involving three aspartates, one of which being bidentate, one cysteine and one arginine residue, has been proposed; the arginine residue is displaced to form the mononitrosyl iron complex (Figure 3) [11].

Other cations, such as Mg^{2+}, Ca^{2+} or Zn^{2+}, are cofactors of PAS domain-containing proteins. Crystal structures of the *Escherichia coli* and *Salmonella typhimurium* periplasmic sensor domains of PhoQ reveal that these structures fold into a PAS domain but with some striking differences, as the insertion of α-helices creates a large negatively charged area that is partially involved in the binding of divalent cations such as Mg^{2+} or Ca^{2+} (Figure 4) [12,13]. In the

presence of Mg^{2+}/Ca^{2+}, a bridge between the negatively charged PhoQ surface and negatively charged bacterial phospholipids can form; upon release of the divalent cations from the PhoQ sensor domain, repulsion between the negatively charged PhoQ surface and the membrane can result in a change in their orientation relative to the membrane. This change corresponds to the initial event in transition from a repressed to an active PhoQ conformation [12]. In the PAS domain of the phosphoglycerate kinase in *Leishmania major*, it has been shown that a single histidine residue is essential for Mg^{2+} binding in the absence of substrate; repression of PGK activity is performed by the Mg^{2+} cation bridge formed between the PAS and PGK domains [14]. Recently, the structure of the PAS domain of the WalK sensor from *Staphylococcus aureus* was resolved [15]. The structure revealed the presence of a Zn^{2+} ion bound to the surface of the PAS domain (Figure 5A). This feature enables Zn^{2+} in the surrounding solvent to access the binding site. The Zn^{2+} ion adopts a slightly distorted tetrahedral coordination geometry by binding to two Nδ1 atoms from two histidine residues, one Oδ1 atom from an aspartate residue and one Oε3 atom from a glutamate residue (Figure 5B). Structural and molecular dynamics simulations of WalK suggested that Zn^{2+} binding directly influences the relative positioning of the cytoplasmic PAS and CAT domains; upon Zn^{2+} binding the dihedral angle between the two domains varied from 136° to 175° and the average distance between the upper and lower helices of the cytoplasmic domain decreased from 21.6 Å to 12.3Å (Figure 5C, [15]). These large conformational changes, induced by Zn^{2+} binding, would regulate the activity of the protein [15].

2.2 Heme-binding PAS domain

Heme cofactor is found in several classes of PAS/GAF domain-containing proteins, including FixL, DosP, Aer2, DosS/DevS [16-19]. Among the heme-containing PAS domains, FixL has been the most extensively studied. The three-dimensional structure of the FixL PAS domain in *Rhizobium meliloti* and *Bradyrhizobium japonicum* revealed that the heme iron is pentacoordinated by a histidine residue, with no other ligand coordinating the sixth position (Figure 6A and 6B) [16,20]. While the porphyrin ring is inserted into a hydrophobic pocket, the ligand binding site remains fairly accessible through an entryway marked by three water molecules that interact with an arginine residue [16]. Oxygen and CO have been shown to bind Fe(II) while CN^- binds Fe(III) on *Rhizobium meliloti* FixL; it has been proposed that the negative shift of the midpoint redox potential observed upon fixation of O_2, CN^- and imidazole is the consequence of a combination of effects including ligand fixation, heme distortion and ionic interactions of the propionate groups [21]. Structural studies of the oxygen-sensing

domain of *B. japonicum* FixL have helped to address important issues relevant to the sensing mechanism [22]. Comparison of the crystal structures of FixL heme domain in its ligand-free and oxygen-bound forms reveals that the binding of dioxygen to the heme iron alters the nonplanarity of the heme, resulting in shifts in the positions of the two heme propionates [22]. The movement of the propionate side chains is, in turn, transmitted to the polypeptide via the rearrangements of salt bridges with arginine and histidine residue side chains, affecting the FG loop that is stabilized in an alternative conformation that lies farther from the heme (Figure 6C). These conformational changes then lead to signal transduction [20,22].

Contrary that observed in FixL, it has been shown that the heme domain of DosP contains a low-spin heme iron hexacoordinated with a sulfur atom of a methionine residue at the sixth position. This iron coordination is displaced upon O_2 binding, leading to the output signal [17]. In the sensor protein DevS from *Mycobacterium tuberculosis*, the ferrous state of the heme Fe in the GAF domain is a typical pentacoordinated Fe(II), while the ferric state is hexacoordinated containing a water molecule ligand at the sixth ligand [19]. Spectro-electrochemical analysis demonstrated that, in contrast to that to FixL, the binding of O_2 to DevS does not change the heme planarity or the propionate interactions with the protein moiety [23].

2.3 Fe-S cluster -containing PAS domains

Fe-S clusters are composed of iron ions and sulfide and are mainly coordinated to the protein moiety via cysteine thiol side chains. These clusters are found in a variety of proteins, including O_2 sensors containing PAS and GAF domains. The sensor kinase NreB from *Staphylococcus carnosus* binds a $[4Fe\text{-}4S]^{2+}$ cluster in a PAS domain liganded by four cysteine residues. Upon reaction with air, the cluster is first degraded to $[2Fe\text{-}2S]^{2+}$ and later to Fe hydroxide [24] in a reaction sequence similar to that of the O_2-sensitive transcriptional regulator FNR [25]. As for FNR, it can be hypothesized that the released sulfide ions are oxidized to sulfane and form a persulfide with two of the cysteine ligands of the cluster, allowing sulfur storage and reversion to a $[4Fe\text{-}4S]^{2+}$ form cluster in NreB by reduction and repair without involvement of iron-sulfur biosynthesis machinery [26,27]. The sensor kinase AirS of the two-component system AirS-AirR from *Staphylococcus aureus* binds a redox-active [2Fe-2S] cluster critical for kinase activity in a GAF domain liganded by four cysteine residues organized in the consensus motif $C\text{-}X_7\text{-}C\text{-}X\text{-}C\text{-}X_{17}\text{-}C$. In the $[2Fe\text{-}2S]^{2+}$ form oxidized by O_2, AirS is fully active, while the reduced form $[2Fe\text{-}2S]^{1+}$ under anaerobic conditions has minimal kinase activity. Destruction of the cluster by stronger oxidizing agents such as H_2O_2 abolishes kinase activity [28]. Both NreB and AirS bind Fe-S clusters in a PAS or GAF domain for oxygen sensing but their

responses to O_2 differ: NreB responds by $[4Fe\text{-}4S]^{2+}/[2Fe\text{-}2S]^{2+}$ conversion while AirS responds by $[2Fe\text{-}2S]^{2+}/[2Fe\text{-}2S]^{1+}$ oxidation.

Recently, a new Fe-S redox regulator OrpR, belonging to the σ^{54}-dependent transcriptional regulator family, was characterized from the anaerobic sulfate reducer *Desulfovibrio vulgaris* Hildenborough [29]. OrpR contains a PAS domain that binds a $[4Fe\text{-}4S]^{2+}$ cluster under anaerobic conditions. Site-directed mutagenesis demonstrated that three conserved cysteine residues in the PAS domain included in the sequence $C\text{-}X_8\text{-}C\text{-}X_3\text{-}C$ were ligands of the Fe-S cluster (Figure 7). The cluster is converted to $[3Fe\text{-}4S]^{1+}$ and Fe^{3+} under mild oxidative conditions, while it is dissembled by O_2. However, both $[4Fe\text{-}4S]^{2+}$ and $[3Fe\text{-}4S]^{1+}$ redox states of OrpR are able to bind DNA with no significantly different affinity [29].

3. Activity, signal sensing and signal transduction of metal-containing PAS/GAF bacterial sensors

How do these disparate metal-binding PAS and GAF sensor units regulate the activity of effector domains? This question is addressed in the second part of the review focusing on metal-containing PAS/GAF domains included in TCS and σ^{54}-dependent transcriptional regulators.

3.1. Two-component systems with input metallic PAS/GAF domains

TCS systems are the main prokaryote actors sensing environmental changes using histidine kinase (HK) sensing domain (Figure 1A) and a cognate response regulator domain (RR) to mediate the cellular response. The sensing module of HK, which is commonly located in the periplasmic or cytoplasmic spaces, modulates the enzymatic activity of the cytoplasmic effector modules (DHp and CA) via the central HAMP domain (Figure 1A). Except for the PhoQ/PhoP TCS, for which divalent cations such as Mg^{2+} serve as direct signals, other TCSs studied to date exhibit b- and c-type hemes, non-heme iron and iron-sulfur metal cofactors bound to the PAS/GAF domain to sense the signal and to mediates it to the output domain to modulate the activity of the protein. In these disparate sensors, O_2 seems to be the main signal perceived by the metal to activate or inactivate the phosphotransferase activity of HK. The state-of-the-art PAS/GAF metal-containing HK sensors are described below, on the basis of the HK activity in the presence of O_2.

3.1.1 Repression of TCS metal-containing PAS/GAF bacterial sensors by O_2

FixL/FixJ, NreB/NreC and DosS/DosT are cytosolic oxygen-sensing TCSs isolated from *Rhizobium*, Firrmicutes species and *Mycobacterium tuberculosis*, respectively [24,30,31].

FixL is the sensing domain of FixL/FixJ TCS conserved in α-proteobacteria that consists of two N-terminal tandem PAS domains and a C-terminal HK domain such as in *Bradyrhizobium japonicum* [16,32]. In the presence of O_2, O_2 is bound to the b-type heme of the second PAS domain, leading to heme distortion (Figure 6C) that turns off the HK activity [16]. Under hypoxia conditions, O_2 dissociates from the heme, inducing a local conformational change that leads to the autophosphorylation of the HK module and the transfer of phosphate from ATP to the RR FixJ which, in turn, stimulates the expression of target genes (Figure 8) [32].

NreB is an O_2 sensor kinase characterized in *Staphylococcus carnosus* and *Staphylococcus aureus* that is composed of an N-terminal PAS domain and an HK domain [8,24,33]. Under anoxic conditions, the PAS domain is in the form of $[4Fe\text{-}4S]^{2+}$, a state in which NreB has high autokinase activity resulting in RR NreC phosphorylation and target gene transcription (Figure 8) [34]. In the presence of O_2, $[4Fe\text{-}4S]^{2+}$ is converted into $[2Fe\text{-}2S]^{2+}$, leading to a decrease in autokinase activity causing low levels of NreC phosphorylation and gene activation (Figure 8) [24,35]. In parallel and in the absence of nitrate, the GAF-containing domain NreA converts the kinase activity of NreB into phosphatase activity that inactivates NreC; however, no cofactor has been described in association with the GAF domain of NreA [36].

DevS and DosT are two cognate O_2 sensor kinases of the DevR transcriptional regulator [37,38]. Two N-terminal GAF domains in tandem are present in both sensors in *M. tuberculosis*. Upon binding of O_2 to the heme-containing GAF module, kinase activity is ceased because of a decrease in kinase activity concomitant with phosphatase-dominant forms of DosT and DevS (Figure 8) [23,39-41]. When the O_2 concentration starts to decrease, DosT first promotes DosR phosphorylation and the transcription of its regulon [23,42]; at lower O_2 concentrations, DosT also activates histidine kinase activity and makes DevR fully phosphorylated. Hence, DosT and DevS work in a two-step adaptation mechanism for hypoxia [23]. NO and CO are also able to activate kinase autophosphorylation [43-45]. In the PAS/GAF metal-containing sensors FixL, NreB, DevS and DosT, the kinase activity is then switched off in the presence of O_2 and is switched on by hypoxia (Figure 8).

3.1.2 Activation of TCS metal-containing PAS/GAF bacterial sensors by O_2

In contrast, three other TCS/PAS metal-containing sensors, AirS/AirR, Aer2 and DosP have been shown to be activated in presence of O_2.

AirS/AirR from *Staphylococcus aureus* is a cytosolic oxygen-sensing TCS containing the sensing domain AirS, which is composed of a GAF domain associated with an HK module (Figure 9). Under anaerobic conditions the $[2Fe\text{-}2S]^{+}$ cluster form in the GAF domain of AirS switch off kinase activity [28]. Under oxidative conditions (in the presence of O_2, NO or CO), AirS responds to oxidation by converting the $[2Fe\text{-}2S]^{+}$ in a $[2Fe\text{-}2S]^{2+}$ cluster, a form fully active for phosphorylation and the expression of target genes of the RR AirR (Figure 9) [28]. However, prolonged oxidation of AirS leads to the apoform of AirS and results in the loss of kinase activity [8,28].

Aer2 is a TCS chemoreceptor combining one (*Pseudomonas aeruginosa*) or two PAS domains (*Vibrio cholerae*) with a poly-HAMP (histidine kinase-adenylyl cyclase-methyl-accepting chemotaxis protein-phosphatase) module [18,46]. The PAS (PAS2 in *V. cholerae*) domain coordinates a b-type heme (Figure 9). In the absence of O_2, the kinase is switched off, whereas the presence of the ligand switches on the kinase, inducing the phosphorylation and activation of chemosensory proteins (Figure 9). The activation is linked to the association of O_2 with the heme group, generating a conformation signal in the PAS domain that is transmitted to the kinase control module [18,46-49].

Last, DosP is a b-type heme O_2-sensing phosphodiesterase in *Escherichia coli* that catalyzes the conversion of cyclic-di-GMP to linear di-GMP (Figure 9). Heme b is bound to the N-terminal PAS domain associated with the C-terminal phosphodiesterase catalytic domain. As described above, the association of the ligand (O_2, CO and NO) with heme displaces the iron coordination and induces an increase in the rigidity of the protein structure, leading to signal transduction to the phosphodiesterase domain which is switched on [50-52].

Hence, in PAS/GAF metal-containing sensors AirS, Aer2 and DosP, activities of the catalytic domain are switched on in the presence of O_2 (Figure 9).

3.1.3 Repression of TCS-containing PAS/GAF bacterial sensors by direct metal-sensing.

The TCSs described above exhibit b- and c-type hemes, non-heme iron and iron-sulfur metal cofactors bound to the PAS/GAF domain to sense the O_2 signal PAS/GAF domain. TCS, PhoQ/PhoP and WalK/WalR are two PAS containing TCSs in which metal serves as a direct signal.

PhoQ is a transmembrane sensor kinase, found in enterobacteria, composed of a periplasmic PAS domain and a C-terminal catalytic HK domain located in the cytoplasm (Figure 10). Under physiological conditions, Mg^{2+} is bound to the PAS domain, dephosphorylates its cognate RR PhoP resulting in the inactivation of PhoQ [53]. In Mg^{2+}-limited conditions, the metal

dissociates from the PAS domain, and a reorientation of PhoQ relative to the membrane is observed. This change corresponds to the activation of PhoP in the cytoplasm through a classic phosphotransfer pathway in these TCSs (Figure 10) [53,54]. Other divalent metal ligands such as Ni^{2+}, Mn^{2+} and Ca^{2+}, when associated to PhoQ, can also inactivate the kinase [55].

WalKR regulates peptidoglycan synthesis and is essential in *Staphylococcus aureus* [56]. WalK, the HK sensor, comprises two N-terminal tandem PAS domains and a C -terminal HK domain. One PAS is located in the extracellular space, and another is located in the cytoplasm (Figure 10) [57]. Cytoplasmic PAS has recently been shown to bind Zn^{2+}, which directly influences the activation status of the protein [15]. In the absence of Zn^{2+} the autophosphorylation of WalK is favored, which leads to phosphotransfer to the RR WalR and binding of the RR on its target sequences. In the presence of Zn^{2+}, cation binding influences the positioning between the PAS and HK domains (Figure 5C), a conformation that decreases the autophosphorylation of WalK [15].

3.2 σ^{54}-Dependent activators with input metallic GAF/PAS domains

σ^{54}-dependent activators control σ^{54}-dependent transcription in response to environmental signals. These activator proteins generally consist of three domains: an N-terminal regulatory domain that senses the signal, a central AAA^+ domain responsible for ATP hydrolysis and a C-terminal DNA-binding domain (Figure 1B). Three σ^{54}-dependent activators contain a PAS/GAF sensory domain, NorR, AdhR and OrpR.

Binding of NO to the non-heme iron of the NorR activator from *E. coli* stimulates the ATPase activity of the central domain of NorR, enabling the activation of transcription by σ^{54}-RNA polymerase (Figure 11) [10]. Prior to NO activation, the GAF domain is located above the AAA+ domain preventing ATP hydrolysis [58]. Upon activation, a mononitrosyl iron complex is formed, and the GAF domain relocates downwards to the periphery of the AAA+ domain, making the AAA+ domain accessible for ATP hydrolysis and σ^{54} RNA polymerase (Figure 11) [10,58].

The AdhR activator from *Clostridium beijerinckii* also possesses a non-heme iron center within its N-terminal GAF domain (Figure 11). Under oxidative stress, the tandem GAF and PAS domains repress the accessibility of the AAA+ domain in AdhR [59]. Binding of Fe^{2+} in anaerobic conditions relieves the repression of AAA+ and stimulates ATPase activity allowing AdhR to stimulate transcription (Figure 11) [59].

Lastly characterized, the OrpR activator in *Desulfovibrio vulgaris* Hildenborough contains a $[4Fe\text{-}4S]^{2+}$ cluster in its N-terminal PAS domain (Figure 11). In anaerobiosis OrpR is activated

and stimulates the OrpR regulon [29,60,61]. Increasing the redox potential turns off the OrpR activity via the redox-sensitive $[4Fe\text{-}4S]^{2+}$ in the PAS domain (Figure 11) [29]. The PAS domain negatively regulates the activity of OrpR under oxidative conditions, but its role on the AAA^{+} domain remains to be discovered [29].

4. Conclusion

Despite their invariant structure, the PAS/GAF domains bind disparate metal cofactors with precise coordination and high specificity concomitant with their extensive sequence diversity. O_2 and redox potential seem to be the favorite signaling stimuli of these sensors, but gases such as NO or CO can also mediate their induction. Stimuli induce subtle conformational changes concentrated in cofactor-binding sites, allowing signal transduction to the effector output modules and leading to cellular response.

PAS/GAF sensors can regulate the activities of very different effector domains through either their redox state or their structural change upon ligand binding, which induces conformational changes. The metal-containing PAS/GAF domains characterized to date are coupled to histidine kinases of two-component signaling systems and σ^{54}-dependent transcription factors. In both cases, signal-induced conformational changes are necessary to transduce the signal to the effector domains. Even if these conformational changes are protein-dependent, the mechanism adopted to regulate the activity of the output domain seems to be completely correlated with the class of effector domain. In HK, the different structural conformations of the cofactor pocket linked to the absence or presence of a ligand modulate the phosphorylation state of HK and its activity. In σ^{54}-dependent activators, signal-induced structural conformation changes in PAS/GAF domains have been shown to impact the accessibility of the AAA+ domain for ATP hydrolysis and σ^{54} RNA polymerase.

Metal-containing PAS/GAS domains, as other PAS/GAF domains, offer great possibilities for coupling a broad range of ligand binding to various cellular responses, and it is very likely that they have not yet revealed all their secrets. For example, 141 PAS domains have been identified in the genome of the deltaproteobacterium *Desulfovibrio* but only two have been characterized to date. Moreover, the first heme-binding PAS domain accommodating enzymatic activity has been reported, which suggests that other PAS enzymes are likely to exist [62].

More generally, in addition to be signal sensing proteins, the bacterial PAS/GAF with and without cofactors have been shown to be involved in signal modulation, transduction, dimerization, protein interaction and cellular localization [63]. Advantageously to other signaling proteins, PAS/GAF-containing proteins are associated to a wide variety of domains

such as HKs, σ^{54}-dependent activators, diguanylate cyclases/phosphodiesterases and methyl-accepting chemotaxis proteins (this list is not exhaustive). Extracellular and intracellular localization, the incredible versatility of signal sensed (light, gases, redox potential, metals, fatty acids, carbohydrates…) make PAS/GAF domains versatile and unique systems for rapid adaptation to environmental changes and ensure survival of microorganisms.

Figure legends:

Figure 1: **Schematic representation of the protein architecture and signal transduction of canonical PAS/GAF-containing sensor histidine kinases and σ^{54}-dependent transcriptional regulators**. (A) Histidine Kinases SHKs are mostly dimeric and they are basically organized in three structural elements: the sensing PAS/GAF core (red) able to bind a metal (yellow) followed by the four-alpha-helical HAMP region connected to the helical effector module which comprises the DHp domain (Dimerization and phospho-accepting Histidine) (pink) and the catalytic domain (CA) (light blue). (B) Canonical σ^{54}-dependent transcriptional regulators are mostly hexameric and contain three domains: the N-terminal sensing domain (red), the central AAA+ module responsable for ATPase activity (blue) and the C-terminal HTH (Helix Turn Helix) DNA binding domain (green).

Figure 2: **Structural comparison PAS/GAF domain.** (A) Proposed three-dimensional PAS domain based on the PYP (Phosphoactive Yellow Protein) structure, obtained from Taylor and colleagues (PDB id: 3PYP) [11]. The PAS domain is organized in four main structural elements: the PAS core (red), the β-scaffold (green), the helical connector (blue) and the N-terminal cap (yellow). (B) Structure of the GAF domain based on the YGK9 protein (PDB id: 3kO6). Regions that can be superimposed between PYP and YGK9 structure within 3 Å are colored in red.

Figure 3: **Proposed structural model of Fe center in the NorR GAF domain**. (A) Overview of the model showing the position of the iron in the GAF domain. (B) View of the ferrous iron center showing the proposed iron ligands. The Arg75 residue is the most likely to undergo ligand displacement upon NO binding. Figure was obtained from Tucker and colleagues [11].

Figure 4: **Dimeric structure of the PhoQ sensor domain from *S. typhimurium***. Red-colored side-chains are negatively charged residues and gray balls represent Ca^{2+} cations (PDB id: 1yax) [12].

Figure 5: Structure of the PAS domain of the WalK sensor from *Staphylococcus aureus*. (A) Entire PAS domain; the bound Zn^{2+} is shown as a gray sphere and its coordinating residues are shown as cyan sticks. (B) Focus on the Zn^{2+}-binding site; the atoms that contribute to the interaction with Zn^{2+} are in spheres (PDB id: 4mn5) [15]. (C) Molecular modelling of WalK in the presence (1) or absence (2) of Zn^{2+} showing the predicted conformational changes induced

in the WalK PAS and catalytic (CAT) domains upon metal binding; obtained from Monk and colleagues [15].

Figure 6: **Structures of ligand-free FixL heme domains**. (A) from *Rhizobium meliloti* (PDB id: 1d06) [20](B) from *Bradyrhizobium japonicum* (PDB id: 1drm) [16]. (C) Superposition of the crystal structures of the *Bradyrhizobium japonicum* oxygen-bound (bronze) (PDB id: 1dp6) and ligand-free (blue) (PDB id: 1drm) FixL heme domain [22]. Dioxygen is in red.

Figure 7: **Structural model of the PAS domain from OrpR**. The cysteine residues involved in cluster binding are indicated; obtained from Fievet and colleagues [29].

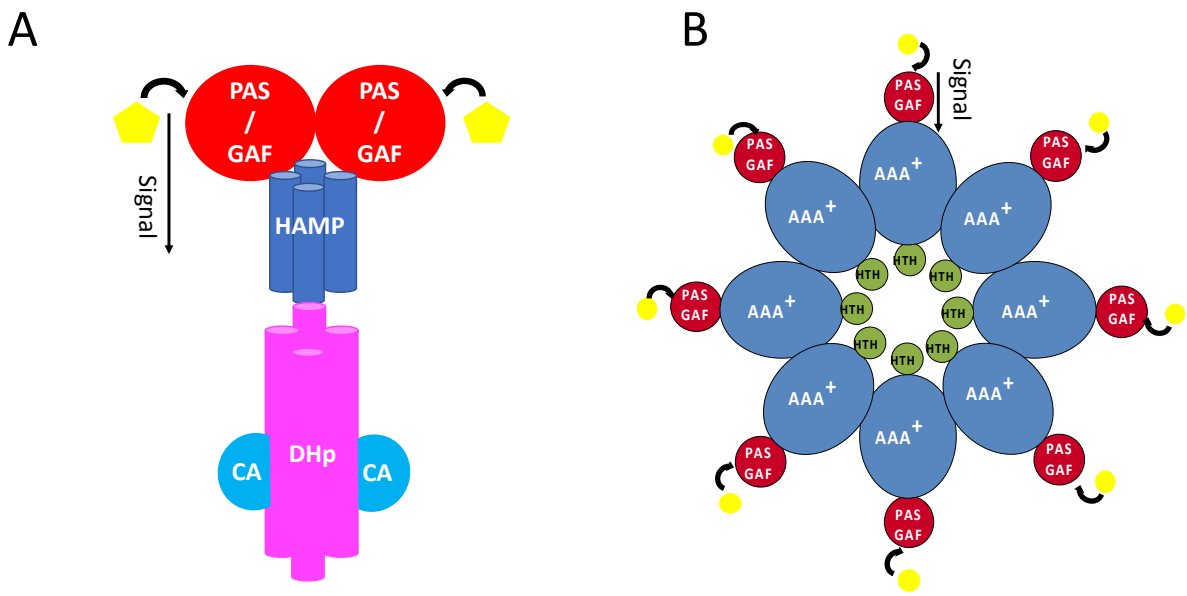
Figure 8: **Organization and O_2 repression of TCS metal-containing PAS/GAF sensors**. Domains organization and model of inactivation by O_2 of the metal-containing PAS/GAF TCS sensors FixL in *Bradyrhizobium japonicum*, NreB in *Staphylococcus carnosus* and DosS/DosT in *Mycobacterium tuberculosis*.

Figure 9: **Organization and O_2 activation of TCS metal-containing PAS/GAF sensors.** Domains organization and model of activation by O_2 of the metal-containing PAS/GAF TCS sensors AirS in *Staphylococcus aureus*, Aer2 in *Pseudomonas aeruginosa* and DosP in *Escherichia coli*.

Figure 10: **Repression of TCS-containing PAS/GAF sensors by direct metal-sensing.** Domains organization and model of inactivation by cations of the metal-containing PAS/GAF TCS sensors PhoQ in enterobacteria and WalK in *Staphylococcus aureus*.

Figure 11: **σ^{54}-Dependent activators with input metallic GAF/PAS domains.** Domains organization and model of activation/inactivation of the metal-containing PAS/GAF σ^{54}-dependent activators NorR in *E. coli*, AdhR in *Clostridium beijerinckii* and OrpR in *Desulfovibrio vulgaris* Hildenborough.

Figure 1 :



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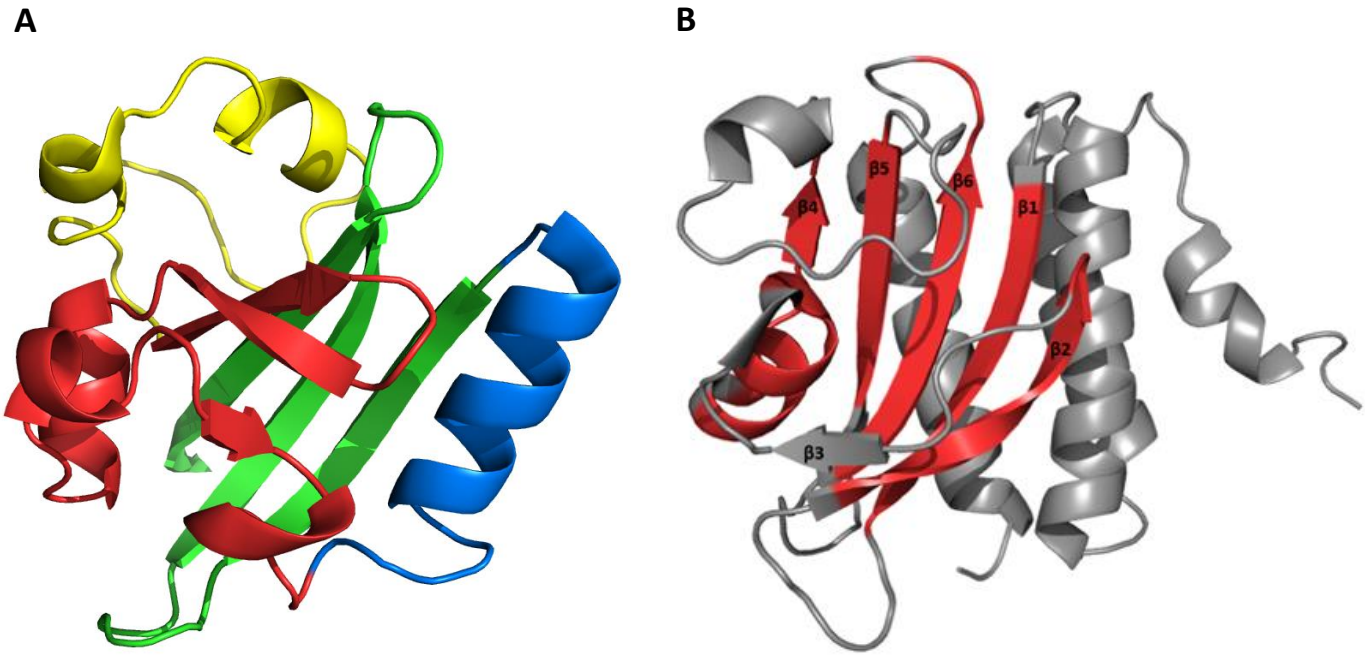

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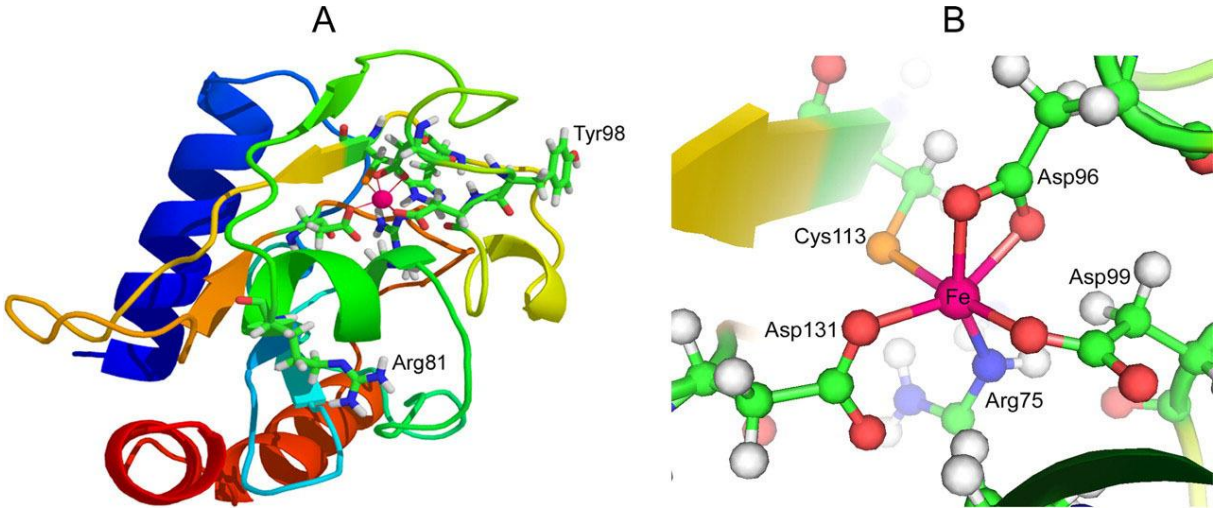


Figure 4

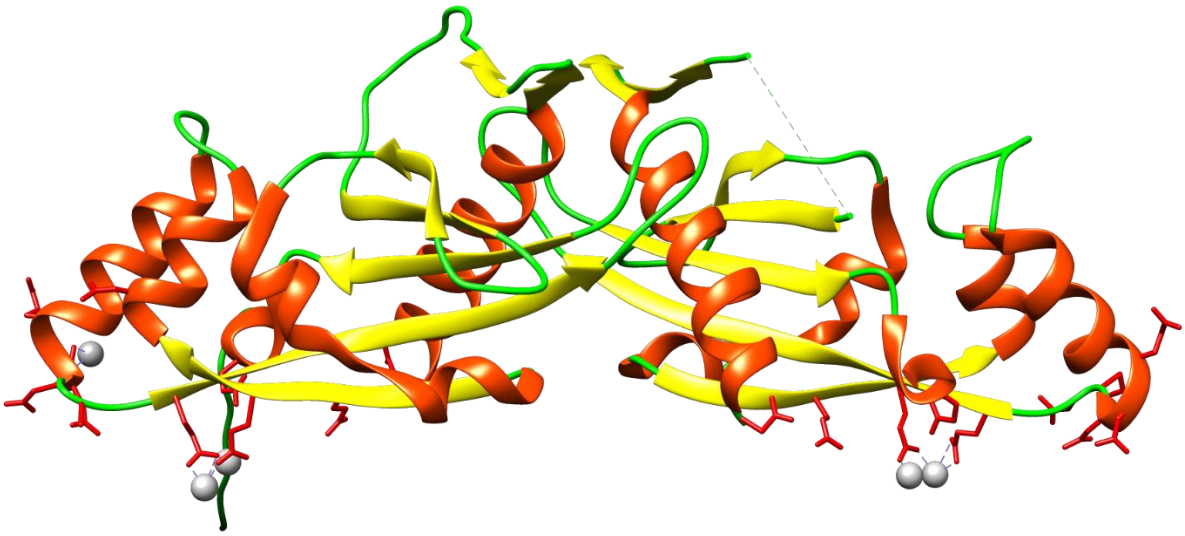


Figure 5

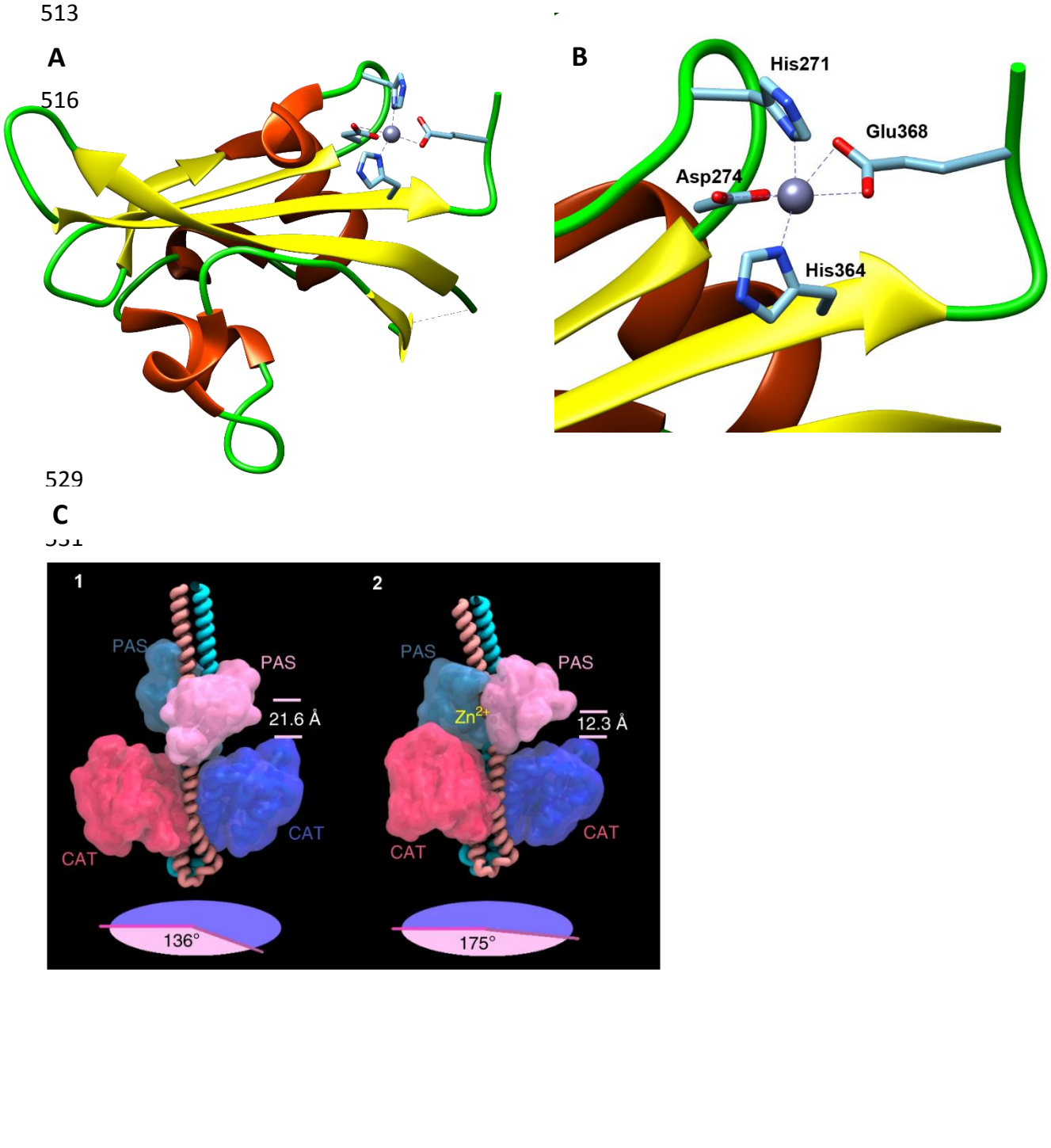

A
B
His271
Glu368
Asp274
His364
C
1
2
PAS
PAS
21.6 Å
CAT
CAT
136°
PAS
PAS
Zn2+
12.3 Å
CAT
CAT
175°

Figure 6 :

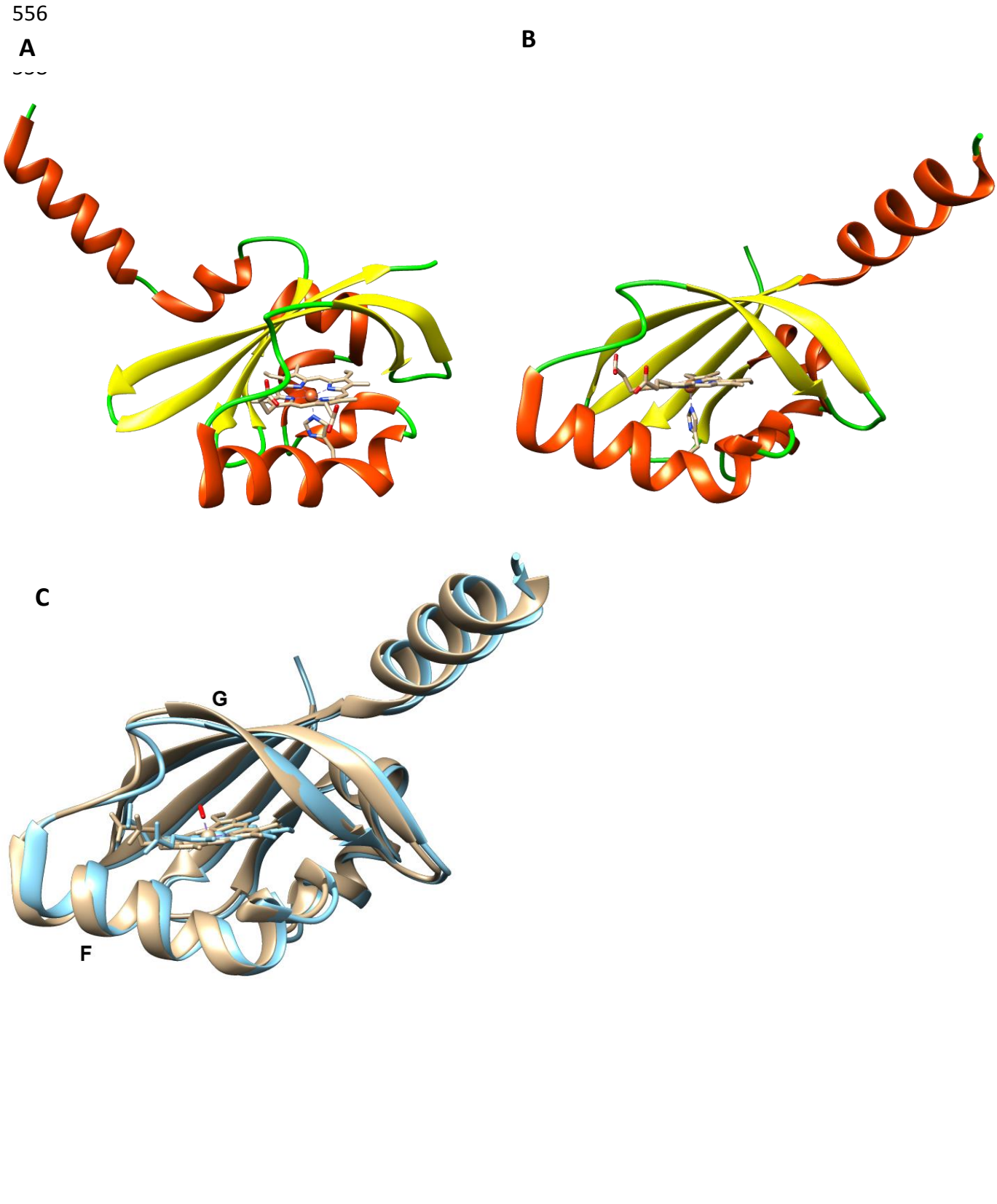


Figure 7

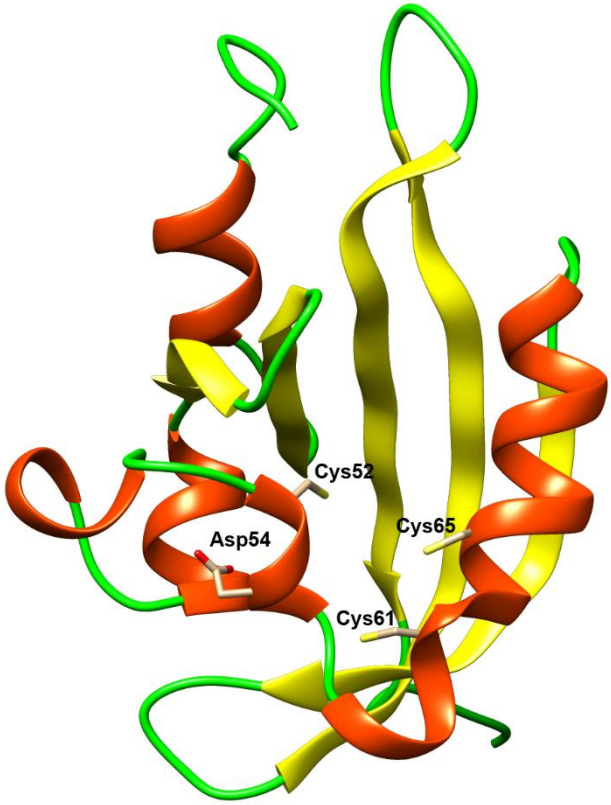


Figure 8:

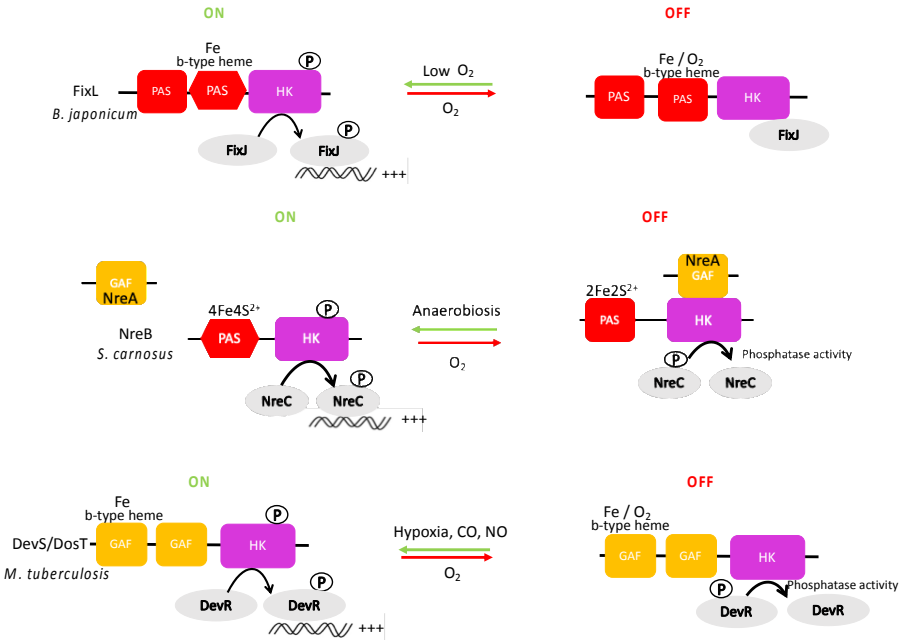

ON
Fe
b-type heme
FixL
B. japonicum
PAS
PAS
HK
P
FixJ
FixJ
+++
Low O2
O2
OFF
Fe / O2
b-type heme
PAS
PAS
HK
FixJ
ON
GAF
NreA
NreB
S. carnosus
4Fe4S2+
PAS
HK
P
NreC
NreC
+++
Anaerobiosis
O2
OFF
NreA
GAF
2Fe2S2+
PAS
HK
P
Phosphatase activity
NreC
NreC
ON
Fe
b-type heme
DevS/DosT
M. tuberculosis
GAF
GAF
HK
P
DevR
DevR
+++
Hypoxia, CO, NO
O2
OFF
Fe / O2
b-type heme
GAF
GAF
HK
P
Phosphatase activity
DevR
DevR

Figure 9:

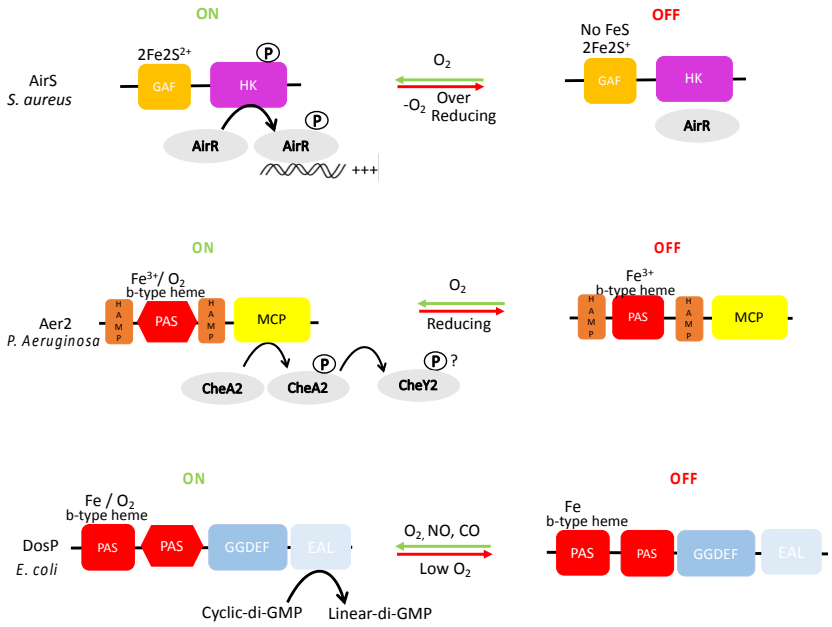

ON
AirS
S. aureus
2Fe2S2+
GAF
HK
P
AirR
AirR
P
+++
O2
-O2
Over
Reducing
OFF
No FeS
2Fe2S+
GAF
HK
AirR
ON
Fe3+/ O2
b-type heme
Aer2
P. Aeruginosa
HAMP
PAS
HAMP
MCP
CheA2
CheA2
P
P
?
CheY2
O2
Reducing
OFF
Fe3+
b-type heme
HAMP
PAS
HAMP
MCP
ON
Fe / O2
b-type heme
DosP
E. coli
PAS
PAS
GGDEF
EAL
Cyclic-di-GMP
Linear-di-GMP
O2, NO, CO
Low O2
OFF
Fe
b-type heme
PAS
PAS
GGDEF
EAL

Figure 10:

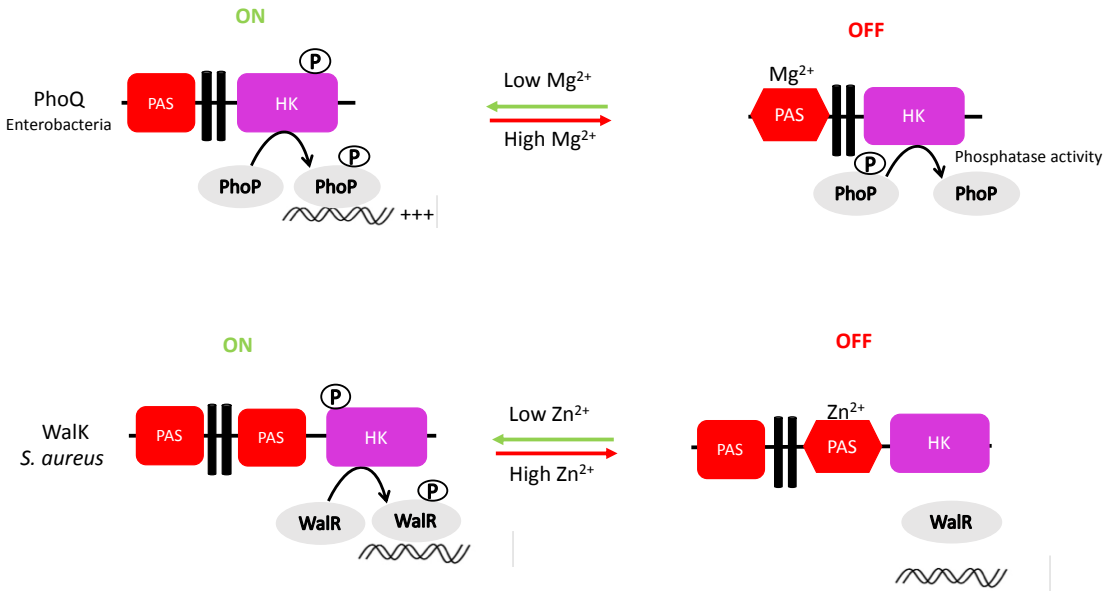
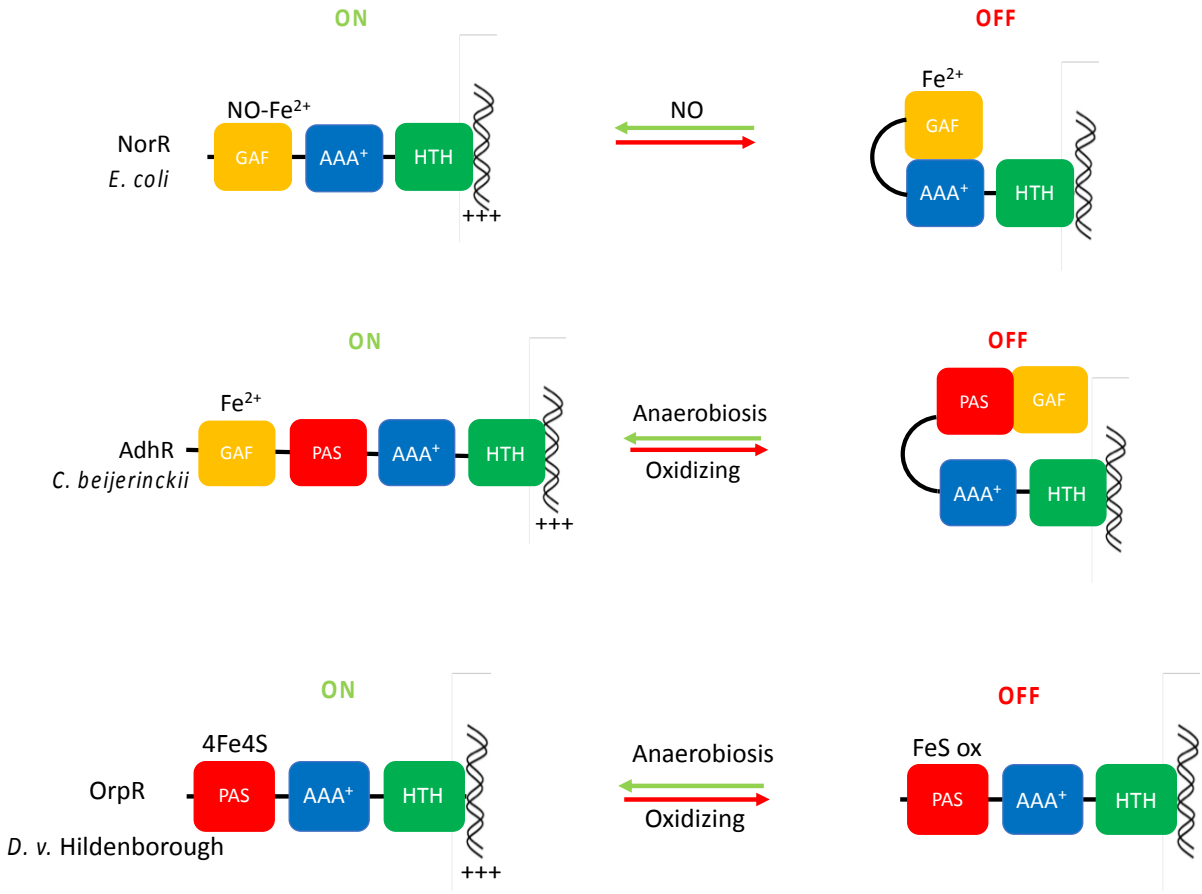

ON
PhoQ
Enterobacteria
PAS
HK
P
PhoP
PhoP
+++
Low Mg2+
High Mg2+
OFF
Mg2+
PAS
HK
P
Phosphatase activity
PhoP
PhoP
ON
WalK
S. aureus
PAS
PAS
HK
P
WalR
WalR
Low Zn2+
High Zn2+
OFF
Zn2+
PAS
PAS
HK
WalR

Figure 11:


ON
OFF
NO-Fe2+
NorR
E. coli
GAF
AAA+
HTH
+++
NO
Fe2+
GAF
AAA+
HTH
ON
OFF
Fe2+
AdhR
C. beijerinckii
GAF
PAS
AAA+
HTH
+++
Anaerobiosis
Oxidizing
PAS
GAF
AAA+
HTH
ON
OFF
4Fe4S
OrpR
D. v. Hildenborough
PAS
AAA+
HTH
+++
Anaerobiosis
Oxidizing
FeS ox
PAS
AAA+
HTH

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