Title
Nitric oxide-sensing H-NOX proteins govern bacterial communal behavior

Journal
Trends in Biochemical Sciences, 38(11)

ISSN
0968-0004

Authors
Plate, Lars
Marletta, Michael A

Publication Date
2013-11-01

DOI
10.1016/j.tibs.2013.08.008

Peer reviewed

Published in final edited form as:
Trends Biochem Sci. 2013 November ; 38(11): . doi:10.1016/j.tibs.2013.08.008.

Nitric Oxide-Sensing H-NOX Proteins Govern Bacterial Communal Behavior

Lars Plate[1,2] and **Michael A. Marletta**[1,2,3]

[1]Department of Chemistry, Scripps Research Institute, 10550 North Torrey Pines Rd, La Jolla, CA 92037

[2]Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720

[3]Department of Chemistry, University of California, Berkeley, CA 94720

Abstract

Heme-Nitric oxide/Oxygen binding (H-NOX) domains function as sensors for the gaseous signaling agent nitric oxide (NO) in eukaryotes and bacteria. Mammalian NO signaling is well characterized and involves the H-NOX domain of soluble guanylate cyclase. In bacteria, H-NOX proteins interact with bacterial signaling proteins in two-component signaling systems or in cyclic-di-GMP metabolism. Characterization of several downstream signaling processes has shown that bacterial H-NOX proteins share a common role in controlling important bacterial communal behaviors in response to NO. The H-NOX pathways regulate motility, biofilm formation, quorum sensing, and symbiosis. Here, we review the latest structural and mechanistic studies that have elucidated how H-NOX domains selectively bind NO and transduce ligand binding into conformational changes that modulate activity of signaling partners. Furthermore, we summarize the recent advances in understanding the physiological function and biochemical details of the H-NOX signaling pathways.

Keywords

H-NOX; nitric oxide; two-component signaling; cyclic-di-GMP; biofilm; quorum sensing

Nitric Oxide Sensing by Bacteria

Nitric oxide (NO) is now a well-established signaling molecule. As a gaseous, chemically reactive free radical, a central role for NO in cell communication was unexpected. Eukaryotes employ this freely diffusible molecule in low transient concentrations as a cardiovascular signaling agent and neurotransmitter [1]. NO is also produced by macrophages in a localized area at much higher, cytotoxic concentrations where it serves as a host-defense against pathogens [2]. Bacteria are exposed to NO from environmental sources at concentrations ranging widely from cytotoxic, micromolar levels (as produced by macrophages) to sub-nanomolar levels. In addition, bacteria produce NO endogenously. It occurs as an intermediate in denitrification through reduction of nitrate and nitrite [3], and a

Corresponding author: Marletta, M.A. (marletta@scripps.edu).

growing number of bacterial species with sequenced genomes have also been found to contain nitric oxide synthases, which generate NO through oxidation of L-arginine [4].

Since high NO concentrations are cytotoxic, many pathogens have evolved means to sense and detoxify NO generated during the innate response [5–7]. Detoxifying enzymes, such as flavohemoglobin (HmpA), flavorubredoxin nitric oxide reductase (NorVW), respiratory nitric oxide reductase (NorB), and cytochrome c nitrite reductases (NrfA), convert NO to less toxic molecules, i.e. nitrate, nitrous oxide, and ammonia [7–11]. The expression of detoxification genes is controlled by a variety of transcription factors [5,12,13]. Consistent with a function in NO clearance, these transcriptional regulators have high-nanomolar to low-micromolar affinity, sufficient to sense toxic levels of NO [14,15].

Recent studies have demonstrated that bacteria employ non-lethal, sub-micromolar NO concentrations as signaling agents to control bacterial communal behavior. NO regulates biofilm formation and dispersal, motility, symbiosis, and quorum sensing [16–20]. The archetypal NO sensors for these processes are *H*eme-*N*itric Oxide/*Ox*ygen binding (H-NOX) proteins, a receptor family that shares high sequence homology with the heme-binding domain of the principal mammalian NO receptor, soluble guanylate cyclase (sGC). H-NOX proteins are present in more than 200 bacterial species, including several pathogens. Rather than exerting direct transcriptional control, these proteins serve as high-affinity NO sensors in more sophisticated multi-step downstream signaling processes. This review addresses the structure, function, and activation mechanism of H-NOX proteins. Furthermore, recent discoveries regarding the physiological role and biochemical details of the diverse downstream signaling pathways are discussed.

Heme-Nitric Oxide/Oxygen Binding Proteins

H-NOX proteins were originally identified in a bioinformatics search for sequence homologs of the heme-binding domain of the mammalian NO receptor sGC [21]. NO binding to the ferrous heme cofactor of sGC activates the cyclase domain to convert GTP to cyclic GMP, the secondary messenger for downstream signaling events [1]. The N-terminal 194 residues of the sGC β subunit constitute the minimal heme-binding domain [22] and contain a conserved His residue required for coordination to the central iron atom [23,24]. Sequences with 15–40% identity to the sGC H-NOX were subsequently identified in bacteria across many phyla, including *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, *Cyanobacteria*, and *Thermotogae* [21,25]. There are now over 250 known bacterial species that contain H-NOX proteins. Nonetheless, certain bacterial classes, such as *Actinobacteria or Bacilli*, lack organisms containing H-NOX genes altogether although these species produce NO. As discussed above, many other types of NO sensors exist and it is currently unknown what evolutionary processes govern the presence of H-NOX genes and associated signaling pathways.

The heme prosthetic group in the Fe(II) state is capable of coordinating several diatomic gas ligands. Consequently, a variety of gas-binding hemoproteins exist for O_2, NO and CO [26,27], but most proteins, such as the globin family or FixL, cannot discriminate between O_2, NO and CO [27,28]. By contrast, sGC has exquisite ligand selectivity for NO and shows no measurable affinity for O_2 [1]. This stringent discrimination against O_2 is a requirement for selective NO receptors, because the proteins must be capable of efficiently coordinating low nanomolar NO in the presence of greater than 1000-fold excess O_2 in aerobic environments. Characterization of several bacterial H-NOX proteins from facultative anaerobes revealed sGC-like ligand selectivity for NO [25,29]. However, a second subfamily of H-NOX proteins from obligate anaerobes is capable of binding both O_2 and NO with high affinity [25,30].

The first crystal structure of an H-NOX family member was from *Thermoanaerobacter tengcongensis* (*Tt* H-NOX) and it revealed a protein fold that was distinct from other heme-based gas sensors [30,31]. H-NOX domains consist of an N-terminal, helical subdomain and a C-terminal subdomain (Fig. 1A). The heme cofactor is deeply buried between the two subdomains and the central iron is coordinated axially to a conserved His residue on α-helix F [30,31]. Furthermore, the propionate groups of the heme form salt-bridges to an absolutely conserved YxSxR motif, which is also required for stable heme binding to the protein and activation in sGC [32] (Fig. 1B).

Downstream effectors of H-NOX proteins

Bacterial H-NOX domains are either encoded as freestanding proteins (76% of known H-NOX sequences) or as domains of transmembrane methyl-accepting chemotaxis proteins (MCP, see glossary) (24%). H-NOX-MCP fusions are present in *Thermotogae*, *Firmicutes*, and the *Desulfovibrio* genus. Intriguingly, all of the H-NOX-MCP fusions are found in obligate anaerobes or microaerotolerant species. Furthermore, the chemotaxis-related H-NOX domains contain a tyrosine in the distal heme pocket, which is a major determinant for O_2-binding [33] (Fig. 2A, yellow shade). The O_2-binding H-NOX-MCP fusion has been proposed to serve as a high-affinity O_2 sensor that directs obligate anaerobes away from O_2 [34]. Alternatively, H-NOX-MCP fusions could function as NO sensors in low O_2 environments where selective pressure to exclude O_2 binding was absent during the evolution of these proteins.

The freestanding, NO-selective H-NOX genes are found in operons with diverse bacterial signaling genes (Fig. 2A). The majority of the operons contain histidine kinase (HK, see glossary) genes directly adjacent to the H-NOX gene (112 of 209 species) (Fig. 2B). Interestingly, no HKs adjacent to H-NOX genes are predicted to be membrane-incorporated, nor do they contain sensor domains. The separately expressed H-NOX sensor functions in trans, substituting for the typical periplasmic sensor domains of HKs [18,35,36].

About one third of H-NOX operons with HKs also contain response regulator (RR) genes (Fig. 2B), which likely form specific interaction(s) with the HK [37]. One such grouping can be observed in the *Bacteroidetes*, where many operons contain RRs with DNA-binding effector domains from the LytTR family (Fig. 2A). The clustering could point to a conserved function for a two-component signaling system that controls gene expression in response to NO. Some other RRs in H-NOX operons contain HD-GYP cyclic-di-GMP phosphohydrolases, GGDEF diguanylate cyclases, or DNA-binding domains from the NtrC family as effector domains. RRs from the chemotaxis-related CheY family lack an effector domain altogether [38]. The wide variety of effector domains in other organisms suggests diverse roles for the H-NOX signaling pathways. Furthermore, the majority of H-NOX associated HKs are orphans, lacking a RR gene in the same operon (Fig. 2B).

Another family of signaling genes frequently occurring in H-NOX operons are diguanylate cyclases (DGCs) and phosphodiesterases (PDEs) (Fig. 2A). These proteins regulate the synthesis and/or degradation of the bacterial secondary messenger cyclic-di-GMP (see glossary). Organisms with DGC/PDE proteins in the H-NOX operon include mostly gammaproteobacteria (Fig. 2A). Interestingly, certain bacteria (e.g. *Legionella pneumophila* or *Sagitulla stellate*) contain two H-NOX genes in separate operons (Fig. 2A). In these organisms, one of the duplicated genes is often associated with a HK, while the other is adjacent to a diguanylate cyclase gene. This is suggestive that both signaling modes are essential for some species.

Ligand selectivity for NO

A combination of structural studies and site-specific mutants has elucidated the basis for ligand discrimination in the H-NOX family. The crystal structure of *Tt* H-NOX, an O_2-binding H-NOX from an obligate anaerobe, shows a hydrogen-bonding network in the distal pocket of the heme, consisting of Y140, W9, and N74. The tyrosine residue forms a direct H-bond with the heme-bound O_2 [30] (Fig. 3A). The H-bonding network is absent in the distal heme pocket of the NO-selective H-NOX protein from *Nostoc sp.* and is instead replaced by non-hydrogen bonding, hydrophobic residues [39] (Fig. 3B). Multiple-sequence alignment shows that the tyrosine and parts of the H-bonding network are conserved in the O_2-binding H-NOX subfamily, whereas hydrophobic residues occupy the same position in the NO-selective H-NOX proteins, including sGC. Mutation of the Tyr to Phe in *Tt* H-NOX weakens the O_2-binding affinity, which can be rescued by introducing a compensatory Tyr [33]. Introduction of a Tyr into the distal heme pocket of the NO-selective H-NOX of *Legionella pneumophila* and an sGC truncation (β1[1–385]) allows the formation of a weak O_2 complex, confirming that the Tyr residue is a key determinant for O_2 binding [33]. Nonetheless, introduction of a Tyr into full-length sGC did not confer O_2-binding [40], suggesting that the Tyr residue is not the sole factor governing ligand selectivity.

Extensive mutagenesis studies of H-NOX proteins have revealed additional elements that influence ligand affinity, including heme distortion, heme pocket conformation, protein dynamics, and a tunnel network for ligand entry and exit [41–44] (Fig. 3C). In H-NOX proteins that have been structurally characterized, the heme cofactor is severely distorted from planarity. A conserved proline residue (P115 in *Tt* H-NOX) and a conserved hydrophobic residue (I5 in *Tt* H-NOX) in the N-terminal α-helix contact the neighboring pyrrole surface from opposite sides, causing a distinct kink in the heme [30,41,45]. Relaxation of heme distortion through a proline-to-alanine substitution leads to tighter O_2 binding [41], demonstrating that heme geometry can influence ligand affinity. The importance of protein conformation and correct positioning of the H-bonding network for efficient O_2-binding has been demonstrated through introduction of steric bulk via engineered phenylalanine residues in the heme pocket [42,43]. Added bulk in the distal pocket causes a ~1 Å upward shift of the H-bonding network and the Tyr residue away from the heme, which weakens the O_2 binding affinity more than 100-fold [42]. Opening of the heme pocket and increasing flexibility of the residues in that pocket also contribute to weaker O_2 affinities [43]. Furthermore, in NO-selective H-NOX proteins, a tunnel network extending from the protein surface to the heme pocket has been implicated in increased ligand flux near the heme, which could facilitate preferential binding of NO in the presence of high concentrations of O_2 [44]. His-ligated heme proteins coordinate NO more tightly compared to O_2, and consequently, the higher ligand flow in the NO-selective H-NOX proteins could lead to larger amounts of NO entering the heme pocket, which could then bind NO more efficiently [44,46].

An important distinction between O_2 and NO binding to H-NOX proteins is the coordination state of the heme. O_2 binds axially to the open coordination site on the Fe(II) forming a 6-coordinate complex, while in most H-NOX proteins, NO forms a 5-coordinate heme complex due to dissociation of the axial His residue [18,19,25,29,30,35,47]. The transition to a 5-coordinate NO species has been described as a mechanism to increase the NO affinity because NO dissociation from the 5-coordinate heme is significantly slower [46,48]. The His-dissociation also has important consequences for the activation mechanism of H-NOX proteins and signaling to downstream effectors.

Activation mechanism

To serve as effective gas sensors in signaling pathways, H-NOX domains must be able to communicate a change in ligation state to their downstream effector proteins. Structural characterization of several bacterial H-NOX proteins in different ligation states has provided important insights into the conformational changes that are triggered by gas binding or dissociation.

A striking observation in the O_2-bound crystal structure of *Tt* H-NOX is the high degree of heme distortion [30]. Similar heme distortion is observed in the NMR structure of *Shewanella oneidensis* (*So*) H-NOX and, to a lesser extent, in the H-NOX protein from *Nostoc* sp. (*Ns*) [39,45]. Intriguingly, the amount of heme distortion correlates with the movement of the N-terminal helical subdomain, which can be displaced with respect to the C-terminal domain by 3–5 Å (Fig. 4A). [30,39,41,45,47,49]. Direct evidence for a connection between heme planarity and N-terminal rotation has been established through mutagenesis of the conserved Pro (P115 in *Tt* H-NOX) to Ala, which is sufficient to relax the heme and cause a large N-terminal shift [41]. A ligand-induced change to the geometry of the heme can therefore be amplified to a larger conformational change between the N and C-terminal subdomains of the H-NOX protein.

NO binding occurs at the open-coordination site of the 5-coordinate Fe(II), forming a transient 6-coordinate complex (Fig. 4B). In most H-NOX proteins, as in sGC, NO association severely weakens the bond between the Fe(II) and the distal His residue, leading to dissociation of the His and formation of a 5-coordinate Fe(II)-NO complex (Fig. 4B) [25,50]. Unfortunately, structural determination for an unliganded H-NOX and a 5-coordinate NO-bound H-NOX has proved difficult. Instead, structural mimics of the 5-coordinate Fe(II)-NO have helped elucidate the conformational changes resulting from NO binding and His dissociation. In both *So* and *Tt* H-NOX, the bonding connection between α-helix F and the heme was severed by mutation of the coordinating His residue to Gly. Structures of the His→Gly variant revealed a similar rotational displacement between the H-NOX subdomains and concomitant heme-relaxation [45,47]. Activity assays of the H-NOX-associated HK in *S. oneidensis* confirmed that the wild-type CO-bound H-NOX mimicked the unliganded state, whereas the CO-bound H103G mutant resembled the activated, 5-coordinate NO-bound H-NOX state [45]. These observations led to a heme strain model for H-NOX activation (Fig. 4B) [45]. In the inactive, unliganded state, the heme is held in a distorted “spring-loaded” conformation through coordination of the iron to the His on α-helix F [47]. NO binding to the iron and subsequent dissociation of the His release the heme, allowing it to adopt a more planar geometry. Tight van-der-Waals contacts with residues in the N-terminal subdomain (e.g. I5) are responsible for translating the heme relaxation to an upward shift and rotational displacement of the entire N-terminal subdomain with respect to α-helix F. Functional validation of the heme strain model has been attained by testing the influence of H-NOX heme conformation on the enzyme activity of its effector protein. The P117A mutation in the H-NOX of *Shewanella woodyi*, which leads to a planar heme, is sufficient to mimic the NO-activated H-NOX state [51].

The rotational displacement of the N-terminal subdomain relative to α-helix F is a probable mechanism for communicating the ligation state of the H-NOX domain with associated effectors. The effector domains conceivably share interaction surfaces with the H-NOX, which include α-helix F and part of the N-terminal subdomain. An involvement of these H-NOX surfaces in inter-domain contacts in sGC has been shown [52].

Nitric Oxide controls diverse communal behaviors

Biofilm dispersal through regulation of cyclic-di-GMP levels

The molecular mechanism how associated effector proteins are activated by H-NOX and ligand-dependent conformational changes still has to be elucidated. Nonetheless, recent biochemical and genetic characterization of the effector proteins and downstream signaling pathways revealed the physiological function of H-NOX proteins in several organisms. Because the genes are often found in operons adjacent to DGC and PDE genes, it was proposed that NO and the H-NOX proteins could be involved in regulation of cyclic-di-GMP dependent processes such as biofilm formation [17,21]. Biofilms are composed of surface-adhered, matrix-encapsulated clusters of bacterial cells, which are difficult to treat with antibiotics and are often associated with chronic infections [53,54]. The switch between planktonic, motile microbial life style and biofilm-associated, sessile life style is controlled by diverse environmental influences [55,56]. A direct role for H-NOX/NO signaling in controlling biofilm formation has been demonstrated in the opportunistic pathogen *Legionella pneumophila*, which causes Legionnaire's disease [57]. Mixed-species biofilm communities in anthropogenic water supplies play an important role in the *Legionella* growth cycle and serve as a nutrient and storage ground [58]. Deletion of the *hnoX*1 gene promotes biofilm formation, consistent with a role for the signaling pathway in biofilm repression [17]. Furthermore, mild overexpression of the H-NOX-associated DGC protein causes elevated biofilm formation proving an involvement of cyclic-di-GMP [17]. Lastly, *in vitro* characterization of the DGC showed that the NO-bound H-NOX state activated cyclic-di-GMP synthesis, although the specific activity of the DGC was relatively weak [17].

The H-NOX-dependent kinetic control of an effector protein containing dual DGC and PDE domains was characterized in more detail in *Shewanella woodyi*, a metabolically versatile marine organism originally isolated from squid ink [59]. The H-NOX-associated DGC/PDE from *S. woodyi* (*Sw* DGC) is capable of both the synthesis and degradation of cyclic-di-GMP [60]. Analysis of steady-state kinetic parameters indicates that unliganded H-NOX activates Sw DGC whereas NO-bound H-NOX does not. On the other hand, NO-bound H-NOX increases PDE activity [19]. One caveat is that the overall k_{cat}/K_M for PDE activity is significantly higher than for DGC activity, even in the presence of unliganded H-NOX. Consequently, the enzyme mostly functions as a PDE and regulation of this activity predominates. The apparent NO-induced switch from DGC to PDE activities is consistent with the physiological response of *S. woodyi* to NO. *S. woodyi* exhibits an H-NOX dependent decrease in intracellular cyclic-di-GMP concentrations and a concomitant lower propensity to form biofilms [19].

Overall, the studies in *L. pneumophila* and *S. woodyi* lead to a model for H-NOX/NO-dependent biofilm dispersal described in Fig. 5A. The H-NOX protein forms a regulatory complex with a DGC/PDE protein. NO binding to H-NOX inhibits cyclic-di-GMP production and sometimes increases PDE activity, lowering cyclic-di-GMP concentrations, which causes biofilm dispersal.

Control of biofilm levels through a multi-component cyclic-di-GMP signaling network

The majority of bacterial H-NOX genes are found adjacent to HK genes indicating that H-NOX proteins function as NO sensors in two-component signaling systems (Fig. 2B). H-NOX-dependent control of HK activity was first demonstrated in *S. oneidensis* [35] and has since been studied in *Vibrio harveyi* and *Pseudoaltermonas atlantica* [18,36]. In all cases, the NO-bound state of the H-NOX inhibits HK autophosphorylation whereas the unliganded state either has no measurable effect on HK activity [18,35] or increases HK

autophosphorylation [36]. Thus, the NO-bound state of the H-NOX is apparently inhibitory to both HKs and DGCs.

As previously discussed, 65% of bacterial species containing a co-cistronic H-NOX/HK pair lack a cognate RR gene in the same operon, including *S. oneidensis*, *V. harveyi*, and *V. cholerae* (Fig. 2). To characterize the physiological function of the H-NOX/HK signaling system in *S. oneidensis*, the cognate RRs of HnoK, the H-NOX associated HK, were identified by phosphotransfer profiling, a kinetic profiling of all possible orphan RR candidates [16,37]. Surprisingly, HnoK targets three different RR: HnoB, HnoC, HnoD (Fig. 5B). HnoB and HnoD are also phosphorylation targets of HnoK in *V. cholerae* [16]. Phosphotransfer to HnoD has been confirmed in *P. atlantica*, although transfer to HnoB and HnoC in this organism was not tested [36]. The similar phosphotransfer patterns and co-occurrences of *hnoK* with *hnoBCD* genes in several gammaproteobacteria points to a broad conservation of this H-NOX signaling network [16].

Biochemical characterization of the RR components has shed light on their function and the physiological output of the multi-component signaling network. Intriguingly, the network is also involved in adjusting cyclic-di-GMP concentrations and controlling biofilm formation. HnoB contains a PDE effector domain and phosphorylation by HnoK stimulates hydrolysis of cyclic-di-GMP [16]. Furthermore, HnoD contains a degenerate HD-GYP PDE, which is catalytically inactive but functions as an allosteric inhibitor of HnoB to fine-tune PDE activity [16]. Together, HnoB and HnoD increase cyclic-di-GMP levels in response to NO stimuli, which leads to increased biofilm formation (Fig. 5B). The third RR, HnoC, has been recently shown to mediate transcriptional feedback control for all of the signaling components in the network to further regulate the dynamics of the NO response (Fig. 5B) [61].

Overall, regulation of cyclic-di-GMP concentration and bacterial motility has emerged as a common theme, through either direct control of DGC or PDE activity, or through more sophisticated multi-component phosphotransfer pathways. The simpler one-component system seems to cause dispersal of biofilms in response to NO, while the more sophisticated system with different layers of feed-forward and feed-back control signals the opposite: increased adhesion and biofilm formation in response to NO. Surface adhesion in biofilms could function as a physical protection mechanism by buffering cytotoxic NO at the surface layers to prevent diffusion into the lower regions of the biofilm [16].

Cross-talk with quorum sensing

In addition to regulating motility and biofilm formation, recent work suggests that H-NOX/NO signaling can influence quorum sensing. Quorum sensing in *Vibrio harveyi*, an opportunistic pathogen of marine organisms, involves three separate autoinducers and three corresponding transmembrane sensor HKs: CqsS, LuxN, and LuxPQ (Fig. 6A). Phosphotransfer from all three HKs to a single Hpt protein, LuxU integrates the quorum sensing signal. Further downstream signaling events regulate expression of quorum sensing genes, which control bioluminescence and other communal responses (Fig. 6A). Autoinducer concentrations above the response threshold switch the HK activity from kinase to phosphatase, promoting dephosphorylation of LuxU and LuxO and subsequent induction of gene expression by LuxR [62].

V. harveyi cultures exhibit increased bioluminescence at low culture density in the presence of low-nanomolar concentrations of NO, suggesting that NO could function as a autoinducer [18]. NO-mediated quorum sensing is dependent on the *hnoX* gene. As in other H-NOX/HK systems, the NO-bound H-NOX inhibits the autophosphorylation activity of the associated hybrid HK, termed HqsK [18]. The discovery that HqsK phosphorylates LuxU established a

link between H-NOX/NO signaling and the quorum sensing phosphorelay pathway [18] (Fig. 6A). Nonetheless, the phosphotransfer kinetics from HqsK to LuxU are slow compared to other signaling partners, for example LuxN to LuxU [63]. This raises the question whether the HsqK/LuxU interaction is cognate or the result of cross-talk between a separate H-NOX phosphorelay system and the quorum sensing network [37,64]. It will be interesting to see whether HqsK can interact with another Hpt protein, linking it to additional RR targets. Likewise, it will be interesting to determine whether NO can influence quorum sensing in other organisms.

Effect on host-colonization by Vibrio fischeri

Evidence that the H-NOX/NO signaling system can influence infection of a host organism has been established in a different *Vibrio* species: *Vibrio fischeri*. This bioluminescent marine organism engages in symbiosis with the Hawaiian bobtail squid *Euprymna scolopes*. *V. fischeri* colonizes the light organ of the squid. The *V. fischeri* derived bioluminescence protects the squid from predators by providing counterillumination at night to avoid casting shadows in moon light [65,66]. A particular challenge in the initiation of symbiosis for a newly hatched *E. scolopes* is the specific acquisition of *V. fischeri* and exclusion of more abundant environmental bacterial species [67]. NO formation by the squid has been shown to be an important factor for symbiont colonization and serves as a specificity determinant for *V. fischeri* [68,69].

The H-NOX/NO signaling pathway in *V. fischeri* downregulates expression of iron uptake and utilization genes [20]. The regulated promoters contain a binding motif for the ferric-uptake regulator (Fur), and regulation by Fur has been confirmed [70]. Many of the genes are involved specifically in hemin uptake as a source of iron, linking H-NOX/NO signaling to repression of hemin acquisition and utilization (Fig. 6B). The *Vf* H-NOX gene is part of an operon containing a HK gene (HnoK). However, how HnoK is linked to regulation of Fur and the iron utilization genes remains to be determined (Fig. 6B).

Further experiments showed that the *hnoX* deletion strain possessed a colonization advantage that stems from more efficient iron utilization in the low-iron environment of the light organ ducts. NO exposure in the presence of hemin as the sole iron source causes a significant slowing of growth in the wild-type strain due to downregulation of hemin uptake [20]. However, this limitation of iron uptake by *V. fischeri* during the course of colonization has been hypothesized to serve as an important survival strategy to avoid damage from hydroxyl radicals generated through Fenton chemistry from Fe(II) and hydrogen peroxide [20]. NO exposure, which occurs even before bacteria enter the light organ ducts, has been suggested to prime *V. fischeri* for ROS exposure by lowering intracellular iron levels thereby slowing growth [20,68]. Consequently, H-NOX signaling is at least partially responsible for the competitive advantage of *V. fischeri* colonization compared to other bacteria present in the seawater.

Concluding remarks

The recent discoveries in H-NOX signaling demonstrate the diversity of behavioral outputs controlled by NO, ranging from motility, biofilm formation, host-colonization, to quorum sensing. The repertoire of physiological functions is likely to expand, as diverse H-NOX pathways from other species are characterized in the future.

Significant progress has been made in characterizing ligand discrimination and activation mechanisms in H-NOX proteins. Further structural and mechanistic studies are required to identify the interfaces between H-NOX and effector proteins and probe the regulation of

effector protein activity. These mechanistic insights into the regulation mechanism of bacterial H-NOX proteins will be broadly applicable.

Given the important role for NO in the mammalian immune response, it will be important to explore H-NOX-mediated countermeasures involved in bacterial pathogenesis. In *V. cholerae*, biofilms have been shown to enhance infectivity [71,72], and defense against reactive nitrogen species is important for host colonization [73,74]. Therefore, defensive biofilm formation in response to host NO assaults could play a significant role in enhancing *V. cholerae* infectivity. Further studies are required to elucidate the exact role of H-NOX/NO signal pathway in the infection process.

Glossary Box

Methyl-accepting chemotaxis protein (MCP)	MCPs are bacterial transmembrane receptors for chemical attractants or repellents, which stimulate a motility response for pursuit or evasion [75]. They interact with the chemotaxis two-component signaling system. Ligand occupancy on the MCP alters phosphorylation of the histidine kinase CheA and the response regulator CheY, which controls the rotational direction of the flagellar motor [75].
Histidine kinase (HK)	HKs function in bacterial two-component signaling systems and are typically transmembrane proteins with periplasmic or extra-cellular sensor domains [76]. The sensory domain transduces a response to a chemical stimuli by modulating HK autophosphorylation activity leading to phosphorylation of a conserved histidine residue [77].
Two-component signaling system	Two-component signaling systems mediate the adaptation of bacteria to environmental changes and stimuli. The signaling system consists of a sensor HK and one or several partner response regulator(s). A particular chemical stimulus controls the autophosphorylation activity of a HK, which transfers the phosphoryl group to a partner response regulator (RR). RRs typically contain an effector domain, and phosphorylation alters the function of the effector domain, controlling the overall output of the signaling system [38,77].
Cyclic-di-GMP	Cyclic-di-GMP has emerged as a crucial bacterial secondary messenger controlling diverse functions such as virulence, cell cycle progression, and motility and biofilm formation [78]. Cyclic-di-GMP is produced from two molecules of GTP by diguanylate cyclase (DGC) domains, which contain a conserved GGDEF sequence motif. In turn, cyclic-di-GMP can be hydrolyzed to 5'-phosphoguanylyl-(3'–5')-guanosine (pGpG) by two different families of phosphodiesterase (PDE) domains: EAL or HD-GYP domains, also named after conserved sequence motifs [78,79].
Quorum sensing	Quorum sensing is an important group behavior in which bacteria sense their cell population density [62]. Many bacteria secrete small molecule chemicals, called autoinducers, whose accumulated concentration can serve as a signal of cell density. Threshold concentrations of autoinducers are detected by specific sensors, which trigger population-synchronized downstream signaling events [62]. Processes regulated by quorum sensing include gene expression, motility, biofilm formation, virulence, and bioluminescence.

References

1. Derbyshire, ER.; Marletta, MA. Biochemistry of soluble guanylate cyclase. In: Schmidt, H., editor. cGMP: Generators, Effectors and Therapeutic Implications. Handbook of experimental pharmacology; 2009. p. 17-31.
2. MacMicking J, et al. Nitric oxide and macrophage function. Annu. Rev. Immunol. 1997; 15:323–350. [PubMed: 9143691]
3. Rodionov DA, et al. Dissimilatory metabolism of nitrogen oxides in bacteria: comparative reconstruction of transcriptional networks. PLoS Comput. Biol. 2005; 1:e55. [PubMed: 16261196]
4. Sudhamsu J, Crane BR. Bacterial nitric oxide synthases: what are they good for? Trends Microbiol. 2009; 17:212–218. [PubMed: 19375324]
5. Spiro S. Regulators of bacterial responses to nitric oxide. FEMS Microbiol. Rev. 2007; 31:193–211. [PubMed: 17313521]
6. Fang FC. Antimicrobial reactive oxygen and nitrogen species: concepts and controversies. Nat. Rev. Microbiol. 2004; 2:820–832. [PubMed: 15378046]
7. Stevanin TM, et al. Flavohemoglobin Hmp protects *Salmonella enterica* serovar typhimurium from nitric oxide-related killing by human macrophages. Infect. Immun. 2002; 70:4399–4405. [PubMed: 12117950]
8. Poole R, Hughes M. New functions for the ancient globin family: bacterial responses to nitric oxide and nitrosative stress. Mol. Microbiol. 2000; 36:775–783. [PubMed: 10844666]
9. Gardner AM, et al. Flavorubredoxin, an inducible catalyst for nitric oxide reduction and detoxification in *Escherichia coli*. J. Biol. Chem. 2002; 277:8172–8177. [PubMed: 11751865]
10. Mills PC, et al. A combination of cytochrome c nitrite reductase (NrfA) and flavorubredoxin (NorV) protects *Salmonella enterica* serovar Typhimurium against killing by NO in anoxic environments. Microbiology (Reading, Engl.). 2008; 154:1218–1228.
11. Stevanin TM, et al. Nitric oxide detoxification systems enhance survival of *Neisseria meningitidis* in human macrophages and in nasopharyngeal mucosa. Infect. Immun. 2005; 73:3322–3329. [PubMed: 15908358]
12. Bush M, et al. Transcriptional regulation by the dedicated nitric oxide sensor, NorR: a route towards NO detoxification. Biochem Soc T. 2011; 39:289–293.
13. Tucker NP, et al. There's NO stopping NsrR, a global regulator of the bacterial NO stress response. Trends Microbiol. 2010; 18:149–156. [PubMed: 20167493]
14. D'Autréaux B, et al. Characterization of the nitric oxide-reactive transcriptional activator NorR. Meth. Enzymol. 2008; 437:235–251. [PubMed: 18433632]
15. Heurlier K, et al. The nitric oxide (NO)-sensing repressor NsrR of *Neisseria meningitidis* has a compact regulon of genes involved in NO synthesis and detoxification. J. Bacteriol. 2008; 190:2488–2495. [PubMed: 18245279]
16. Plate L, Marletta MA. Nitric oxide modulates bacterial biofilm formation through a multicomponent cyclic-di-GMP signaling network. Mol Cell. 2012; 46:449–460. [PubMed: 22542454]
17. Carlson HK, et al. H-NOX regulation of c-di-GMP metabolism and biofilm formation in *Legionella pneumophila*. Mol. Microbiol. 2010; 77:930–942.
18. Henares BM, et al. Discovery of a nitric oxide responsive quorum sensing circuit in *Vibrio harveyi*. ACS Chem Biol. 2012; 7:1331–1336. [PubMed: 22606970]
19. Liu N, et al. Nitric oxide regulation of cyclic di-GMP synthesis and hydrolysis in *Shewanella woodyi*. Biochemistry. 2012; 51:2087–2099. [PubMed: 22360279]
20. Wang Y, et al. H-NOX-mediated nitric oxide sensing modulates symbiotic colonization by *Vibrio fischeri*. Proc. Natl. Acad. Sci. USA. 2010; 107:8375–8380. [PubMed: 20404170]
21. Iyer LM, et al. Ancient conserved domains shared by animal soluble guanylyl cyclases and bacterial signaling proteins. BMC Genomics. 2003; 4:5–12. [PubMed: 12590654]
22. Karow DS, et al. Characterization of functional heme domains from soluble guanylate cyclase. Biochemistry. 2005; 44:16266–16274. [PubMed: 16331987]

23. Zhao Y, et al. Identification of histidine 105 in the beta1 subunit of soluble guanylate cyclase as the heme proximal ligand. Biochemistry. 1998; 37:4502–4509. [PubMed: 9521770]
24. Foerster J, et al. A functional heme-binding site of soluble guanylyl cyclase requires intact N-termini of alpha 1 and beta 1 subunits. Eur. J. Biochem. 1996; 240:380–386. [PubMed: 8841402]
25. Karow DS, et al. Spectroscopic characterization of the soluble guanylate cyclase-like heme domains from *Vibrio cholerae* and *Thermoanaerobacter tengcongensis*. Biochem. 2004; 43:10203–10211. [PubMed: 15287748]
26. Farhana A, et al. Environmental heme-based sensor proteins: implications for understanding bacterial pathogenesis. Antioxid Redox Signal. 2012; 17:1232–1245. [PubMed: 22494151]
27. Gilles-Gonzalez M-A, Gonzalez G. Heme-based sensors: defining characteristics, recent developments, and regulatory hypotheses. Journal of Inorganic Biochemistry. 2005; 99:1–22. [PubMed: 15598487]
28. Gilles-Gonzalez MA, et al. Heme-based sensors, exemplified by the kinase FixL, are a new class of heme protein with distinctive ligand binding and autoxidation. Biochemistry. 1994; 33:8067–8073. [PubMed: 8025112]
29. Boon EM, et al. Nitric oxide binding to prokaryotic homologs of the soluble guanylate cyclase beta1 H-NOX domain. J. Biol. Chem. 2006; 281:21892–21902. [PubMed: 16728401]
30. Pellicena P, et al. Crystal structure of an oxygen-binding heme domain related to soluble guanylate cyclases. Proc. Natl. Acad. Sci. USA. 2004; 101:12854–12859. [PubMed: 15326296]
31. Nioche P, et al. Femtomolar sensitivity of a NO sensor from *Clostridium botulinum*. Science. 2004; 306:1550–1553. [PubMed: 15472039]
32. Schmidt PM, et al. Identification of residues crucially involved in the binding of the heme moiety of soluble guanylate cyclase. J. Biol. Chem. 2004; 279:3025–3032. [PubMed: 14570894]
33. Boon EM, et al. A molecular basis for NO selectivity in soluble guanylate cyclase. Nat. Chem. Biol. 2005; 1:53–59. [PubMed: 16407994]
34. Boon EM, Marletta MA. Ligand specificity of H-NOX domains: from sGC to bacterial NO sensors. Journal of Inorganic Biochemistry. 2005; 99:892–902. [PubMed: 15811506]
35. Price MS, et al. *Shewanella oneidensis* MR-1 H-NOX regulation of a histidine kinase by nitric oxide. Biochem. 2007; 46:13677–13683. [PubMed: 17988156]
36. Arora DP, Boon EM. Nitric oxide regulated two-component signaling in *Pseudoalteromonas atlantica*. Biochem Biophys Res Commun. 2012; 421:521–526. [PubMed: 22521885]
37. Laub MT, et al. Phosphotransfer profiling: systematic mapping of two-component signal transduction pathways and phosphorelays. Meth. Enzymol. 2007; 423:531–548. [PubMed: 17609150]
38. Galperin MY. Diversity of structure and function of response regulator output domains. Curr. Opin. Microbiol. 2010; 13:150–159. [PubMed: 20226724]
39. Ma X, et al. NO and CO differentially activate soluble guanylyl cyclase via a heme pivot-bend mechanism. EMBO J. 2007; 26:578–588. [PubMed: 17215864]
40. Martin E, et al. Ligand selectivity of soluble guanylyl cyclase: effect of the hydrogen-bonding tyrosine in the distal heme pocket on binding of oxygen, nitric oxide, and carbon monoxide. J. Biol. Chem. 2006; 281:27836–27845. [PubMed: 16864588]
41. Olea C, et al. Probing the function of heme distortion in the H-NOX family. ACS Chem Biol. 2008; 3:703–710. [PubMed: 19032091]
42. Weinert EE, et al. Determinants of ligand affinity and heme reactivity in H-NOX domains. Angew Chem Int Ed Engl. 2010; 49:720–723. [PubMed: 20017169]
43. Weinert EE, et al. Controlling conformational flexibility of an O(2)-binding H-NOX domain. Biochemistry. 2011; 50:6832–6840. [PubMed: 21721586]
44. Winter MB, et al. Tunnels modulate ligand flux in a heme nitric oxide/oxygen binding (H-NOX) domain. Proc. Natl. Acad. Sci. USA. 2011; 108:E881–E889. [PubMed: 21997213]
45. Erbil WK, et al. A structural basis for H-NOX signaling in *Shewanella oneidensis* by trapping a histidine kinase inhibitory conformation. Proc. Natl. Acad. Sci. USA. 2009; 106:19753–19760. [PubMed: 19918063]

46. Tsai A-L, et al. A“sliding scale rule” for selectivity among NO, CO, and O_2 by heme protein sensors. Biochemistry. 2012; 51:172–186. [PubMed: 22111978]
47. Olea C Jr, et al. Structural insights into the molecular mechanism of H-NOX activation. Protein Science. 2010; 19:881–887. [PubMed: 20162612]
48. Yoo B-K, et al. Picosecond binding of the His ligand to four-coordinate heme in cytochrome c': a one-way gate for releasing proximal NO. J Am Chem Soc. 2013; 135:3248–3254. [PubMed: 23373628]
49. Olea C Jr, et al. Modulating Heme Redox Potential through Protein-Induced Porphyrin Distortion. J Am Chem Soc. 2010; 132:12794–12795. [PubMed: 20735135]
50. Stone JR, Marletta MA. Spectral and kinetic studies on the activation of soluble guanylate cyclase by nitric oxide. Biochemistry. 1996; 35:1093–1099. [PubMed: 8573563]
51. Muralidharan S, Boon EM. Heme flattening is sufficient for signal transduction in the H-NOX family. J Am Chem Soc. 2012; 134:2044–2046. [PubMed: 22257139]
52. Underbakke ES, et al. Higher-order interactions bridge the nitric oxide receptor and catalytic domains of soluble guanylate cyclase. Proc. Natl. Acad. Sci. USA. 2013; 110:6777–6782. [PubMed: 23572573]
53. Costerton JW, et al. Bacterial biofilms: a common cause of persistent infections. Science. 1999; 284:1318–1322. [PubMed: 10334980]
54. Römling U, Balsalobre C. Biofilm infections, their resilience to therapy and innovative treatment strategies. J. Intern. Med. 2012; 272:541–561. [PubMed: 23025745]
55. Stanley NR, Lazazzera BA. Environmental signals and regulatory pathways that influence biofilm formation. Mol. Microbiol. 2004; 52:917–924. [PubMed: 15130114]
56. McDougald D, et al. Should we stay or should we go: mechanisms and ecological consequences for biofilm dispersal. Nat. Rev. Microbiol. 2011; 10:39–50. [PubMed: 22120588]
57. Swanson MS, Hammer BK. *Legionella pneumophila* pathogesesis: a fateful journey from amoebae to macrophages. Annu Rev Microbiol. 2000; 54:567–613. [PubMed: 11018138]
58. Declerck P. Biofilms: the environmental playground of *Legionella pneumophila*. Environ Microbiol. 2010; 12:557–566. [PubMed: 19678829]
59. Makemson JC, et al. *Shewanella woodyi* sp. nov., an exclusively respiratory luminous bacterium isolated from the Alboran Sea. Int. J. Syst. Bacteriol. 1997; 47:1034–1039. [PubMed: 9336902]
60. Liu N, et al. Characterization of a diguanylate cyclase from *Shewanella woodyi* with cyclase and phosphodiesterase activities. Mol Biosyst. 2010; 6:1561–1564. [PubMed: 20467666]
61. Plate L, Marletta MA. Phosphorylation-dependent Derepression by the Response Regulator HnoC in the *Shewanella oneidensis* Nitric Oxide Signaling Network. under review. 2013
62. Ng W-L, Bassler BL. Bacterial quorum-sensing network architectures. Annu. Rev. Genet. 2009; 43:197–222. [PubMed: 19686078]
63. Timmen M. AI-1 Influences the Kinase Activity but Not the Phosphatase Activity of LuxN of *Vibrio harveyi*. Journal of Biological Chemistry. 2006; 281:24398–24404. [PubMed: 16807235]
64. Laub MT, Goulian M. Specificity in two-component signal transduction pathways. Annu. Rev. Genet. 2007; 41:121–145. [PubMed: 18076326]
65. Jones BW, Nishiguchi MK. Counterillumination in the Hawaiian bobtail squid, *Euprymna scolopes* Berry (Mollusca: Cephalopoda). Marine Biology. 2004; 144:1151–1155.
66. Visick KL, Ruby EG. *Vibrio fischeri* and its host: it takes two to tango. Curr. Opin. Microbiol. 2006; 9:632–638. [PubMed: 17049299]
67. Nyholm SV, McFall-Ngai MJ. The winnowing: establishing the squid-vibrio symbiosis. Nat. Rev. Microbiol. 2004; 2:632–642. [PubMed: 15263898]
68. Wang Y, Ruby EG. The roles of NO in microbial symbioses. Cell Microbiol. 2011; 13:518–526. [PubMed: 21338463]
69. Davidson SK, et al. NO means “yes” in the squid-vibrio symbiosis: nitric oxide (NO) during the initial stages of a beneficial association. Cell Microbiol. 2004; 6:1139–1151. [PubMed: 15527494]
70. Septer AN, et al. The haem-uptake gene cluster in *Vibrio fischeri* is regulated by Fur and contributes to symbiotic colonization. Environ Microbiol. 2011; 13:2855–2864. [PubMed: 21883801]

71. Zhu J, Mekalanos JJ. Quorum sensing-dependent biofilms enhance colonization in *Vibrio cholerae*. Dev. Cell. 2003; 5:647–656. [PubMed: 14536065]

72. Faruque SM, et al. Transmissibility of cholera: in vivo-formed biofilms and their relationship to infectivity and persistence in the environment. Proc. Natl. Acad. Sci. USA. 2006; 103:6350–6355. [PubMed: 16601099]

73. Davies BW, et al. DNA damage and reactive nitrogen species are barriers to *Vibrio cholerae* colonization of the infant mouse intestine. PLoS Pathog. 2011; 7:e1001295. [PubMed: 21379340]

74. Stern AM, et al. The NorR regulon is critical for *Vibrio cholerae* resistance to nitric oxide and sustained colonization of the intestines. MBio. 2012; 3:e00013–e00112. [PubMed: 22511349]

75. Hazelbauer GL, et al. Bacterial chemoreceptors: high-performance signaling in networked arrays. Trends in Biochemical Sciences. 2008; 33:9–19. [PubMed: 18165013]

76. Cheung J, Hendrickson WA. Sensor domains of two-component regulatory systems. Curr. Opin. Microbiol. 2010; 13:116–123. [PubMed: 20223701]

77. Stock AM, et al. Two-component signal transduction. Annu. Rev. Biochem. 2000; 69:183–215. [PubMed: 10966457]

78. Hengge R. Principles of c-di-GMP signalling in bacteria. Nat. Rev. Microbiol. 2009; 7:263–273. [PubMed: 19287449]

79. Schirmer T, Jenal U. Structural and mechanistic determinants of c-di-GMP signalling. Nat. Rev. Microbiol. 2009; 7:724–735. [PubMed: 19756011]

80. Letunic I, Bork P. Interactive Tree Of Life v2: online annotation and display of phylogenetic trees made easy. Nucleic Acids Res. 2011; 39:W475–W478. [PubMed: 21470960]

Highlights

- We describe Heme-Nitric oxide/Oxygen binding (H-NOX) domains function as sensors nitric oxide (NO) in bacteria
- H-NOX proteins interact with bacterial signaling proteins in two-component signaling systems or in cyclic-di-GMP metabolism
- Structural and mechanistic studies have elucidated how H-NOX domains selectively bind NO and transduce ligand binding into function
- H-NOX proteins share a common role in reorganizing important bacterial communal behaviors in response to nitric oxide
- H-NOX pathways control motility, biofilm formation, quorum sensing, and symbiosis

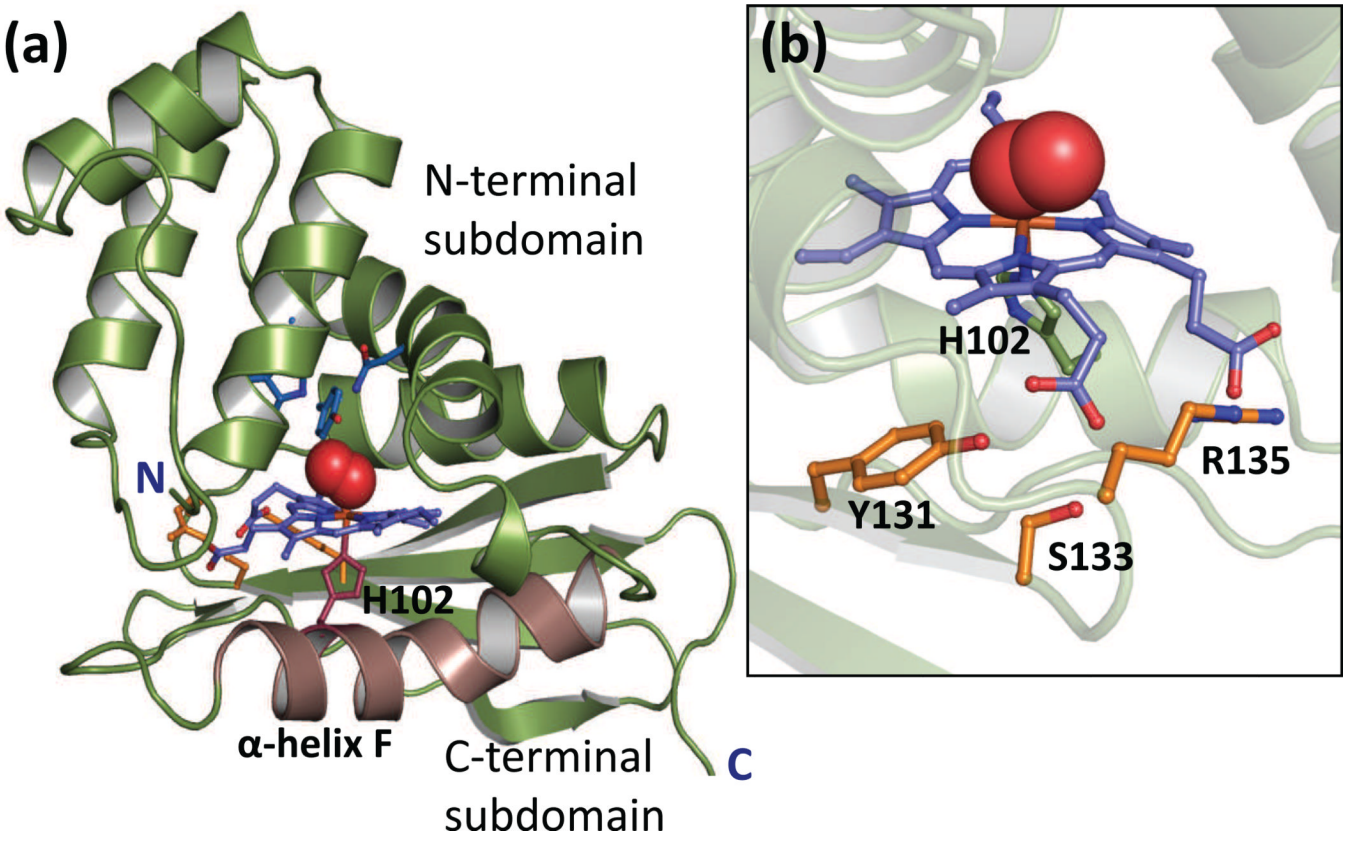

Fig. 1. X-ray crystal structure of *Thermoanaerobacter tengcongensis* (Tt) H-NOX

A. Structure of the H-NOX domain showing the N-terminal subdomain on top and C-terminal subdomain on the bottom with the heme cofactor buried in a pocket. The heme-coordinating H102 residue and α-helix F are at the bottom (light red).

B. Close-up of the heme-binding pocket. The YxSxR residues are shown in orange.

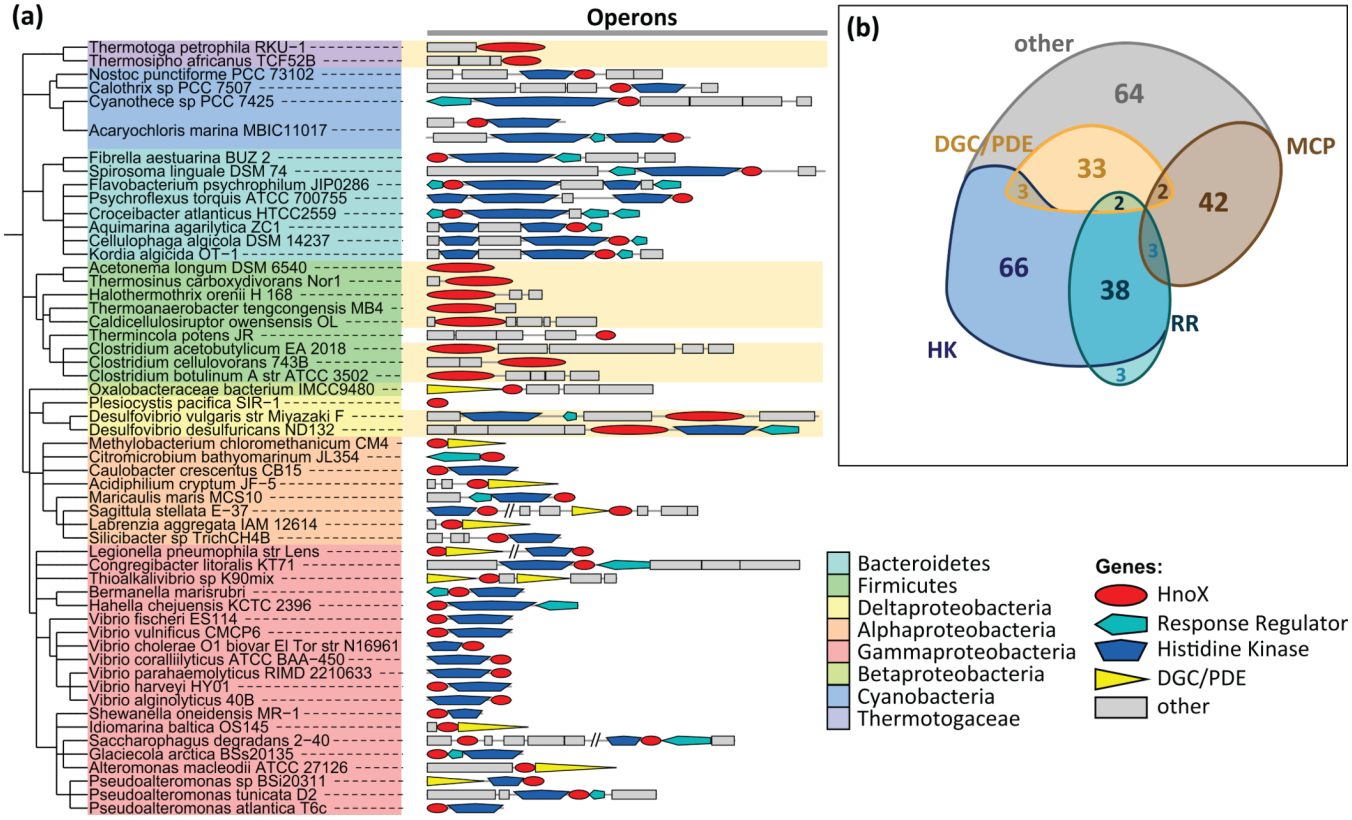

Fig. 2. Distribution of selected bacterial H-NOX genes and operon organization

A. Phylogenetic tree of select bacterial species containing H-NOX genes. To the right, the gene organization of the H-NOX containing operons is schematized. Operons shaded in yellow contain predicted O_2-binding H-NOX proteins based on the presence of a Tyr residue in the distal pocket. The figure was generated using the Interactive Tree of Life [80].

B. Euler diagram displaying the number of H-NOX containing species that possess each of the listed downstream effector genes in the H-NOX operon.

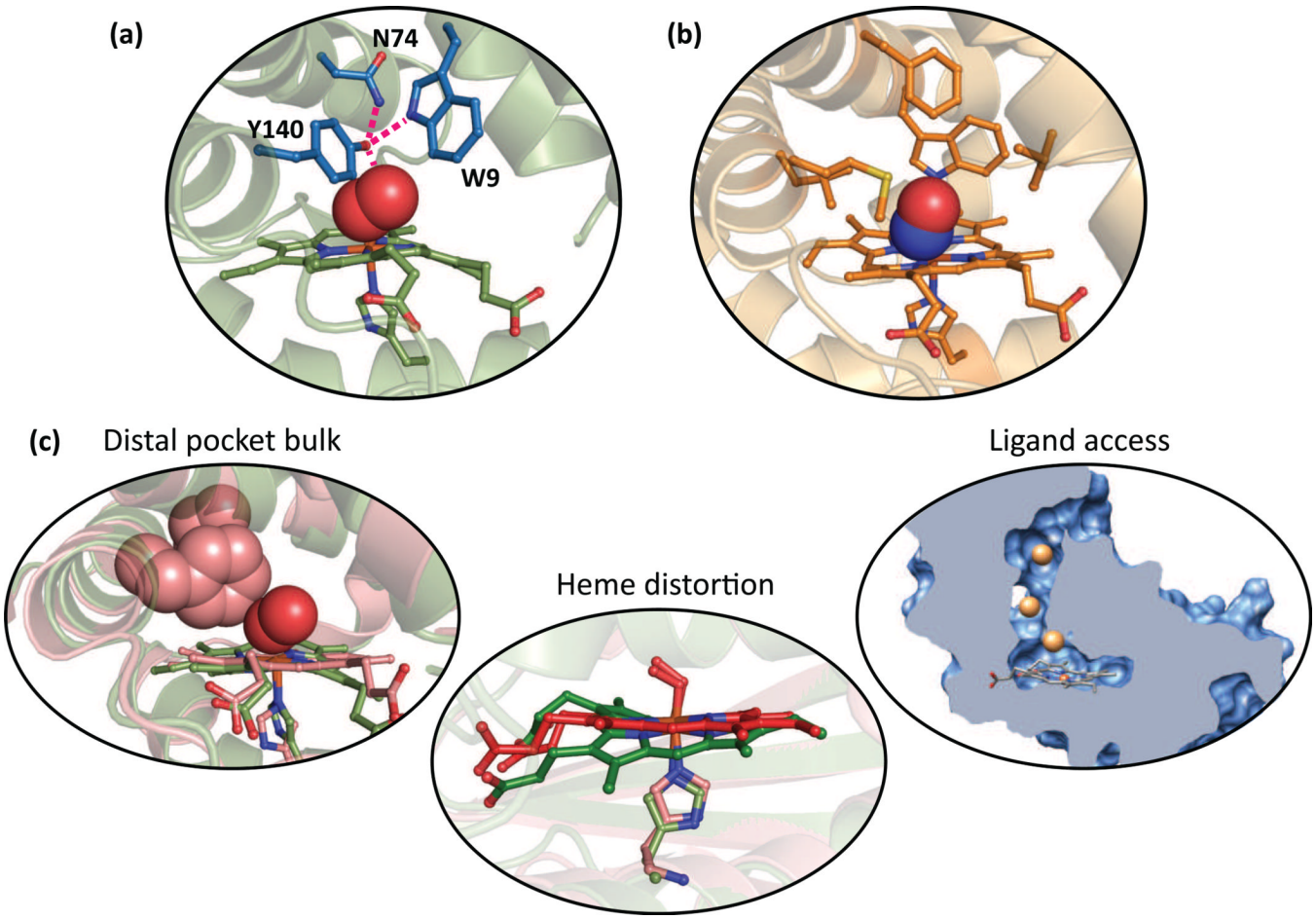

Fig. 3. Determinants of ligand selectivity for O_2 and NO binding

A. The heme pocket of the O_2-binding H-NOX protein from *T. tengcongensis* (PDB: 1U55) containing the H-bonding network consisting of Y140, W9, N74.

B. The hydrophobic heme-pocket of the NO-selective H-NOX protein from *Nostoc sp.* (PDB: 2O09).

C. Additional factors involved in tuning the ligand binding affinity of H-NOX proteins include heme distortion, distal pocket bulk and protein flexibility, as well as ligand access through tunnels into the heme pocket.

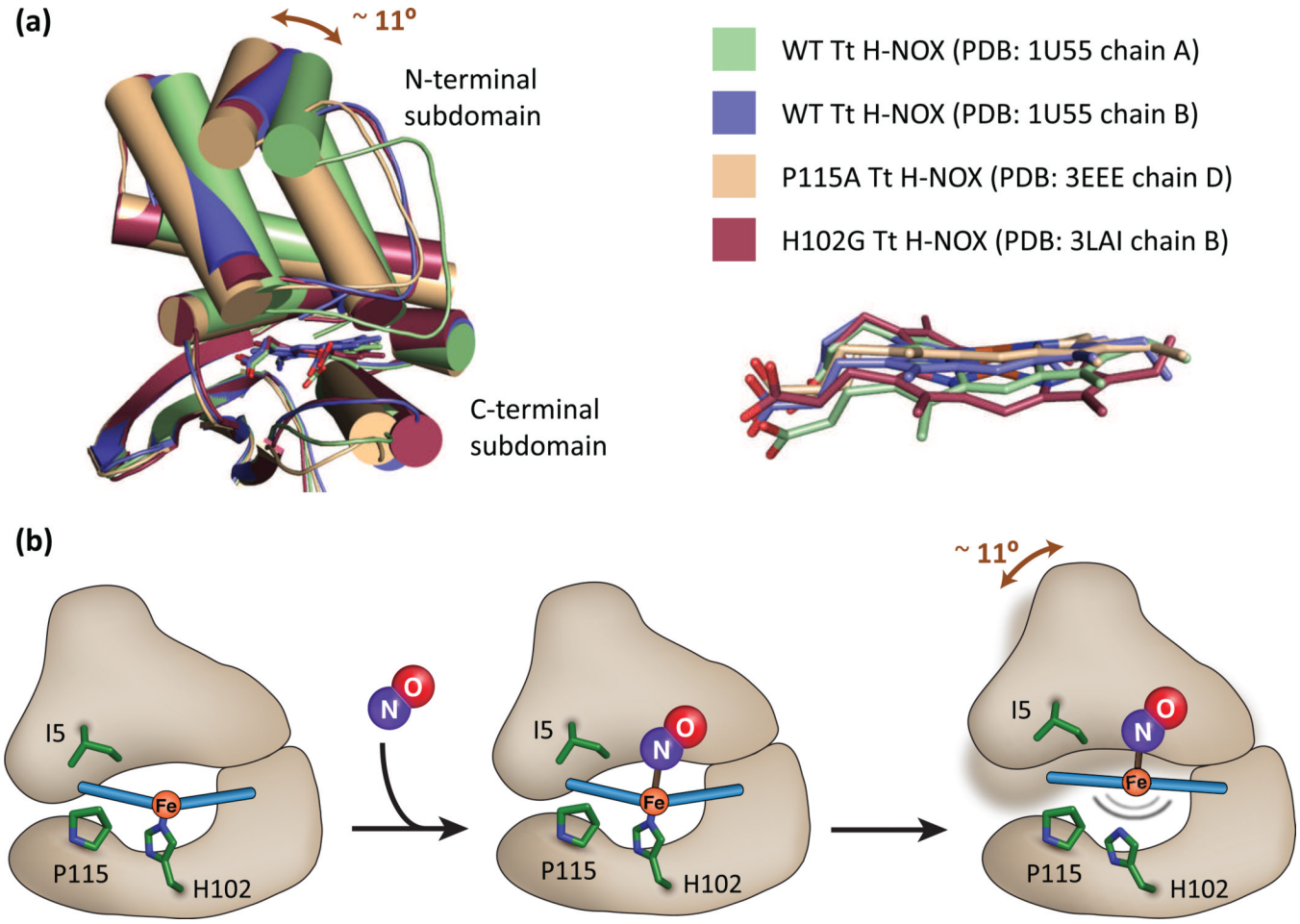

Fig. 4. Ligand-induced activation mechanism

A. Structural alignment of selected *Tt* H-NOX crystal structures (left) displaying the rotational displacement of the N-terminal subdomain with respect to the C-terminal subdomain. Alignment of the heme cofactors on the right displays the varying degree of heme distortion associated with each protein conformation.

B. Heme-strain model for H-NOX activation. In the unliganded H-NOX state, the heme is highly distorted due to van-der-Waals interaction between P115 and I5 (*Tt* H-NOX numbering) with two of the heme pyrroles. Initial formation of a six-coordinate Fe(II)-NO complex weakens the iron-His bond leading to His dissociation. Formation of the 5-coordinate Fe(II)-NO complex allows relaxation of the heme into a more planar geometry. Contacts between the N-terminal helix and the heme, in particular through I5, trigger an upward rotational displacement of the N-terminal subdomain (top) relative to the C-terminal subdomain (bottom).

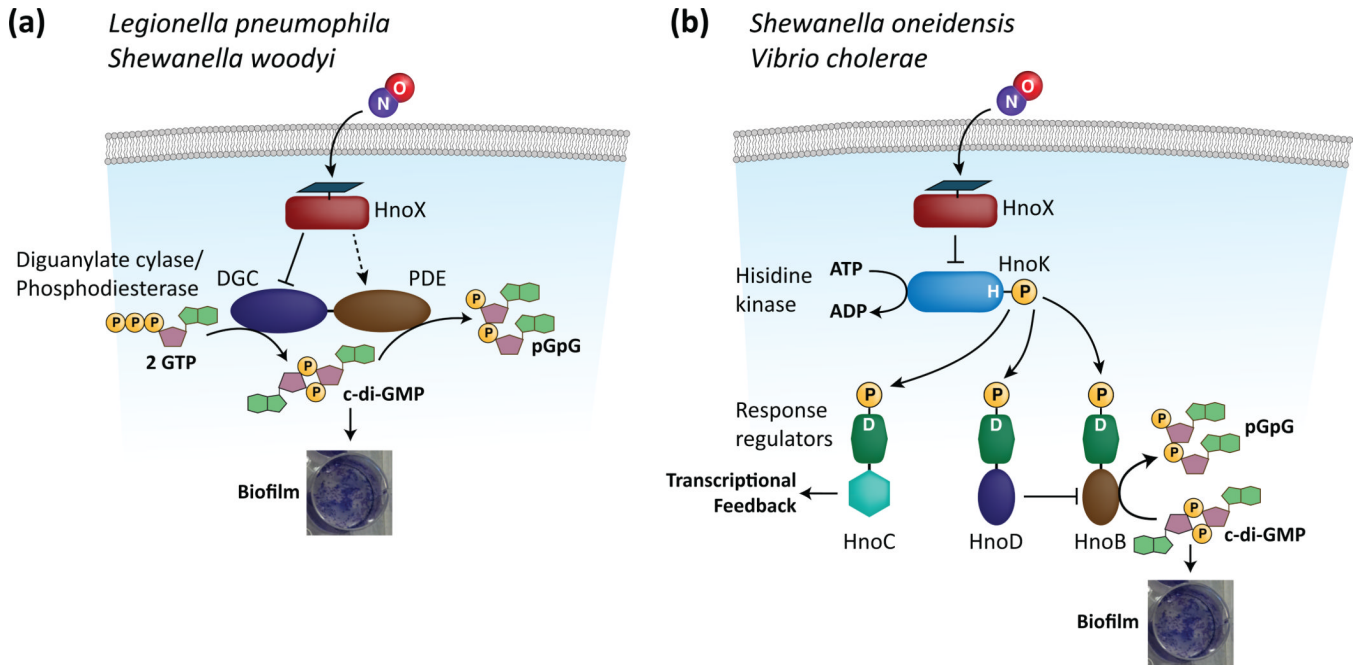

Fig. 5. H-NOX-dependent control of biofilm formation through regulation of cyclic-di-GMP levels

A. H-NOX signaling in *L. pneumophila* and *S. woodyi*. The H-NOX protein HnoX interacts with a dual diguanylate cyclase/phosphodiesterase (DGC/PDE) enzyme. The NO-bound H-NOX state inhibits DGC activity, and in the case of *S. woodyi*, also activates PDE activity, leading to increased c-di-GMP hydrolysis and lower biofilm formation in reponse to NO. B. H-NOX signaling promotes biofilm formation in response to NO in *S. oneidensis* and *V. cholerae*. The NO-bound H-NOX state inhibits autophosphorylation of the associated histidine kinase HnoK. HnoK possesses three phosphotransfer targets: HnoB, HnoC, and HnoD. HnoB contains a phosphorylation-activated PDE domain that hydrolyzes c-di-GMP. HnoD functions as a phosphorylation-dependent allosteric inhibitor of HnoB to fine-tune c-di-GMP hydrolysis. HnoC serves as a transcriptional regulator of the signaling genes in the network for further feedback control.

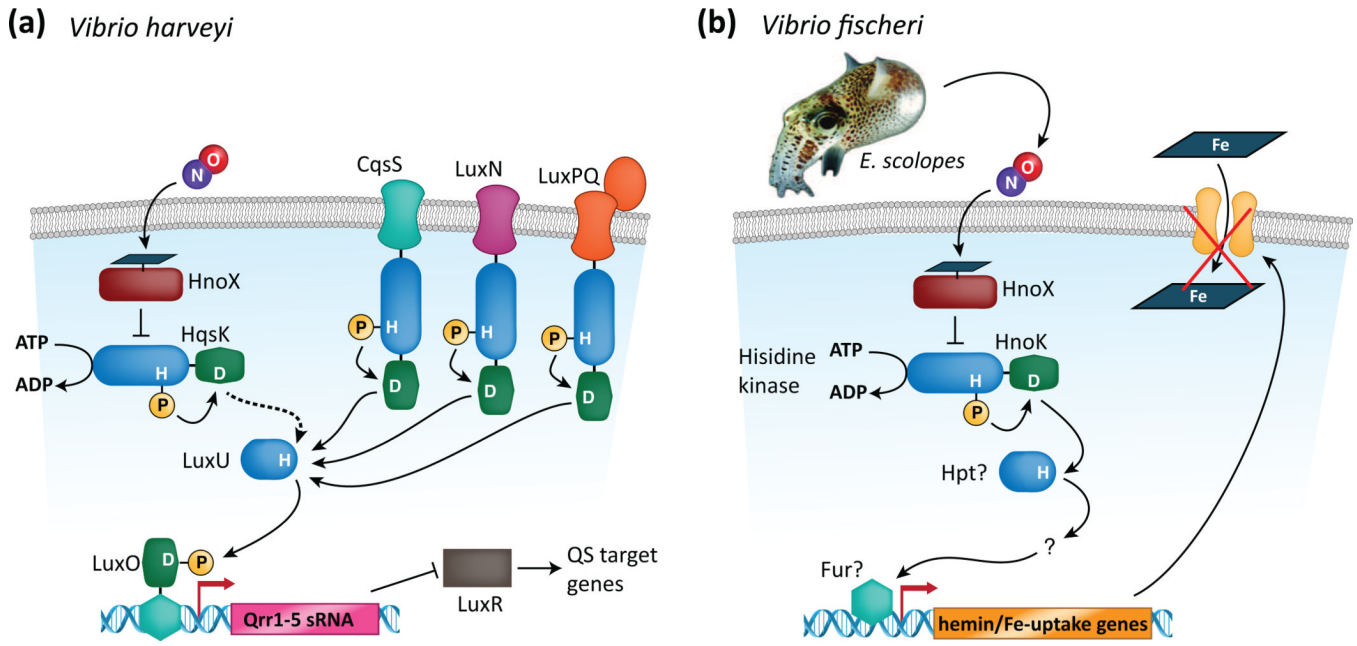

Fig. 6. H-NOX-dependent control of host colonization in *V. fischeri* and cross-talk with quorum sensing in *V. harveyi*

A. H-NOX signaling in *V. fischeri* in the symbiosis with the Hawaiian bobtail squid *E. scolopes*. Bacteria encounter NO during colonization of the squid light organ. NO-bound H-NOX inhibits autophosphorylation of the associated HK. Through an uncharacterized phosphorelay system, the NO-bound HnoX downregulates the expression of a gene set containing a Fur-binding motif in the promoter region. A subset of genes repress hemin-specific iron-uptake and utilization genes. Downregulation of intracellular iron levels in response to NO primes *V. fischeri* for exposure to ROS during the course of colonization. B. Interaction between the H-NOX signaling and quorum sensing pathway in *V. harveyi*. Quorum sensing autoinducers control the activity of three sensor HKs (CqsS, LuxN and LuxPQ). Phosphotransfer from all three HKs to a single Hpt protein, LuxU, and a RR, LuxO, integrates the quorum sensing signal. LuxO controls the expression of small regulatory RNAs Qrr1–5, which in turn repress LuxR expression. LuxU is the final transcriptional activator of quorum sensing genes, regulating bioluminescence and other communal responses. NO-bound HnoK inhibits the activity of the associated HqsK histine kinase. HqsK is capable of also phosphorylating LuxU, linking NO sensing to expression of quorum sensing genes.