REVIEW

Flavo-haemoglobin: the pre-eminent nitric oxide–detoxifying machine of microorganisms [version 1; peer review: 6 approved]

Robert K. Poole

Department of Molecular Biology and Biotechnology, The University of Sheffield, Firth Court, Sheffield, S10 2TN, UK

V1 First published: 08 Jan 2020, 9(F1000 Faculty Rev):7
<https://doi.org/10.12688/f1000research.20563.1>

Latest published: 08 Jan 2020, 9(F1000 Faculty Rev):7
<https://doi.org/10.12688/f1000research.20563.1>

Abstract

Flavo-haemoglobins were first described in yeast as early as the 1970s but their functions were unclear. The surge in interest in nitric oxide biology and both serendipitous and hypothesis-driven discoveries in bacterial systems have transformed our understanding of this unusual two-domain globin into a comprehensive, yet undoubtedly incomplete, appreciation of its pre-eminent role in nitric oxide detoxification. Here, I focus on research on the flavo-haemoglobins of microorganisms, especially of bacteria, and update several earlier and more comprehensive reviews, emphasising advances over the past 5 to 10 years and some controversies that have arisen. Inevitably, in light of space restrictions, details of nitric oxide metabolism and globins in higher organisms are brief.

Keywords

flavo-haemoglobin, nitric oxide, microbiology

Open Peer Review

Approval Status

	1	2	3	4	5	6
version 1						
08 Jan 2020						

Faculty Reviews are review articles written by the prestigious Members of **Faculty Opinions**. The articles are commissioned and peer reviewed before publication to ensure that the final, published version is comprehensive and accessible. The reviewers who approved the final version are listed with their names and affiliations.

1. **Andrés Vázquez-Torres**, University of Colorado School of Medicine, Aurora, USA
2. **Mark P. Brynildsen**, Princeton University, Princeton, USA
3. **John S. Olson**, Rice University, Houston, USA
4. **Marie-Alda Gilles-Gonzalez**, University of Texas Southwestern Medical Center, Dallas, USA
5. **Elizabeth M. Boon**, Stony Brook University, Stony Brook, USA
6. **Vinai C. Thomas**, University of Nebraska

Medical Center, Omaha, USA

Any comments on the article can be found at the end of the article.

Corresponding author: Robert K. Poole (r.poole@sheffield.ac.uk)

Author roles: Poole RK: Writing – Original Draft Preparation, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: Work in my laboratory has been continuously supported by the UK Biotechnology and Biological Sciences Research Committee via numerous research grants, for which I am most grateful.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Copyright: © 2020 Poole RK. This is an open access article distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: Poole RK. **Flavohaemoglobin: the pre-eminent nitric oxide-detoxifying machine of microorganisms [version 1; peer review: 6 approved]** F1000Research 2020, 9(F1000 Faculty Rev):7 <https://doi.org/10.12688/f1000research.20563.1>

First published: 08 Jan 2020, 9(F1000 Faculty Rev):7 <https://doi.org/10.12688/f1000research.20563.1>

Nitric oxide in biology and a caveat

The importance of the small gas nitric oxide (NO) can hardly have escaped the attention of most scientists in the fields of clinical medicine, physiology, biochemistry, microbiology and environmental science. “Popular” textbooks describe this science and medicine^{1,2}. Total citations of “nitric” AND “oxide” in the Web of Science Core Collection approach 300,000. Until the past few decades, the literature was dominated by chemistry but now the largest category is biochemistry and molecular biology (>42,000 citations, representing more than 14% of the total; Figure 1). This clearly demonstrates the pervasive impact of NO in biology. This short review aims to link these impacts with one of the several enzymes known to destroy this invaluable yet potentially toxic molecule.

NO is a free radical species; it may therefore be written formally as NO· (NO-dot) but conventionally simply as NO. The chemistry of NO is complex and, as a result, there are numerous intricacies, misunderstandings and sometimes errors in the literature. It is soluble in water (approximately 1.6 mM at 37 °C and 1.94 mM at 25 °C³) but does not react with it. The difficulties stem from its short lifetime in cellular environments: if generated at 10⁻⁷ M (for example, from NO synthases, or NOSs), NO has a lifetime of 30 min if its fate is oxidation

to NO₂ but may be as low as 1 s on reaction with biological targets, especially haems, thiols and superoxide anion⁴. A multitude of redox-related species may be generated from NO in biological situations and each may have different targets. This chemistry is not detailed here except where it is required for clarity, but excellent reviews exist⁵⁻⁷. In brief, nitrosonium cation (NO⁺), nitroxyl anion (NO⁻, although HNO is dominant at pH 7), nitrogen dioxide (NO₂), and dinitrogen trioxide (N₂O₃, the product of NO reacting with O₂) and peroxynitrite (ONOO⁻, the product of NO reacting with superoxide radical) are not “forms of NO” but products of NO reactions. There is only one NO^{4,8}!

The major source of NO *in vivo* is via the activity of NOSs. The complex biology, chemistry and medical significance of NOS are outside the scope of this commentary, but excellent reviews and articles cover mammalian⁹, microbial¹⁰ and the elusive plant NOS-like¹¹ activities. The roles of NO in signalling and “gasotransmitters” in higher organisms are also beyond the scope of this article, but see 7. In higher organisms, NO plays a key role in cellular immunity, where the gas, generated primarily by inducible NOS, attacks diverse macromolecules in invading microbes⁶. The only organisms in which flavohaemoglobins are found are microbes, including pathogens. Flavohaemoglobins

Figure 1. Publications per annum found by using the search term “nitric AND oxide” in Web of Science Core Collection, December 2019. The inset shows the number of citations in the search categorised by subject area.

are arguably the most important, but not the only, mechanism by which the microbe strikes back.

Why then do higher organisms not also possess flavohaemoglobins? The answer may be that the high concentrations of other (non-flavo) globins protect animal cells from excessive NO; the combination of methaemoglobin reductase and very high haemoglobin concentrations in red blood cells provides an effective NO removal mechanism, functionally equivalent to the NO dioxygenase activities of flavohaemoglobin¹².

The term “nitrosative stress” appears to have been introduced to this field in 1996¹³ to describe specifically the reaction of S-nitrosothiols (RSNOs, such as S-nitrosocysteine) with intracellular thiols via S-nitrosation (that is, the transfer of the nitrosonium group NO⁺ to biomolecules)⁴. It is important to note that the term should not be used, as it sometimes appears to be, to describe all NO chemistry in biology: “NO cannot act as a nitrosating agent, unless there are oxidizing agents present, such as a transition metal species or oxygen. Thus, NO cannot nitrosate thiols. Reports to the contrary result from the presence, sometimes adventitiously, of an oxidizing agent or from an imprecise description of the reaction”⁴. Thus, for example, the *Escherichia coli* flavohaemoglobin (Hmp), the subject of this article, cannot *directly* protect against nitrosating agents since only NO reacts in a physiologically useful way with this globin. “Nitrosative” is not an adjectival form of NO!

The broad reactivity of NO in biology implies that certain cellular components will be more susceptible to NO damage than others. A comprehensive kinetic model that encompasses this reactivity in *E. coli* that incorporates spontaneous and enzymatic reactions as well as damage and repair of biomolecules has been developed¹⁴. This model, informed by experimental measurements of NO dynamics, allows a detailed analysis of how NO distributes in *E. coli* cultures and identification of the control parameters of the NO distribution. The simulation predicted that Hmp functions as a dominant NO consumption pathway at O₂ concentrations as low as 35 μM (that is, microaerobic conditions): virtually all (99.85%) of the NO consumed by the cells was predicted to be through Hmp detoxification, and most of the remainder through oxidation by O₂ and reaction with superoxide anion. Surprisingly, Hmp loses utility as the NO delivery rate increases, as a result of substrate inhibition¹⁵. Such models are valuable for rigorously investigating NO stress in microbes and may identify novel strategies to potentiate the effects of NO¹⁴.

The discovery of the flavohaemoglobin Hmp

Hmp was discovered in bacteria in 1991¹⁶, only a year after key articles from Furgott, Ignarro and Murad identified the endothelium-derived relaxing factor (EDRF) as a gas with a molecular mass of only 30 and a year before the recognition of NO as “Molecule of the Year” by *Science* in 1992. Between 1989 and 1998, when the Nobel Prize for Physiology or Medicine was awarded to Furgott, Ignarro and Murad^{17–19}, the citation count per annum increased almost 70-fold. In fact, the discovery that EDRF was NO was also made by Salvador Moncada, then at

the Wellcome Research Laboratories in the UK, but, astonishingly, the Nobel award did not include Moncada. At the time, those involved in this work might not have foreseen how NO research would be so sustained (currently running at about 12,000 citations per annum) and all-encompassing in biology (Figure 1), nor could we have known that the flavohaemoglobin Hmp would assume the role of the pre-eminent NO-detoxifying enzyme in microbes. However, other contenders exist (see below).

Hmp was not the first bacterial globin to be identified and sequenced. Rather, the first was the haemoglobin of *Vitreoscilla*, which is an obscure bacterium whose soluble haemoprotein (Vgb) is dramatically increased in concentration under the microaerobic conditions that the organism encounters²⁰. The function or functions of this protein are still unknown: despite evidence that its expression in heterologous hosts can confer some protection from nitrosative stress²¹ and perhaps acts as an oxidase (although this is disputed²²), the generally accepted view is that Vgb facilitates oxygen utilisation; numerous articles have claimed biotechnological applications for this effect (see below). Vgb is unlike flavohaemoglobin: in a recent attempt to classify and logically name the globin family^{23,24}, we proposed that one family should be the 3/3 myoglobin-like proteins. One sub-family comprises the two-domain flavohaemoglobins (having haem and reductase modules), and the second comprises the single-domain globins, of which the *Vitreoscilla* protein is one example that comprises only the haem domain.

Hmp was the first microbial globin for which a gene sequence was obtained, for which modes of regulation were established and, most importantly, for which a function was unequivocally demonstrated. The serendipitous discovery of the *hmp* gene by the author and colleagues¹⁶ showed it to be a 44-kDa monomer with a haem domain that was almost half (46%) identical to Vgb. The C-terminal domain closely resembles ferredoxin-NADP⁺ reductase in that both have highly conserved binding sites for NAD(P)H and FAD²⁵. Purified Hmp possesses haem B and FAD^{26,27}, the presence of which was confirmed by the crystal structures of bacterial flavohaemoglobins^{28–30}. This reductase domain, which transfers electrons from NAD(P)H to haem-bound ligands (and soluble molecules^{25,31}), is essential for the function of Hmp^{32,33}.

Here, the original names Hmp (intentionally and cautiously suggesting only a haemoprotein nature and not rashly assuming a function) and *hmp* are used for the protein and gene, respectively, in enterobacteria¹⁶. Yhb in yeasts³⁴ and the more general abbreviation Fhb (flavohaemoglobin) are used elsewhere.

The functions of flavohaemoglobin

The first clue to function came from our discovery that solutions of NO gas (not a nitrosating agent) were potent inducers of *hmp* gene transcription³⁵. Shortly after, Gardner and colleagues demonstrated an enzymic function for Hmp, named NO dioxygenase (that is, the conversion of NO and O₂ to innocuous NO₃[−])³⁶. An alternative interpretation of this critical inducible reaction is that Hmp is not a dioxygenase (that is, in which the

two O atoms are used to oxygenate NO^{36,37}) but a denitrosylase^{38,39}; here, the haem-bound NO (Fe^{III}NO⁻) reacts with an oxygen molecule to produce nitrate. However, later evaluation of the reaction mechanism confirmed the NO dioxygenase mechanism⁴⁰: analysis of the stoichiometric product (nitrate) showed more than 99% double O-atom incorporation from Hmp¹⁸O₂. The NO dioxygenation mechanism involves (1) rapid reaction of NO with a Fe^{III}-O₂⁻ intermediate (the product of the facile reaction of O₂ with Hmp Fe^{II} to form Fe^{III}-OONO) and (2) rapid isomerization of this intermediate to form nitrate. The O-O bond homolyzes to form a protein-caged [Fe^{IV} = O .NO₂] intermediate, and ferryl oxygen attacks .NO₂ to form nitrate. This mechanism appears common to all higher haemoglobins and myoglobin that have been examined^{40,41} (Figure 2).

Therefore, NO detoxification by Hmp is optimal when oxygen is abundant. If oxygen is low (0–50 μM), NO defences are severely compromised, exhibiting a roughly 30-fold increase in NO clearance time compared with anaerobic and aerobic conditions for the same addition of an NO donor compound (50 μM DPTA NONOate)⁴². Modelling suggested that a steep drop in anoxic activity of NorV, a flavorubredoxin with NO removal activity, as [O₂] fell, combined with impaired translational and Hmp activities at low [O₂], results in suboptimal overlap of these two detoxification systems, resulting in up to a roughly 60% loss in their combined NO detoxification activities. In addition, at low [O₂] conditions, the concentrations of NO and O₂ oscillated, arising from kinetic competition for O₂ between the aerobic respiratory oxidases and Hmp⁴². Other candidates for NO detoxification are described later.

Although the NO dioxygenase activity of Hmp is the key mechanism for NO removal aerobically, an anoxic lower activity has been described⁴³. This exhibits a rate that is orders of magnitude slower than the O₂-dependent reaction, and the physiological relevance is a matter of debate⁴⁴. Nevertheless, NO binds Fe(III) Hmp to generate a nitrosyl adduct that is stable anoxically but decays in air to reform the Fe(III) protein⁴³. NO displaces CO bound to Fe(II) Hmp but CO recombines after only 2 s at room temperature, indicative of NO reduction and dissociation from the haem. Direct demonstration by membrane-inlet mass spectrometry of NO consumption and nitrous oxide production during anoxic incubation of NADH-reduced Hmp confirm the reaction *in vitro*⁴³ (Figure 2).

NO at nanomolar levels induces biofilm dispersal in numerous bacteria (for example^{45,46}). Consequently, NO-charged catheters have been investigated to prevent bacterial colonization⁴⁷. Thus, production of Fhb in *Pseudomonas aeruginosa* inhibits dispersal while imidazoles (see below) attenuate the prevention of dispersal⁴⁸.

Interestingly, rendering Hmp inactive in *Salmonella enterica* Gallinarum, in which RpoS and the SsrA/B regulator were also mutated, generated a hyper-susceptible strain that caused no mortality on injection into chickens. Vaccination of chickens with this strain conferred complete protection against challenge with virulent bacteria comparable to that achieved with a conventional vaccine strain⁴⁹. Disabling NO defences as a strategic utility is also suggested by the finding that elimination of ClpP (a major ATP-dependent protease) largely eliminated NO

Figure 2. Flavohaemoglobin as a pre-eminent nitric oxide (NO)-detoxifying protein. A typical (Gram-negative) envelope is shown that allows ready access of extracellular NO to intracellular Hmp. A small contribution to the cellular NO pool from intracellular sources is indicated. Hmp comprises a haem domain (red), an NAD(P)H-oxidizing domain (green) and an FAD domain (cyan). The redox centres are shown. The primary reaction catalysed is the conversion, by a dioxygenase mechanism, of O₂ and NO to form nitrate. Minor reactions also reported are shown below in the blue box. Transcriptional regulators identified thus far are shown at the right (green box), and the numerous consequences of Hmp activity are indicated at the right (orange box).

detoxification by *E. coli*⁵⁰. The effect is due to deficient transcript levels of *hmp* and widespread perturbations in other NO-responsive genes.

Recently, a new function was proposed (Figure 2): scavenging of the mild oxidant sulfur trioxide anion radical (STAR), a product in cells of (bi)sulfite oxidation⁵¹. The reaction of STAR with ferrous globins is rapid, and STAR reacts 260- and 1000-fold faster with Ngb (neuroglobin) and Fhb, respectively, than with glutathione, suggesting a detoxification function. The Fhbs of yeast and bacteria exhibit this activity, and a flavohaemoglobin mutant of *Saccharomyces cerevisiae* was slow-growing in the presence of sulfide attributed to mitochondrial damage⁵¹.

Flavohaemoglobins are widely distributed in microorganisms

Although much of what we have learned about these globins has come from bacteria, predominantly *E. coli* (sequence, function, gene regulation, and three-dimensional structure), the first reports of a microbial globin were in yeast^{52–55}. No involvement in NO chemistry was then suspected. Sequences for Fhbs are among the most numerous globin genes in bacteria of diverse taxons (533 sequences reported), exceeded only by class 2 truncated globins (622 sequences)²⁴. A newer survey identified 3318 Fhb sequences⁵⁶, comprising 2363 in bacteria and 204 in eukaryotes. Fhbs appear to be absent from Archaea⁵⁷. The bacterial sequences were distributed across 10 bacterial divisions with the highest number in the Proteobacteria. Interestingly, other divisions appear devoid of these proteins or they are uncommon, as in Bacteroidetes and Cyanobacteria.

Eukaryotic flavohaemoglobins are also found in protozoa, other fungi and two trypanosomes of insects. The protozoan parasite *Giardia intestinalis* possesses only five known haem proteins, one of which is flavohaemoglobin; this protein is expressed when trophozoites are exposed to NO or nitrite stresses and acts as an NO dioxygenase^{58,59}. The key roles of flavohaemoglobins in NO homeostasis in filamentous fungi and yeast are now widely recognised^{60–62}.

Both eukaryotic and bacterial flavohaemoglobins are generally considered soluble enzymes. Although bacterial flavohaemoglobins are normally recovered for purification from cytoplasmic fractions, around 30% are periplasmic in *E. coli* on the basis of Western immunoblotting⁶³, but the haem holoenzyme appears to be uniquely cytoplasmic. In yeast, Yhb is located in the cytosol, mitochondrial matrix and the intermembrane space but also in the inner membrane⁶⁴; however, the CO-binding fraction of Yhb is not present in inner membrane vesicles.

The manner as to how this important protein has become so widely distributed has recently been addressed^{23,24,56}. We proposed that the flavohaemoglobin gene family arose from an ancestral globin and later spread to eukaryotes via horizontal gene transfer^{23,24}. Such transfers between the domains of life are infrequent in biology, but “single-protein metabolic modules” (for example, Fhb and its self-contained NO detoxification

function) are prone to gene duplication (see below) and such horizontal gene transfer during evolution. A striking example of gene transfer from bacteria is afforded by a study of the acquisition by the eczema-causing fungus *Malassezia* of an Fhb from *Corynebacterium* and a concomitant increase in NO resistance⁵⁶.

Physiological aspects

It is now accepted that Hmp detoxifies NO, primarily aerobically, supported by the following key observations, many of which are from the older literature; illustrative examples are given.

- Null *hmp* mutants of *Salmonella* and *E. coli* are hyper-sensitive to the antimicrobial activity of NO or S-nitrosoglutathione (GSNO)^{65–69}.
- Hmp catalyses redox chemistry with NO and O₂ at the haem, and the haem of Hmp is readily reducible by physiological substrate (NAD(P)H) by means of electron transfer from FAD^{31–33,70}.
- The level of Hmp correlates with the level of NO resistance of respiration in *E. coli*. Respiration of an *hmp* mutant is highly sensitive to sub-micromolar NO, whereas respiration in cells pre-induced by treatment with sodium nitroprusside (SNP) is resistant to NO concentrations up to 50 μM⁷¹.
- Null *hmp* mutants of *Salmonella* and *E. coli* are hyper-sensitive to killing by human macrophages^{66,72,73}, and *hmp* mutants of *Yersinia pestis* are attenuated for virulence in the NO-rich infection bubo⁷⁴.
- Bacteria growing on the exterior of spleen microcolonies respond to soluble signals and induce synthesis of Hmp, thus eliminating inward NO diffusion and protection of interior bacterial population from NO-derived inducing signals⁷⁵.
- The Fhb of the plant pathogen *Erwinia chrysanthemi* confers NO tolerance on the fungus but also, by intercepting plant-derived NO, attenuates the hyper-sensitive response⁷⁶.
- *Salmonellae* experiencing nitrosative stress generate a burst of the alarmone nucleotide guanosine tetraphosphate (ppGpp). This activates transcription of valine biosynthetic genes, thereby re-establishing branched-chain amino acid biosynthesis that enables the translation of Hmp⁷⁷.
- The genome of the yeast *Candida albicans* contains three genes encoding flavohaemoglobin-related proteins but, based on studies of mutants lacking each of these genes, only one, CaYHB1, is responsible for NO consumption and detoxification⁷⁸. Loss of CaYHB1 increases the sensitivity of *C. albicans* to NO-mediated growth inhibition and decreases virulence in mice compared with that in wild-type strains.
- Duplicate flavohaemoglobins may have distinctive functions, and one has the established NO dioxygenase function. For example, a gene duplication event in the

Actinobacteria is suggested to have given rise to a second clade of type II flavohaemoglobins with unusual structural and functional properties, including D-lactate metabolism^{79,80}.

- Similarly, gene duplication seems to have generated fungal Fhb clades with different locations. In *Aspergillus oryzae*⁸¹, Fhb1 is located in the cytosol and the clade 4 Fhb2 in mitochondria, so that mechanisms for NO depletion in each cellular compartment are effected.

Regulation of flavohaemoglobin gene expression

NO or nitrosating agents up-regulate the *hmp* gene. In fact, *hmp* is consistently among the most highly up-regulated genes seen in genome-wide transcription profiling of *E. coli*, *Bacillus subtilis* and *Salmonella* cultures exposed to NO and nitrosating agents^{82–84}, in *Salmonella* following infection and induction of NO synthesis in J774 cells⁷³, and in the plant symbiont *Sinorhizobium meliloti*⁸⁵. Recently, NO₂, an air pollutant, was also reported to up-regulate *hmp* expression in *Pseudomonas* strains⁸⁶.

Although our understanding of *hmp* regulation is incomplete, several mechanisms have been identified and studied so far. These include several described in early studies^{87–90} and, more recently, a sigma-dependent small RNA⁹¹. In *S. aureus*, the two-component regulator SrrAB, generally considered an oxygen sensor, regulates *hmp* under low-oxygen conditions or on exposure to NO^{92–94}. In *Salmonella*, DksA recently emerged as an important factor for full expression of *hmp* transcription following NO exposure⁹⁵.

A major mechanism is undoubtedly via NsrR^{73,96–98}, a transcriptional repressor in the Rrf2 family containing an NO-sensitive FeS cluster (probably [4Fe-4S]). Reaction of the cluster with NO decreases its DNA-binding affinity and relieves repression at sensitive promoters that control expression of not only *hmp* but also *poxB* (via read-through from the upstream *hcp-hcr* genes) and the *sufABCDSE* cluster involved in iron-sulfur biogenesis and repair^{99,100}. Recent work shows that *nsrR* is expressed from a strong promoter but that translation is inefficient. This is important since target promoters with low affinity for NsrR may partially escape repression⁹⁹. When H₂O₂ and NO coexist (as they do in the phagolysosome), NO detoxification is delayed, an effect attributed to inhibition by H₂O₂ of *hmp* gene transcription and translation under the control of NsrR¹⁰¹.

In eukaryotic fungi such as *Aspergillus*, two proteins, FhbA and FhbB, are differentially induced to catabolise NO^{61,102}. NO is produced endogenously by a nitrate reductase early in the transition from vegetative growth to development. NO homeostasis is critical since NO levels influence the balance between conidiation and sexual reproduction.

Inhibitors of flavohaemoglobin activity and their utility

Since flavohaemoglobins confer a degree of pathogen resistance to NO generated by the immune system, including within the macrophage and its cocktail of reactive species (see above), inhibitors that target the haem prosthetic group of flavohaemoglobins are potential antimicrobial agents. Imidazoles having

bulky aromatic substituents fit into the globin haem pocket and coordinate the ferric iron with a K_d of 333 μM³⁰. Structural studies confirm that azole binds the Fhb haem and reveal major conformational reorganisation^{103,104}.

Others (miconazole, econazole, clotrimazole and ketoconazole) have similar activities against Fhbs¹⁰⁵ and inhibit NO metabolism in bacteria and yeast. However, they do not achieve the NO-induced stasis seen in flavohaemoglobin-null mutants. One of these agents, miconazole, is the most effective azole against *Staphylococcus* and ligates to both ferric and ferrous globin¹⁰⁶. Over 20 years ago^{107,108}, we reported that, in the absence of NO, Hmp generates superoxide anion by single electron transfer from NAD(P)H to haem-bound oxygen. Interestingly, miconazole enhanced superoxide production by the *S. aureus* enzyme, so that, in macrophages, bacteria possessing flavohaemoglobin are compromised in survival compared with flavohaemoglobin-deficient bacteria¹⁰⁶. This presumably is attributed to the inhibitor binding to haem and diverting electrons to oxygen^{107,108}. Other acceptors³¹ may also be reduced when haem function is blocked, as occurs with CO³¹.

Alternative inhibitors might be found among quinones and nitroaromatic compounds. *S. aureus* flavohaemoglobin rapidly reduces these compounds, which may act as subversive substrates, diverting electron flux from FAD and enhancing the toxicity of NO by formation of superoxide¹⁰⁹.

Flavohaemoglobins in biotechnology?

There have been countless reports of the ability of the *Vitreoscilla* globin, when expressed in heterologous hosts, to enhance aerobic cell yields or product formation^{110–112}. The mechanistic basis of these diverse effects remains unclear. However, a recent report¹¹³ claims that both an exogenously introduced *Vitreoscilla* globin and the native Fhb enhance pullulan production in the yeast *Aureobasidium*. Fhb gene expression in the yeast was elevated 3.5-fold over native levels, based on reverse transcription polymerase chain reaction (RT-PCR) data, but CO difference spectra show only a modest increase in a pigment with some of the characteristics of globins or other haem proteins; the broad absorbance spectra and noisy traces should not be interpreted as unequivocal evidence of high globin expression. Expression of either globin increased oxygen uptake, but the data are not quantified and no mechanism for enhanced pullulan yields is presented.

E. coli Hmp, anchored to electrodes, electrolytically interconverts NADH and NAD⁺ by transfer of electrons to the FAD moiety where NADH/NAD⁺ is transformed. It is suggested that this might be employed in NAD-dependent bioelectrodes for biosyntheses, biosensors and biofuel cells¹¹⁴.

Flavohaemoglobin is not unique as a nitric oxide–detoxifying machine

Many other globins convert NO to NO₃[–] via an NO dioxygenase activity. These include the truncated globin of *Mycobacterium tuberculosis*, trHbN¹¹⁵, the single-domain globin Cgb of *Campylobacter jejuni*¹¹⁶, *M. tuberculosis* HbN¹¹⁵, mammalian cytoglobin¹¹⁷ and *Arabidopsis* cytoglobin 3¹¹⁸.

When oxygen is available, the catalytic efficiency of the Fhb reaction, k_{cat}/K_m , is very high: up to $2400 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$ ¹¹⁹. However, under anoxic or low-oxygen conditions, where the activity of Hmp is dramatically reduced, NO tolerance in *Salmonella* is affected additionally by a combination of three enzymes, flavorubredoxin (NorV), and cytochrome *c* nitrite reductase (NrfA). A study of the effects of all eight possible combinations of *norV*, *hmp* and *nrfA* single, double and triple mutations suggested an important additive role for both NorV and NrfA^{120,121}. None of the NO detoxification systems—Hmp, NorV and NrfA—is solely responsible for nitrosative stress tolerance of *S. typhimurium* in raw sausages where sodium nitrite is used as a curing agent¹²². Somewhat different conclusions were reached in a study of a uropathogenic strain of *E. coli* (UPEC) that induces a variety of defence mechanisms in response to NO, including direct NO detoxification (Hmp, NorV, NrfA), iron-sulfur cluster repair (YtfE), and the expression of the NO-tolerant cytochrome *bd-I* respiratory oxidase (CydAB)^{123,124}. During UPEC growth and survival during infection, loss of the flavohaemoglobin Hmp and cytochrome *bd-I* elicited the greatest sensitivity to NO-mediated growth inhibition, whereas all but the periplasmic nitrite reductase NrfA provided protection against neutrophil killing and promoted survival within activated macrophages. Intriguingly, cytochrome *bd-I* was the only system that augmented UPEC survival in a mouse model, suggesting that maintaining aerobic respiration under conditions of nitrosative stress is a key factor for host colonisation. In *Salmonella enterica* also, cytochrome *bd* augments defences against NO in systemic tissues¹²⁵. Thus, cytochrome *bd* emerges as a major contributor to bacterial NO tolerance and host colonisation under microaerobic conditions. The hybrid cluster protein Hcp and its NADH-dependent cognate reductase Hcr were not tested in this study, but it is striking

that a role for this system as a high-affinity NO reductase could be demonstrated only when the Hcp reductase was introduced into a strain deleted for the *nirBD*, *nrfAB*, *norVW*, *hmp* and *hcp* genes¹²⁶. Other non-globin contenders include the flavorubredoxin and nitrite reductase NrfA of *E. coli*¹²⁷, various flavodiiron proteins, *Paracoccus denitrificans* NO reductase NorBC, and the NO reductase of *S. aureus*¹²⁸.

Conclusions and outlook

The flavohaemoglobins of numerous bacterial species and groups, yeasts, fungi and protozoa continue to fascinate those devoted to understanding globin functions, NO homeostasis in biology and clinical medicine. Our knowledge has exploded since their discovery in 1991 (at least at a molecular level) and has revealed new paradigms of enzyme mechanisms, gene regulatory mechanisms and physiological significance. Undoubtedly, much remains to be learned. It is striking, though, that throughout this period (almost 30 years), no clear evidence has emerged for a flavohaemoglobin in higher organisms. This continues to offer the hope that such a protein, a “single protein metabolic module”, might represent a useful target for antimicrobial therapies. Imidazoles with great efficacy have been identified as inhibitors and these efforts, in concert with increasing understanding of protein function, ligand and electron migration within such flavoproteins, may yet give us new antimicrobial weapons.

Acknowledgements

I am indebted to the many talented and industrious postdoctoral scientists, PhD students and overseas collaborators with whom I have enjoyed working over the years.

References

- Ignarro LJ: **NO More Heart Disease**. St. Martin's Griffin, New York. 2005.
- Butler A, Nicholson R: **Life, Death and Nitric Oxide**. Royal Society of Chemistry, Cambridge. 2003.
[Reference Source](#)
- Zacharia IG, Deen WM: **Diffusivity and solubility of nitric oxide in water and saline**. *Ann Biomed Eng*. 2005; **33**(2): 214–22.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Poole RK, Hughes MN: **New functions for the ancient globin family: bacterial responses to nitric oxide and nitrosative stress**. *Mol Microbiol*. 2000; **36**(4): 775–83.
[PubMed Abstract](#) | [Publisher Full Text](#)
- F** Fukuto JM, Carrington SJ, Tantillo DJ, *et al.*: **Small molecule signaling agents: the integrated chemistry and biochemistry of nitrogen oxides, oxides of carbon, dioxygen, hydrogen sulfide, and their derived species**. *Chem Res Toxicol*. 2012; **25**(4): 769–93.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | **F1000 Recommendation**
- Bowman LA, McLean S, Poole RK, *et al.*: **The diversity of microbial responses to nitric oxide and agents of nitrosative stress close cousins but not identical twins**. *Adv Microb Physiol*. 2011; **59**: 135–219.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Wareham LK, Southam HM, Poole RK: **Do nitric oxide, carbon monoxide and hydrogen sulfide really qualify as 'gasotransmitters' in bacteria?** *Biochem Soc Trans*. 2018; **46**(5): 1107–18.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Hughes MN: **Chemistry of nitric oxide and related species**. *Meth Enzymol*. 2008; **436**: 3–19.
[PubMed Abstract](#) | [Publisher Full Text](#)
- F** Stuehr DJ, Vasquez-Vivar J: **Nitric oxide synthases—from genes to function**. *Nitric Oxide*. 2017; **63**: 29.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | **F1000 Recommendation**
- F** Kinkel TL, Ramos-Montañez S, Pando JM, *et al.*: **An essential role for bacterial nitric oxide synthase in *Staphylococcus aureus* electron transfer and colonization**. *Nat Microbiol*. 2016; **2**: 16224.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | **F1000 Recommendation**
- F** Santolini J, André F, Jeandroz S, *et al.*: **Nitric oxide synthase in plants: Where do we stand?** *Nitric Oxide*. 2017; **63**: 30–8.
[PubMed Abstract](#) | [Publisher Full Text](#) | **F1000 Recommendation**
- Gladwin MT, Ognibene FP, Pannell LK, *et al.*: **Relative role of heme nitrosylation and beta-cysteine 93 nitrosation in the transport and metabolism of nitric oxide by hemoglobin in the human circulation**. *Proc Natl Acad Sci U S A*. 2000; **97**(18): 9943–8.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Hausladen A, Privalle CT, Keng T, *et al.*: **Nitrosative Stress: Activation of the Transcription Factor OxyR**. *Cell*. 1996; **86**(5): 719–29.
[PubMed Abstract](#) | [Publisher Full Text](#)
- F** Robinson JL, Brynildsen MP: **A kinetic platform to determine the fate of nitric oxide in *Escherichia coli***. *PLoS Comput Biol*. 2013; **9**(5): e1003049.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | **F1000 Recommendation**
- Gardner PR, Gardner AM, Martin LA, *et al.*: **Nitric-oxide dioxygenase activity and**

- function of flavohemoglobins. sensitivity to nitric oxide and carbon monoxide inhibition. *J Biol Chem.* 2000; 275(41): 31581–7.
[PubMed Abstract](#) | [Publisher Full Text](#)
16. Vasudevan SG, Armarego WL, Shaw DC, *et al.*: Isolation and nucleotide sequence of the hmp gene that encodes a hemoglobin-like protein in *Escherichia coli* K-12. *Mol Gen Genet.* 1991; 226(1–2): 49–58.
[PubMed Abstract](#) | [Publisher Full Text](#)
 17. Murad F: Discovery of Some of the Biological Effects of Nitric Oxide and its Role in Cell Signaling. *Biosci Rep.* 1999; 19(3): 133–54.
[PubMed Abstract](#) | [Publisher Full Text](#)
 18. Furchtgott RF: Endothelium-Derived Relaxing Factor: Discovery, Early Studies, and Identification as Nitric Oxide (Nobel Lecture). *Angew Chem Int Ed.* 1999; 38(13–14): 1870–80.
[Publisher Full Text](#)
 19. Ignarro LJ: Nitric Oxide: A Unique Endogenous Signaling Molecule in Vascular Biology (Nobel Lecture). *Angew Chem Int Ed.* 1999; 38(13–14): 1882–92.
[Publisher Full Text](#)
 20. Webster DA, Orii Y: Physiological role of oxygenated cytochrome o: Observations on whole-cell suspensions of *Vitreoscilla*. *J Bacteriol.* 1978; 135(1): 62–7.
[PubMed Abstract](#) | [Free Full Text](#)
 21. Frey AD, Farrés J, Bollinger CJ, *et al.*: Bacterial hemoglobins and flavohemoglobins for alleviation of nitrosative stress in *Escherichia coli*. *Appl Environ Microbiol.* 2002; 68(10): 4835–40.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 22. Dikshit RP, Dikshit KL, Liu Y, *et al.*: The bacterial hemoglobin from *Vitreoscilla* can support the aerobic growth of *Escherichia coli* lacking terminal oxidases. *Arch Biochem Biophys.* 1992; 293(2): 241–5.
[PubMed Abstract](#) | [Publisher Full Text](#)
 23. Vinogradov SN, Bailly X, Smith DR, *et al.*: Microbial eukaryote globins. *Adv Microb Physiol.* 2013; 63: 391–446.
[PubMed Abstract](#) | [Publisher Full Text](#)
 24. Vinogradov SN, Tinajero-Trejo M, Poole RK, *et al.*: Bacterial and archaeal globins - a revised perspective. *Biochim Biophys Acta.* 2013; 1834(9): 1789–800.
[PubMed Abstract](#) | [Publisher Full Text](#)
 25. Andrews SC, Shipley D, Keen JN, *et al.*: The haemoglobin-like protein (HMP) of *Escherichia coli* has ferrisiderophore reductase activity and its C-terminal domain shares homology with ferredoxin NADP⁺ reductases. *FEBS Lett.* 1992; 302(3): 247–52.
[PubMed Abstract](#) | [Publisher Full Text](#)
 26. Ioannidis N, Cooper CE, Poole RK: Spectroscopic studies on an oxygen-binding haemoglobin-like flavohaemoprotein from *Escherichia coli*. *Biochem J.* 1992; 288(Pt 2): 649–55.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 27. Poole RK, D'mello R, Hill S, *et al.*: The oxygen reactivity of bacterial respiratory haemoproteins: oxidases and globins. *Biochim Biophys Acta.* 1994; 1187(2): 226–31.
[PubMed Abstract](#) | [Publisher Full Text](#)
 28. Ermler U, Siddiqui RA, Cramm R, *et al.*: Crystal structure of the flavohemoglobin from *Alcaligenes eutrophus* at 1.75 Å resolution. *EMBO J.* 1995; 14(24): 6067–77.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 29. Ollesch G, Kaunzinger A, Juchelka D, *et al.*: Phospholipid bound to the flavohemoprotein from *Alcaligenes eutrophus*. *Eur J Biochem.* 1999; 262(2): 396–405.
[PubMed Abstract](#) | [Publisher Full Text](#)
 30. Ilari A, Bonamore A, Farina A, *et al.*: The X-ray structure of ferric *Escherichia coli* flavohemoglobin reveals an unexpected geometry of the distal heme pocket. *J Biol Chem.* 2002; 277(26): 23725–32.
[PubMed Abstract](#) | [Publisher Full Text](#)
 31. Poole RK, Rogers NJ, D'mello RA, *et al.*: *Escherichia coli* flavohaemoglobin (Hmp) reduces cytochrome c and Fe(III)-hydroxamate K by electron transfer from NADH via FAD: sensitivity of oxidoreductase activity to haem-bound dioxygen. *Microbiology.* 1997; 143(Pt 5): 1557–65.
[PubMed Abstract](#) | [Publisher Full Text](#)
 32. Poole RK, Ioannidis N, Orii Y: Reactions of the *Escherichia coli* flavohaemoglobin (Hmp) with oxygen and reduced nicotinamide adenine dinucleotide: evidence for oxygen switching of flavin oxidation and a mechanism for oxygen sensing. *Proc Biol Sci.* 1994; 255(1344): 251–8.
[PubMed Abstract](#) | [Publisher Full Text](#)
 33. Poole RK, Ioannidis N, Orii Y: Reactions of the *Escherichia coli* flavohaemoglobin (Hmp) with NADH and near-micromolar oxygen: oxygen affinity of NADH oxidase activity. *Microbiology.* 1996; 142(Pt 5): 1141–8.
[PubMed Abstract](#) | [Publisher Full Text](#)
 34. Cassanova N, O'Brien KM, Stahl BT, *et al.*: Yeast flavohemoglobin, a nitric oxide oxidoreductase, is located in both the cytosol and the mitochondrial matrix: effects of respiration, anoxia, and the mitochondrial genome on its intracellular level and distribution. *J Biol Chem.* 2005; 280(9): 7645–53.
[PubMed Abstract](#) | [Publisher Full Text](#)
 35. Poole RK, Anjum MF, Membrillo-Hernández J, *et al.*: Nitric oxide, nitrite, and Fnr regulation of hmp (flavohemoglobin) gene expression in *Escherichia coli* K-12. *J Bacteriol.* 1996; 178(18): 5487–92.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 36. Gardner PR, Gardner AM, Martin LA, *et al.*: Nitric oxide dioxygenase: an enzymic function for flavohemoglobin. *Proc Natl Acad Sci U S A.* 1998; 95(18): 10378–83.
[PubMed Abstract](#) | [Free Full Text](#)
 37. Hausladen A, Gow AJ, Stamler JS: Nitrosative stress: metabolic pathway involving the flavohemoglobin. *Proc Natl Acad Sci U S A.* 1998; 95(24): 14100–5.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 38. Hausladen A, Stamler JS: Is the flavohemoglobin a nitric oxide dioxygenase? *Free Radic Biol Med.* 2012; 53(5): 1209–10.
[PubMed Abstract](#) | [Publisher Full Text](#)
 39. Hausladen A, Gow A, Stamler JS: Flavohemoglobin denitrosylase catalyzes the reaction of a nitroxyl equivalent with molecular oxygen. *Proc Natl Acad Sci U S A.* 2001; 98(18): 10108–12.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 40. Gardner PR, Gardner AM, Brashear WT, *et al.*: Hemoglobins dioxygenate nitric oxide with high fidelity. *J Inorg Biochem.* 2006; 100(4): 542–50.
[PubMed Abstract](#) | [Publisher Full Text](#)
 41. Gardner P: Nitric oxide dioxygenase function and mechanism of flavohemoglobin, hemoglobin, myoglobin and their associated reductases. *J Inorg Biochem.* 2005; 99(1): 247–66.
[PubMed Abstract](#) | [Publisher Full Text](#)
 42. Robinson JL, Brynildsen MP: Discovery and dissection of metabolic oscillations in the microaerobic nitric oxide response network of *Escherichia coli*. *Proc Natl Acad Sci U S A.* 2016; 113(12): E1757–E1766.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 43. Kim SO, Orii Y, Lloyd D, *et al.*: Anoxic function for the *Escherichia coli* flavohaemoglobin (Hmp): reversible binding of nitric oxide and reduction to nitrous oxide. *FEBS Lett.* 1999; 445(2–3): 389–94.
[PubMed Abstract](#) | [Publisher Full Text](#)
 44. Gardner AM, Gardner PR: Flavohemoglobin detoxifies nitric oxide in aerobic, but not anaerobic, *Escherichia coli*. Evidence for a novel inducible anaerobic nitric oxide-scavenging activity. *J Biol Chem.* 2002; 277(10): 8166–71.
[PubMed Abstract](#) | [Publisher Full Text](#)
 45. Arora DP, Hossain S, Xu Y, *et al.*: Nitric Oxide Regulation of Bacterial Biofilms. *Biochemistry.* 2015; 54(24): 3717–28.
[PubMed Abstract](#) | [Publisher Full Text](#)
 46. Barraud N, Schleheck D, Klebensberger J, *et al.*: Nitric oxide signaling in *Pseudomonas aeruginosa* biofilms mediates phosphodiesterase activity, decreased cyclic di-GMP levels, and enhanced dispersal. *J Bacteriol.* 2009; 191(23): 7333–42.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 47. Margel D, Mizrahi M, Regev-Shoshani G, *et al.*: Nitric oxide charged catheters as a potential strategy for prevention of hospital acquired infections. *PLoS One.* 2017; 12(4): e0174443.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 48. Zhu X, Oh HS, Ng YCB, *et al.*: Nitric Oxide-Mediated Induction of Dispersal in *Pseudomonas aeruginosa* Biofilms Is Inhibited by Flavohemoglobin Production and Is Enhanced by Imidazole. *Antimicrob Agents Chemother.* 2018; 62(3): pii: e01832–17.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 49. Cho Y, Park YM, Barate AK, *et al.*: The role of *rpoS*, *hmp*, and *ssrAB* in *Salmonella enterica* Gallinarum and evaluation of a triple-deletion mutant as a live vaccine candidate in Lohmann layer chickens. *J Vet Sci.* 2015; 16(2): 187–94.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 50. Robinson JL, Brynildsen MP: An ensemble-guided approach identifies ClpP as a major regulator of transcript levels in nitric oxide-stressed *Escherichia coli*. *Metab Eng.* 2015; 31: 22–34.
[PubMed Abstract](#) | [Publisher Full Text](#)
 51. Gardner PR, Gardner DP, Gardner AP: Globins Scavenge Sulfur Trioxide Anion Radical. *J Biol Chem.* 2015; 290(45): 27204–14.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 52. Oshino R, Oshino N, Chance B: The oxygen equilibrium of yeast hemoglobin. *FEBS Lett.* 1971; 19(2): 96–100.
[PubMed Abstract](#) | [Publisher Full Text](#)
 53. Oshino R, Asakura T, Tamura M, *et al.*: Yeast hemoglobin-reductase complex. *Biochem Biophys Res Commun.* 1972; 46(3): 1055–60.
[PubMed Abstract](#) | [Publisher Full Text](#)
 54. Oshino R, Oshino N, Chance B: Studies on yeast hemoglobin. The properties of yeast hemoglobin and its physiological function in the cell. *Eur J Biochem.* 1973; 35(1): 23–33.
[PubMed Abstract](#) | [Publisher Full Text](#)
 55. Oshino R, Asakura T, Takio K, *et al.*: Purification and molecular properties of yeast hemoglobin. *Eur J Biochem.* 1973; 39(2): 581–90.
[PubMed Abstract](#) | [Publisher Full Text](#)
 56. Wisecaver JH, Alexander WG, King SB, *et al.*: Dynamic Evolution of Nitric Oxide Detoxifying Flavohemoglobins, a Family of Single-Protein Metabolic Modules in Bacteria and Eukaryotes. *Mol Biol Evol.* 2016; 33(8): 1979–87.
[PubMed Abstract](#) | [Publisher Full Text](#)
 57. Forrester MT, Foster MW: Protection from nitrosative stress: a central role for microbial flavohemoglobin. *Free Radic Biol Med.* 2012; 52(9): 1620–33.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 58. Rafferty SP, Dayer G: Heme proteins of *Giardia intestinalis*. *Exp Parasitol.* 2015;

- 159: 13–23.
[PubMed Abstract](#) | [Publisher Full Text](#)
59. **F** Lukaszewicz B, McCol E, Yee J, *et al.*: Resonance Raman studies on the flavohemoglobin of the protist *Giardia intestinalis*: evidence of a type I/II-peroxidase-like heme environment and roles of the active site distal residues. *J Biol Inorg Chem*. 2017; **22**(7): 1099–108.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 60. Merhej J, Delaveau T, Guitard J, *et al.*: Yap7 is a transcriptional repressor of nitric oxide oxidase in yeasts, which arose from neofunctionalization after whole genome duplication. *Mol Microbiol*. 2015; **96**(5): 951–72.
[PubMed Abstract](#) | [Publisher Full Text](#)
 61. Cánovas D, Marcos JF, Marcos AT, *et al.*: Nitric oxide in fungi: is there NO light at the end of the tunnel? *Curr Genet*. 2016; **62**(3): 513–8.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 62. Zhou S, Fushinobu S, Nakanishi Y, *et al.*: Cloning and characterization of two flavohemoglobins from *Aspergillus oryzae*. *Biochem Biophys Res Commun*. 2009; **381**(1): 7–11.
[PubMed Abstract](#) | [Publisher Full Text](#)
 63. Vasudevan SG, Tang P, Dixon NE, *et al.*: Distribution of the flavohaemoglobin, HMP, between periplasm and cytoplasm in *Escherichia coli*. *FEMS Microbiol Lett*. 1995; **125**(2–3): 219–24.
[PubMed Abstract](#) | [Publisher Full Text](#)
 64. **F** Björck ML, Zhou S, Rydstrom Lundin C, *et al.*: Reaction of *S. cerevisiae* mitochondria with ligands: Kinetics of CO and O₂ binding to flavohemoglobin and cytochrome c oxidase. *Biochim Biophys Acta Bioenerg*. 2017; **1858**(2): 182–8.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 65. Svensson L, Poljakovic M, Sæve S, *et al.*: Role of flavohemoglobin in combating nitrosative stress in uropathogenic *Escherichia coli*—implications for urinary tract infection. *Microb Pathog*. 2010; **49**(3): 59–66.
[PubMed Abstract](#) | [Publisher Full Text](#)
 66. Stevanin TM, Poole RK, Demoncheaux EA, *et al.*: Flavohemoglobin Hmp protects *Salmonella enterica* serovar typhimurium from nitric oxide-related killing by human macrophages. *Infect Immun*. 2002; **70**(8): 4399–405.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 67. Membrillo-Hernández J, Coopamah MD, Anjum MF, *et al.*: The flavohemoglobin of *Escherichia coli* confers resistance to a nitrosating agent, a “Nitric oxide Releaser,” and paraquat and is essential for transcriptional responses to oxidative stress. *J Biol Chem*. 1999; **274**(2): 748–54.
[PubMed Abstract](#) | [Publisher Full Text](#)
 68. Hernández-Urzúa E, Mills CE, White GP, *et al.*: Flavohemoglobin Hmp, but not its individual domains, confers protection from respiratory inhibition by nitric oxide in *Escherichia coli*. *J Biol Chem*. 2003; **278**(37): 34975–82.
[PubMed Abstract](#) | [Publisher Full Text](#)
 69. Crawford MJ, Goldberg DE: Role for the *Salmonella* flavohemoglobin in protection from nitric oxide. *J Biol Chem*. 1998; **273**(20): 12543–7.
[PubMed Abstract](#) | [Publisher Full Text](#)
 70. El Hammi E, Houée-Lévin C, Řezáč J, *et al.*: New insights into the mechanism of electron transfer within flavohemoglobins: tunnelling pathways, packing density, thermodynamic and kinetic analyses. *Phys Chem Chem Phys*. 2012; **14**(40): 13872–80.
[PubMed Abstract](#) | [Publisher Full Text](#)
 71. Stevanin TM, Ioannidis N, Mills CE, *et al.*: Flavohemoglobin Hmp affords inducible protection for *Escherichia coli* respiration, catalyzed by cytochromes bo’ or bd, from nitric oxide. *J Biol Chem*. 2000; **275**(46): 35868–75.
[PubMed Abstract](#) | [Publisher Full Text](#)
 72. **F** Bang IS, Liu L, Vazquez-Torres A, *et al.*: Maintenance of nitric oxide and redox homeostasis by the *salmonella* flavohemoglobin hmp. *J Biol Chem*. 2006; **281**(38): 28039–47.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 73. Gilberthorpe NJ, Lee ME, Stevanin TM, *et al.*: NsrR: a key regulator circumventing *Salmonella enterica* serovar Typhimurium oxidative and nitrosative stress in vitro and in IFN-gamma-stimulated J774.2 macrophages. *Microbiology*. 2007; **153**(Pt 6): 1756–71.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 74. **F** Sebbane F, Lemaître N, Sturdevant DE, *et al.*: Adaptive response of *Yersinia pestis* to extracellular effectors of innate immunity during bubonic plague. *Proc Natl Acad Sci U S A*. 2006; **103**(31): 11766–71.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 75. **F** Davis KM, Mohammadi S, Isberg RR: Community behavior and spatial regulation within a bacterial microcolony in deep tissue sites serves to protect against host attack. *Cell Host Microbe*. 2015; **17**(1): 21–31.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 76. Boccara M, Mills CE, Zeier J, *et al.*: Flavohaemoglobin HmpX from *Erwinia chrysanthemi* confers nitrosative stress tolerance and affects the plant hypersensitive reaction by intercepting nitric oxide produced by the host. *Plant J*. 2005; **43**(2): 226–37.
[PubMed Abstract](#) | [Publisher Full Text](#)
 77. **F** Fitzsimmons LF, Liu L, Kim JS, *et al.*: *Salmonella* Reprograms Nucleotide Metabolism in Its Adaptation to Nitrosative Stress. *mBio*. 2018; **9**(1): pii: e00211–18.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 78. Ullmann BD, Myers H, Chirananand W, *et al.*: Inducible defense mechanism against nitric oxide in *Candida albicans*. *Eukaryot Cell*. 2004; **3**(3): 715–23.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 79. Gupta S, Pawaria S, Lu C, *et al.*: Novel flavohemoglobins of mycobacteria. *IUBMB Life*. 2011; **63**(5): 337–45.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 80. Thakur N, Kumar A, Dikshit KL: Type II flavohemoglobin of *Mycobacterium smegmatis* oxidizes d-lactate and mediate electron transfer. *Int J Biol Macromol*. 2018; **112**: 868–75.
[PubMed Abstract](#) | [Publisher Full Text](#)
 81. Zhou S, Fushinobu S, Kim SW, *et al.*: Functional analysis and subcellular location of two flavohemoglobins from *Aspergillus oryzae*. *Fungal Genet Biol*. 2011; **48**(2): 200–7.
[PubMed Abstract](#) | [Publisher Full Text](#)
 82. **F** Mukhopadhyay P, Zheng M, Bedzyk LA, *et al.*: Prominent roles of the NorR and Fur regulators in the *Escherichia coli* transcriptional response to reactive nitrogen species. *Proc Natl Acad Sci U S A*. 2004; **101**(3): 745–50.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 83. Moore CM, Nakano MM, Wang T, *et al.*: Response of *Bacillus subtilis* to nitric oxide and the nitrosating agent sodium nitroprusside. *J Bacteriol*. 2004; **186**(14): 4655–64.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 84. **F** Flatley J, Barrett J, Pullan ST, *et al.*: Transcriptional responses of *Escherichia coli* to S-nitrosoglutathione under defined chemostat conditions reveal major changes in methionine biosynthesis. *J Biol Chem*. 2005; **280**(11): 10065–72.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 85. Meilhoc E, Cam Y, Skapski A, *et al.*: The response to nitric oxide of the nitrogen-fixing symbiont *Sinorhizobium meliloti*. *Mol Plant Microbe Interact*. 2010; **23**(6): 748–59.
[PubMed Abstract](#) | [Publisher Full Text](#)
 86. Kondakova T, Catovic C, Barreau M, *et al.*: Response to Gaseous NO₂ Air Pollutant of *P. fluorescens* Airborne Strain MFAF76a and Clinical Strain MFN1032. *Front Microbiol*. 2016; **7**: 379.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 87. Membrillo-Hernández J, Coopamah MD, Channa A, *et al.*: A novel mechanism for upregulation of the *Escherichia coli* K-12 hmp (flavohaemoglobin) gene by the ‘NO releaser’, S-nitrosoglutathione: nitrosation of homocysteine and modulation of MetR binding to the *glyA-hmp* intergenic region. *Mol Microbiol*. 1998; **29**(4): 1101–12.
[PubMed Abstract](#) | [Publisher Full Text](#)
 88. Cruz-Ramos H, Crack J, Wu G, *et al.*: NO sensing by FNR: regulation of the *Escherichia coli* NO-detoxifying flavohaemoglobin, Hmp. *EMBO J*. 2002; **21**(13): 3235–44.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 89. Spiro S: Nitric oxide-sensing mechanisms in *Escherichia coli*. *Biochem Soc Trans*. 2006; **34**(Pt 1): 200–2.
[PubMed Abstract](#) | [Publisher Full Text](#)
 90. Hernández-Urzúa E, Zamorano-Sánchez DS, Ponce-Coria J, *et al.*: Multiple regulators of the Flavohaemoglobin (*hmp*) gene of *Salmonella enterica* serovar Typhimurium include RamA, a transcriptional regulator conferring the multidrug resistance phenotype. *Arch Microbiol*. 2007; **187**(1): 67–77.
[PubMed Abstract](#) | [Publisher Full Text](#)
 91. Hao Y, Updegrave TB, Livingston NN, *et al.*: Protection against deleterious nitrogen compounds: role of σ⁵⁴-dependent small RNAs encoded adjacent to *sdhA*. *Nucleic Acids Res*. 2016; **44**(14): 6935–48.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 92. Richardson AR, Dunman PM, Fang FC: The nitrosative stress response of *Staphylococcus aureus* is required for resistance to innate immunity. *Mol Microbiol*. 2006; **61**(4): 927–39.
[PubMed Abstract](#) | [Publisher Full Text](#)
 93. Kinkel TL, Roux CM, Dunman PM, *et al.*: The *Staphylococcus aureus* SrrAB two-component system promotes resistance to nitrosative stress and hypoxia. *mBio*. 2013; **4**(6): e00696–13.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 94. Oogai Y, Kawada-Matsuo M, Komatsuza H: *Staphylococcus aureus* SrrAB Affects Susceptibility to Hydrogen Peroxide and Co-Existence with *Streptococcus sanguinis*. *PLoS One*. 2016; **11**(7): e0159768.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 95. **F** Chou WK, Brynildsen MP: Loss of DksA leads to multi-faceted impairment of nitric oxide detoxification by *Escherichia coli*. *Free Radic Biol Med*. 2019; **130**: 288–96.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 96. **F** Bodenmiller DM, Spiro S: The *yjeB* (*nsrR*) gene of *Escherichia coli* encodes a nitric oxide-sensitive transcriptional regulator. *J Bacteriol*. 2006; **188**(3): 874–81.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 97. Nakano MM, Geng H, Nakano S, *et al.*: The nitric oxide-responsive regulator NsrR controls ResDE-dependent gene expression. *J Bacteriol*. 2006; **188**(16): 5878–87.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 98. **F** Wang Y, Dunn AK, Wilneff J, *et al.*: *Vibrio fischeri* flavohaemoglobin protects against nitric oxide during initiation of the squid-*Vibrio* symbiosis. *Mol*

- Microbiol. 2010; **78**(4): 903–15.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
99. Chhabra S, Spiro S: Inefficient translation of *nsrR* constrains behaviour of the *NsrR* regulon in *Escherichia coli*. *Microbiology*. 2015; **161**(10): 2029–38.
[PubMed Abstract](#) | [Publisher Full Text](#)
 100. Filenko N, Spiro S, Browning DF, *et al.*: The *NsrR* regulon of *Escherichia coli* K-12 includes genes encoding the hybrid cluster protein and the periplasmic, respiratory nitrite reductase. *J Bacteriol*. 2007; **189**(12): 4410–7.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 101. **F** Adolfsen KJ, Chou WK, Brynildsen MP: Transcriptional Regulation Contributes to Prioritized Detoxification of Hydrogen Peroxide over Nitric Oxide. *J Bacteriol*. 2019; **201**(14): pii: e00081-19.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 102. Marcos AT, Ramos MS, Marcos JF, *et al.*: Nitric oxide synthesis by nitrate reductase is regulated during development in *Aspergillus*. *Mol Microbiol*. 2016; **99**(1): 15–33.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 103. El Hammi E, Warkentin E, Demmer U, *et al.*: Structure of *Ralstonia eutropha* flavohemoglobin in complex with three antibiotic azole compounds. *Biochemistry*. 2011; **50**(7): 1255–64.
[PubMed Abstract](#) | [Publisher Full Text](#)
 104. El Hammi E, Warkentin E, Demmer U, *et al.*: Active site analysis of yeast flavohemoglobin based on its structure with a small ligand or econazole. *FEBS J*. 2012; **279**(24): 4565–75.
[PubMed Abstract](#) | [Publisher Full Text](#)
 105. Helmick RA, Fletcher AE, Gardner AM, *et al.*: Imidazole antibiotics inhibit the nitric oxide dioxygenase function of microbial flavohemoglobin. *Antimicrob Agents Chemother*. 2005; **49**(5): 1837–43.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 106. Nobre LS, Todorovic S, Tavares AF, *et al.*: Binding of azole antibiotics to *Staphylococcus aureus* flavohemoglobin increases intracellular oxidative stress. *J Bacteriol*. 2010; **192**(6): 1527–33.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 107. Membrillo-Hernández J, Ioannidis N, Poole RK: The flavohaemoglobin (HMP) of *Escherichia coli* generates superoxide *in vitro* and causes oxidative stress *in vivo*. *FEBS Lett*. 1996; **382**(1–2): 141–4.
[PubMed Abstract](#) | [Publisher Full Text](#)
 108. Wu G, Corker H, Orii Y, *et al.*: *Escherichia coli* Hmp, an “oxygen-binding flavohaemoprotein”, produces superoxide anion and self-destructs. *Arch Microbiol*. 2004; **182**(2–3): 193–203.
[PubMed Abstract](#) | [Publisher Full Text](#)
 109. **F** Moussaoui M, Misevičienė L, Anusevičius Ž, *et al.*: Quinones and nitroaromatic compounds as subversive substrates of *Staphylococcus aureus* flavohemoglobin. *Free Radic Biol Med*. 2018; **123**: 107–15.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 110. Frey AD, Shepherd M, Jokipii-Lukkari S, *et al.*: The single-domain globin of *Vitreoscilla*: augmentation of aerobic metabolism for biotechnological applications. *Adv Microb Physiol*. 2011; **58**: 81–139.
[PubMed Abstract](#) | [Publisher Full Text](#)
 111. Stark BC, Dikshit KL, Pagilla KR: Recent advances in understanding the structure, function, and biotechnological usefulness of the hemoglobin from the bacterium *Vitreoscilla*. *Biotechnol Lett*. 2011; **33**(9): 1705–14.
[PubMed Abstract](#) | [Publisher Full Text](#)
 112. Bollinger CJ, Bailey JE, Kallio PT: Novel hemoglobins to enhance microaerobic growth and substrate utilization in *Escherichia coli*. *Biotechnol Prog*. 2001; **17**(5): 798–808.
[PubMed Abstract](#) | [Publisher Full Text](#)
 113. Xue SJ, Jiang H, Chen L, *et al.*: Over-expression of *Vitreoscilla* hemoglobin (VHb) and flavohemoglobin (FHB) genes greatly enhances pullulan production. *Int J Biol Macromol*. 2019; **132**: 701–9.
[PubMed Abstract](#) | [Publisher Full Text](#)
 114. Shipovskov S, Bonamere A, Boffi A, *et al.*: Electrocatalytic interconversion of NADH and NAD⁺ by *Escherichia coli* flavohemoglobin. *Chem Commun (Camb)*. 2015; **51**(89): 16096–8.
[PubMed Abstract](#) | [Publisher Full Text](#)
 115. **F** Carabet LA, Guertin M, Lagüe P, *et al.*: Mechanism of the Nitric Oxide Dioxygenase Reaction of *Mycobacterium tuberculosis* Hemoglobin N. *J Phys Chem B*. 2017; **121**(37): 8706–18.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 116. Elvers KT, Wu G, Gilberthorpe NJ, *et al.*: Role of an inducible single-domain hemoglobin in mediating resistance to nitric oxide and nitrosative stress in *Campylobacter jejuni* and *Campylobacter coli*. *J Bacteriol*. 2004; **186**(16): 5332–41.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 117. **F** Zhou D, Hemann C, Boslett J, *et al.*: Oxygen binding and nitric oxide dioxygenase activity of cytoglobin are altered to different extents by cysteine modification. *FEBS Open Bio*. 2017; **7**(6): 845–53.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
 118. **F** Mukhi N, Kundu S, Kaur J: NO dioxygenase- and peroxidase-like activity of Arabidopsis phytohemoglobin 3 and its role in *Sclerotinia sclerotiorum* defense. *Nitric Oxide*. 2017; **68**: 150–62.
[PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
 119. Gardner AM, Martin LA, Gardner PR, *et al.*: Steady-state and transient kinetics of *Escherichia coli* nitric-oxide dioxygenase (flavohemoglobin). The B10 tyrosine hydroxyl is essential for dioxygen binding and catalysis. *J Biol Chem*. 2000; **275**(17): 12581–9.
[PubMed Abstract](#) | [Publisher Full Text](#)
 120. Mills PC, Rowley G, Spiro S, *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*. 2008; **154**(Pt 4): 1218–28.
[PubMed Abstract](#) | [Publisher Full Text](#)
 121. Prior K, Hautefort I, Hinton JC, *et al.*: All stressed out. *Salmonella* pathogenesis and reactive nitrogen species. *Adv Microb Physiol*. 2009; **56**: 1–28.
[PubMed Abstract](#) | [Publisher Full Text](#)
 122. Mühlhölz A, Kabisch J, Pichner R, *et al.*: Contribution of the NO-detoxifying enzymes HmpA, NorV and NrfA to nitrosative stress protection of *Salmonella* Typhimurium in raw sausages. *Food Microbiol*. 2014; **42**: 26–33.
[PubMed Abstract](#) | [Publisher Full Text](#)
 123. Mason MG, Shepherd M, Nicholls P, *et al.*: Cytochrome bd confers nitric oxide resistance to *Escherichia coli*. *Nat Chem Biol*. 2009; **5**(2): 94–6.
[PubMed Abstract](#) | [Publisher Full Text](#)
 124. Shepherd M, Achard ME, Idris A, *et al.*: The cytochrome bd-I respiratory oxidase augments survival of multidrug-resistant *Escherichia coli* during infection. *Sci Rep*. 2016; **6**: 35285.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 125. Jones-Carson J, Husain M, Liu L, *et al.*: Cytochrome bd-Dependent Bioenergetics and Antinitrosative Defenses in *Salmonella* Pathogenesis. *mBio*. 2016; **7**(6): pii: e02052-16.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 126. Wang J, Vine CE, Balasany BK, *et al.*: The roles of the hybrid cluster protein, Hcp and its reductase, Hcr, in high affinity nitric oxide reduction that protects anaerobic cultures of *Escherichia coli* against nitrosative stress. *Mol Microbiol*. 2016; **100**(5): 877–92.
[PubMed Abstract](#) | [Publisher Full Text](#)
 127. Mehta HH, Liu Y, Zhang MQ, *et al.*: Genome-wide analysis of the response to nitric oxide in uropathogenic *Escherichia coli* CFT073. *Microb Genom*. 2015; **1**(4): e000031.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 128. Lewis AM, Matzdorf SS, Endres JL, *et al.*: Examination of the *Staphylococcus aureus* nitric oxide reductase (saNOR) reveals its contribution to modulating intracellular NO levels and cellular respiration. *Mol Microbiol*. 2015; **96**(3): 651–69.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Open Peer Review

Current Peer Review Status:

Editorial Note on the Review Process

Faculty Reviews are review articles written by the prestigious Members of **Faculty Opinions**. The articles are commissioned and peer reviewed before publication to ensure that the final, published version is comprehensive and accessible. The reviewers who approved the final version are listed with their names and affiliations.

The reviewers who approved this article are:

Version 1

- 1. Vinai C. Thomas**
Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, Nebraska, USA
Competing Interests: No competing interests were disclosed.
- 2. Elizabeth M. Boon**
Department of Chemistry and Institute of Chemical Biology and Drug Design, Stony Brook University, Stony Brook, NY, USA
Competing Interests: No competing interests were disclosed.
- 3. Marie-Alda Gilles-Gonzalez**
Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, Texas, USA
Competing Interests: No competing interests were disclosed.
- 4. John S. Olson**
The BioSciences Department, Rice University, Houston, Texas, USA
Competing Interests: No competing interests were disclosed.
- 5. Mark P. Brynildsen**
Department of Chemical and Biological Engineering, Princeton University, Princeton, NJ, USA
Competing Interests: No competing interests were disclosed.
- 6. Andrés Vázquez-Torres**
Department of Immunology and Microbiology, University of Colorado School of Medicine, Aurora, CO, USA
Competing Interests: No competing interests were disclosed.

The benefits of publishing with F1000Research:

- Your article is published within days, with no editorial bias
- You can publish traditional articles, null/negative results, case reports, data notes and more
- The peer review process is transparent and collaborative
- Your article is indexed in PubMed after passing peer review
- Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com

F1000Research