

Published in final edited form as:

Curr Opin Microbiol. 2014 June ; 0: 16–24. doi:10.1016/j.mib.2014.05.018.

Deciphering Nitric Oxide Stress in Bacteria with Quantitative Modeling

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Abstract

Many pathogens depend on nitric oxide (NO•) detoxification and repair to establish an infection, and inhibitors of these systems are under investigation as next-generation antibiotics. Due to the broad reactivity of NO• and its derivatives with biomolecules, a deep understanding of how pathogens sense and respond to NO•, as an integrated system, has been elusive. Quantitative kinetic modeling has been proposed as a method to enhance analysis and understanding of NO• stress at the systems-level. Here we review the motivation for, current state of, and future prospects of quantitative modeling of NO• stress in bacteria, and suggest that such mathematical approaches would prove equally useful in the study of other broadly reactive antimicrobials, such as hydrogen peroxide (H₂O₂).

Introduction

NO• is a potent antimicrobial produced by immune cells to combat pathogens [1,2]. The importance of NO• to immunity is evidenced by the many pathogens, including *Mycobacterium tuberculosis*, *Neisseria meningitides*, *Vibrio cholerae*, *Salmonella enterica* serovar Typhimurium, *Pseudomonas aeruginosa*, and enterohemorrhagic *Escherichia coli* (EHEC), whose virulence depends on NO• detoxification and repair systems (Table 1) [3–8]. Collectively, these studies suggest that knowledge of how pathogens sense and respond to NO• could illuminate antibacterial strategies that synergize with host immunity. Research on NO• stress has continued for over two decades, and the cumulative picture that has emerged is immensely complex [1,9–12]. This derives from the broad reactivity of NO• and its reactive intermediates (reactive nitrogen species: RNS) with biomolecules [1,9,12]. Depending on the environment, dosage, and delivery rate, NO• will destroy iron-sulfur (Fe-S) clusters, reversibly bind heme, directly react with O₂ and superoxide (O₂^{•-}), and/or be enzymatically detoxified, whereas its derivatives (NO₂•, N₂O₃, N₂O₄, HNO, and ONOO⁻) damage thiols, tyrosine residues, and DNA bases (Figure 1) [1,9,12–14]. This systems-level stress becomes even further complicated when one considers that Fe-S clusters and thiols are

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used for a broad range of enzymatic and regulatory functions throughout the cellular network [15–19]. To decipher this response and understand how bacteria, as an integrated system, sense and respond to NO•, a quantitative understanding of intracellular NO• reactivity is required. NO• has many available reaction paths upon entering a cell, and the biological outcome of NO• exposure, whether it is continued growth, bacteriostasis, expression of virulence factors, transition to an antibiotic-tolerant state and/or cell death [17, 20– 22], is governed by a complex, kinetic competition. Quantitative knowledge of this competition and the factors that control it will reveal novel targets within the NO• response network for the discovery and development of therapeutics that synergize with host-derived NO•.

Due to the complexity of the competition for NO• among biomolecules, mathematical models are required to quantitatively analyze and understand data from NO•-stressed environments [13,14,23,24]. Beyond data interpretation, these models enable identification of emergent properties of the NO• response network and formulation of testable predictions concerning the impact of genetic and environmental perturbations. Here, we summarize evidence that suggests quantitative modeling will facilitate the discovery and development of NO•-based antibiotics, review the current state of NO• models along with their contributions to the present understanding of NO• stress, and reflect upon the future prospects of quantitative modeling to enhance the study of systems-level stresses from not only NO•, but other broadly reactive antimicrobials as well, such as H₂O₂.

NO• detoxification and repair systems are prevalent virulence factors

The ability to withstand NO• stress has been linked to the virulence of an impressive number of pathogens, several of which are presented in Table 1. Notably, in *S. Typhimurium*, Stevanin and colleagues found that a mutant defective in NO• dioxygenase (*hmp*) exhibited reduced survival in human macrophages, and that the effect was eliminated upon treatment with an inhibitor of inducible nitric oxide synthase (iNOS) [25]. More recently, this effect was corroborated *in vivo*, where *hmp* *S. Typhimurium* had attenuated virulence in mice, and iNOS inhibition restored virulence [26]. In EHEC, a genomic study of clinical isolates found that the presence of a functional *norV* gene, which encodes NO• reductase, correlated with an increased frequency of hemolytic-uremic syndrome (HUS) [27]. This connection was substantiated by a study demonstrating that EHEC possessing an inactive *norV* gene exhibited reduced survival in mouse macrophages compared to those with an active *norV* [4]. Recently, the genome of the EHEC strain responsible for the 2011 outbreak in Germany, which resulted in the highest incidence of HUS on record [28], was found to contain a functional *norV* [29], lending even further support for the previous genomic study. For *Yersinia pestis*, a microarray analysis of a model rat infection identified *hmp* expression to be significantly up-regulated, and subsequent experiments revealed that a *hmp* mutant exhibited attenuated virulence [30]. Beyond NO• detoxification, microbial repair systems have also been found to be important for resisting NO• stress and were shown to contribute to virulence. A transposon screen in *M. tuberculosis* found that mutations in proteasome components (*mpa* and *pafA*) and a nucleotide excision repair gene (*uvrB*) increased killing by NO• produced from acidified nitrite *in vitro*, and reduced virulence in mouse infection models [3,31]. In *S. Typhimurium*, Richardson and colleagues found that base excision

repair mutants (*xth nfo*) had attenuated virulence in mice, which was fully restored upon administration of an iNOS inhibitor [32]. These and related studies support a role for NO• and its derivatives as key mediators of host defense, and suggest that targeting the NO• defenses of pathogens may be an effective way to inhibit infection [33].

Therapeutic potential of NO•-based antibiotics

Several studies have found chemical inhibitors of the NO• response network that increase the sensitivity of pathogens to NO• [3,33,34]. Two chemical inhibitors were shown to block activity of the *M. tuberculosis* proteasome, and successfully reproduced the NO•-sensitive phenotype of proteasome-deficient mutants [3]. Helmick and colleagues found that imidazoles could inhibit NO• dioxygenase *in vitro*, and whole-cell NO• detoxification in *Staphylococcus aureus* and *E. coli* cultures, though the effects were far less pronounced in *E. coli* due to the poor Gram-negative membrane permeability of imidazoles [33]. By performing a screen to identify inhibitors of DlaT, an enzyme important for *M. tuberculosis* to tolerate NO•-stress, Bryk and colleagues discovered that rhodanines enhance killing of non-replicating *M. tuberculosis* treated with NO• by several orders of magnitude [34]. Further, D157070 (DlaT inhibitor) reduced *M. tuberculosis* viability in murine bone-marrow macrophages. These studies demonstrate the potential of targeting the NO• response network for the discovery of novel antibiotics, and suggest that a deeper understanding of NO• stress will reveal additional therapeutic strategies for investigation, since all targets are not equally accessible, as demonstrated with imidazoles and *E. coli* [33]. It is also worth noting that, in addition to potentiating immune-derived NO•, chemicals that target the NO• response network would prove useful for therapies that directly administer exogenous NO• to infection sites. Direct administration techniques have been garnering attention in recent years, due to the ability of NO• to eliminate antibiotic resistant pathogens [35–38], and numerous delivery mechanisms, including nanoparticles [35,39], probiotic patches [37], and dendrimers [38], have been explored. Several excellent reviews on the topic have recently been published [40–42], so here we will only highlight an important design constraint. Specifically, the delivery method must achieve NO• concentrations high enough to be antibacterial but low enough to remain non-toxic to eukaryotic cells. This therapeutic window can be as small as 5-fold [41], thereby presenting a significant challenge for direct delivery methods. One way to relieve this constraint is to couple direct delivery with agents that increase the sensitivity of pathogens to NO•, effectively expanding the therapeutic window.

NO• elicits a complex, systems-level stress response

A comprehensive, quantitative understanding of NO• stress has been elusive due to the reactivity of NO• and its derivatives with a wide range of biomolecules [1,9,10,12], and the depth to which these perturbations propagate through cellular networks. This complexity is best illustrated by the findings of transcriptomic [7,17,19,43–47], proteomic [15,18], and metabolomic [48,49] studies, which have collectively demonstrated diverse, systems-wide responses to NO•. Hyduke and colleagues conducted a DNA microarray analysis of NO•-stressed *E. coli*, and found that 709 genes were significantly perturbed, affecting a diverse range of cellular functions, including branched-chain amino acid synthesis, respiration, Fe-S

assembly, and energy metabolism [17]. This study also included a chemoinformatic analysis of the *E. coli* proteome, wherein 554 proteins were identified as potential RNS targets based on the presence of possible NO•-reactive features (such as Fe-S clusters, heme groups, or reactive thiol motifs). A transcriptomic study of anaerobically-grown *E. coli* found that approximately 4% of the genome (173 genes) exhibited a significant change in expression upon NO• treatment [43]. Again, the perturbed genes spanned a diversity of functions beyond NO• detoxification, including DNA metabolism, cofactor and prosthetic group synthesis, fatty acid metabolism, cell structure maintenance, metal ion and multidrug transport, purine and pyrimidine synthesis, and energy metabolism. The transcriptome of oral pathogen *Porphyromonas gingivalis* was measured following NO• exposure, and it too demonstrated an expansive, systems-level response where expression of approximately 19% of its genome (380 genes) was significantly perturbed [44]. Although the majority of the affected genes were of unknown function, those associated with energy metabolism, translation, and regulatory functions were found to be altered by NO•. A recent metabolomic study of NO•-treated *V. cholerae* found extensive metabolic distress, as demonstrated by an accumulation of upper glycolytic metabolites, impairment of arginine synthesis, and accumulation of citrate, thereby suggesting TCA cycle dysfunction [48]. Similarly, Auger and colleagues studied NO•-stressed *Pseudomonas fluorescens* and observed obstruction of the TCA cycle [49]. Proteomic analyses have also revealed systems-level perturbations by NO•; for example, Rhee and colleagues measured S-nitrosation of *M. tuberculosis* proteins by acidified nitrite- or macrophage-derived NO•, and found 29 enzymes whose functions included amino acid biosynthesis, energy metabolism, antioxidant defense, heat shock response, RNA polymerase, and lipid metabolism to be nitrosation targets [15]. Analysis of S-nitrosation in *E. coli* revealed some similarities, as well as novel targets, including a subunit of pyruvate dehydrogenase [18]. These studies demonstrate the extent to which NO• perturbs numerous facets of bacterial physiology, and highlight the challenges associated with gaining a complete understanding of how a pathogen, as an integrated system, responds to NO•.

Quantitative modeling of NO• stress

On a molecular level, the complex bacterial responses to NO• all originate from the NO• biochemical reaction network. Therefore, a deeper understanding of NO• stress must begin with a quantitative examination of intracellular NO• dynamics. The broad reactivity of NO• and derived RNS, culminating in the breadth of physiological perturbations identified by -omics studies, underscores the need for a quantitative, mathematical approach to deconvolute the effects of NO• stress and the ensuing microbial response. Furthermore, the dynamics of these processes span multiple time scales, ranging from fractions of a second (spontaneous chemical reactions) to minutes (regulatory responses), thus requiring a computational approach for their integration. The need for a model-based approach to quantitatively study the complex reaction network of NO• in biological systems has motivated the construction of kinetic models to simulate the NO• biochemical network and gain insight into its biological roles.

Initial models to examine NO• stress in biological systems

Initial attempts to model NO• biochemical reaction networks were largely motivated by a desire to understand its role in mammalian signaling. Lancaster constructed a kinetic model of NO• chemistry accounting for the oxidation, nitration, and nitrosation reaction types governing the fate of NO• and its reactive intermediates, and was able to make predictions regarding the relative importance of each pathway under different biological conditions (*e.g.*, inflammatory and non-inflammatory levels of NO• production) [13]. This model was reformulated by Lim and colleagues to describe intracellular NO• chemistry of inflamed tissue at steady-state, and was expanded to include additional antioxidants, amino acids, and lipid-phase reactions [14]. Simulations provided valuable information on approximate concentrations of RNS that are generally too unstable and/or scarce to measure experimentally, as well as their major intracellular sinks [14]. Bagci and colleagues used mathematical modeling to explore the participation of NO• in apoptosis by integrating an NO• chemical network with a mitochondrial apoptotic signaling network, thus providing quantitative insight into the pro- and anti-apoptotic activity of NO• [23]. Though these methods laid the foundation for the quantitative study of NO• in biological systems, little consideration was given to cellular responses to NO•, such as repair of damaged biomolecules and regulatory responses, which we have recently found to be critical in simulating the dynamics of NO• stress in microbes [24].

Recent advances in model-driven analyses of microbial NO• stress

Recent work in our laboratory has demonstrated the feasibility and utility of a model-based approach in studying NO• stress in a microbial system [24]. Drawing upon existing kinetic models and the available body of literature, a comprehensive kinetic model of NO• biochemistry in *E. coli* was constructed and experimentally validated. The model encompassed processes such as damage and repair of Fe-S clusters, DNA, and thiols, as well as enzymatic NO• detoxification, autoxidation, and reversible inhibition of respiratory cytochromes. Model simulations exhibited excellent predictive accuracy with regard to major system perturbations, such as the deletion of *hmp*, which encodes the primary aerobic NO• detoxification system in *E. coli*. In addition, parametric analysis identified the rate of NO• delivery to the system as a control parameter having strong influence on the distribution of NO• consumption, which was confirmed experimentally. Finally, the model was found to accurately capture NO• dynamics under microaerobic O₂ concentrations, and successfully predict the importance of the major aerobic (Hmp) and anaerobic (NorV) detoxification systems in this medically important regime.

Potential of quantitative modeling to transform the study of NO• stress in bacteria

As discussed above, bacteria mount a systems-level response to NO• that spans energy metabolism, amino acid biosynthesis, translation, transcription, respiration, DNA metabolism, protein cofactor synthesis, and direct detoxification [1]. Quantitative modeling offers a means to interpret NO• responses and investigate their underlying architecture. For example, network analysis techniques, such as parameter variation [24] or metabolic control analysis [50], can be used to identify species, pathways, or other network components that significantly alter the NO• distribution upon perturbation. In this way, emergent systems

properties of the NO• stress response can be discovered, painting a more complete picture of the complex network, and offering novel therapeutic targets. In addition, quantitative models provide an excellent framework to integrate diverse types of data, such as metabolite, transcript, and protein levels, since explicit variables for concentrations of cellular components are used. Further, a rigorously-constructed model represents the current knowledgebase, and observed phenomena that disagree with simulations represent a knowledge gap to be filled. For example, a screen may identify a novel gene or chemical that modifies how a bacterium processes NO•, resulting in an unexplained NO• dynamic. To understand the basis of such novel phenotypes, analyses can be performed to identify parameters whose modulation reconciles simulations with experiments. These model adjustments provide readily testable hypotheses, such as altered gene expression or protein degradation, to explain the mechanism by which a mutation or chemical perturbs the NO• response network.

Challenges facing quantitative modeling of NO• stress

Although model-based approaches to study NO• stress offer numerous benefits, they are all inherently limited by the availability of kinetic data and knowledge of the system architecture, such as the repertoire of NO• detoxification enzymes present. For microbes where this information is scarce, coarse-grained models, where reaction pathways and parameters have been lumped together, can be trained on experimental data and used for quantitative analyses, until more detailed information becomes available. Another challenge is related to rapid quantification of unstable, short-lived intermediates that often exist in trace quantities, such as N₂O₃ and NO₂• [14]. The lack of precise measurements of these species prevents direct validation of those components in the model, and therefore conclusions based on simulation of those species should be handled with caution. One approach to reconcile the lack of a direct measurement, however, is to use a reliable proxy, such as a stable and measurable downstream product. Overall, these challenges limit the accuracy of quantitative models of NO• stress, but it is important to note that failure of a rigorously-constructed model to capture a phenotypic response represents an opportunity to discover novel biology not contained within the available knowledgebase.

Beyond NO•

The broad reactivity of NO• makes quantitative modeling an attractive tool for studying its systems-level effects on bacteria. This quality of NO• is mirrored in other immune antimicrobials, such as H₂O₂ [51]. The importance of H₂O₂ to immunity has also been supported by the many pathogens that require H₂O₂ detoxification systems to establish or sustain an infection, such as *S. Typhimurium* [52], *M. tuberculosis* [53,54], *S. aureus* [55], *Helicobacter pylori* [56], *Streptococcus pyogenes* [57], and *Enterococcus faecalis* [58] (Table 2). In phagocytic cells, H₂O₂ is derived from the dismutation of O₂•⁻ that is produced by NADPH oxidase [59], and readily diffuses into bacterial cells [60] to react with cysteine [61] and methionine [62,63] residues, Fe-S clusters [64], transition metals [65], and α-keto acids [66,67], or undergo enzymatic detoxification by catalases [68], and hydroperoxidases [69] (Figure 2). H₂O₂ can also be reduced by Fe²⁺ to form the stronger oxidant, HO•, which reacts with most biomolecules at diffusion-limited rates [70]. Given this broad reactivity, it

is not surprising that transcriptomic studies have shown that H₂O₂ treatment results in systems-level changes in the expression of genes involved in DNA repair, virulence, membrane function, metabolism, and peroxide detoxification [71–74].

The complexity of the H₂O₂ biochemical reaction network suggests that quantitative modeling could provide a deeper understanding of how bacteria sense and respond to H₂O₂ as an integrated system. Currently, the best models of H₂O₂ biochemistry are specific to mammalian systems due to its importance as a cellular signaling molecule and implication in a number of diseases [75–77]. These models have included H₂O₂ elimination by the antioxidants glutathione and thioredoxin, enzymes catalase, glutathione peroxidase, glutathione reductase, glutaredoxin, and peroxiredoxin, as well as processing of oxidized protein thiols [75–77], but are incomplete due to the lack of reactions describing damage and repair of many biomolecules and the exclusion of transcriptional regulation. Analogous to NO• stress, quantitative modeling has the potential to provide a deeper understanding of H₂O₂ stress, and thereby illuminate therapeutic targets to sensitize pathogens to oxidative immune attack.

Conclusion

NO• is an important immune antimicrobial that produces a systems-level stress that is difficult to understand quantitatively without the use of mathematical models. These models offer utilities far beyond data interpretation, such as a platform to investigate systems-level control of NO• metabolism, and an ability to mechanistically dissect novel phenotypes of the NO• response network. Recent advances in this area include a detailed model of NO• stress in *E. coli* [24], which led to the identification of an emergent property of the NO• response network, and increased understanding of NO• stress under the physiologically relevant microaerobic regime. While important knowledge can be gained through analysis and expansion of this model, it can also serve as a template to develop models for less well-characterized bacteria, which is a necessary step to transform quantitative modeling into a common practice for investigations of stress caused by NO• and other broadly-reactive antimicrobials, such as H₂O₂.

Acknowledgments

This work was supported in part by the National Science Foundation Graduate Research Fellowship under Grant No. DGE 1148900, and the National Institute of Allergy and Infectious Diseases of the National Institutes of Health under award number R21AI105342.

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- * Of special interest
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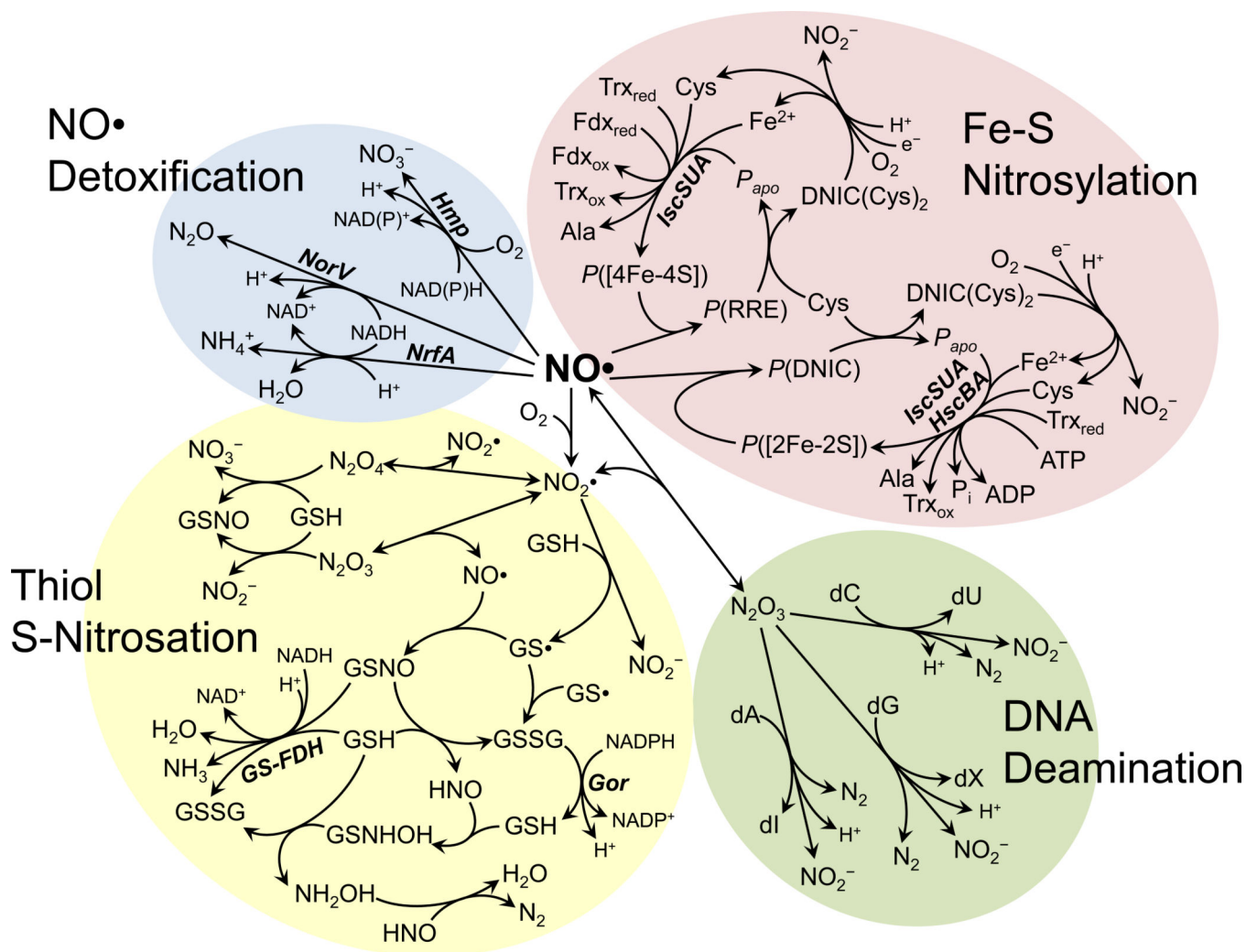
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Highlights

- NO• detoxification and repair have been linked to virulence in many pathogens.
- NO• and its derivatives are broadly reactive and elicit a systems-level response.
- Quantitative models can enhance understanding of the NO• response network.
- Quantitative models can improve study of other broadly-reactive antimicrobials.

**Figure 1.**

Biochemical reaction network of NO in *E. coli*. The diagram illustrates several key pathways involved in NO metabolism. Reactions are grouped into categories of NO detoxification, Fe-S nitrosylation and repair, DNA deamination, and thiol S-nitrosation and denitrosation. Enzymes catalyzing a reaction are shown in bold italics next to the reaction arrow. Enzyme abbreviations: *NorV*, NO reductase; *Hmp*, NO dioxygenase; *NrfA*, formate-dependent nitrite reductase; *GS-FDH*, glutathione-dependent formaldehyde dehydrogenase; *Gor*, glutathione reductase; *IscSUA*, Fe-S cluster assembly system; *HscBA*, Fe-S assembly chaperone. Metabolite abbreviations: GSH, glutathione; GS•, glutathionyl radical; GSNO, S-nitrosoglutathione; GSSG, glutathione disulfide; dA, deoxyadenosine; dG, deoxyguanosine; dC, deoxycytidine; dI, deoxyinosine; dX, deoxyxanthosine; dU, deoxyuridine; P([2Fe-2S]), protein-bound [2Fe-2S] cluster; P([4Fe-4S]), protein-bound [4Fe-4S] cluster; P(DNIC), protein-bound dinitrosyl-iron complex; P(RRE), protein-bound Roussins' red ester; DNIC(Cys)₂, L-cysteine-bound dinitrosyl-iron complex; P_{apo}, apo-protein (lacking Fe-S cluster); Trx_{red}, reduced thioredoxin; Trx_{ox}, oxidized thioredoxin; Fdx_{red}, reduced ferredoxin; Fdx_{ox}, oxidized ferredoxin.

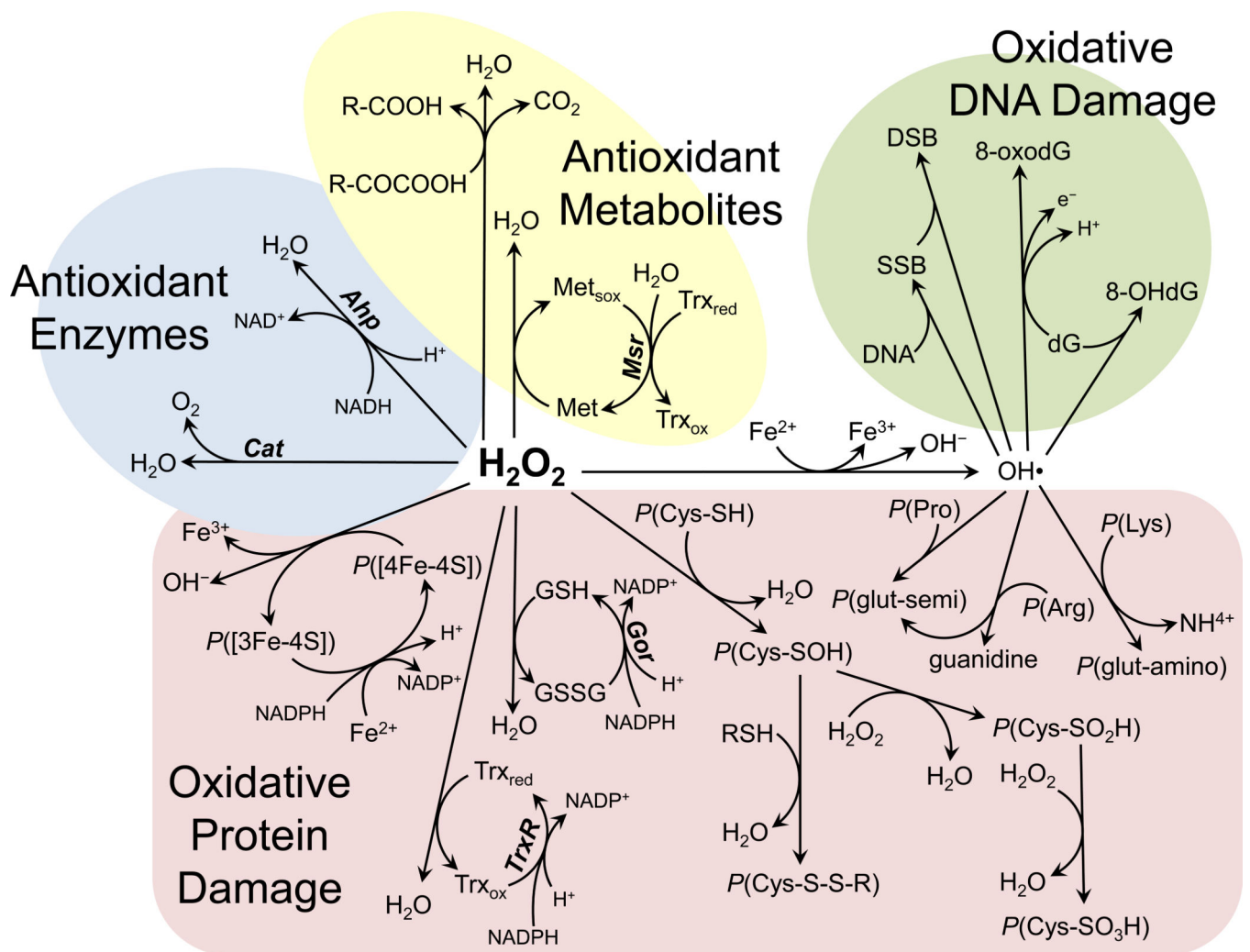


Figure 2.

Biochemical reaction network of H_2O_2 in *E. coli*. The diagram highlights the complexity of the intracellular H_2O_2 reaction network. Reactions are grouped into categories of antioxidant enzymes, antioxidant metabolites, oxidative DNA damage, and oxidative protein damage. Enzyme abbreviations: Cat, catalase; Ahp, alkyl hydroperoxidase; Msr, methionine sulfoxide reductase; TrxR, thioredoxin reductase; Gor, glutathione reductase. Metabolite abbreviations: R-CO-COOH, generic α -keto acid; R-COOH, carboxylic acid; Met_{sox} , L-methionine-S-oxide; SSB, DNA single strand break; DSB, DNA double strand break; dG, deoxyguanosine; 8-OHdG, 8-hydroxy-2-deoxyguanosine; 8-oxodG, 8-oxo-7,8-dihydro-2'-deoxyguanosine; Trx_{red} , reduced thioredoxin; Trx_{ox} , oxidized thioredoxin; GSH, reduced glutathione; GSSG, glutathione disulfide; $P([4\text{Fe-4S}])$, protein with [4Fe-4S] cluster; $P([3\text{Fe-4S}])$, damaged protein with [3Fe-4S] cluster; $P(\text{Cys-CH}_2\text{-SH})$, protein-bound L-cysteine; -SOH, sulfenic acid; -SO₂H, sulfinic acid; -SO₃H, sulfonic acid; $P(\text{Pro})$, protein-bound L-proline; $P(\text{Arg})$, protein-bound L-arginine; $P(\text{Lys})$, protein-bound L-lysine; $P(\text{glut-semi})$, protein-bound glutamic semialdehyde; $P(\text{glut-amino})$, protein-bound aminoadipic semialdehyde.

Table 1

Pathogens for which NO• detoxification or repair has been identified as a virulence factor.

Pathogen	Gene(s)	Description	Ref.
<i>E. coli</i> (enterohemorrhagic)	<i>norV</i>	Strains harboring an inactive <i>norV</i> gene (<i>norVs</i>) exhibited reduced survival in murine macrophages.	[4]
<i>E. coli</i> (uropathogenic)	<i>hmp</i>	Isolates from patients with urinary tract infection had increased <i>hmp</i> expression, and <i>hmp</i> mutants were outcompeted by the wild-type in a mouse infection model.	[78]
<i>M. tuberculosis</i>	<i>mpa</i> , <i>paqA</i> <i>uvrB</i> , <i>dlaT</i>	Mutants deficient in proteasome components (<i>mpa</i> or <i>paqA</i>) [3] or nucleotide excision repair (<i>uvrB</i>) [31] exhibited attenuated virulence in mice.	[3,31]
<i>N. meningitidis</i>	<i>cycP</i> , <i>norB</i>	Mutants lacking cytochrome <i>c'</i> (<i>cycP</i>) or NO• reductase (<i>norB</i>) exhibited reduced survival in human macrophages and human nasopharyngeal mucosa organ cultures.	[6]
<i>P. aeruginosa</i>	<i>norCBD</i>	A mutant deficient in NO• reductase (<i>norCBD</i>) exhibited reduced viability in murine macrophages.	[8]
<i>S. Typhimurium</i>	<i>hmp</i> , <i>xth</i> , <i>nfo</i> , <i>ytfE</i> , <i>STM1808</i>	Mutants lacking <i>hmp</i> exhibited reduced survival in human macrophages [25] and attenuated virulence in mice [26]. Mutations in base excision repair (<i>xth</i> and <i>nfo</i>) [32], Fe-S assembly (<i>ytfE</i>) [7], or previously-unidentified <i>STM1808</i> [7] each caused attenuated virulence in mice.	[7,25,26,32]
<i>S. aureus</i>	<i>hmp</i>	Mutants deficient in <i>hmp</i> exhibited attenuated virulence in mice.	[79]
<i>V. cholerae</i>	<i>hmpA</i>	Mutants lacking <i>hmpA</i> were outcompeted in a mouse intestine colonization assay.	[5]
<i>Y. pestis</i>	<i>hmp</i>	A mutation in <i>hmp</i> resulted in longer incubation times and attenuated virulence in rats.	[30]

Table 2

Pathogens for which H₂O₂ detoxification has been identified as a virulence factor.

Pathogen	Gene(s)	Description	Ref.
<i>E. faecalis</i>	<i>tpx, npr, ahp</i>	Mutants lacking thiol peroxidase (<i>tpx</i>) have attenuated virulence in a mouse peritonitis model. Triple mutants lacking Tpx, NADH peroxidase (<i>npr</i>), and alkyl hydroperoxide reductase (<i>ahp</i>) were more significantly attenuated.	[58]
<i>H. pylori</i>	<i>katA, kapA</i>	Mutants lacking <i>katA</i> and <i>kapA</i> were less able to sustain long-term infection in mice.	[56]
<i>M. tuberculosis</i>	<i>katG</i>	Mutations in <i>katG</i> decreased persistence within infected mice [53] and human monocytes <i>in vitro</i> [54].	[53,54]
<i>S. Typhimurium</i>	<i>katE, katG, katN, ahpC, tsaA</i>	Inactivation of all five catalase and hydroperoxidase genes resulted in high sensitivity to H ₂ O ₂ and decreased survival within murine macrophages.	[52]
<i>S. aureus</i>	<i>katA, ahpC</i>	Mutations in <i>katA</i> and <i>ahpC</i> decreased ability to colonize the nasal cavities of cotton rats.	[55]
<i>S. pyogenes</i>	<i>gpoA</i>	<i>S. pyogenes</i> requires glutathione peroxidase (GpoA) for virulence in several mouse models.	[57]