CHAPTER 17.6

Ensemble modeling enables quantitative exploration of bacterial nitric oxide stress networks

Jonathan L. Robinson and Mark P. Brynildsen
Department of Chemical and Biological Engineering, Princeton University, Princeton, New Jersey, USA

17.6.1 Introduction

The increasing incidence of antibiotic-resistant infections combined with a deteriorating pipeline of new antibiotics have continued to worsen an already prevalent global health crisis (Centers for Disease Control and Prevention (CDC), 2013; Nathan and Cars, 2014; Taubes, 2008). The situation has recently culminated in the White House issuing an executive order to strengthen and coordinate efforts to combat antibiotic resistance (Obama, 2014), which has intensified the search for alternatives to antibiotics. One such class of anti-infectives is antivirulence therapies, which target host–pathogen interactions essential to pathogenesis (Fernebro, 2011). By inhibiting virulence factors such as cell adhesion, toxin production, biofilm formation, detoxification of immune antimicrobials, and quorum sensing, antivirulence therapies fight infection in a manner that limits selective pressure to the host environment and avoids damage to commensal microbiota (Cegelski *et al.*, 2008; Escaich, 2008; Fernebro, 2011). Because resistance derives from selective pressure, agents that inhibit infection without perturbing bacterial growth beyond the host will inherently have slower resistance development than conventional antibiotics (Rasko and Sperandio, 2010). Bacterial defenses against nitric oxide (NO•), a potent antimicrobial synthesized by the innate immune response (Fang, 2004; Nathan, 1992), have been identified as virulence factors for a number of pathogens (Robinson *et al.*, 2014a), and are thus being explored for the development of new anti-infectives (Bryk *et al.*, 2008; Darwin *et al.*, 2003; Helmick *et al.*, 2005). Compounds that impair bacterial NO• response networks could be used to synergize with host-derived NO• to prevent infection and/or facilitate immune-mediated clearance. However, known inhibitors of bacterial NO• detoxification systems, such as cyanide (Gardner *et al.*, 1998), carbon monoxide (Gardner *et al.*, 2000), and antimycin (Carr *et al.*, 1989), are highly toxic toward humans, thereby precluding their use as antibacterials. Antimicrobial imidazoles have also been shown to inhibit bacterial NO• detoxification systems, but their poor permeability into Gram-negative bacteria limits their utility (Helmick *et al.*, 2005). Identification of alternative inhibitors of bacterial NO• defense systems is therefore desirable to facilitate antivirulence development. Two complementary approaches to identify such inhibitors are to perform chemical screens to identify compounds with more favorable properties than current agents, or study the bacterial NO• stress response to identify other network components that can be targeted to enhance bacterial sensitivity toward NO•. Although mutants and chemicals can be identified through the use of screens, the mechanisms underlying how genetic and chemical perturbations impact NO• defenses may be elusive due to the complexity of NO• stress responses.

NO• exposure inhibits, damages, and activates an extensive and diverse collection of biomolecules within bacteria, resulting in systems-level perturbations (Bowman *et al.*, 2011; Toledo and Augusto, 2012). The kinetic competition between reaction targets and microbial NO• detoxification and repair systems contribute to a complex reaction network dictating the outcome of NO• exposure (Robinson and Brynildsen, 2013). In order to quantitatively study this network, a computational approach is required (Lim *et al.*, 2008; Robinson *et al.*, 2014a). Previously, we demonstrated the ability of kinetic models to capture the dynamics of NO• in cultures of *E. coli*, and accurately predict the influence of environmental and genetic perturbations on the behavior of its NO• response network (Robinson and Brynildsen, 2013; Robinson *et al.*, 2014b). With the proven potential to quantitatively interpret and predict NO• dynamics and perturbations to the bacterial response network, we hypothesize that these models have the capacity to be used in an ensemble framework for mechanistic dissection of any chemical treatment or genetic mutation. The implementation of an ensemble modeling approach holds promise in enhancing and broadening the scope of quantitative models as tools for extracting therapeutically important information about bacterial NO• response networks. By collectively analyzing an ensemble of different network

Stress and Environmental Regulation of Gene Expression and Adaptation in Bacteria, First Edition. Edited by Frans J. de Bruijn.

configurations and/or parameterizations, and iteratively reducing the ensemble with model-guided experimentation, the mechanism underlying the effect of a chemical or mutation found to sensitize bacteria toward NO• stress could be determined.

In this chapter, we summarize the current state of the art in modeling bacterial NO• stress, and the accomplishments achieved using these models thus far. We then discuss how to implement an ensemble approach with these quantitative models, and its potential to provide mechanistic insight into NO•-sensitive phenotypes caused by chemical or genetic perturbations. Given the existing studies that have demonstrated the feasibility and utility of using kinetic models to study NO• stress in bacteria, we extrapolate that ensemble modeling will be highly successful when applied to the mechanistic dissection of genetic and chemical perturbations to bacterial NO• response networks.

17.6.2 Current models of bacterial NO• stress

Within bacteria, NO• and its reaction products can interact with a wide range of biochemical species, including iron–sulfur ([Fe-S]) clusters, O_2, thiols, DNA bases, hemes, tyrosine residues, and transition metals (Bowman *et al.*, 2011; Toledo and Augusto, 2012), resulting in the inhibition of major cellular processes ranging from aerobic respiration (Stevanin *et al.*, 2000) and TCA cycle function (Richardson *et al.*, 2011) to amino acid biosynthesis (Hyduke *et al.*, 2007) and glycolysis (Mohr *et al.*, 1999). To counter this stress, bacteria have evolved defensive machinery to detoxify NO• (Carr *et al.*, 1989; Gardner *et al.*, 1998) and repair NO•-damaged biomolecules (Spek *et al.*, 2001; Yang *et al.*, 2002). The result is a complex web of reactions that extends to the far reaches of metabolism, transcriptional regulation, and macromolecular biosynthesis (Bowman *et al.*, 2011; Robinson *et al.*, 2014a). As such, the dynamics of NO• stress are dictated by the kinetics of the reactions that comprise this network, and can be simulated using quantitative models. The use of a kinetic model permits the analysis of dynamic, highly complex cellular networks, where the changes in species concentrations and the timescales of these changes can vary by orders of magnitude. Our previous work has demonstrated that this approach can accurately capture the dynamics of the NO• stress network in *E. coli*, and reveal interesting and important emergent system properties (Robinson and Brynildsen, 2013; Robinson *et al.*, 2014b).

A kinetic model of the NO• biochemical network in *E. coli* has been constructed and includes processes such as NO• autoxidation, thiol nitrosation, DNA base deamination, enzymatic NO• detoxification, nitrosylation and repair of [Fe-S] clusters, and reversible inhibition of cytochrome terminal oxidases (Robinson and Brynildsen, 2013). Experimental measurements of [NO•] dynamics in a culture of NO•-treated *E. coli* were in quantitative agreement with simulations, and they validated the model prediction that increasing the rate of NO• delivery decreases the utility of NO• dioxygenase (Hmp), thus defining a kinetic regime where the major aerobic NO• detoxification system in *E. coli* is dispensable (Robinson and Brynildsen, 2013). More recently, the model has been used to explore the influence of two important design parameters (release rate and total payload) of therapeutics that directly deliver NO• to infection sites on the efficacy of NO• treatment (Robinson *et al.*, 2014b). Simulations predicted that a faster NO• delivery rate would correspond to greater therapeutic efficacy (longer duration of bacterial respiration inhibition) for lower NO• payloads, while slower release rates were predicted to be more effective at higher total NO• payloads. Experimental measurements of [NO•] and [O_2] quantitatively confirmed the model-predicted relationship (Robinson *et al.*, 2014b). Figure 17.6.1 illustrates the use of a kinetic model to capture NO• dynamics in a bacterial culture and validate model predictions with experimental measurements.

Given these accomplishments, models of bacterial NO• stress are ready to be put to use in drug and target discovery by providing a framework to mechanistically dissect perturbations that impact the network. The identification of a genetic mutant or small molecule that enhances bacterial NO• sensitivity does not necessarily illuminate its mechanism of action. By using a quantitative model, one can interpret the experimental observations in a manner that takes into account the existing knowledge of the governing bacterial NO• response network. In cases where the NO•-sensitizing mutation or chemical is novel, and thus extends beyond the current knowledge base upon which the model was constructed, it will require flexibility in the model to capture the observed phenotype. An approach that has proven effective in accounting for uncertainty in network structure and/or parameterization is ensemble modeling (Alves and Savageau, 2000; Brown and Sethna, 2003; Kuepfer *et al.*, 2007), where an ensemble of different models can be generated to span the breadth of potential mechanisms.

17.6.3 Ensemble modeling enables quantitative exploration of uncertain systems

The use of ensemble modeling allows quantitative analysis of models with a high degree of uncertainty in parameter values (e.g., reaction rates and species concentrations), system components (e.g., metabolites and enzymes), and/or network architecture (e.g., available reactions and their mechanisms) due to scarce or conflicting experimental data (Alves and Savageau, 2000; Battogtokh *et al.*, 2002; Kuepfer *et al.*, 2007). The system can instead be represented by a diversity of models (the ensemble), where each possesses different parameter values and/or network structures. Through an iterative process of *in silico* predictions and experimental measurements, the confidence and predictive strength of the ensemble increase as it converges to a single mechanism.

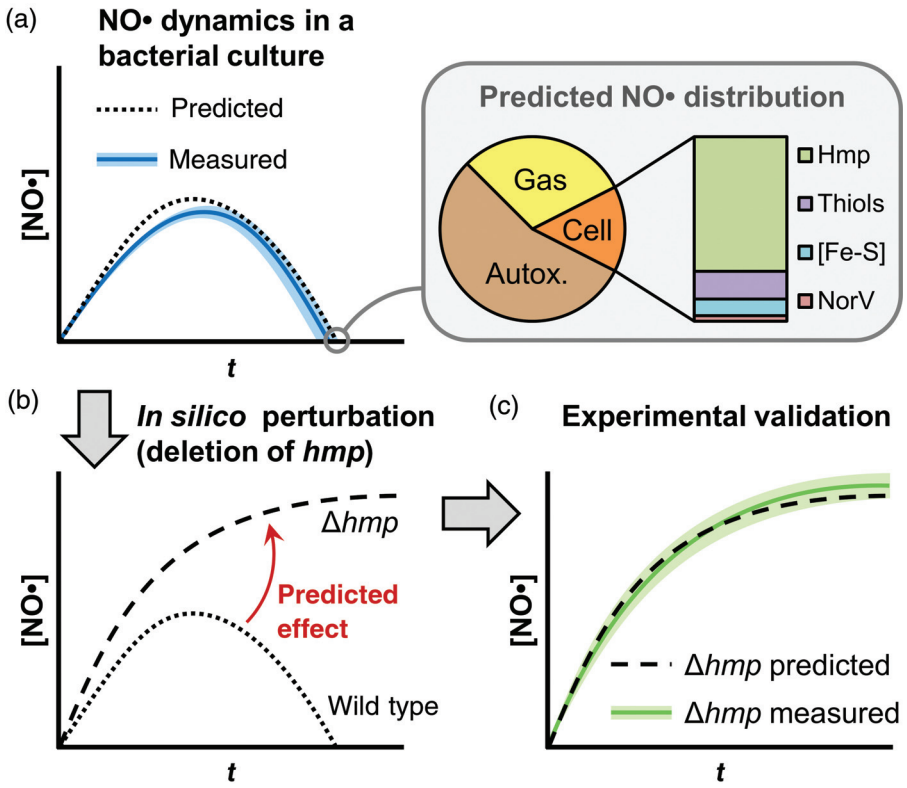
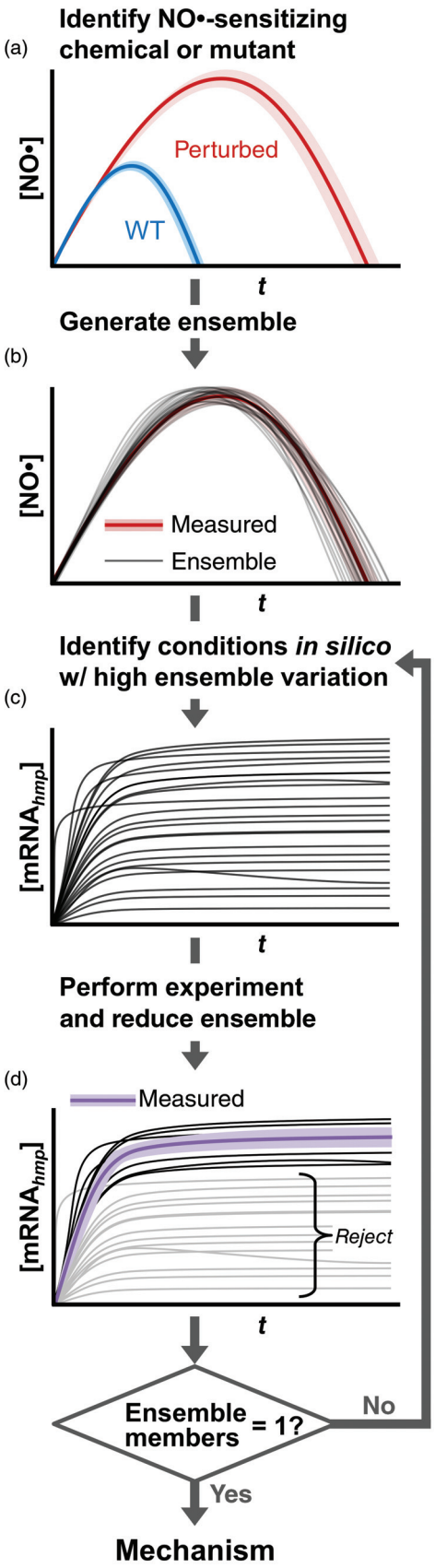


Figure 17.6.1 Kinetic modeling can be used to quantitatively capture and predict NO• dynamics in bacterial cultures. (a) A hypothetical measured [NO•] curve (solid blue curve) following NO• treatment of a bacterial culture compared with a simulation (dotted black curve) from a kinetic model of the bacterial NO• stress network. To the right of the curves is a representative distribution of NO• consumption by different network pathways in *E. coli*, predicted by the model (*Autox.* and *Gas* are NO• autoxidation in the culture media and loss to the gas phase, respectively, whereas cellular consumption is broken down into Hmp-mediated detoxification, thiol nitrosation, [Fe-S] nitrosylation, and NorV-mediated detoxification). (b) Models can be used to analyze the network *in silico*, including the prediction of system perturbations such as the deletion of the dominant intracellular NO• sink (*E. coli*'s Hmp in this example). (c) Hypothetical experimental validation (solid green curve) of the model-predicted result (dashed black line) of the network perturbation. Data and simulations shown are purely illustrative; for actual measurements and model simulations, see Robinson and Brynildsen (2013) and Robinson *et al.* (2014b).

Ensemble generation can be accomplished using a number of different approaches (Alves and Savageau, 2000; Battogtokh *et al.*, 2002; Brown and Sethna, 2003; Hettling and van Beek, 2011). For brevity, we focus on two here: one that is hypothesis driven, and another involving a systematic relaxation of model parameters. In the first approach, competing hypotheses regarding the structure of a particular reaction pathway exist, and therefore, different models can be created to represent each individual hypothesis (Kuepfer *et al.*, 2007; Schaber *et al.*, 2012). In the second approach, a systematic relaxation of model parameters can be performed to yield mechanistically distinct systems. In this case, each member of the ensemble would share the same network architecture but differ in their parameter values (Alves and Savageau, 2000; Rizk and Liao, 2009; Tran *et al.*, 2008). Once the ensemble has been generated, experiments can be performed to reduce the ensemble size and ultimately arrive at a single mechanism that describes the system of interest.

To begin the reduction process, the ensemble is used to identify conditions *in silico* that yield substantial disagreement between ensemble members. Conditions promoting large variation among ensemble predictions are ideal for discriminating between possible mechanisms, and are therefore used to guide experimental design. The corresponding experiments are performed, and models whose predictions are in disagreement with the new experimental data are discarded, reducing the ensemble size (Tan *et al.*, 2011). This feedback cycle of guided experimentation and ensemble reduction can be iterated until convergence to a single mechanism is achieved.

A number of previous studies have implemented ensemble modeling to computationally explore systems where considerable uncertainty existed, or to investigate the robustness of predictions to parameter uncertainty (Battogtokh *et al.*, 2002; Hettling and van Beek, 2011; Jia *et al.*, 2012; Kuepfer *et al.*, 2007; Rizk and Liao, 2009; Tan *et al.*, 2011). For example, Kuepfer and colleagues applied ensemble modeling to evaluate 19 different network structures describing the target-of-rapamycin (TOR) pathway of *Saccharomyces cerevisiae* (Kuepfer *et al.*, 2007). Although the ensemble was not reduced to a single model, the



quantification of relative training errors and prediction errors with experimental data narrowed it to seven models whose shared features provided insight into the underlying mechanism, such as the importance of the Tap42p–Tip41p complex formation in signal control (Kuepfer *et al.*, 2007). In the context of modeling metabolic networks, Tran and colleagues generated an ensemble of models of *E. coli* central metabolism that differed in their dynamics but shared the same steady-state flux and metabolite concentrations (Tran *et al.*, 2008). Their analysis highlights the fact that the path taken (i.e., the order of experiments) to reduce the ensemble does not affect the final model to which the ensemble converges, but can impact the number of experiments required to achieve convergence. More recently, ensemble modeling has been applied to analyze the production of aromatic amino acids in *E. coli*, where phenotypic data obtained from enzyme overexpressions were used to obtain a reduced ensemble capable of accurately predicting the dynamics and enzymatic bottlenecks of the 3-deoxy-D-arabinoheptulosonate-7-phosphate (DAHP) synthesis pathway (Rizk and Liao, 2009). Hettling and van Beek have used a mathematical model to evaluate two hypothesized roles of creatine kinase (CK) in heart muscle, taking an ensemble approach to account for error associated with experimentally measured parameter values (Hettling and van Beek, 2011). An algorithm that accounts for model "sloppiness" (Brown and Sethna, 2003; Gutenkunst *et al.*, 2007) has been used to generate and analyze an ensemble of parametrically distinct models that can identify confidence intervals not only on parameters, but also on predictions (Hettling and van Beek, 2011). Without reducing the size of the ensemble, the predictions were able to rule out the hypothesis that shuttling of high-energy phosphate groups is an important role of CK in heart muscle (Hettling and van Beek, 2011).

Figure 17.6.2 Flowchart illustrating the application of ensemble modeling to dissect the mechanism underlying a perturbation found to enhance NO• sensitivity. (a) A mutation or chemical is identified that impairs bacterial NO• detoxification through an unknown mechanism of action, resulting in a perturbed [NO•] profile. Shown are hypothetical measured [NO•] curves demonstrating the effect of the perturbation (red curve) to "normal" wild-type (WT) NO• consumption dynamics (blue curve). (b) An ensemble of models is generated by releasing parameter constraints and/or varying network structure, and re-optimizing the model to capture the perturbed [NO•] curve. (c) The ensemble is used to perform *in silico* analyses to determine which experimental conditions will yield a large variation in dynamics among ensemble members. In this example, predicted *hmp* (messenger RNA) profiles are illustrated to vary substantially among ensemble members. (d) The corresponding experiment is performed, and models exhibiting incorrect predictions are eliminated from the ensemble. Steps (C) and (D) are iterated until the ensemble has been reduced to a single model, which represents the mechanism underlying the NO•-sensitizing perturbation. Data and simulations shown are purely illustrative; for actual measurements and model simulations, see Robinson and Brynildsen (2013) and Robinson *et al.* (2014b).

17.6.4 Prospects of ensemble modeling of NO• stress

Given the complexity and breadth of the bacterial NO• stress response network, a quantitative model is necessary to study its dynamics as a collective system. Upon discovery of a chemical or mutant that perturbs the NO• response network, we hypothesize that an ensemble approach could be used to systematically delineate the mechanism underlying the observed phenotype (Figure 17.6.2).

The ensemble could be used to simulate experimentally accessible conditions *in silico* to rapidly screen for those that result in large variation among ensemble members, facilitating the elimination of mechanisms that are inconsistent with the new data. Through an iterative process, the ensemble is reduced to a single model, representing the mechanism through which the chemical or mutation is perturbing the bacterial NO• response network. Knowledge of the mechanism will illustrate how resistance can be obtained and illuminate possible synergies with other strategies, while also providing a deeper understanding of the pathogen's physiology.

17.6.5 Conclusion

Antibiotic resistance is a threat to global health, and it has motivated the search for alternative approaches to conventional antimicrobials (Arias and Murray, 2009; Bush *et al.*, 2011; CDC, 2013; Taubes, 2008). Antivirulence is a promising alternative that targets the host–pathogen interactions essential for establishing infection, such as pathogen defenses against the immune system (Cegelski *et al.*, 2008; Escaich, 2008; Fernebro, 2011). NO• is a potent antimicrobial produced by the immune system (Fang, 2004; Nathan, 1992), and NO• defense systems are common virulence factors for pathogens (Darwin and Nathan, 2005; Richardson *et al.*, 2006; Robinson *et al.*, 2014a). Disrupting bacterial NO• defense networks therefore represents a promising antivirulence approach that could facilitate immune-mediated clearance (Bryk *et al.*, 2008; Darwin *et al.*, 2003; Helmick *et al.*, 2005). Moreover, agents found to sensitize bacteria toward NO• could be used synergistically with exogenous NO• delivery platforms, which are currently under investigation for the treatment and prevention of antibiotic-resistant infections (Friedman *et al.*, 2011; Jones *et al.*, 2010; Schairer *et al.*, 2012). Because the promiscuous reactivity of NO• and its oxidation products yields a highly complex reaction network, it can be difficult to decipher the mechanism underlying a chemical or mutation found to sensitize a pathogen toward NO•. Kinetic modeling has been shown to quantitatively capture NO• dynamics in *E. coli* cultures, and successfully predict the result of system perturbations or changes in experimental conditions. We hypothesize that the use of an ensemble approach, which has been shown to be effective in handling parameter or network structure uncertainty, could be used with kinetic models of NO• stress to delineate the mechanism underlying a chemical or mutant identified to sensitize bacteria toward NO•. Through an iterative process of *in silico* ensemble predictions and guided experimentation, the ensemble could be reduced to a single model that describes the mechanism of the NO•-sensitizing compound or mutation. Furthermore, we assert that ensemble modeling, when coupled with high-throughput mutant or chemical screens that search for enhanced NO• sensitivity, will provide a robust platform to identify targets or compounds with antivirulence potential, and their mechanisms of action.

Acknowledgements

This work was supported in part by the National Science Foundation with a CAREER award to MPB (CBET-1453325) and Graduate Research Fellowship to JLR (DGE 1148900), and by Princeton University (startup funds and the Forese Family Fund for Innovation).

References

Alves R, Savageau MA. 2000. Systemic properties of ensembles of metabolic networks: application of graphical and statistical methods to simple unbranched pathways. *Bioinformatics* 16(6): 534–547.

Arias CA, Murray BE. 2009. Antibiotic-resistant bugs in the 21st century – a clinical super-challenge. *The New England Journal of Medicine* 360(5): 439–443.

Battogtokh D, Asch DK, Case ME, Arnold J, Schuttler HB. 2002. An ensemble method for identifying regulatory circuits with special reference to the *qa* gene cluster of *Neurospora crassa*. *Proceedings of the National Academy of Sciences of the USA* 99(26): 16904–16909.

Bowman LAH, McLean S, Poole RK, Fukuto JM. 2011. The diversity of microbial responses to nitric oxide and agents of nitrosative stress: Close cousins but not identical twins. *Advances in Microbial Physiology* 59: 135–219.

Brown KS, Sethna JP. 2003. Statistical mechanical approaches to models with many poorly known parameters. *Physical Review E* 68(2 Pt 1): 021904.

Bryk R, Gold B, Venugopal A, Singh J, Samy R, *et al.* 2008. Selective killing of nonreplicating Mycobacteria. *Cell Host & Microbe* 3(3): 137–145.

Bush K, Courvalin P, Dantas G, Davies J, Eisenstein B, *et al.* 2011. Tackling antibiotic resistance. *Nature Reviews Microbiology* 9(12): 894–896.

Carr GJ, Page MD, Ferguson SJ. 1989. The energy-conserving nitric-oxide-reductase system in *Paracoccus denitrificans*. Distinction from the nitrite reductase that catalyses synthesis of nitric oxide and evidence from trapping experiments for nitric oxide as a free intermediate during denitrification. *European Journal of Biochemistry* 179(3): 683–692.

Cegelski L, Marshall GR, Eldridge GR, Hultgren SJ. 2008. The biology and future prospects of antivirulence therapies. *Nature Reviews Microbiology* 6(1): 17–27.

Centers for Disease Control and Prevention (CDC), US Department of Health and Human Services. 2013. Antibiotic resistance threats

in the United States, 2013. http://www.cdc.gov/drugresistance/threat-report-2013/

Darwin KH, Ehrt S, Gutierrez-Ramos JC, Weich N, Nathan CF. 2003. The proteasome of *Mycobacterium tuberculosis* is required for resistance to nitric oxide. *Science* 302(5652): 1963–1966.

Darwin KH, Nathan CF. 2005. Role for nucleotide excision repair in virulence of *Mycobacterium tuberculosis*. *Infection and Immunity* 73(8): 4581–4587.

Escaich S. 2008. Antivirulence as a new antibacterial approach for chemotherapy. *Current Opinion in Chemical Biology* 12(4): 400–408.

Fang FC. 2004. Antimicrobial reactive oxygen and nitrogen species: concepts and controversies. *Nature Reviews Microbiology* 2(10): 820–832.

Fernebro J. 2011. Fighting bacterial infections-future treatment options. *Drug Resistance Updates* 14(2): 125–139.

Friedman A, Blecher K, Sanchez D, Tuckman-Vernon C, Gialanella P, *et al.* 2011. Susceptibility of Gram-positive and -negative bacteria to novel nitric oxide-releasing nanoparticle technology. *Virulence* 2(3): 217–221.

Gardner PR, Gardner AM, Martin LA, Dou Y, Li T, *et al.* 2000. Nitric-oxide dioxygenase activity and function of flavohemoglobins. sensitivity to nitric oxide and carbon monoxide inhibition. *The Journal of Biological Chemistry* 275(41): 31581–31587.

Gardner PR, Gardner AM, Martin LA, Salzman AL. 1998. Nitric oxide dioxygenase: an enzymic function for flavohemoglobin. *Proceedings of the National Academy of Sciences of the USA* 95(18): 10378–10383.

Gutenkunst RN, Waterfall JJ, Casey FP, Brown KS, Myers CR, *et al.* 2007. Universally sloppy parameter sensitivities in systems biology models. *PloS Computational Biology* 3(10): 1871–1878.

Helmick RA, Fletcher AE, Gardner AM, Gessner CR, Hvitved AN, *et al.* 2005. Imidazole antibiotics inhibit the nitric oxide dioxygenase function of microbial flavohemoglobin. *Antimicrobial Agents and Chemotherapy* 49(5): 1837–1843.

Hettling H, van Beek JHGM. 2011. Analyzing the functional properties of the creatine kinase system with multiscale "sloppy" modeling. *PLoS Computational Biology* 7(8): e1002130.

Hyduke DR, Jarboe LR, Tran LM, Chou KJ, Liao JC. 2007. Integrated network analysis identifies nitric oxide response networks and dihydroxyacid dehydratase as a crucial target in *Escherichia coli*. *Proceedings of the National Academy of Sciences of the USA* 104(20): 8484–8489.

Jia G, Stephanopoulos G, Gunawan R. 2012. Ensemble kinetic modeling of metabolic networks from dynamic metabolic profiles. *Metabolites* 2(4): 891–912.

Jones ML, Ganopolsky JG, Labbe A, Wahl C, Prakash S. 2010. Antimicrobial properties of nitric oxide and its application in antimicrobial formulations and medical devices. *Applied Microbiology and Biotechnology* 88(2): 401–407.

Kuepfer L, Peter M, Sauer U, Stelling J. 2007. Ensemble modeling for analysis of cell signaling dynamics. *Nature Biotechnology* 25(9): 1001–1006.

Lim CH, Dedon PC, Deen WA. 2008. Kinetic analysis of intracellular concentrations of reactive nitrogen species. *Chemical Research in Toxicology* 21(11): 2134–2147.

Mohr S, Hallak H, de Boitte A, Lapetina EG, Brune B. 1999. Nitric oxide-induced S-glutathionylation and inactivation of glyceraldehyde-3-phosphate dehydrogenase. *The Journal of Biological Chemistry* 274(14): 9427–9430.

Nathan C. 1992. Nitric oxide as a secretory product of mammalian cells. *FASEB Journal* 6(12): 3051–3064.

Nathan C, Cars O. 2014. Antibiotic resistance – problems, progress, and prospects. *The New England Journal of Medicine* 371(19): 1761–1763.

Obama B. 2014. *Combating antibiotic-resistant bacteria.* Executive Order No. 13676, 3 CFR.

Rasko DA, Sperandio V. 2010. Anti-virulence strategies to combat bacteria-mediated disease. *Nature Reviews Drug Discovery* 9(2): 117–128.

Richardson AR, Dunman PM, Fang FC. 2006. The nitrosative stress response of *Staphylococcus aureus* is required for resistance to innate immunity. *Molecular Microbiology* 61(4): 927–939.

Richardson AR, Payne EC, Younger N, Karlinsey JE, Thomas VC, *et al.* 2011. Multiple targets of nitric oxide in the tricarboxylic acid cycle of *Salmonella enterica* serovar Typhimurium. *Cell Host & Microbe* 10(1): 33–43.

Rizk ML, Liao JC. 2009. Ensemble modeling for aromatic production in *Escherichia coli*. *PLoS One* 4(9): e6903.

Robinson JL, Adolfsen KJ, Brynildsen MP. 2014a. Deciphering nitric oxide stress in bacteria with quantitative modeling. *Current Opinion in Microbiology* 19: 16–24.

Robinson JL, Brynildsen MP. 2013. A kinetic platform to determine the fate of nitric oxide in *Escherichia coli*. *PLoS Computational Biology* 9(5): e1003049.

Robinson JL, Miller RV, Brynildsen MP. 2014b. Model-driven identification of dosing regimens that maximize the antimicrobial activity of nitric oxide. *Metabolic Engineering Communications* 1: 12–14.

Schaber J, Baltanas R, Bush A, Klipp E, Colman-Lerner A. 2012. Modelling reveals novel roles of two parallel signalling pathways and homeostatic feedbacks in yeast. *Molecular Systems Biology* 8: 622.

Schairer DO, Chouake JS, Nosanchuk JD, Friedman AJ. 2012. The potential of nitric oxide releasing therapies as antimicrobial agents. *Virulence* 3(3): 271–279.

Spek EJ, Wright TL, Stitt MS, Taghizadeh NR, Tannenbaum SR *et al.* 2001. Recombinational repair is critical for survival of *Escherichia coli* exposed to nitric oxide. *Journal of Bacteriology* 183(1): 131–138.

Stevanin TM, Ioannidis N, Mills CE, Kim SO, Hughes MN, *et al.* 2000. Flavohemoglobin Hmp affords inducible protection for *Escherichia coli* respiration, catalyzed by cytochromes *bo*' or *bd*, from nitric oxide. *The Journal of Biological Chemistry* 275(46): 35868–35875.

Tan Y, Rivera JG, Contador CA, Asenjo JA, Liao JC. 2011. Reducing the allowable kinetic space by constructing ensemble of dynamic models with the same steady-state flux. *Metabolic Engineering* 13(1): 60–75.

Taubes G. 2008. The bacteria fight back. *Science* 321(5887): 356–361.

Toledo JC, Augusto O. 2012. Connecting the chemical and biological properties of nitric oxide. *Chemical Research in Toxicology* 25(5): 975–989.

Tran LM, Rizk ML, Liao JC. 2008. Ensemble modeling of metabolic networks. *Biophysical Journal* 95(12): 5606–5617.

Yang W, Rogers PA, Ding H. 2002. Repair of nitric oxide-modified ferredoxin [2Fe-2S] cluster by cysteine desulfurase (IscS). *The Journal of Biological Chemistry* 277(15): 12868–12873.