Agent-Based Modeling of Oxygen-Responsive Transcription Factors in *Escherichia coli*

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Abstract

In the presence of oxygen (O₂) the model bacterium *Escherichia coli* is able to conserve energy by aerobic respiration. Two major terminal oxidases are involved in this process - Cyo has a relatively low affinity for O₂ but is able to pump protons and hence is energetically efficient; Cyd has a high affinity for O₂ but does not pump protons. When *E. coli* encounters environments with different O₂ availabilities, the expression of the genes encoding the alternative terminal oxidases, the *cydAB* and *cynABCDE* operons, are regulated by two O₂-responsive transcription factors, ArcA (an indirect O₂ sensor) and FNR (a direct O₂ sensor). It has been suggested that O₂-consumption by the terminal oxidases located at the cytoplasmic membrane significantly affects the activities of ArcA and FNR in the bacterial nucleoid. In this study, an agent-based modeling approach has been taken to spatially simulate the uptake and consumption of O₂ by *E. coli* and the consequent modulation of ArcA and FNR activities based on experimental data obtained from highly controlled chemostat cultures. The molecules of O₂, transcription factors and terminal oxidases are treated as individual agents and their behaviors and interactions are imitated in a simulated 3-D *E. coli* cell. The model implies that there are two barriers that dampen the response of FNR to O₂, i.e. consumption of O₂ at the membrane by the terminal oxidases and reaction of O₂ with cytoplasmic FNR. Analysis of FNR variants suggested that the monomer-dimer transition is the key step in FNR-mediated repression of gene expression.

Introduction

The bacterium *Escherichia coli* is a widely used model organism to study bacterial adaptation to environmental change. As an enteric bacterium, *E. coli* has to cope with an O₂-starved niche in the host and an O₂-rich environment when excreted. In order to exploit the energetic benefits that are conferred by aerobic respiration, *E. coli* has two major terminal oxidases: cytochrome *bd*-I (Cyd) and cytochrome *bo* (Cyo) that are encoded by the *cydAB* and *cynABCDE* operons, respectively [1,2]. Cyd has a high affinity for O₂ and is isolated at low O₂ concentrations (micro-aerobic conditions), whereas Cyo has a relatively low affinity for O₂ and is predominant at high O₂ concentrations (aerobic conditions) [3]. These two terminal oxidases contribute differentially to energy conservation because Cyo is a proton pump, whereas Cyd is not [1,2], however, the very high affinity of Cyd for O₂ allows the bacterium to maintain aerobic respiration at nanomolar concentrations of O₂, thereby maintaining aerobic respiratory activity rather than other, less favorable, metabolic modes [4–6].

The transcription factors, ArcA and FNR, regulate *cydAB* and *cynABCDE* expression in response to O₂ supply [7]. FNR is an iron-sulfur protein that senses O₂ in the cytoplasm [8,9]. In the absence of O₂ the FNR iron-sulfur cluster is stable and the protein forms dimers that are competent for site-specific DNA-binding and regulation of gene expression [10]. The FNR iron-sulfur cluster reacts with O₂ in such a way that the DNA-binding dimeric form of FNR is converted into a non-DNA-binding monomeric species [10]. Under anaerobic conditions, FNR acts as a global regulator in *E. coli* [11–13], including the *cydAB* and *cynABCDE* operons, which are repressed by FNR when the O₂ supply is restricted [7]. Under aerobic conditions, repression of *cydAB* and *cynABCDE* is relieved and Cyd and Cyo proteins are synthesized [3]. In contrast, ArcA responds to O₂ availability indirectly via the membrane-bound sensor ArcB. In the absence of O₂ ArcB responds to changes in the redox state of the electron transport chain and the presence of fermentation products by autoprophorylation [14–16]. Phosphorylated ArcB is then able to transfer phosphate to the cytoplasmic ArcA regulator (ArcA→P₂), which then undergoes oligomerization to form a tetra-phosphorylated octomer that is capable of binding at multiple sites in the *E. coli* genome [17,18], including those in the promoter regions of *cydAB* and *cynABCDE* to enhance synthesis of Cyd and inhibit production of Cyo [7,17]. Because the terminal oxidases (Cyd and Cyo) consume O₂ at the cell membrane, a feedback loop is formed that links the activities of the oxidases to the regulatory activities of ArcA and FNR (Figure 1). These features of the system -
The model bacterium Escherichia coli has a modular electron transport chain that allows it to successfully compete in environments with differing oxygen (O₂) availabilities. It has two well-characterized terminal oxidases, Cyd and Cyo. Cyd has a very high affinity for O₂, whereas Cyo has a lower affinity, but is energetically more efficient. Expression of the genes encoding Cyd and Cyo is controlled by two O₂-responsive regulators, ArcBA and FNR. However, it is not clear how O₂ molecules enter the E. coli cell and how the locations of the terminal oxidases and the regulators influence the system. An agent-based model is presented that simulates the interactions of O₂ with the regulators and the oxidases in an E. coli cell. The model suggests that O₂ consumption by the oxidases at the cytoplasmic membrane and by FNR in the cytoplasm protects FNR bound to DNA in the nucleoid from inactivation and that dimerization of FNR in response to O₂ depletion is the key step in FNR-mediated repression. Thus, the focus of the agent-based model on spatial events provides information and new insight, allowing the effects of dysregulation of system components to be explored by facile addition or removal of agents.

Results/Discussion

The starting point for this work was the suggestion that spatial effects play an important role in controlling the response of the E. coli transcription factor FNR to changes in O₂ availability [15]. Early work showed that when dissolved O₂ was detectable in the culture medium the activity of FNR decreased, exhibiting ∼50% activity in the range 2–5 μM dissolved O₂ and that under these conditions the external concentration of O₂ was equivalent to that in the bacterial cytoplasm [19,20]. However, when the cultures were supplied with low concentrations of O₂ in the input gas, dissolved O₂ in the culture medium could not be measured conventionally, because the O₂ is consumed by the respiratory activity of the bacteria. Therefore, a physiological measure of O₂ availability (the aerobicosis scale) was adopted to investigate the effects of low O₂ concentrations on bacteria [21]. On the aerobicosis scale, the minimum O₂ input that results in undetectable excretion of the fermentation product acetate under carbon-limiting conditions is defined as 100% aerobicosis (100% AU). As the amount of O₂ supplied to cultures decreases the specific rate of acetate production (qacetate) increases to a maximum under anaerobic conditions (0% AU). Between these limits (0–100% AU) lies the micro-aerobic range, defined by the linear decrease in qacetate as the O₂ transfer rate increases, i.e. there is an inverse correlation between qacetate and aerobicosis [21]. When the O₂ supply exceeds the minimum required to abolish acetate excretion the AU value extends beyond 100%, reaching 217% when the culture medium is O₂ saturated. This physiological measurement of O₂ availability is reliable for cultures grown at values as low as 4% AU [21]. In previous experiments using this approach steady-state chemostat cultures were established at fixed points on the aerobicosis scale and samples were taken for measurement of the numbers of Cyd and Cyo molecules per bacterial cell (Table 1) [5]. In addition, Western blotting showed that the concentration of FNR in the cell was constant at 3000 protomers per bacterium across the aerobicosis scale (Table 1).

The agent-based model was constructed to simulate interactions between three groups of agents: O₂ molecules, terminal oxidases, (Cyd and Cyo) and regulators (FNR and ArcBA), which interact with O₂ directly or indirectly. As shown in Figure 2, the initial state of the model is defined in the file 0.xml, which contains information on agent properties such as Id, type, status, position etc. – the data in Table 1 was used to provide numbers of Cyd, Cyo, ArcA and FNR molecules. There is no information available on the abundance of ArcB in the cell and thus it was assumed that there are 1000 ArcB molecules per cell based on the ~10:1 ratio of response-regulator to sensor kinase of another E. coli two-component system, PhoBR [22]. At the beginning of the simulation, the 0.xml file is read to establish the 3-D E. coli cell. From the initial state, the agents start moving randomly within their 3-D activity space, and interact with each other according to a set of pre-defined interaction rules and interaction radii (Tables 2 and 3), leading to an emergent state.

The algorithm of supplying O₂ to the cell responds to given AU levels and automatically calculates the rate of O₂ molecules supplied to the cell. Whilst the model runs, agent information is updated and stored as a series of XML files for further analysis and visualization.

Modeling the regulatory response to O₂ availability

The dynamics of the system were investigated by running the simulation through two cycles of transitions from 0–217% AU. Figure 3a shows a top view of a 3-D E. coli cell at 0% AU (steady-state anaerobic conditions). Under these conditions, the FNR molecules are present as dimers, all ArcB molecules are phosphorylated and the ArcA is octameric. The DNA binding sites for ArcA (120 in the model) and FNR (350 in the model) in the nucleoid are fully occupied. The number of ArcA sites was chosen from the data reported by Liu and De Wulf [18]. The model must include a mechanism for ArcA~P to leave regulated promoters. Upon introduction of O₂ into an anaerobic steady-state chemostat cultures ∼5 min was required to inactivate ArcA-mediated transcription [15]. In the agent-based model presented here, each iteration represents 0.2 sec. Therefore, assuming that ArcA~P leaving the 120 DNA sites is a first order process, then t₁/₂ is ∼45 sec, which is equivalent to ∼0.3% ArcA~P leaving the DNA per iteration (Table 3). The number of FNR binding sites was based on ChiP-seq and ChiP-Chip measurements, which detected ∼220 FNR sites and a genome sequence analysis that predicted ∼450 FNR sites; thus a mid-range value of 350 was chosen [23–25]. Interaction with O₂ causes FNR to dissociate from the DNA (Table 3). Under fully aerobic conditions (217% AU) the FNR dimers are disassembled to monomers, and the different forms of ArcA coexist (Figure 3b). The ArcA- and FNR-DNA binding sites in the nucleoid are mostly unoccupied due to the lower concentrations of FNR dimers and ArcA octamers.
Examination of the system as it transits from 0% to 217% AU showed that the DNA-bound, transcriptionally active FNR was initially protected from inactivation by consumption of O2 at the cell membrane by the terminal oxidases and by reaction of O2 with the iron-sulfur clusters of FNR dimers in the bacterial cytoplasm - the progress of this simulation is shown in Video S1. This new insight into the buffering of the FNR response could serve a useful biological purpose by preventing premature switching off of anaerobic genes when the bacteria are exposed to low concentration O2 pulses in the environment.

In the various niches occupied by E. coli, the bacterium can experience the full range of O2 concentrations from zero, in the Table 1. Numbers of Cyd, Cyo, ArcA and FNR molecules per E. coli cell at different points on the aerobiosis scale.

| AU (%) | Cyd (molecules per cell) | Cyo (molecules per cell) | Total ArcA monomer (molecules per cell) | Total FNR monomer (molecules per cell) |
|--------|--------------------------|--------------------------|----------------------------------------|---------------------------------------|
| 0      | 11442                    | 4336                     | 8000                                   | 3000                                  |
| 31     | 51403                    | 6202                     | 8000                                   | 3000                                  |
| 85     | 66729                    | 14017                    | 8000                                   | 3000                                  |
| 115    | 19102                    | 10985                    | 8000                                   | 3000                                  |
| 217    | 10284                    | 9036                     | 8000                                   | 3000                                  |

The Cyd, Cyo and ArcA numbers are those reported by Rolfe et al. [3]. The number of FNR molecules per cell was calculated from analysis of Western blots developed with anti-FNR serum for whole cell samples taken from steady-state cultures at the indicated aerobiosis units (AU). doi:10.1371/journal.pcbi.1003595.t001

Figure 1. Components of the agent-based model. The diagram shows the interactions between and locations of the components of the model. Oxygen molecules (O2) cross the cytoplasmic membrane and enter the bacterial cell where they are reduced to water (H2O) by the action of the membrane-bound terminal oxidases (Cyo and Cyd). The transcription regulator, FNR is located in the cytoplasm and is inactivated by reaction with O2. The active form of FNR represses the expression of both the cydAB and cyaABCDE operons located in the nucleoid. The ArcBA two-component system responds to O2 indirectly (dashed line). The availability of O2 alters the redox state of the electron transport chain and the production of fermentation products. These changes are sensed by the membrane-bound sensor ArcB, which autophosphorylates when O2 is restricted. ArcB transfers phosphate to the cytoplasmic regulator ArcA, which acts to repress expression of cyaABCDE and activate expression of cydAB. doi:10.1371/journal.pcbi.1003595.g001
anaerobic regions of a host alimentary tract, to full O₂ saturation (~200 μM, equivalent to ~120,000 O₂ molecules per cell), but fully aerobic metabolism is supported when the O₂ supply exceeds 1,000 O₂ molecules per cell. The profiles of five repetitive simulations for each agent in the model are presented in Figure 4. From iteration 1 to 5000 and iteration 15000 to 20000, O₂ was supplied at a constant value of 6,500 molecules per cell such that the total number of O₂ molecules entering the cell increased linearly; when the O₂ supply was stopped (5000 to 15000 and 20000 to 30000 iterations) no more O₂ entered the cell and thus the number of O₂ molecules that had entered the cell remained unchanged during these periods (Figure 4a). When O₂ became available to the cell (from iteration 1), the sensor ArcB was de-phosphorylated and started to de-phosphorylate ArcA. Consequently, the number of ArcA octamers bound at their cognate sites in the nucleoid decreased rapidly. The ArcA tetramers and dimers produced during de-phosphorylation of the ArcA octamer were transformed to inactive (de-phosphorylated) ArcA dimers, (Figure 4d–f). Under aerobic conditions (iteration 2. Components and process of agent-based model simulation. The file 0.xml contains all the settings for the agents involved to provide the initial model state. The FNR system, ArcBA system and terminal oxidases are displayed in light grey, light blue and light red ellipses respectively. The interaction rules and parameters are pre-defined, which determines how and when the interactions take place. For each of the iterations the number of O₂ molecules supplied to modelled cell is calculated. While model runs, updated information is generated in the same format as the initial file and stored in a series of xml files for further analysis. Experimental data, where available, were used for preparing the initial file and designing the interaction rules and parameters.

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5000) all the ArcA was decomposed to inactive ArcA dimers. When the O2 supply was stopped (from iteration 5001), the number of inactive ArcA dimers decreased rapidly as shown in Figure 4f, being transformed into phosphorylated ArcA dimers, tetramers and octamers (Figure 4c–e). Due to the phosphorylated ArcA dimers and tetramers combining to form ArcA octamers, their numbers dropped after initially increasing. The rate at which the ArcA octomers accumulated (ArcA activation) after O2 withdrawal was slower than the rate of ArcA inactivation (Figures 4b and c). In this implementation of the modeled transition cycle, the numbers of

Table 2. Agent properties.

| Molecule   | Form            | Initial number | Step length (nm) | Initial location | Activity space            |
|------------|-----------------|----------------|------------------|------------------|---------------------------|
| O2         | Molecule        | 0              | Variable¹       | Outside the cell | From outside to inside of the cell |
| FNR        | Monomer         | 0              | 15               | n/a              | Inside the cell           |
|            | Free dimer      | 1150           | 15               | Evenly distributed in the cell | Inside the cell |
| ArcA²      | Octamer         | 1000           | 18               | Evenly distributed in the cell | Inside the cell |
|            | Octamer bound to DNA | 120         | 0                | Inside the nucleoid space | Stationary on DNA |
|            | Tetramer        | 0              | 10               | n/a              | Inside the cell           |
| ArcB³      | Protein molecule | 1000           | 5                | Inner membrane   | Inner membrane            |
| Cyo        | Oxidase molecule | Vary at different AU levels⁴ | 5   | Inner membrane   | Inner membrane            |
| Cyd        | Oxidase molecule | Vary at different AU levels⁴ | 5   | Inner membrane   | Inner membrane            |

The third column lists the number of molecules used to initiate the model.
¹See Table 4.
²The ArcA numbers were reported by Rolfe et al. [3].
³The ArcB numbers are assumed based on the 10:1 ratio of response-regulator to sensor-kinase for another E. coli two-component system PhoB-PhoR [22].
⁴Numbers are listed in Table 1.
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Table 3. Agent interaction rules.

| Molecules | Interaction rules¹ |
|-----------|--------------------|
| O2        | Interacting with FNR dimer, ArcB, Cyo and Cyd |
| FNR dimer | FNR dimer + O2 → FNR monomer + FNR monomer |
| FNR dimer bound to DNA | FNR dimer + binding site → FNR dimer bound to binding site |
| FNR monomer | FNR monomer + FNR monomer + FNR dimer |
| ArcB      | ArcB + P + O2 → ArcB + O2 |
| ArcA octamer | ArcA octamer + ArcB → ArcA dimer + ArcB + P |
| ArcA tetramer | ArcA tetramer + ArcA dimer → ArcA octamer |
| ArcA dimer | ArcA dimer + ArcB → ArcA dimer + ArcB + P |
| ArcA dimer + P | ArcA dimer + P + ArcB |
| ArcA octamer bound to DNA | ArcA octamer bound to DNA is assigned a probability of 0.3% to leave the DNA in every iteration. This ‘off rate’ is required because ArcA→P dephosphorylation occurs by the action of ArcB at the cell membrane (see text). |
| Cyo       | Cyo + O2 → Cyo + H2O |
| Cyd       | Cyd + O2 → Cyd + H2O |

The interaction radii (nm) were defined and refined as described in the Methods.
¹Additional descriptions of the ArcBA and FNR interaction rules are provided in the Supporting Information.
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ArcA octamers in the cytoplasm and bound to DNA did not reach that observed in the initial state before the second cycle of O₂ supply began, indicating that a longer period is required to return to the fermentation state.

The numbers of FNR dimer bound to binding sites and free FNR dimer (cytoplasmic FNR dimer) decreased when O₂ was supplied to the system (Figures 4g–h), but the rate was slower than that for ArcA inactivation, consistent with O₂ consumption at the

Figure 3. The initial and final states of model with no O₂ and with excess O₂ supplied. (a) The initial state of model with no O₂ (0% AU) supplied to the cell. (b) The final state of model with excess O₂ (217%) supplied to the cell. Each type of molecule is represented by a different color as shown in the key.

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Agent-Based Modeling of E. coli Respiration

a) Cumulative number of oxygen molecules that have entered cell

b) Number of AceA octamers bound to binding sites

c) Number of free AceA octamers

d) Number of AceA tetramers

e) Number of active AceA dimers

f) Number of inactive AceA dimers

g) Number of FNR dimers bound to binding sites

h) Free FNR dimers

i) Number of FNR monomers

- Model simulation 1
- Model simulation 2
- Model simulation 3
- Model simulation 4
- Model simulation 5
membrane, which can be sensed by ArcB to initiate inactivation of ArcA, but lowers the signal for inactivation of FNR. When O2 was removed from the system (from iteration 5001) FNR was activated over a similar timeframe to ArcA (Figures 4b and g), which was again consistent with previous observations [15]. As with ArcA, free FNR dimers and FNR monomers did not fully return to their original states after O2 supply was withdrawn in the model, indicating that further iterations are required to reach steady-state (Figure 4h–i). These results clearly indicate that the model is self-adaptive to the changes in O2 availability, and the reproducible responses prove the reliability and robustness of the model. The ArcBA system simulated in this model is based on a preliminary biological assumption, and the agent-based model presented here should prove a reliable and flexible platform for exploring the key components of the system and testing new experimental findings.

Model validation

In order to validate the model with biological measurements of FNR DNA-binding activity estimated using an FNR-dependent lacZ reporter, the ArcBA system agents were removed from the model by setting their agent numbers to zero. The ArcBA system is an indirect O2 sensor and does not consume O2, hence the FNR system was not affected by withdrawing ArcBA from the model, but this simplification increased simulation speed.

The O2 step length and other model parameters were estimated using the experimental data obtained at 31% AU. Using the estimated O2 step length at 31% AU and defining the step length of O2 molecules, $S_{O_2}$, as 0 at 0% AU, a linear model, $S_{O_2} = k \times C_{O_2}$, was constructed to predict the step lengths of O2 at other AU levels, where $k=2.1$ and $C_{O_2}$ represents the O2 concentration at different AU levels (Table 4). The O2 step length predicted by this model were used to validate the model at 85%, 115% and 217% AU, and the accuracy of the linear model was shown by the good correlation between the model and experimental data.

Profiles of five repetitive simulations in which the simplified model was used to predict the numbers of active FNR dimers in steady-state cultures of bacteria grown at different AU values are presented in Figure 5. At 31% AU, the model implied that FNR-mediated gene expression is unaffected compared to an anaerobic culture (0% AU), i.e. the number of FNR binding sites occupied in the nucleoid remained unchanged (Figures 5a and c). Even at 85% AU, ~80% of the FNR-binding sites remained occupied (Figures 5b and f). It was only when the O2 supply was equivalent to >115% AU that occupation of the FNR-binding sites in the nucleoid decreased (Figures 5 e, d, g and h). These outputs matched the FNR activities calculated from the measurements of an FNR-dependent reporter (Table 5) and thus demonstrate the abilities of the model to simulate the general behavior of FNR dimers in steady-state cultures of E. coli.

A second validation approach using two FNR variants that are compromised in their ability to undergo monomer-dimer transitions was adopted. The FNR variant FNR I151A can acquire an iron-sulfur cluster in the absence of O2, but subsequent dimerization is impaired [26]. The FNR D154A variant can also acquire an iron-sulfur cluster under anaerobic conditions, but does not form monomers in the presence of O2 [26]. To mimic the behavior of these two FNR variants the interaction radius for FNR dimer formation was changed in the model. Thus, the interaction distance for wild-type FNR monomers, which was initially set at 6 nm ($r_3$, Table 3) was increased to 2000 nm for the FNR D154A variant, essentially fixing the protein as a dimer, or decreased to 2.5 nm for the FNR I151A variant, making this protein predominantly monomeric under anaerobic conditions. The results of simulations run under aerobic (217% aerobic) and anaerobic conditions (0% aerobic) suggested that under aerobic conditions wild-type FNR and FNR I151A should be unable to inhibit transcription from an FNR-repressed promoter (i.e. the output from the reporter system is 100%), whereas FNR D154A should retain ~50% activity (Table 6). Under anaerobic conditions, wild-type FNR was predicted to exhibit maximum repressive activity (i.e. 0% reporter output), whereas FNR I151A and FNR D154A mediated slightly enhanced repression compared to the simulated aerobic conditions (Table 6). To test the accuracy of these predictions, the ability of wild-type FNR, FNR I151A and FNR D154A to repress transcription of a synthetic FNR-regulated promoter (FfjgalA4) under aerobic and anaerobic conditions was tested [27]. The choice of a synthetic FNR-repressed promoter was made to remove complications that might arise due to iron-sulfur cluster incorporation influencing the protein-protein interactions between FNR and RNA polymerase; in the reporter system chosen FNR simply occludes the promoter of the reporter gene and as such DNA-binding by FNR controls promoter

| AU (%) | O2 in input gas (%) | O2 molecules per cell per iteration | Step length of O2 molecule (nm) |
|--------|---------------------|-----------------------------------|--------------------------------|
| 0      | 0                   | 0                                 | 0                              |
| 31     | 2.8                 | 13                                | 6                              |
| 85     | 8.1                 | 37                                | 14.5±2.5                       |
| 115    | 11.1                | 51                                | 25.5±0.5                       |
| 217    | 21.1                | 97                                | 41.5±1.5                       |

The percentages of O2 in the input gas at different AU levels (first column) are listed in the second column. The third column presents the calculated numbers of O2 molecules supplied to a cell. The step length values are listed in the fourth column are obtained from a set of model tests at 31% AU only and were validated at 85%, 115% and 217% AU.

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Table 4. Relative parameters for O2 molecules.
Figure 5. Profiles of FNR dimers at different AU levels. (a–d) Variation of the numbers of total FNR dimers and (e–h) numbers of FNR dimers bound to DNA at AU levels 31%, 85%, 115% and 217% respectively. doi:10.1371/journal.pcbi.1003595.g005
activity. The experimental data obtained matched the general response of the FNR variants in the simulation, but not very precisely for FNR D154A, with the experimental data indicating more severe repression by FNR D154A under both aerobic and anaerobic conditions than predicted (Table 6). This suggested that the interaction radius \( r_2 = 5 \text{ nm} \) (Table 3), which controls the binding of FNR to its DNA target required adjustment to enhance DNA-binding of the FNR D154A variant. Therefore, the simulations were rerun after adjusting \( r_2 \) to 7 nm for all the DNA-binding of the FNR D154A variant. Therefore, the binding of FNR to its DNA target required adjustment to enhance monomer-dimer transition is the primary determinant controlling transcriptional repression, but for wild-type FNR the upstream dimeric state, the rate of binding to the target DNA governs analyses imply that for FNR D154A, which is essentially fixed in a monomer-dimer transition is the primary determinant controlling the output from the reporter.

### Table 5. Comparison between experimental and simulation results for wild-type FNR.

| Aerobiosis units (%) | Total FNR dimer (molecules per cell) | FNR dimer bound to binding sites (molecules per cell) | Predicted output from an FNR-dependent promoter (% of maximum) | Total FNR dimer (molecules per cell) | Measured output from an FNR-dependent promoter (% of maximum) |
|----------------------|------------------------------------|-----------------------------------------------------|---------------------------------------------------------------|------------------------------------|---------------------------------------------------------------|
| 0                    | 1500                               | 350                                                 | 100                                                           | 1500                               | 100±3                                                         |
| 31                   | 1350±6                             | 350                                                 | 100                                                           | 1377                               | 95±4                                                          |
| 85                   | 1033±16                            | 287±16                                              | 82±4.6                                                        | 1020                               | 67±7                                                          |
| 115                  | 280±15                             | 7±3                                                 | 2±0.9                                                         | 248                                | 13±3                                                          |
| 217                  | 73±8                               | 0                                                   | 0                                                             | 60                                 | 0                                                             |

The model simulation data was taken by averaging the model outputs at steady-state. For these results, the values shown are the averages and standard deviations. The last column shows experimentally determined transcription from the FNR-dependent *FF-41.5-lacZ* promoter; the transcriptional output at 0% AU was set to 100.

1. The total numbers of FNR dimers were calculated from the Western blots and FNR-dependent promoter activities.
2. The measured output from an FNR-dependent promoter was reported by [15,38].

### Table 6. Comparison between experimental and simulation results for FNR variants.

| Strain       | Aerobic          | Anaerobic         |
|--------------|------------------|-------------------|
|              | Simulation \( r_2 = 5 \text{ nm} \) | Experiment | Simulation \( r_2 = 7 \text{ nm} \) | Simulation \( r_2 = 5 \text{ nm} \) | Experiment | Simulation \( r_2 = 7 \text{ nm} \) |
| FNR          | 100              | 108.2±2.2         | 99.7               | 0                                | 13.0±1.2 | 0                                            |
| FNR I151A    | 100              | 126.7±2.8         | 100                | 87.5±1.4                         | 83.3±4.4 | 79.6±0.7                                      |
| FNR D154A    | 52.0±1.2         | 26.2±1.2          | 30.4±0.6           | 52.0±2.1                         | 32.6±3   | 31.5±1.3                                      |

1. For the simulations, the standard deviations were calculated from 5 repeats for FNR and 13 repeats for FNR I151A and FNR D154A. The aerobic condition in simulation was modelled at AU level 217%, and the anaerobic condition was modelled at AU level 0%. \( r_2 \) is the interaction radius between the FNR dimer and its cognate DNA-binding site.
2. For the experimental data 100% expression was set as the β-galactosidase activity obtained in the absence of FNR. Measurements were made from three independent cultures.

### Concluding remarks

The FNR switch has been the subject of several attempts to integrate extensive experimental data into coherent models that account for changes in FNR activity and target gene regulation in response to \( O_2 \) availability [15,28–31]. These models have provided estimates of active and inactive FNR in *E. coli* cells exposed to different \( O_2 \) concentrations and the dynamic behavior of the FNR switch. The ability of FNR to switch rapidly between active and inactive forms is essential for it to fulfill its physiological role as a global regulator and the models are able to capture this dynamic behavior. Thus, it is thought that the ‘futile’ cycling of FNR between inactive and active forms under aerobic conditions has evolved to facilitate rapid activation of FNR upon withdrawal of \( O_2 \) and hence the physiological imperative for rapid activation has determined the structure of the FNR regulatory cycle [30,31]. However, it is less clear from these approaches how the system avoids undesirable switching between active and inactive states at low \( O_2 \) availabilities (micro-aerobic conditions, >0%–<100% AU). To achieve rapid FNR response times it has been suggested that minimizing the range of \( O_2 \) concentrations that constitute a micro-aerobic environment, from the viewpoint of FNR, is advantageous [31]. Unlike previous models of the FNR switch,
the agent-based model described here recognizes the importance of geometry and location in biology. This new approach reveals that spatial effects play a role in controlling the inactivation of FNR in low O2 environments. Consumption of O2 by terminal oxidases at the cytoplasmic membrane and reaction of O2 with the iron-sulfur clusters of FNR in the cytoplasm present two barriers to inactivation of FNR bound to DNA in the nucleoid, thereby minimizing exposure of FNR to micro-aerobic conditions by maintaining an essentially anaerobic cytoplasm for AU values up to ~85%. It is suggested that this buffering of FNR response makes the regulatory system more robust by preventing large amplitude fluctuations in FNR activity when the bacteria are exposed to micro-aerobic conditions or experience environments in which they encounter short pulses of low O2 concentrations. Furthermore, investigation of FNR variants with altered oligomerization properties suggested that the monomer-dimer transition, mediated by iron-sulfur cluster acquisition, is the primary regulatory step in FNR-mediated repression of gene expression. It is expected that the current model will act as a foundation for future investigations, e.g. predicting the effects of adding or removing a class of agent to identify the significant regulatory components of the system.

Methods

Measurement of the rate of O2 supply

Knowledge of the rate of O2 supply, RO2, to the E. coli cells was required in order to simulate the response of the regulators of cydAB and cyoABCDE to different O2 availabilities. Therefore, uninoculated chemostat vessels were used to measure dissolved O2 concentrations, DO2, as a function of the percentage O2 in the input gas, Pi, in the absence of bacteria. This allowed the rate at which O2 dissolves in the culture medium to be calculated from the equation: DO2 = RO2 × Pi, yielding RO2 = 5.898 μmol/L/min. The number of O2 molecules distributed to a single bacterial cell was then calculated from the following equation: NumO2 = DO2 × NA × Vcell × n (where, NA is the Avogadro constant (6.022×1023); Vcell is the volume of E. coli cell (0.3925 μm3)) and as a constant for this equation, n (3.3×10-10) includes the unit transformations, min to sec (60-1) and μmol to mol (10-3), and the time unit represented by an iteration (0.2 sec).

Control of agent mobility

In the model the individual agents (Cyd, Cyo, ArcB, ArcA, FNR and O2) are able to move and interact within the confines of their respective locations in a 3-D-cylinder representing the E. coli cell. To control the velocity of agents, the maximal distances they can move in 3-D space during one iteration (step length) were pre-defined (Table 4). Thus, a step length is pre-defined in program header file (.h) and for each movement, this is multiplied by a randomly generated value within [0,1] to obtain a random moving distance, which in turn is directed towards a 3-D direction (movement vector) that was also randomly generated within defined spatial regions. An example is shown in Figure 6 to illustrate the movements of an O2 molecule when it enters the cell.

Estimating interaction radii

Interactions between agents depend upon the biological rules governing their properties and being in close enough proximity to react. The interaction radius of an agent encapsulates the 3-D space within which reactions occur. As the interaction radii cannot be measured, they were first estimated on the basis of known biological properties. For the radii r1, r6, r12, and r13 (Table 3), arbitrary values were initially set at 31% AU, and the model was then trained to match the experimental result for the number of FNR dimers at 31% AU (Table 5). The modeled output of FNR dimer number at steady-state was compared with the experimental data, and the difference suggested re-adjustment of interaction radii. The adjusted radii were then tested against the FNR dimer number at 31% AU (Table 5). The modeled output of FNR dimer number at steady-state was compared with the experimental data, and the difference suggested re-adjustment of interaction radii. The adjusted radii were then validated with the experimental data and the results indicate that the interaction radii values are capable of describing the behavior of the system. The

| Table 7. The effect of interaction distance (r3) for binding of FNR dimers to target DNA on wild-type FNR activity. |
|---------------------------------------------------------------|
| Interaction radius | 31% AU | 85% AU | 115% AU | 217% AU |
| r3 = 5 nm1 | 100 | 82±4.6 | 2±0.9 | 0 |
| r3 = 7 nm | 100 | 89.8±0.7 | 4.5±0.6 | 0.3 |

The standard deviations for r3 = 5 nm were obtained from 5 repeats and for r3 = 7 nm 10 repeats of the simulation.

1 r3 is the interaction radius between the FNR dimer and its cognate DNA-binding site.

Figure 6. The 3-D movement of an O2 molecule during five successive iterations. Within a pre-defined limit (step length) the agent moves a random distance per iteration. For O2 this is also used to imitate the diffusion along the concentration gradient according to Fick’s first law, in which the flux goes from regions of high concentration to regions of low concentration with a magnitude that is proportional to the concentration gradient (spatial derivative). The adjustment of step length of O2 (Table 4) affects the spatial moving speed, which is simply represented as the greater the step length, the faster the movement. The angle range was defined for O2 molecules that are outside the cell to enable them move towards cell. The O2 molecules inside the cell and all other molecules move in any direction within their defined spatial regions.

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interaction radii of Cyd and Cyo with O2 reflect their relative affinities for O2 (i.e. Cyd has a high O2 affinity and thus reacts more readily, 7 nm interaction radius, than Cyo, which has a lower affinity for O2, 3 nm interaction radius). As, thus far, no accurate biological data is available for ArcBA system, the radii \( r_{5...11} \) were arbitrarily defined and were refined by training the model to match current biological expectations.

Model description

The rod-shaped E. coli cell was modeled as a cylinder (500 nm x 2000 nm) [32] with the nucleoid represented as a sphere with a diameter of 250 nm at the centre of the cell. The experimentally-based parameters and locations of the agents in their initial state are listed in Table 2. As the number of 

| Strain | Description |
|--------|-------------|
| JRG4642 | an

mutant strain | containing a pRW50-based

plasmid | carrying the

reporter plasmid |

were grown until mid-exponential phase (OD600 = 0.35) before assaying for \( \beta \)-galactosidase activity.

Supporting Information

Figure S1 Stategraph for \( \text{O}_2 \) molecules. In order to describe the model clearly, every agent is given a formal description to illustrate its states, memory, functions, and relevant messages that it sends or receives from other agents (see Table S1). The stategraph for an oxygen agent is shown in the diagram. (TIF)

Figure S2 Building a FLAME simulation file. The agent definition (written in XMMML) is parsed by a FLAME model parser, called sparser, which generates the simulation code. In the GCC environment, the code is compiled with the message board library, libmbboard. The initial agent population settings are set in .xml file as the starting status of the model. (TIF)

Table S1 Agent description for the \( \text{O}_2 \) molecule. (DOCX)

Text S2 Additional description of interaction rules for the regulatory systems, ArcBA and FNR. (DOCX)

Video S1 Simulation of ArcBA and FNR activities in response to \( \text{O}_2 \) over two 0–217% AU cycles. (MP4)

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Author Contributions

Conceived and designed the experiments: HB MDR WJ RKP JG MH. Performed the experiments: HB MDR. Analyzed the data: HB MDR. Contributed reagents/materials/analysis tools: SC. Wrote the paper: HB WJ JG MH.

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