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Orientational control is an efficient control mechanism for phase switching in the *E. coli fim* system

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Abstract

The *fim* system in *E. coli* controls the expression of type-1 fimbriae. These are hair-like structures that can be used to attach to host cells. Fimbriation is controlled by a mechanism called "orientational control." We present two families of models for orientational control to understand the details of how it works. We find that the main benefits of orientational control are that (i) it allows rapid adjustment of fimbriation levels in response to a change of environmental conditions while (ii) keeping the overall frequencies with which a cell switches between the fimbriate state and the afimbriate state low. The main reason for the efficiency of orientational control in regulation of fimbriation levels is that it keeps the system far from its steady state.

Keywords: Phase variation; *Fim*; Steady-state; *E. coli*

1. Introduction

Strains of *E. coli* can be in either of two phases characterized by the presence or absence of type-1 fimbriae. Fimbriae allow bacteria to attach to host cells, and are a virulence factor in urinary tract infections and possibly in meningitis, yet are also produced by many commensal (i.e. non-disease causing) strains (Teng, 2005; Bahrani-Mougeot et al., 2002; Connell et al., 1996). Typically colonies of *E. coli* are a mix of fimbriate (i.e. expressing fimbriae) and afimbriate cells. In the urinary tract, high levels of fimbriation in the population colonizing the host triggers an inflammatory host response, with the risk of elimination by host defenses (Godaly et al., 1998; Hedlund et al., 2001; Fischer et al., 2006). If none of the parasites are fimbriate there will be little or no host response; as the fimbriation levels increase, there will be a point at which the host response will rapidly reach full levels (Fischer et al., 2006; Gunther et al., 2002).

While a full blown host response has devastating effects on the parasite colony, it is thought that a moderate response might be advantageous to *E. coli*. There are at least two strong indications for this.

1. As a by-product of the inflammation response the host releases sialic acid that can in turn be converted into GlcNAc-6-P a high energy metabolite (Plumbridge and Vimr, 1999). Hence, a moderate host response leads to a host-based nutrient release.
2. The host response creates a favorable environment for the parasite in that it potentially helps to reduce the population levels of other (competing) parasitic species. Thus a moderate host response eliminates competitors to some degree.

The *fim* system in *E. coli* is thought to be adapted to keep the population levels of fimbriation at a point that avoids the full host response, while evoking a tolerable response (El-Labany et al., 2003; Sohanpal et al., 2004). This is achieved by controlling the fimbriation levels of the population through *phase variation*. Phase variation is a *stochastic* control mechanisms whereby given a specific set

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of ambiental conditions individual cells can be in either of two states. By stochastic we mean that given a set of ambiental conditions every individual cell takes a certain state (or phase) according to a probability distribution determined by the external conditions (van der Woude and Bäumler, 2004). In the present case, this state is the presence or absence of fimbriae (Fig. 1).

1.1. Mechanism controlling phase variation in the *fim* system

In the *fim* system, phase variation is achieved by inversion of a 314 bp long stretch of DNA (*fimS* located between genes *fimE* and *fimA*); inversion literally means that the stretch of DNA in question is excised and re-inserted in the opposite orientation. Inversion of *fimS* is directly catalysed by FimB and FimE (we will henceforth refer to a protein that catalyses the inversion of *fimS* as "recombinase"). These proteins both bind to double sites flanking the 9 bp inverted repeats that demarcate the invertible region. Phase inversion likely requires that both double sites are fully occupied by the same recombinase species, i.e. the double binding sites are occupied by FimB only or by FimE only. Fimbriae are only expressed if the invertible element is on (the "on phase"), and *fimE* expression is likewise enhanced in on phase cells (McClain et al., 1993; Sohanpal et al., 2001; Joyce and Dorman, 2001). FimE in turn will eventually lead to the cell being switched off again. We will henceforth refer to this mechanism as *orientational control* (OC).

The *fim* system is a rather complex regulatory system that integrates a number of ambiental signals (indicators of the host response) into one single value, namely the amount of FimB in the system. There is one caveat: Lrp and IHF are additional factors that influence switching frequencies. Current experimental evidence, however, suggests that these molecules play a minor regulatory role if compared to FimE and FimB. In what follows we will therefore ignore the influence of Lrp and IHF.

Currently, there is little quantitative information about central parameters of the system, such as the recombinase-nucleotide affinities and dissociation rates and particle numbers. Experimental evidence, however, is consistent with the following qualitative picture:

- Binding to double sites is strongly cooperative.
- Fully occupied double sites are unstable (both for FimB and FimE).
- FimE and FimB affinities to the double binding sites are comparable.
- FimB numbers are low.
- Forward (on to off) and backward switching rates of FimB are approximately equal.
- FimE has a very low backward switching rate but switches much faster forward than FimB.

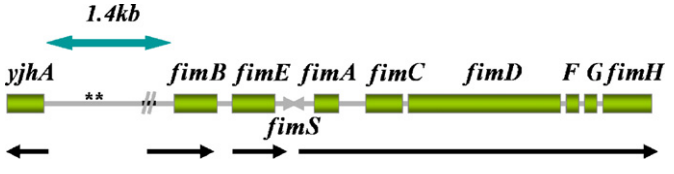

Fig. 1. Outline of the *fim* system. This article focuses on the interplay between *fimE* and *fimB*. The latter is itself controlled by a number of external signals, including sialic acid and GlcNAc-6-P. The genes necessary for the development of the fimbriae are *fimA*, *fimC*, *fimD*, *fimH*. The invertible element is located between *fimE* and *fimA*.

This lack of quantitative detail is a potential problem for the modelling of the system. In a computational simulation it is always necessary to commit to a specific set of parameters. If the validity of the simulations was crucially dependent on finding the specific correct set of parameters, then computational modelling would be impossible. Fortunately, it appears that this is not the case here: the results presented in this contribution are not sensitive to small parameter changes; the conclusions of this article will only rely on the overall relations between parameters, rather than absolute parameter values.

1.2. Aim of this article

The question we want to address in this article is whether OC has specific characteristics that make it (in some respects) superior to other (in particular simpler) alternative mechanisms (see in this context Blomfield, 2002). This question is only meaningful if the mechanism underlying the control of the *fim* system is the result of an adaptive process, rather than just an evolutionary "frozen accident". Previous attempts to model OC came to the conclusion that OC is not such a frozen accident but serves a specific function: Wolf and Arkin (2002, p. 99–100) cite four main benefits of OC over a possible simpler system: (i) OC provides a source of "memory" for how long the invertible element was in a specific position, (ii) keeps the phase switched on sufficiently long to allow building fimbriae and (iii) increases the sensitivity of the fimbriation levels to the FimB levels. Furthermore, Wolf and Arkin claim that (iv) two recombinases are necessary in order to achieve a sharp sigmoidal response of the steady-state fimbriation levels to a change of the FimB levels.

While we acknowledge that Wolf and Arkin's model captures the relevant aspects of the *fim* system, we do think that there are limitations to their conclusions about the optimality of OC as a mechanism to control *fim* phase variation. Firstly, their work is largely based on a steady-state analysis of the *fim* system; this is useful for some applications of the model but, as will be argued below, in the current context non-steady-state models are essential. Secondly, their conclusions about the superiority of OC over other systems is based on comparisons to a (modelled) $fimE^-$ mutant, but ignores other possible single-recombinase alternatives to OC. Thirdly and most importantly, Wolf and Arkin failed to recognize what we think is the main benefit of OC over simpler systems: by operating far from steady state it allows rapid regulation of the

Table 1
A summary of the interaction between the genetic elements in the *fim*-system

fimACDFGH	Genes necessary for the expression of fimbriae
fimE	A recombinase; co-expressed with FimA, suppresses itself (and FimA) by switching off *fimS* with high frequency
fimS	Invertible element of DNA located between *fimE* and *fimB*. Proteins FimB and FimE are expressed only if *fimS* is in the on-direction. We say *fimS* is in the "off position/phase" or simply off when it is oriented in such a way that FimE is not expressed. Otherwise it is in the "on phase"
fimB	A recombinase; catalyses switching of *fimS* with low frequency (yet in both directions)

fimbriation levels in response to changes of the ambiental conditions.

The primary aim of this article is to show how OC enables the population to efficiently regulate fimbriation levels without being hindered by a number of conflicting requirements; we will also address the question why evolution has chosen this mechanism, rather than a functionally apparently equivalent, but simpler alternative. Furthermore, we will propose ways to experimentally test our model.

1.3. Outline of the main argument

Before describing our models in detail, we will outline our conclusions about the mechanism that controls the fim system. It is helpful to think about the system both at the level of the individual cell and at the population level.

If an individual cell is in the off phase (i.e. it is afimbriate) then the FimE levels are negligible and the cell can in the long run be expected to be on and off equally often and for equal amounts of time (because FimB switches with equal frequency in both directions (see Table 1)). In reality this is not what is observed because once the cell switches to the on phase the FimE levels will rise and bias the probability towards the off state (because FimE preferentially switches off). At the aggregate level this results in a population that is heterogeneous with respect to the phase and most importantly also with respect to the [FimB]/[FimE] ratios of the individual cells. This indicates already that steady state descriptions based on this ratio are not very useful to understand the *fim* system.

In this contribution, we will suggest that the proportion of fimbriate cells in the population is controlled by the dynamic balance between two antagonistic "forces" each pushing towards a different steady state: the subpopulation of all cells that are in the off state will tend to relax towards a steady state with half of the population in the on phase, whereas the subpopulation of cells that are in the on phase will relax towards a steady state where most cells are in the off phase. The balance between those opposing "forces" (and with it the fimbriation levels of the population) is controlled by the amount of FimB in the cells, which in turn is determined by the external conditions of the cells. As will be discussed below, this arrangement has a number of biologically relevant advantages over somewhat simpler alternative mechanisms.

2. Methods

The results presented in this article are largely based on two main methods for computational modelling: stochastic models and continuous time markov chains. In stochastic models molecules are represented as discrete quantities in contrast to differential equation models where molecules are represented as continuous concentrations. As such stochastic models can show the effects of random fluctuations. In markov chain models the system is represented as a set of state transitions. Similar to stochastic models, the markov chain models used here take into account that molecules are discrete entities. In what follows the models will be described in more detail.

2.1. Stochastic models

We constructed two families of models. The first set of models is implemented using the Gibson–Bruck stochastic algorithm (we used the implementation of the "Dizzy" simulation package, Ramsey et al., 2005). In these models the invertible element and the double binding sites were represented as a single molecule that can take two states and bind to up to four molecules of FimE and FimB. State change (inversion) requires quadruple occupation by a single recombinase species.

The formation/destruction of a protein nucleotide compound was modelled as a reaction whereby each binding state was represented as a separate molecular species (distinguished by the symbol in the superscript): $X^{\flat} + FimE \underset{k_2}{\overset{k_1}{\rightleftharpoons}} X^{\natural}$, where $X^{\flat}$ and $X^{\natural}$ each represent one of the possible binding states and orientations of the invertible element-recombinase compound. For example if $X^{\flat}$ stands for a state where the invertible element is in the on phase and one molecule of FimB binds to the left double site and the right double site is unoccupied, then $X^{\natural}$ would represent a state where the element is on, one FimB binds to the left double site and one FimE binds to the right double site. The reaction rates, k_1 and k_2 are parameters of the model. In all simulations presented in this article the amount of FimB is fixed, so only FimE varies.

The model explicitly represents the DNA $\rightarrow$ RNA $\rightarrow$ FimE transitions. RNA is transcribed at similar rates in both the on ("RNAOn") and off position ("RNAOff"), yet

Table 2
The set of reactions used in the stochastic model

$X^l + R \rightleftharpoons X^n$	Binding/dissociation of recombinase
$eeX^{on}ee \rightleftharpoons eeX^{off}ee$	FimE mediated switching
$bbX^{on}bb \rightleftharpoons bbX^{off}bb$	FimB mediated switching
$**X^{on}** \Longrightarrow **X^{on}*** + \text{RNAOn}$	Translation of RNA
$**X^{off}** \Longrightarrow **X^{off}*** + \text{RNAOff}$	Translation of RNA
$\text{RNAOn} \Longrightarrow \text{FimE}$	Transcription of FimE
$\text{RNAOff} \Longrightarrow \text{FimE}$	Transcription of FimE
$\text{RNAOff} \Longrightarrow \emptyset$	Degrading RNA
$\text{RNAOn} \Longrightarrow \emptyset$	Degrading RNA
$\text{FimE} \Longrightarrow \emptyset$	Degrading FimE

The asterisks to the left and right of X symbolize the left and right double binding sites. The second and third reaction is the inversion catalysed by FimE and FimB, respectively. The last three reactions are the breakdown reactions for RNA and FimE. RNAOff has a very high breakdown rate and contributes almost nothing to the FimE levels.

RNAOff is broken down at a very high rate. See Table 2 for a summary of the reactions.

2.2. Continuous time markov chains

The second modelling approach was based on the construction of continuous time markov chains (CTMCs). The CTMC-models reported in this article are not simulations of a system (in the sense that a specific pathway of the system's evolution is followed) but provide exact results about the long term behavior of the system and how this long term behavior is approached. We used the PRISM package (Kwiatkowska et al., 2001) to solve the CTMCs.

In CTMCs chemical reactions are modelled as state transitions of variables: the invertible element was explicitly represented as a binary variable whose state transition rates depend on the states of the four ternary variables el, bl, er, br. These represent how many FimE and FimB are bonded on either of the double binding sites. For example, the state $el = 1, bl = 0, br = 2, er = 0$ represents the situation where one FimE binds to the left double site and two FimB bind to the right double site. Any states where $br > 0 \wedge er > 0$ or $el > 0 \wedge bl > 0$ (or both) are disallowed, i.e. there are no possible state transitions leading to them. Switching can only occur if the state satisfies $(br = 2 \wedge bl = 2) \vee (er = 2 \wedge el = 2)$, that is when both double sites are fully occupied and both are occupied by the same molecule. In Fig. 2 this is symbolized by the AND gates. The respective amounts of FimE and FimB in the system are represented by separate state variables.

3. Simulating OC

In this section, we will present example simulations of OC. This will provide the reader with a better understanding of the underlying dynamics of the system and indicate some general dependencies between various variables of the system; these dependencies will later be discussed in more detail.

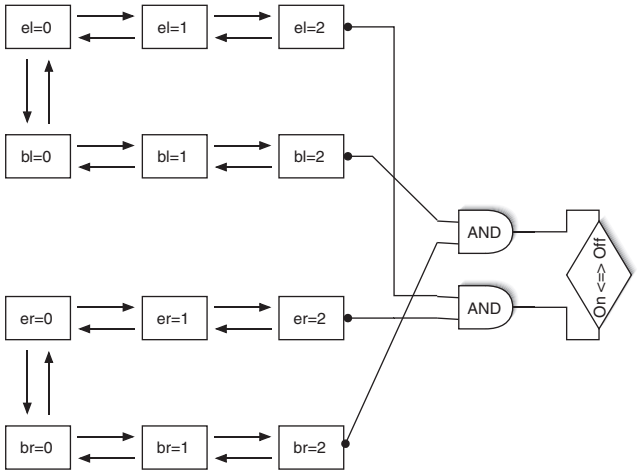

Fig. 2. Schematic outline of the state transitions in the CTMC base model. Every binding event is represented as state transitions. This diagram represents the state transitions corresponding to a binding event of FimE/FimB to one of the double binding sites. Each of those state transitions is synchronized with a corresponding state transition representing an increase/decrease of the number of free FimE/FimB. For example the transition $el = 1 \rightarrow el = 2$ is synchronized with the transition $FimE = k \rightarrow FimE = k - 1$ where k counts the number of FimE at the relevant time. Switching can only take place when both double sites are fully occupied and both are occupied by the same molecular species.

Fig. 3 shows the results of three example simulations. The number of FimB was kept constant during the simulation, the system was initialized with no FimE and the phase set to off. In the example simulations shown in Fig. 3 the steady-state amount of FimE was controlled by adjusting the breakdown rate in the model. The levels labelled high, low and no FimE correspond to approximately 300–400, 30–50 and 0–1 FimE molecules in steady state during the on phase, respectively; this corresponds to FimE breakdown rates of 0.0005, 0.05 and 10000.

In Fig. 3 the top graph displays the results of a run with high steady-state levels of FimE; there are five on phases each of which lasts relatively short time only. The middle graph shows four periods of switching on, yet each period lasts longer than in the case of high FimE levels. In the case of the low FimE steady state, the FimE levels are reduced by a factor of approximately 100. Hence, FimE is less effective at switching off. However, comparison of the low steady state with the case where FimE is essentially absent from the system (i.e. the bottom graph in Fig. 3) indicates that even low levels still are efficient at switching off the system: the graph at the bottom of Fig. 3 shows three distinct periods where the phase is on, each of those lasting for a long time compared to the case of low and high FimE levels.

In comparison, Fig. 4 shows an example run with the same parameters as the middle graph in Fig. 3, yet with the fundamental switching rate of FimB being increased by a factor of 100. As expected increasing the switching rate

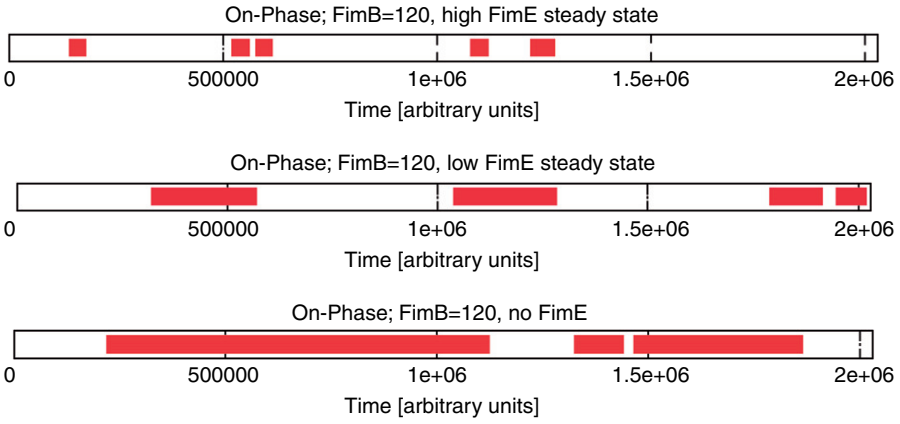

Fig. 3. *Stochastic* (*Gibson–Bruck*) *simulation of OC.* This graph shows three simulations each over 2 m time units; the bar indicates that the phase was switched to on in the corresponding time interval. The parameters of the simulations were identical in the three simulations, except for the breakdown rate of FimE which was lowest in the top graph and highest in the bottom graph. The following parameters were used: FimB = 120, switch-rate FimB = 0.0002, switch-rate FimE = 0.02 (forward) and 0.00001 (backward). The binding/dissociation rates of the recombinases are as follows: first FimB: 0.0123/0.00123; second FimB: 0.02/0.2; identical binding/dissociation rates were chosen for FimE.

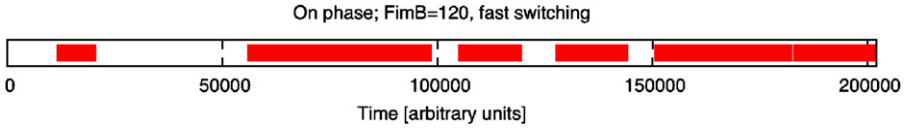

Fig. 4. Stochastic simulation of OC. Same as Fig. 3 (bottom) but the switching rate for FimB increased by a factor of 100. In order to allow better viewing, this graph only shows a tenth of the period shown in Fig. 3. The remaining $\frac{9}{10}$ of the simulation show similar switching rates. This demonstrates that the switching rate is mainly determined by the fundamental switching rate of FimB. The following parameters were used: FimB = 120, switch-rate FimB = 0.02, switch-rate FimE = 0.02 (forward) and 0.00001 (backward).

leads to more and shorter on phases. Note that (for better viewing) Fig. 4 only shows the first 200000 time units of the simulation, which corresponds to a tenth of the time shown in Fig. 3; the remaining $\frac{9}{10}$ of the simulation showed similar behavior.

These simulations give a first indication of how OC works.

- The amount of FimE strongly influences the duration of the on phases. High steady-state levels lead to short phases, lower levels lead to longer on phases (see Fig. 3 (top to bottom)).
- Backward switching (i.e. from off to on) is nearly exclusively actuated by FimB. This is evidenced by the fact that backward switching takes place at times when there is no FimE in the system. See Fig. 5. Hence, the frequency of switching on depends on the switching frequency of FimB.
- At low levels of FimE, FimB will actuate both forward and backward switching. In this case one would expect the system to spend equal amounts of time in both phases; see Fig. 4.

These simulations provide a first crude understanding of how OC works. In the remainder of this article we will further refine this understanding; particularly, we will show

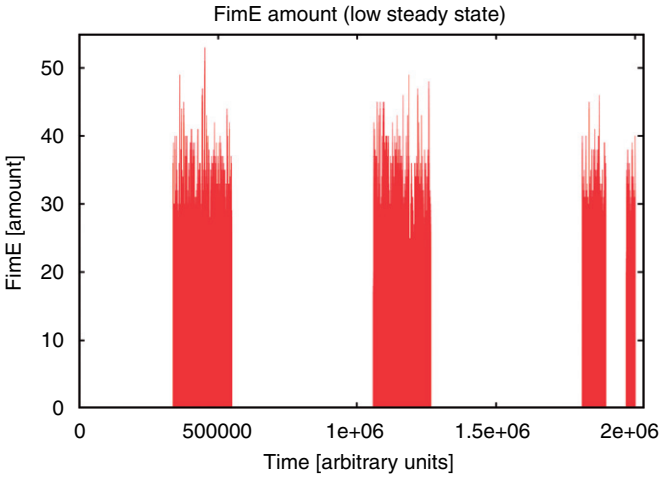

Fig. 5. Level of FimE from the simulation corresponding to Fig. 3. This graph shows the low level of steady-state FimE; the data is taken from the same run as the middle Fig. 3. These low levels of steady-state FimE are not sufficient to efficiently switch off the system. The high steady-state level of FimE would correspond to about ten time as much FimE (data not shown) and results in rapid switching off of the system.

that OC is an efficient mechanism that allows the cell to rapidly adjust fimbriation levels in response to a change of ambiental conditions (such as the onset of a host-based inflammation response).

4. Empirical and fundamental switching rates

In this section, we will discuss in more detail the factors that determine the overall switching rate of the system. Experimentally, the overall switching rate is usually determined by counting how the number of fimbriate individuals in a population changes under various conditions. In a system consisting of one recombinase only (or a system where one recombinase is dominant) the observed switching rate is a result of the following components:

- The fundamental (underlying) switching rate, i.e. the rate of switching once the recombinases are bound to the double binding sites.
- The affinity of the recombinase and the stability of the nucleoprotein compound.
- The recombinase concentration.

The overall, observed switching rate becomes independent of the second and the third criterion if the binding sites are saturated with the recombinase. This will be the case at very high recombinase concentrations. Below this saturation point the overall switching rate will crucially depend on the amount of time recombinases are bound to the binding sites; this in turn depends on the affinity and the concentration.

Fig. 6 (left) demonstrates the situation in a CTMC model of the 2-recombinase system. There the switching rate will also depend on the relative amount of the recombinases. At low numbers of FimE the switching rate is dominated by FimB, whereas at high numbers the rate is dominated by FimE. As can be seen from Fig. 6, the switching rate in the FimE dominated regime is significantly lower than in the FimB dominated regime, although the forward switching rate of FimE is 10 times higher than the switching rate of FimB. The reason for this effect is that in the FimE dominated parts there is a great bias towards forward switching and hence nearly all cells are switched off; since the backward switching rate of FimE is very low, overall little switching goes on. If the backward to forward switching ratio is kept fixed, then the switching rate in the FimE dominated regime depends linearly on the absolute switching rate (assuming that both switching rates are scaled by the same factor); note that the observed switching rate is essentially independent of the absolute backward rate if the forward rate is kept fixed and low.

In summary, this shows that the empirically observed switching rate might be very different from the actual underlying switching rate that drives the system. In particular, up to a certain point it is dependent on the recombinase concentration. As it will turn out, *E. coli* uses this to control fimbriation levels.

5. Three crucial requirements of the *fim* system

We will now focus the discussion on the mechanism that actuates the phase switching. Given the role of phase switching within the wider context of the *fim* system, any such mechanism (at the very least) needs to have the following three properties:

1. Fimbriation levels (or equivalently the steady-state probability of a cell to be in the on phase) are controlled by one recombinase that summarizes the external conditions (FimB in the specific case here).
2. There is a dynamical area where the fimbriation levels are highly sensitive to the level of a recombinase.

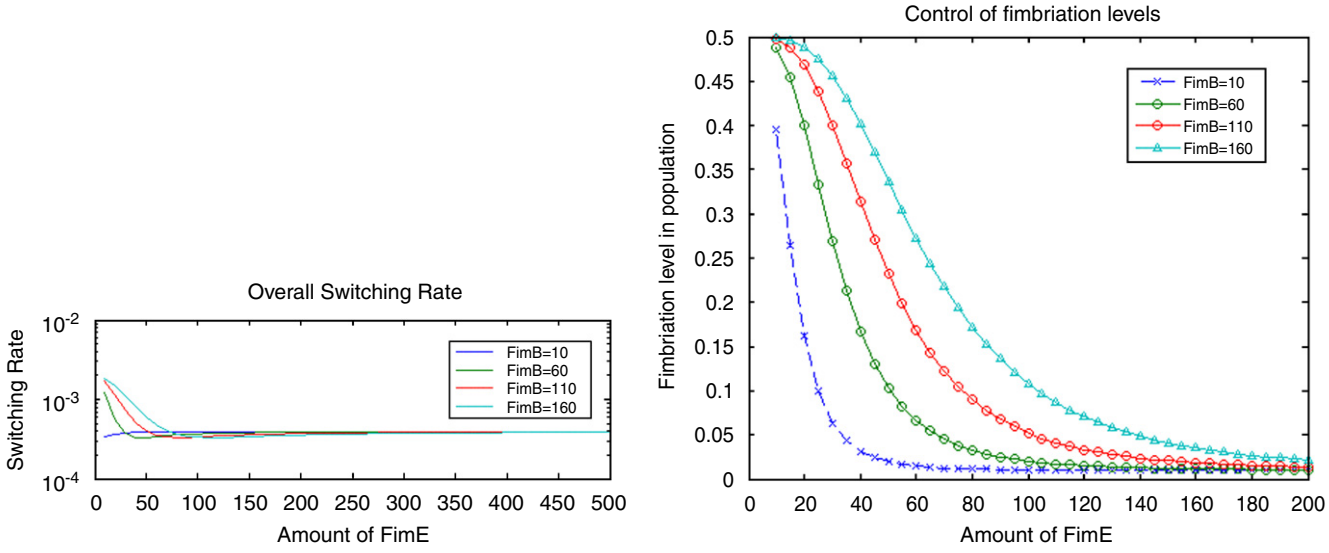

Fig. 6. Left: The total switching rate (i.e. both directions) as a function of various FimB/FimE amounts in the system. The particular parameter values here are: Switching rate FimB = 0.002; for FimE the on to off rate is 0.02 and the rate in the opposite direction is 0.0002. Right: The steady-state fimbriation levels as a function of the amount of FimE. FimB is kept fixed at values ranging from 10 to 160 molecules. This illustrates how the fimbriation levels can be controlled by the recombinase ratios.

3. If cells are switched on, then they must not switch back before a certain period of time has passed in order to allow the fimbriae to be developed; at the same time, the overall probability for a cell to be fimbriate should be low.

5.1. A steady-state model

Let us first consider a model that assumes the fimbriation levels to be in a steady state determined by the [FimB]/[FimE] ratio in the cells. As long as one recombinase is dominant the fimbriation level is independent of the affinity or dissociation rates and also independent of the recombinase concentration (assuming there are at least four molecules necessary to switch). Hence, although the switching rate is dependent on the recombinase concentration, the fimbriation level only depends on the backward to forward switching ratio; this is also true below the saturation concentration. In a 2-recombinase system where neither recombinase is dominant, the fimbriation levels will be somewhere between the steady-state levels of the FimB and FimE-dominated systems.

Assume the FimB levels are kept fixed at some medium high number: In the concrete case of the *fim* system one would then expect the system to make the transition from half of the population being in the on phase to almost no fimbriation as the FimE levels are increased from zero to very high. The sharpness of the transition among other things will be determined by the absolute amount of FimB in the system; Fig. 6 (right) shows this transition for various amounts of FimB. For low amounts of FimB the switch from a FimB dominated to a FimE dominated regime can be quite sudden. This aspect of the model is quite consistent with experimental evidence: Blomfield and coworkers (Gally et al., 1993) observed that a depression of the FimB levels by a factor of 3 led to a reduction of the (overall) backward switching rate by a factor of more than 35 (and a corresponding change in the fimbriation levels).

5.2. Problems of steady-state models

On closer inspection, however, it becomes clear that there are at least two reasons why the above theoretical model (transition between FimE and FimB dominated regimes) cannot account for the empirical data. Firstly, the population of cells is not homogeneous with respect to the [FimB]/[FimE] ratio. If a cell has been in the on phase for a long time, then there will be high levels of FimE whereas only negligible levels in those cells that have been off for a longer time. Therefore, at the population level this ratio is not a well defined quantity and cannot be used to model a population effect (fimbriation).

Secondly, in wild type *E. coli* it is never observed that a population has fimbriation levels of 50% as would be predicted by this model. Quite to the contrary, fimbriation levels are always very low. In the above model, however, a sharp sigmoidal transition can only be observed at the transition from the FimE dominated regime to the FimB dominated regime. Hence we conclude that the transition depicted in Fig. 6 is not relevant for the explanation of the experimentally observed sensitivity of fimbriation to FimB levels.

5.3. Developing fimbriae takes time

In order to understand the role of OC another biological constraint needs to be taken into account: the process of developing fimbriae takes a certain amount of time. For a cell it is therefore not useful to be in the on phase for a very short time only. Hence, there needs to be some way for the cell to ensure that the on phase is maintained for a minimum amount of time. It will turn out that meeting this requirement also puts constraints on the speed with which the fimbriation levels at the population level can be adjusted. In the next section we will consider a single recombinase system as a hypothetical alternative to the actually observed double recombinase mechanism of OC. The analysis of this system will highlight that sensitive control of steady-state fimbriation levels is very well possible in a single recombinase system within the broad parameter range of the *fim* system; yet we will also highlight a distinctive disadvantage of the single recombinase system. This will then lead us to a better explanation of the role of OC.

6. A hypothetical single recombinase system

As discussed above, in a single recombinase system, the fimbriation levels will only depend on the (fundamental) backward to forward switching ratio of the recombinase, but not on the affinity, dissociation rates or absolute switching rates. In a single recombinase system, one would therefore need to introduce additional dependencies in order to have the fimbriation levels depend on the amount of recombinase in the system.

For example, if the affinity of the recombinase depends on the orientation of *fimS*, then the forward to backward switching ratio would become a function of the recombinase concentration; in this case a single recombinase would be sufficient to control fimbriation.

Consider as an example the hypothetical single recombinase systems shown in Fig. 7. There the recombinase has equal fundamental switching rates in both directions. The figure shows steady-state fimbriation levels as a function of recombinase levels; the graphs represent hypothetical single recombinase systems where the affinity of the recombinase for binding to the switch in off-position is reduced by factors ranging from 0.5 to 0.1. See the figure caption for the parameter values used.

Control of recombinase levels in this system is sensitive. For example if the affinity in the off phase is reduced by a factor of 0.5 then increasing the number of recombinase molecules from 10 to 50 causes an increase of the

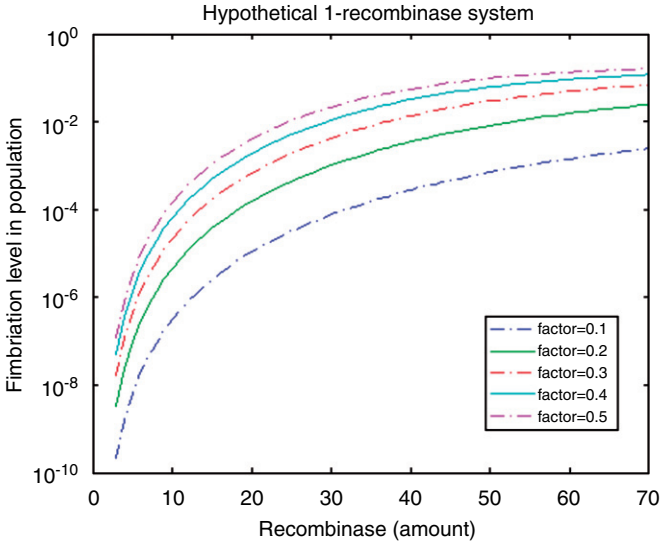

Fig. 7. *CTMC model*: The steady-state fimbriation levels for hypothetical single recombinase systems. The *x*-axis shows the level of the control recombinase. The curves correspond to various differences in the affinity of the recombinase to the off and the on state; the affinity in the off state is reduced by factors of 0.5–0.1. The recombinase switching rate is 0.2 in both directions.

fimbriation levels by three orders of magnitude. Hence, fimbriation levels can be sensitive to recombinase concentrations also in the case of single recombinase systems. Hence, the sensitivity requirement (mentioned in the last section) can be fulfilled by a single recombinase system.

6.1. Time to switch the system off and the overall switching rate

Another question is whether or not it is possible in a single recombinase system to ensure that once the phase is switched to on, it will remain so for a certain amount of time. The time a switch stays on is mainly controlled by the absolute switching rate. Assuming the average rate of switching per time unit is λ, then (assuming a Poisson model for switching events) the probability $P(u)$ that the switch is turned off at time u is given by

$$P(u) = \int_0^u e^{-\lambda t}\lambda \, dt = -e^{-\lambda u} + 1 = \lambda u + O(u^2). \quad (1)$$

For small times u this probability is well approximated by a function that is linear in λ and u. By making λ small (reducing the switching rate) one can increase the corresponding time u without changing the probability that the phase switches back. Hence, an obvious strategy for a hypothetical single recombinase system is to simply reduce the switching rate in order to reduce the probability that the phase is switched off within a short time period u of switching on.

This solution is perfectly feasible in theory, but has a problem in practice: while it is true that the steady-state fimbriation levels of the population are unaffected by the absolute forward and backward switching rates, the time required to reach this steady state is not. The higher the switching rate the faster steady state is reached and vice versa. For slow rates the time required to come near the steady state might be unrealistically high. In a single recombinase system the cell would have to find the trade-off between the requirement of being in the on state for a sufficient amount of time and the ability to rapidly adjust the fimbriation levels in response to ambiental change.

7. OC allows rapid adjustment of fimbriation levels

Wild type *E. coli* never reach the FimB-dominated steady state of half the population being fimbriate; in fact *E. coli* never even come close to it (normal fimbriation levels of wild-type *E. coli* do normally not exceed 10%). This despite the fact that most of the individuals in a population are likely to be in a FimB-dominated regime most of the time. The question then is: why is the population as a whole so far away from the FimB steady state? The answer to this is that OC acts as an effective "force" that pushes the system away from the FimB-dominated steady state. This force is constant in the sense that the transcription rate, breakdown rate and switching rates of FimE are not influenced by environmental conditions (at least in first order approximation); hence the tendency of on phase cells to switch off again is fixed. The observed fimbriation level is a dynamic balance between the tendency of the system to relax towards the FimB-dominated steady state and the antagonistic force of OC that pushes the system in the opposite direction.

The question that still remains to be answered is as follows: why is this (rather contrived) mechanism better than the straightforward single recombinase system? The answer is that OC allows cells to remain in the on phase for a sufficient period of time while also being able to rapidly adjust fimbriation levels in response to changes in ambiental conditions.

In the single recombinase system, there is a trade-off between the time required to adjust the population to a new steady state and the typical time a given cell stays on, once it is turned on (see Section 6). In a 2-recombinase system this is no longer the case, if one recombinase (FimB in our case) is used to switch the system on and the other to switch it off, each with different rates. However, even a hypothetical 2-recombinase system that tracks the steady states determined by the [FimB]/[FimE] ratio of a homogenous population would not be consistent with experimental evidence (see Section 5).

There is also a theoretical reason why steady-state explanations are not optimal for the cell: approaching the steady state is getting slower the closer one is to the steady state. In the case of the *fim* system the required adjustments of the fimbriation levels are small in absolute terms. So, a steady state control would be very inefficient.

To see this consider a system where FimB is dominant (assuming no OC): if the population of bacteria is reasonably big, then the system can be described by a

differential equation. The proportion of bacteria in a specific phase (say on) at any time is given by the solution to

$$\frac{\mathrm{d}}{\mathrm{d}t}x = a(1-x) - ax, \tag{2}$$

where $x(t)$ is the proportion of the population in the off state and a is the overall switching rate. Taking into account that at time $t_0 = 0$ all cells are off, then $x(t)$ is given by

$$x(t) = \tfrac{1}{2}(1 - \exp(-2ta)). \tag{3}$$

Hence the rate with which the system approaches the steady state, $\dot{x}(t) = a\exp(-2ta)$, falls exponentially with time and is in first order approximation linear in a for small times t, the overall switching rate. As discussed in Section 4, the overall switching rate itself is a function of the concentration of FimB, its affinities to the binding sites and the corresponding dissociation rates. The latter two dependencies are fixed in the system. The cell can only regulate the speed with which steady state is approached by adjusting the number of FimB in the system. This is precisely what is observed.

Fig. 8 shows (for small times) the results of a CTMC model of how steady state is approached by a cell in the FimB dominated state. The graph on the l.h.s. displays the probability of the cell being fimbriate (or equivalently the expected fimbriation levels of a population of cells) as a function of time for various levels of FimB; the r.h.s. shows the slopes of the graph on the l.h.s. as a function of the FimB level. As expected, the speed with which steady state is approached correlates positively with FimB levels. Note that for sufficiently long times all curves in Fig. 8 (l.h.s.) would approach 0.5.

7.1. The overall picture

The following overall picture emerges: in most of the cells the FimE levels are negligible compared to the FimB levels. Hence, the system is driven towards the FimB-dominated steady state where half of the cells are fimbriate. The speed with which this steady state is approached depends on the levels of FimB. In real cells the amount of FimB summarizes the relevant external conditions of the cell, i.e. it would indicate the degree of the host response (see Section 1). Thus, ultimately, the speed with which the FimB steady state is approached is regulated by external conditions.

The drive towards the FimB steady state is opposed by OC which acts as a constant antagonistic force. As the external conditions change, so does the dynamic balance between OC and the relaxation towards the FimB steady state; this results in a change of the overall fimbriation levels in the population. The important advantage of this arrangement over a steady-state system is that the adjustments can happen much faster.

Hence, the key to understanding the system is to see that it operates far from the steady state, in fact nearly always close to $x = 0$, kept there by the antagonistic effect of FimE. Fimbriation levels are not regulated by tracking a steady state but by small adjustments to the dynamic balance between FimB and FimE.

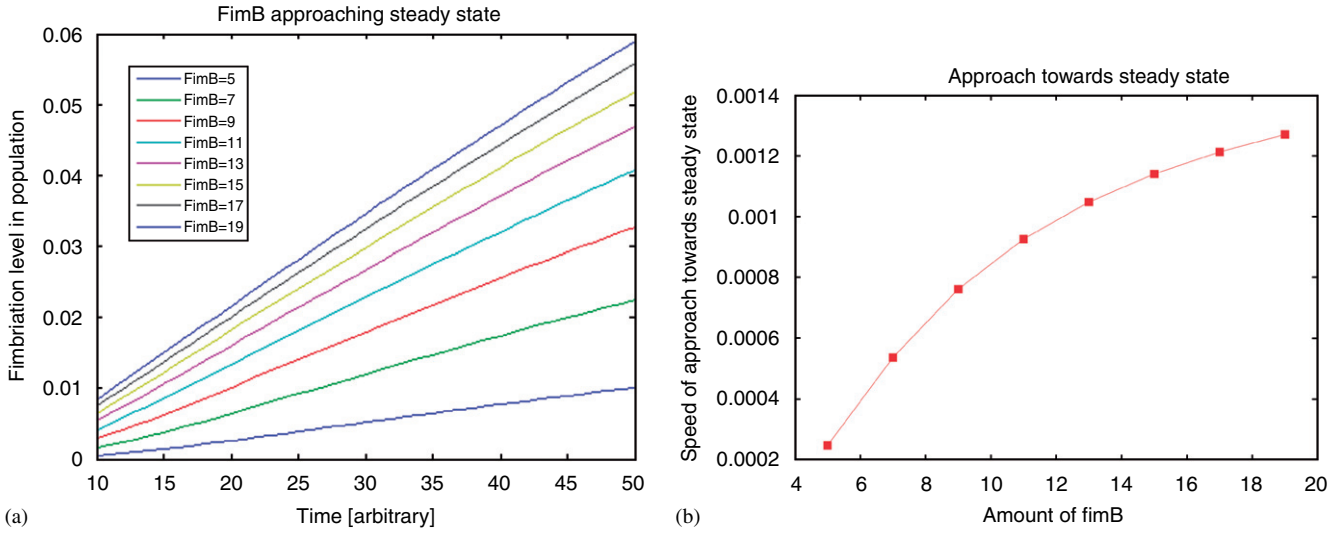

Fig. 8. *Left*: CTMC model showing the probability of the cell being in the on phase as a function of time for various amounts of FimB in the system. This graph shows only short times hence the dependence is essentially linear. *Right*: The estimated slopes of the graphs on the left-hand side as a function of the amount of FimB. This graph shows clearly that in the example system the speed with which steady state is approached increases with the amount of FimB. This graph also suggests that once a certain amount of FimB is present in the system, a saturation effect occurs. From this point on the speed of approach will be insensitive to further increases of FimB. The saturation level is dependent on a number of factors, including affinity and dissociation rates, as well as the volume of the cell. It is unknown where the saturation effect occurs in the real cells.

8. Experimental test

Whether or not control of the *fim* system optimizes the speed of adjustment of fimbriation rates depends on the value of several parameters being in the correct ranges. In particular, if the *fim* system is optimized for rapid adjustment of FimB levels (as we suggest) then physiological FimB-levels must not saturate the double binding sites. This is so because the closer the recombinase levels are to saturation, the smaller the effect of the changing FimB concentrations on the occupation probability of the double binding sites. One would thus expect that a cell that is under a selection pressure for an efficient control mechanism would evolve into the high responsive/low saturation part of parameter space.

The degree to which physiological FimB levels saturate the double binding sites can be determined experimentally and can act as an experimental falsification for the explanation put forward here. It is worth pointing out that saturation of the double binding site is closely related to Fig. 8 (r.h.s). This figure shows that in the model an increase of FimB levels leads to a near linear increase of the speed with which the FimB-dominated steady state is approached. As FimB levels come closer to the saturation point this is no longer true. Also this aspect could be experimentally determined. This indirect approach, however, has the disadvantage that in the absence of a benchmark it would be difficult to understand from the measured data how close the system is to its maximal responsiveness.

Apart from this crucial experiment there are other details of the system that require some experimental clarification. In particular, the interpretation of FimE as a molecular memory that gives information for how long the cell has been in the on phase (as suggested by Wolf and Arkin (see above)). In the illustrative simulations (see Fig. 5 in Section 3) we assumed that the FimE levels reach steady state in a period of time that is short compared to the typical time an on phase lasts. If this is the case, then the FimE levels do not give any useful information about the time the cell had been in the on phase. However, if the FimE levels continuously increase in on phase cells, then the levels of FimE would indicate for how long a cell had been switched on. Using this additional information could improve the efficiency with which phase switching occurred. Whether or not the cells use this additional mechanism of control is unclear at present. Ultimately, this question can only be settled experimentally.

9. Discussion and conclusion

The overall interpretation of the *fim* system as being adapted to a rapid adjustment of fimbriation levels of the parasite colony is consistent with the current biological understanding of the system as provoking moderate host responses while avoiding full scale inflammatory reactions. Experimental evidence suggests (Schilling et al., 2001) that the host response as a function of fimbriation levels is highly nonlinear. For low levels of fimbriation the response is small and slowly increasing; at a critical fimbriation level, however, the host response quickly attains full levels likely to be highly detrimental to the parasite colony. The ability to quickly downgrade fimbriation levels in the wake of an incipient inflammation reaction is therefore essential for the survival of the colony. Hence the need for a fast regulation mechanism.

A question for future research is to what extent the control mechanism we described here generalizes to other systems. We conjecture that the type of non-steady-state control described here is not unique to the *fim* system but might also be found in other phase varying systems with similar adaptive constraints (i.e. need to rapidly adjust the composition of the population in response to a change of external conditions). As such, the proposed mechanism could be a more general *motif*.

However, we think that this motif will be restricted to phase varying systems. The reason is that OC is (at the level of the individual cell) not at all robust. Reduction of noise and robustness of genetic control in the context of a noisy cytoplasmic chemistry is a well recognized problem (Rao et al., 2002; Samoilov et al., 2005). There are strong indications that many genetic systems (Li et al., 2004; Alon et al., 1999) are very well coping with noise. Phase variation of the *fim* system is different. Not only does the cell not attempt to reduce noise, but actively uses the randomness of the underlying chemistry. As such the *fim* phase variation essentially functions as a cellular random number generator (with the phase being the random variable); the cell only controls the distribution of the random numbers the cell outputs (via the FimB levels). Deterministic control is achieved in the "thermodynamic limit" i.e. at the population level where random micro-behavior translates into deterministic fimbriation levels.

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References

Alon, U., Surette, M., Barkai, N., Leibler, S., 1999. Robustness in bacterial chemotaxis. Nature 397, 168–171.

Bahrani-Mougeot, F., Buckles, E., Lockatell, C., Hebel, J., Johnson, D., Tang, C., Donnenberg, M., 2002. Type 1 fimbriae and extracellular polysaccharides are preeminent uropathogenic *Escherichia coli* virulence determinants in the murine urinary tract. Mol. Microbiol. 45, 1079–1093.

Blomfield, I., 2002. The regulation of Pap and Type 1 fimbriation in *Escherichia coli*. Adv. Microbial Physiol. 45, 1–49.

Connell, I., Agace, W., Klemm, P., Schembri, M., Marild, S., Svanborg, C., 1996. Type 1 fimbrial expression enhances *Escherichia coli* virulence for the urinary tract. Proc. Natl Acad. Sci. USA 93, 9827–9832.

El-Labany, S., Sohanpal, B., Lahooti, M., Akerman, R., Blomfield, I.C., 2003. Distant cis-active sequences and sialic acid control the expression of fimB in *Escherichia coli* K-12. Mol. Microbiol. 49, 1109–1118.

Fischer, H., Akira, M.Y.S., Beutler, B., Svanborg, C., 2006. Mechanism of pathogen-specific TLR4 activation in the mucosa: fimbriae, recognition receptors and adaptor protein selection. Eur. J. Immunol. 36, 267–277.

Gally, D., Bogan, J., Eisenstein, B., Blomfield, I., 1993. Environmental regulation of the *fim* switch controlling type 1 fimbrial phase variation in *Escherichia coli* K-12: effects of temperature and media. J. Bacteriol. 175, 6186–6193.

Godaly, G., Frendeus, B., Proudfoot, A., Svensson, M., Klemm, P., Svanborg, C., 1998. Role of fimbriae-mediated adherence for neutrophil migration across *Escherichia coli*-infected epithelial cell layers. Mol. Microbiol. 30, 725–735.

Gunther, I., Snyder, J., Lockatell, V., Blomfield, I., Johnson, D., Mobley, H., 2002. Assessment of virulence of uropathogenic *Escherichia coli* type 1 fimbrial mutants in which the invertible element is phase-locked on or off. Infection and Immunity 70, 3344–3354.

Hedlund, M., Frendéus, B., Wachtler, C., Hang, L., Fischer, H., Svanborg, C., 2001. Type 1 fimbriae deliver an LPS- and TLR4-dependent activation signal to CD14-negative cells. Mol. Microbiol. 39, 542–552.

Joyce, S., Dorman, C., 2001. A Rho-dependent phase-variable transcription terminator controls expression of the FimE recombinase in *Escherichia coli*. Mol. Microbiol. 45, 1107–1117.

Kwiatkowska, M., Norman, G., Parker, D., 2001. In: Kemper, P. (Ed.), Proceedings of the Tools Session of Aachen 2001 International Multiconference on Measurement, Modelling and Evaluation of Computer-Communication Systems, pp. 7–12, available as Technical Report 760/2001, University of Dortmund.

Li, F., Lu, Y., Tang, C., 2004. The yeast cell-cycle network is robustly designed. Proc. Natl Acad. Sci. USA 101 (14), 4781–4786.

McClain, M., Blomfield, I., Eberhardt, K., Eisenstein, B., 1993. Inversion-independent phase variation of type 1 fimbriae in *Escherichia coli*. J. Bacteriol. 175, 4335–4344.

Plumbridge, J., Vimr, E., 1999. Convergent pathways for utilization of the amino sugars N-acetylglucosamine, N-acetylmannosamine, and N-acetylneuraminic acid by *Escherichia coli*. J. Bacteriol. 181, 47–54.

Ramsey, S., Orrell, D., Bolouri, H., 2005. Dizzy: stochastic simulation of large-scale genetic regulatory networks. J. Bioinform. Comput. Biol. 3, 415–436.

Rao, C., Wolf, D., Arkin, A., 2002. Control, exploitation and tolerance of intracellular noise. Nature 420, 231–237.

Samoilov, M., Plyasunov, S., Arkin, A., 2005. Stochastic amplification and signaling in enzymatic futile cycles through noise-induced bistability with oscillations. Proc. Natl Acad. Sci. USA 102 (7), 2310–2315.

Schilling, J.D., Mulvey, M.A., Vincent, C.D., Lorenz, R.G., Hultgren, S.J., 2001. Bacterial invasion augments epithelial cytokine responses to *Escherichia coli* through a lipopolysaccharide-dependent mechanism. J. Immunol. 166 (2), 1148–1155 URL: ⟨http://www.jimmunol.org/cgi/content/abstract/166/2/1148⟩.

Sohanpal, B., Kulasekara, D., Bonnen, A., Blomfield, I., 2001. Orientational control of fimE expression in *Escherichia coli*. Mol. Microbiol. 42, 483–494.

Sohanpal, B., El-Labany, S., Plumbridge, J., Blomfield, I., 2004. Integrated regulatory responses of fimB to N-acetylneuraminic (sialic) acid and GlcNAc in *Escherichia coli* K-12. Proc. Natl Acad. Sci. USA 101, 16322–16327.

Teng, C., 2005. *Escherichia coli* K1 RS218 interacts with human brain microvascular endothelial cells via type 1 fimbria bacteria in the fimbriated state. Infection and Immunity 73, 2923–2931.

van der Woude, M., Bäumler, A., 2004. Phase and antigenic variation in bacteria. Clin. Microbiol. Rev. 17 (3), 581–611.

Wolf, D., Arkin, A., 2002. Fifteen minutes of fim: control of type 1 Pili expression in *E. coli*. Omics 6 (1), 91–114.