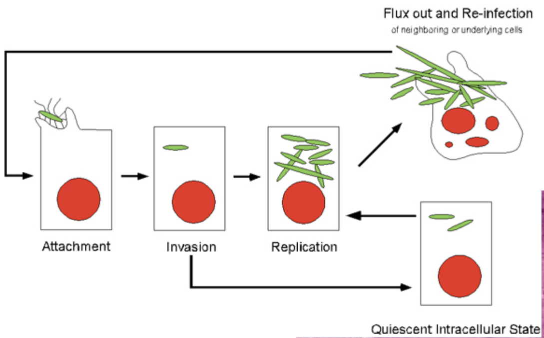
Control of the Pathogenic *fim* Switch in E. coli

Denise Wolf and Adam Arkin

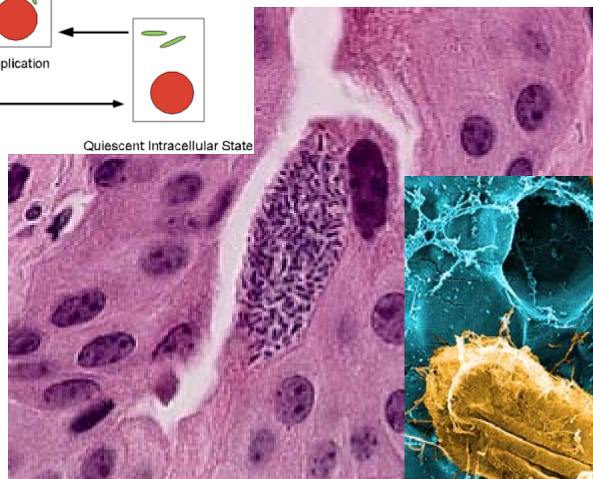
From: Abraham, S.N, *et al.* (1988) *Infection and Immunity*. ***56****(5):1023-1029*

Molecular Control of Infection Dynamics



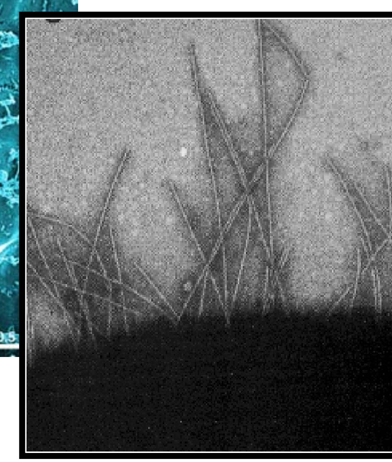
•30% of women will have at least one urinary tract infection during their life

•Urinary Tract Infection (UTI) accounts over 7 million office visits per year

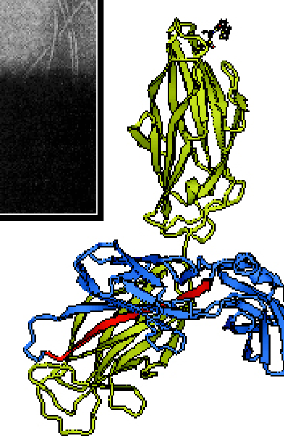


•25% recurrence rate- 85% are caused by the initial strain.

•**80% caused by uropathic *Escherichia coli***



Host defenses: micturition, exfoliation, anti-microbials, neutrophil influx, 2nd, 3rd waves of immune system.



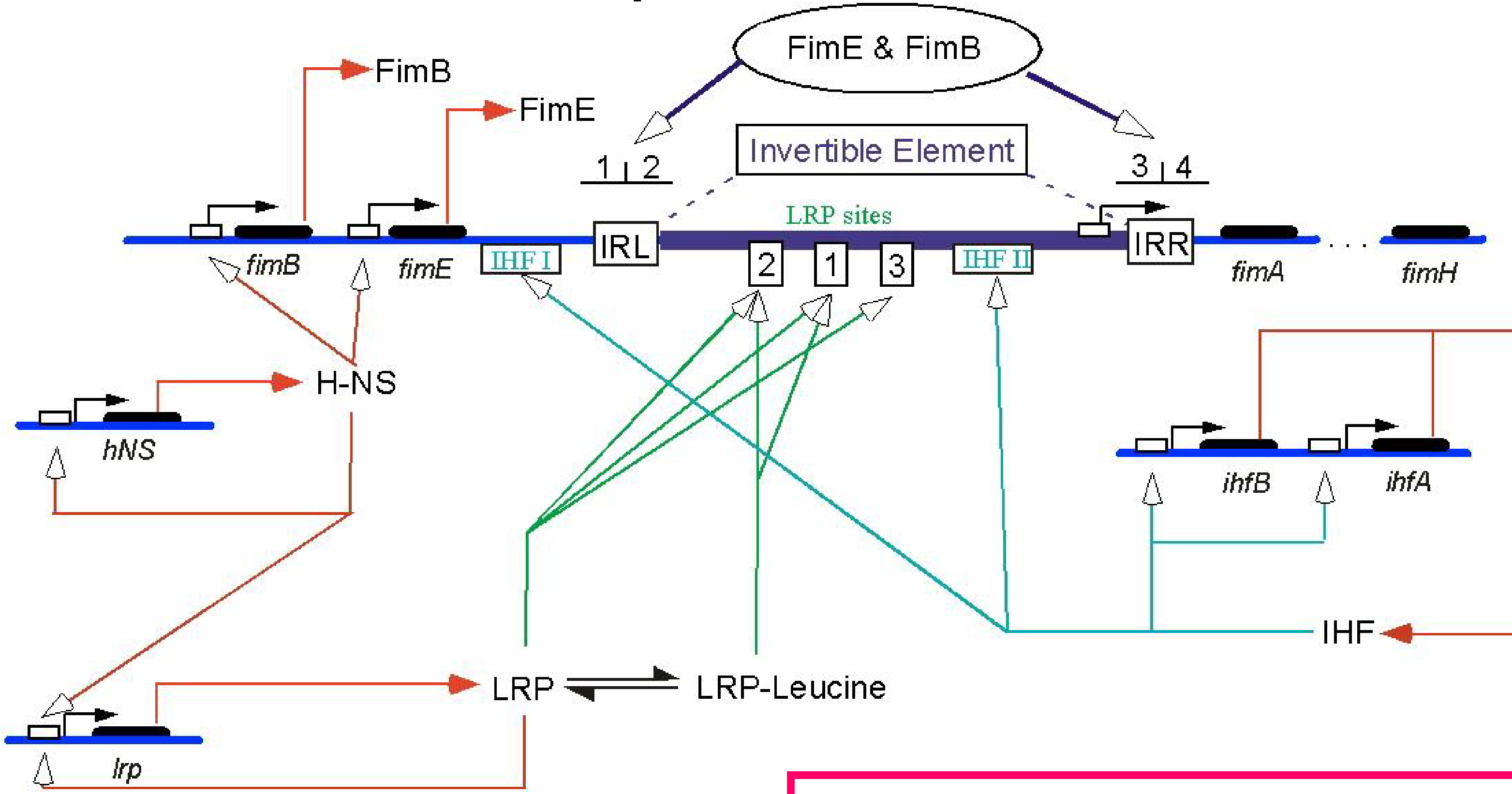
Type-1 pili virulence factors, enabling (1) adhesion, and (2) invasion

Type-1 Pili

- Benefits
  - Avoid being washed away (*adherence*)
  - Opportunities for rapid replication (*invasion, factory mode*)
  - Opportunities for recurrence (*invasion, quiescent mode)*
- Costs
  - Activation of immune system, rapid clearance of infection
  - Detract from survival outside host – agglutination, lack of good target
  - Energy requirements

- *fim* control circuit balances conflicting demands for survival
  - Heterogenous, two-state population, stochastic phase variation
  - Switching rate, piliation level, attachment, detachment, growth rates adapt s.t.
    - Persistent infection
    - Avoidance of neutrophil spike
    - Quiescent pockets for recurrence
    - Locally responsive to state of environment, host, disease process phase

Genetic Make-Up of the Fimbriation Switch



•Invertible element, 314 bp, w/fimA promoter
•**Recombinases** FimB, FimE;
FimE w/marked ON-to-OFF preference
•IHF-necessary for switching (+)
•Lrp - greatly accelerate switching (+)
•H-NS - repressor, Temp dep [ ], activity (-)
•*fimE* orientational control

This compact integrated circuit can

- Mediate the % of cells that have pili.
- Control the rate at which a population changes piliation state
- Sense the temperature of the host to mediate switching
- Sense the amount of nutrient in the medium.

System-level Questions

How does network architecture accomplish phase variation control?

•Basic switch operation:

How does circuit sense the environment and control piliation level, attachment, detachment, invasion rates to balance demands of infection?

•***Design questions:***

•**Why *two* independent recombinases?**

•**Source of ON-to-OFF *specificity of FimE*?**
(hypothesized binding affinities, orient control)

•**Role of *orientational control* of *fimE*?**

•**How is temperature control achieved?**
(***tuned to mammalian body temperature***)

Regulatory motifs?

Mathematical model

Invertible element

Master equation form

$$\frac{dP_{on}}{dt} = f(1-P_{on}) - gP_{on} \qquad (1)$$

Equilibrium statistical thermodynamics for f, g

with

$$f = \frac{\sum_{s\in OFF} \alpha_s e^{-\Delta G_s/RT}[IHF]^{n(s)}[FimE]^{j(s)}[FimB]^{k(s)}[Lrp*]^{m(s)}[Lrp]^{l(s)}}{1+\sum_{s\in OFF} e^{-\Delta G_s/RT}[IHF]^{n(s)}[FimE]^{j(s)}[FimB]^{k(s)}[Lrp*]^{m(s)}[Lrp]^{l(s)}},$$

and

$$g = \frac{\sum_{s\in ON} \alpha_s e^{-\Delta G_s/RT}[IHF]^{n(s)}[FimE]^{j(s)}[FimB]^{k(s)}[Lrp*]^{m(s)}[Lrp]^{l(s)}}{1+\sum_{s\in ON} e^{-\Delta G_s/RT}[IHF]^{n(s)}[FimE]^{j(s)}[FimB]^{k(s)}[Lrp*]^{m(s)}[Lrp]^{l(s)}}.$$

(1) : evolution of **Pon, probability of piliation switch being ON**. **Assume:** two state Markov process, regulatory protein binding/unbinding fast relative to switch rate. **Consequence**: Master eq form, stat thermo f and g.

.

State table for invertible element

IHF binding site

FimE/B binding site

Lrp binding sites

Free energies of binding

Switching rate

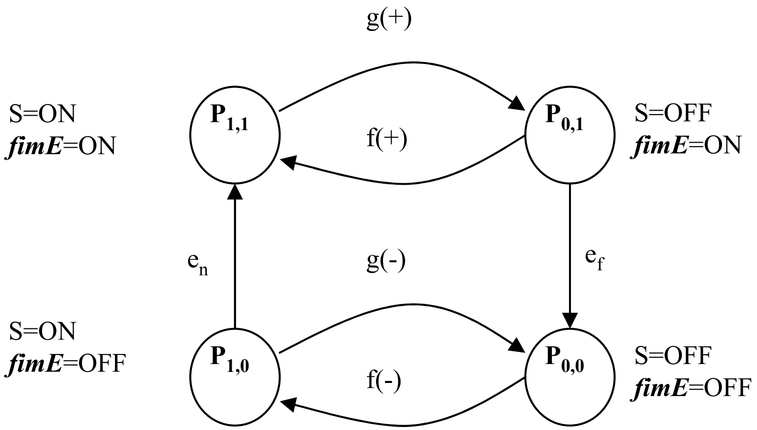
State	P_{IHF}	$P_{FimE/B}$	Lrp-A	Lrp-3	ΔG	α	n	j	k	m	l
1/9	-	-	-	-	0	0	0	0	0	0	0
2/10	IHF	-	-	-	$\Delta G_2/\Delta G_2$	0	1	0	0	0	0
3/11	IHF	FimE	-	-	$\Delta G_3/\Delta G_7$	α_1/α_4	1	0	1	0	0
4/12	IHF	FimB	-	-	$\Delta G_4/\Delta G_8$	α_2/α_3	1	1	0	0	0
5/13	IHF	FimE	Lrp*	-	$\Delta G_{3la}/\Delta G_{7la}$	$\alpha_{1la}/\alpha_{4la}$	1	0	1	4	0
6/14	IHF	FimE	Lrp*	Lrp	$\Delta G_{3lb}/\Delta G_{7lb}$	$\alpha_{1lb}/\alpha_{4lb}$	1	0	1	4	2
7/15	IHF	FimB	Lrp*	-	$\Delta G_{4la}/\Delta G_{8la}$	$\alpha_{2la}/\alpha_{3la}$	1	1	0	4	0
8/16	IHF	FimB	Lrp*	Lrp	$\Delta G_{4lb}/\Delta G_{8lb}$	$\alpha_{2lb}/\alpha_{3lb}$	1	1	0	4	2
17/27	-	FimE	-	-	$\Delta G_{33}/\Delta G_{77}$	0	0	0	1	0	0
18/28	-	FimB	-	-	$\Delta G_{44}/\Delta G_{88}$	0	0	1	0	0	0
19/29	-	FimE	Lrp*	-	$\Delta G_{33la}/\Delta G_{77la}$	0	0	0	1	0	0
20/30	-	FimE	Lrp*	Lrp	$\Delta G_{33lb}/\Delta G_{77lb}$	0	0	0	1	4	2
21/31	-	FimB	Lrp*	-	$\Delta G_{44la}/\Delta G_{88la}$	0	0	1	0	4	0
22/32	-	FimB	Lrp*	Lrp	$\Delta G_{44lb}/\Delta G_{88lb}$	0	0	1	0	4	2
23/33	-	-	Lrp*	-	$\Delta G_{3a}/\Delta G_{7a}$	0	0	0	0	4	0
24/34	-	-	Lrp*	Lrp	$\Delta G_{33a}/\Delta G_{77a}$	0	0	0	0	4	2
25/35	IHF	-	Lrp*	-	$\Delta G_{33b}/\Delta G_{77b}$	0	1	0	0	4	0
26/36	IHF	-	Lrp*	Lrp	$\Delta G_{3la}/\Delta G_{7la}$	0	1	0	0	4	2

e.g.,

$$p(3)=\frac{e^{-\Delta G_3/RT}[IHF][FimE]}{1+e^{-\Delta G_2/RT}[IHF]+e^{-\Delta G_3/RT}[IHF][FimE]+e^{-\Delta G_4/RT}[IHF][FimB]+e^{-\Delta G_{3la}/RT}[Lrp]^4[IHF][FimE]\ldots}.$$

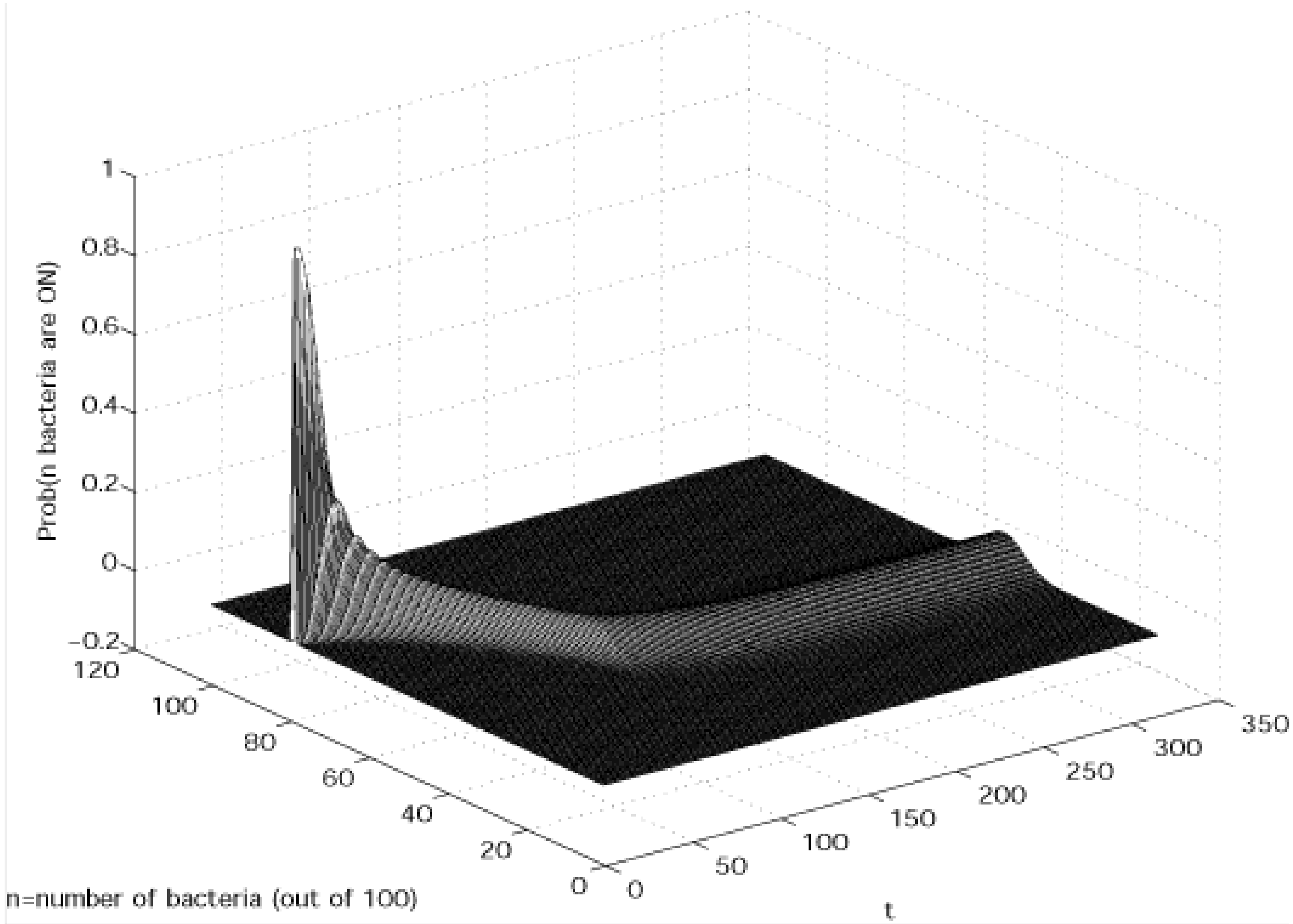
Complete phase variation network model

(with *fimE* orientational control)



$$\frac{d}{dt}\begin{bmatrix} p_{1,1} \\ p_{0,1} \\ p_{0,0} \\ p_{1,0} \end{bmatrix} = \begin{bmatrix} -g(+) & f(+) & 0 & e_n \\ g(+) & -f(+)-e_f & 0 & 0 \\ 0 & e_f & -f(-) & g(-) \\ 0 & 0 & f(-) & -g(-)-e_n \end{bmatrix} \begin{bmatrix} P_{1,1} \\ P_{0,1} \\ P_{0,0} \\ P_{1,0} \end{bmatrix}$$

Single Cell → Population

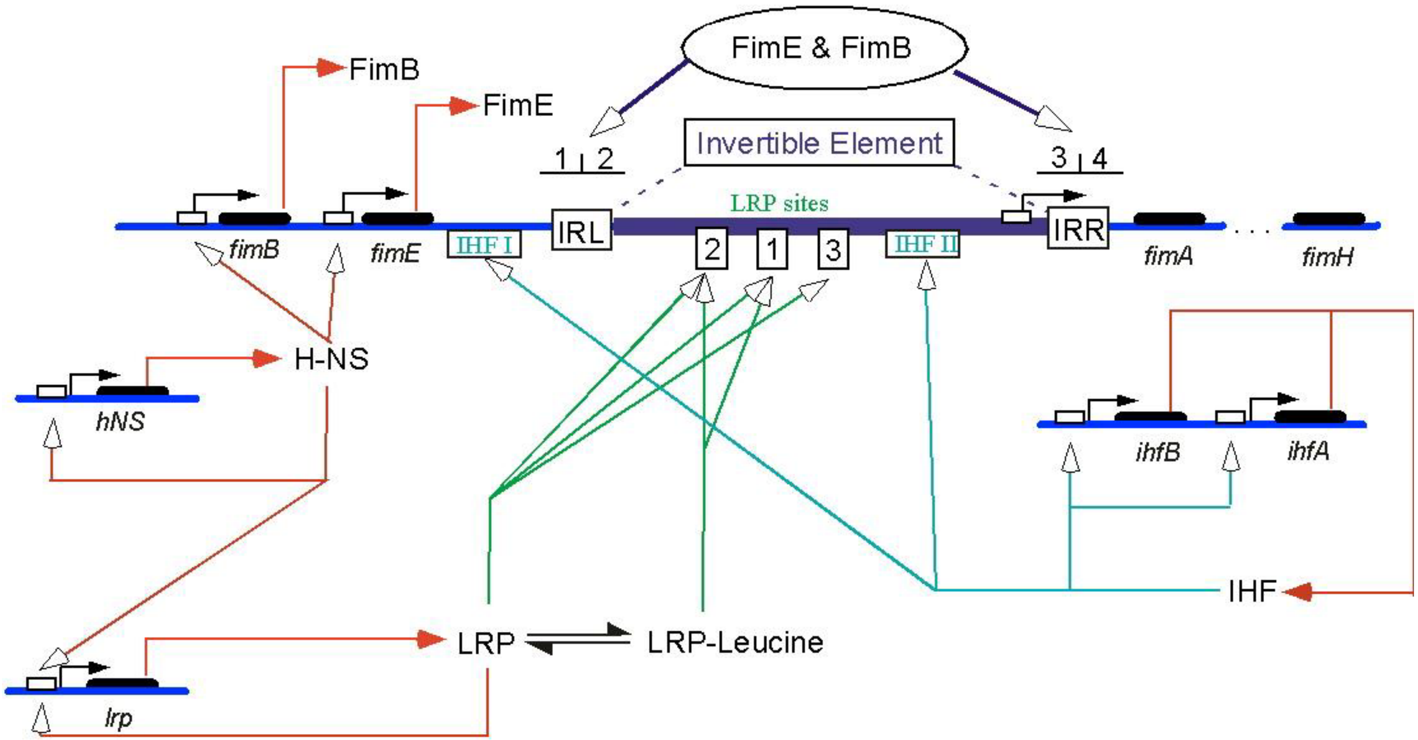


mean ON=$NP_{on}(t)$

standard dev.=$NP_{on}(t)(1-P_{on}(t))$

Results

Question Addressed



•Basic switch operation: sense environment, actuate weakly **ON** ⟺ strongly **OFF**

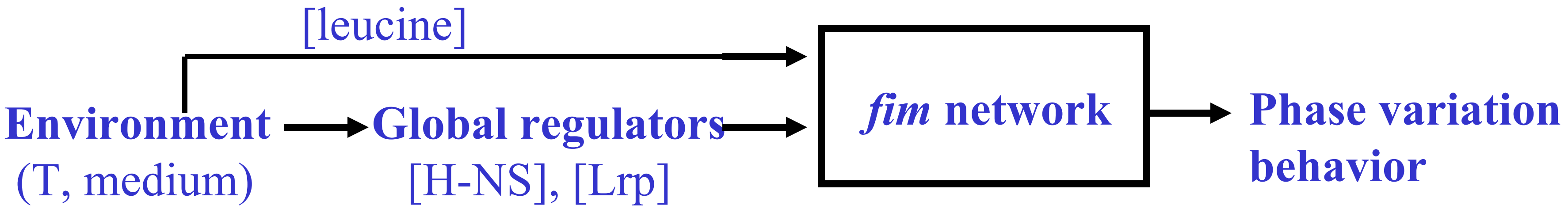
•Source of ON-to-OFF *specificity of FimE*?

•Why *two* independent recombinases when one would suffice?

•Role of *orientational control* of *fimE*?

•How is temperature control achieved? (*tuned to mammalian body temperature*)

The *fim* network: from environment to behavior

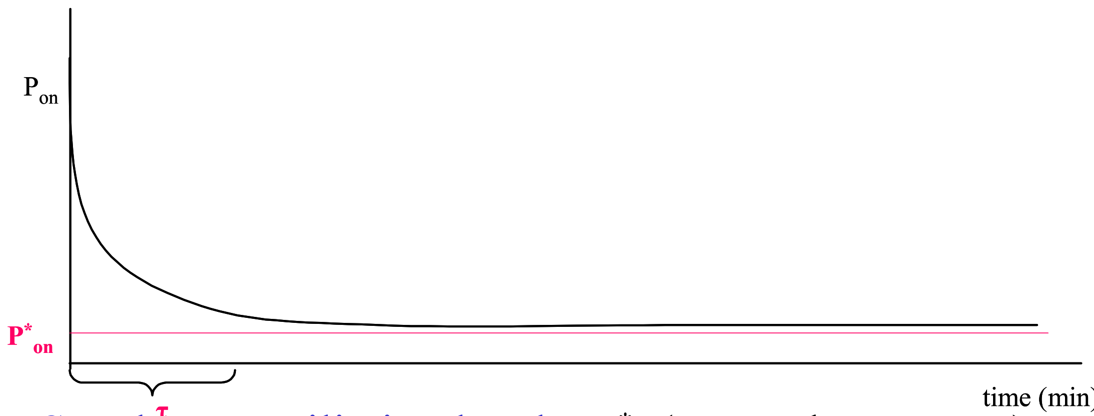


The *fim* network: controllable behaviors

[leucine] → *fim* network

Environment (T, medium) → **Global regulators** [H-NS], [Lrp] → ***fim* network** → **Phase variation behavior**

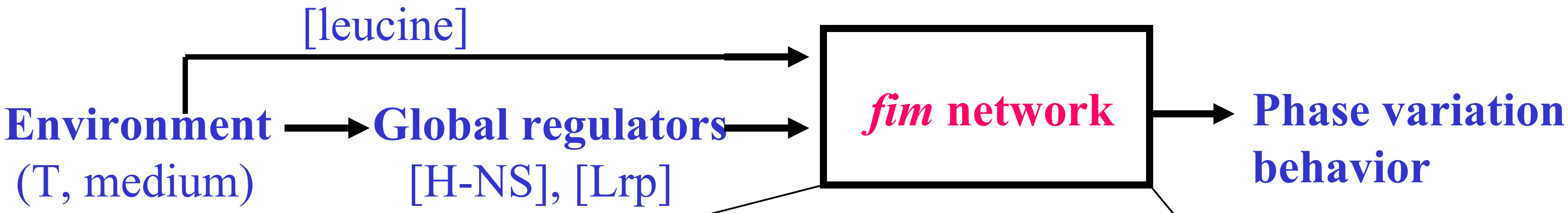
Population level control variables: P_{on}^* and τ



- Steady state piliation level P_{on}^* (∝ attachment rate)
- Time constant τ, a measure of *speed* of response to ΔEnv

Cell level: switch-off distribution p(t') (∝ detachment rate)

The *fim* network: primary mechanisms



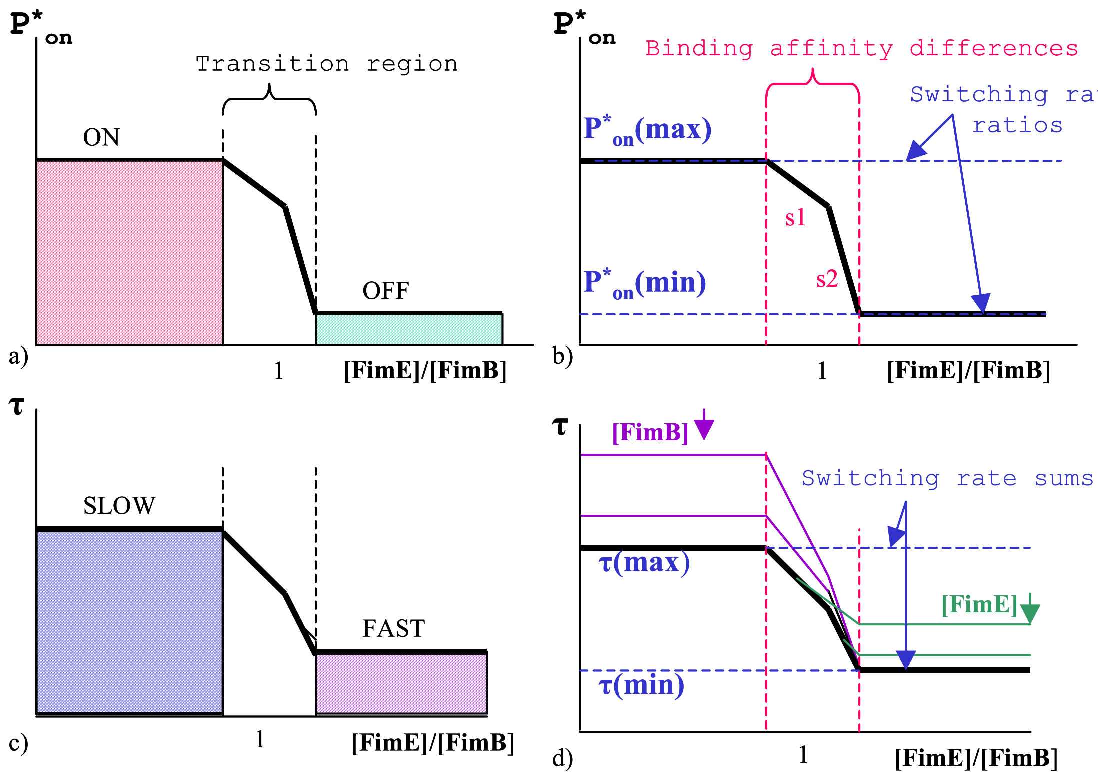
1. *A recombinase ratio controlled switch*

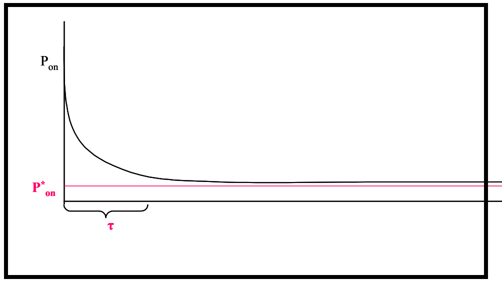
2. *A temperature tuning motif*

3. *A time delay*

Mechanism 1: A recombinase ratio controlled switch

Environment → [H-NS] → [FimE]/[FimB] → ON or OFF, Fast or Slow



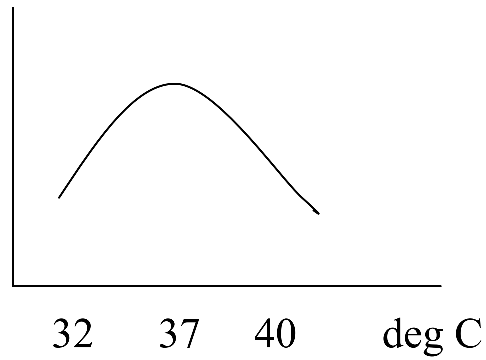


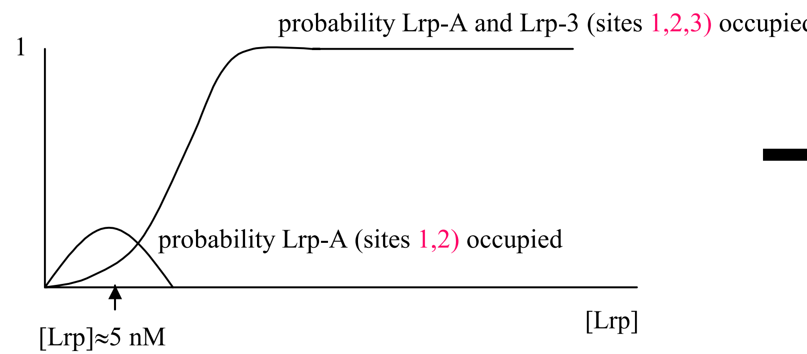
- Implemented by **(1)** differential H-NS repression of *fimB, fimE*, **(2)** competitive binding of FimB, FimE to switch, **(3)** FimE's strong ON-to-OFF bias

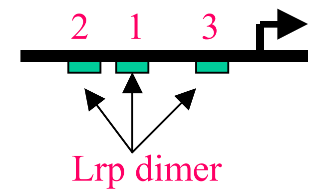
- Robust P_{on}^{*} vs. sensitive τ, decoupled control *(can control for changes in response time, detachment without changing %ON, attachment)*

Mechanism 2: A temperature tuning motif

Temp, medium → [Lrp],[leucine] → Lrp-A, 3 occupancy → max in piliation level at 37degC

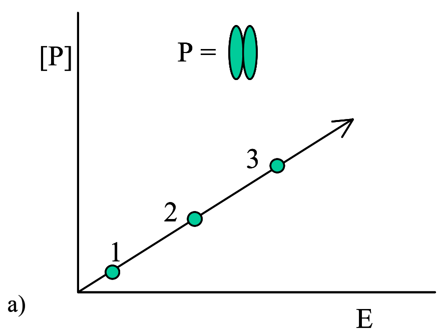


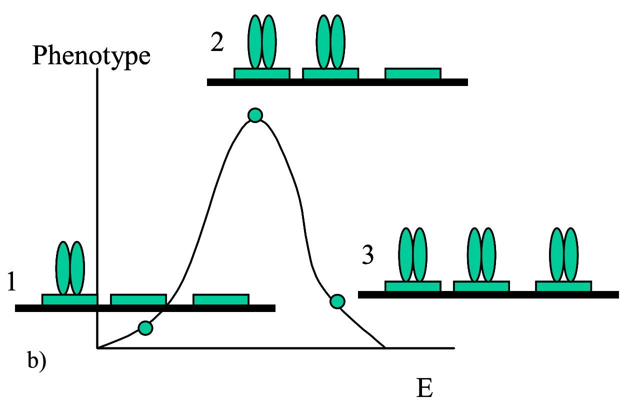




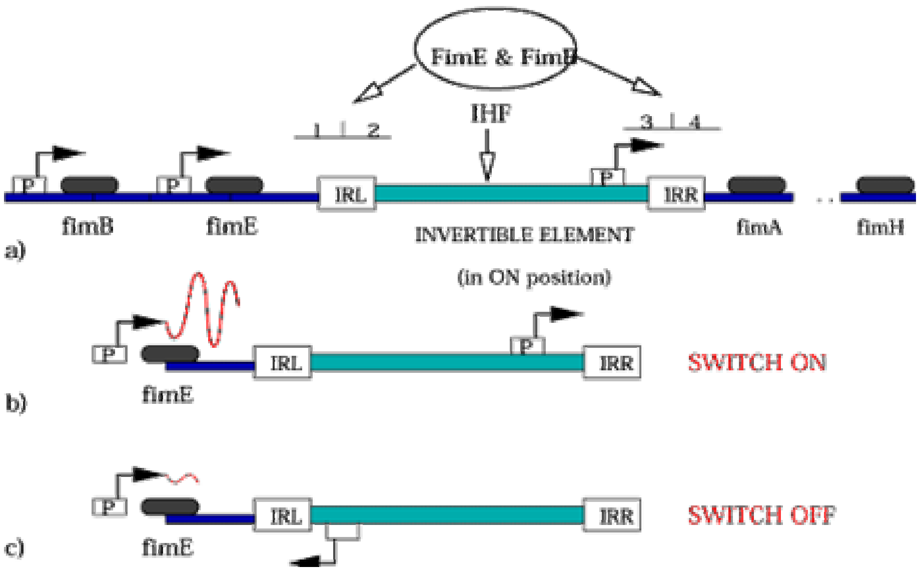
- ***Local maximum in switch rate corresponds to local maximum in Lrp site-1,2 occupancy***

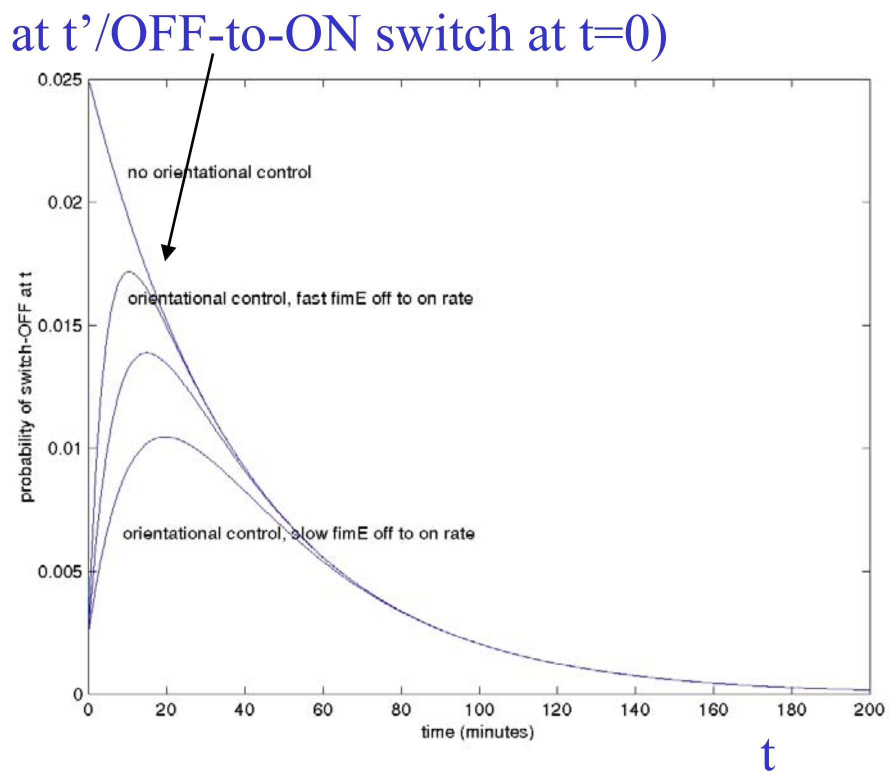
- ***Common regulatory motif (e.g., OmpF, gltBDF,…)***



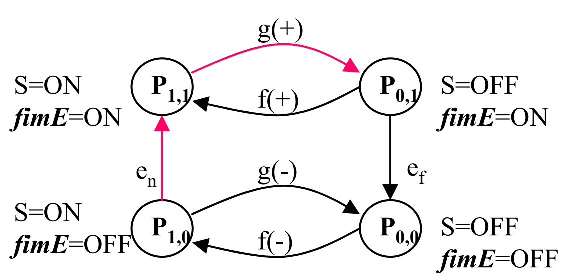


Mechanism 3: A time delay

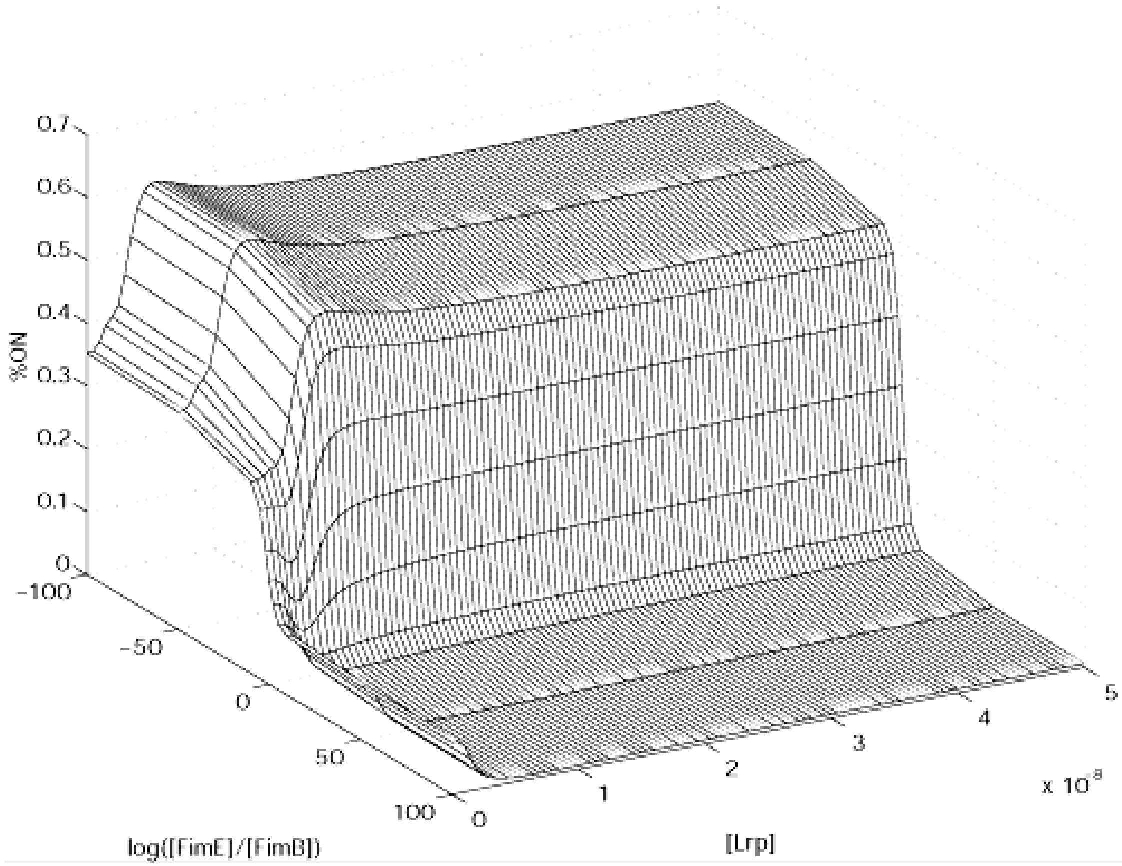




• ***fimE*** **orientational control acts as switch memory, delay (keeps switch on long enough to build pili), and prevents chatter.**



Synergy between mechanisms

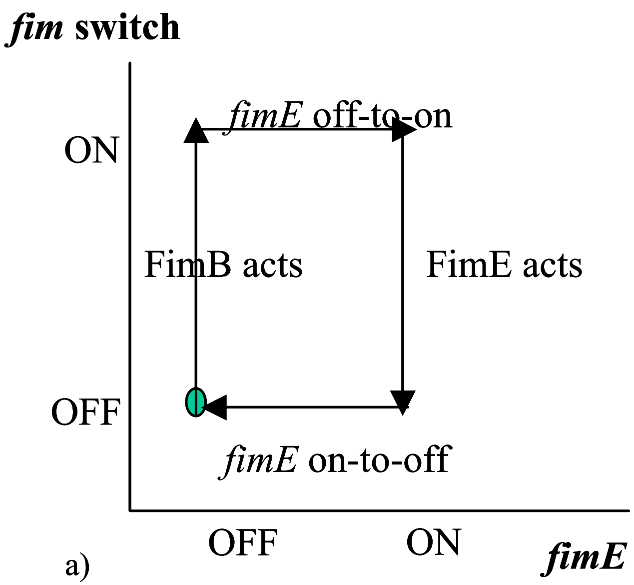


•Recombinase ratio based control and the phenotype tuning motif intersect through the ***heights of the sigmoid asymptotes and slopes of transition regions***.

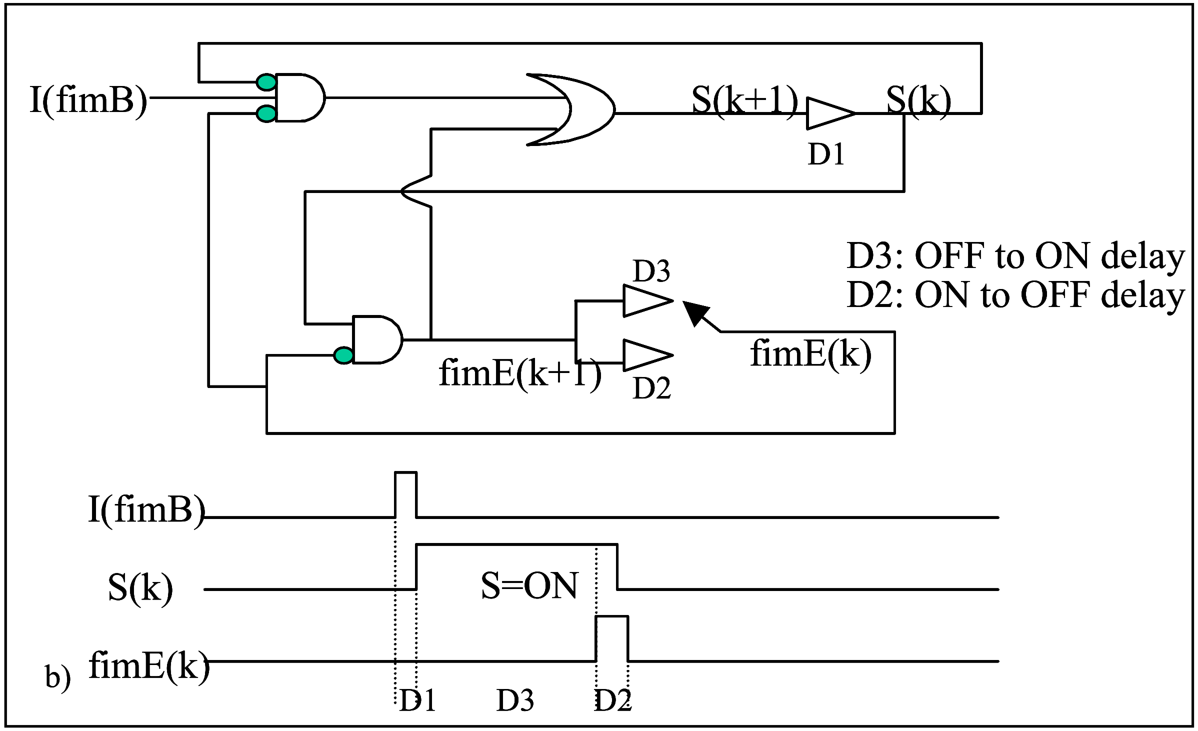
•Sigmoidal along [FimE]/[FimB] axis, inverted parabolic along [Lrp] axis .

•*fimE* orientational control increases sensitivity to environment, through [FimB].

fim as digital circuit: a stochastic pulse generator

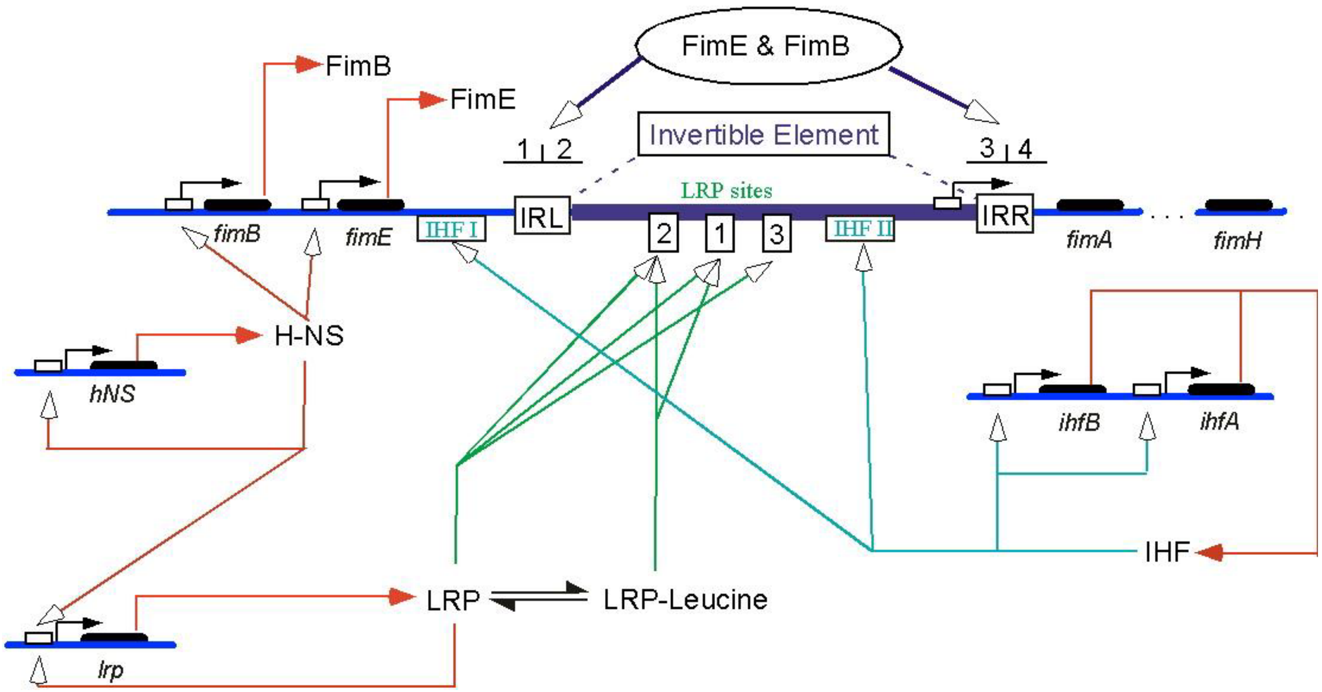


a)



b)

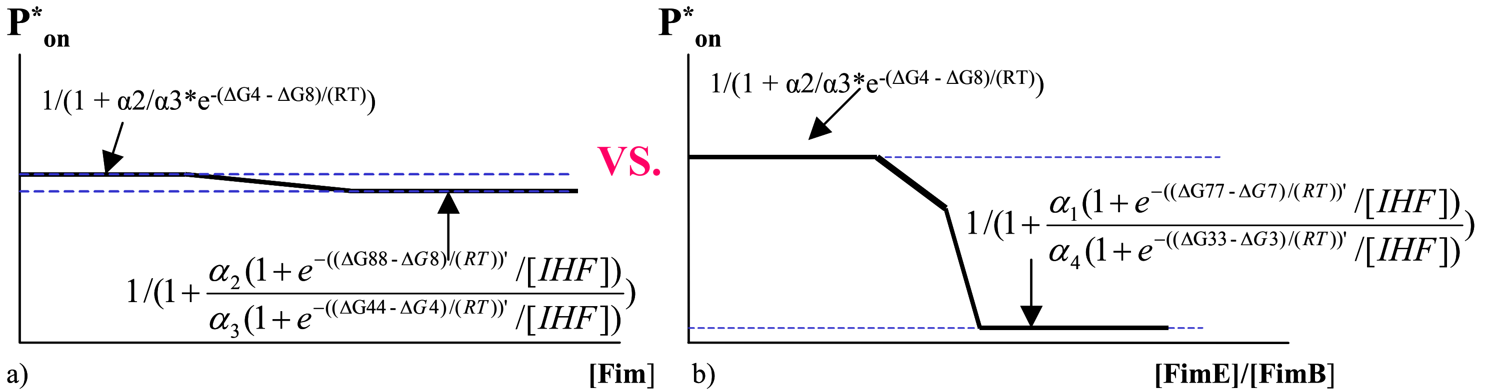
Design questions from literature



- **Why *two* independent recombinases when one would suffice?**
- **Role of *orientational control* of *fimE*?**
- **How is temperature control achieved? (*tuned to mammalian body temperature*)**

Why Two Recombinases?

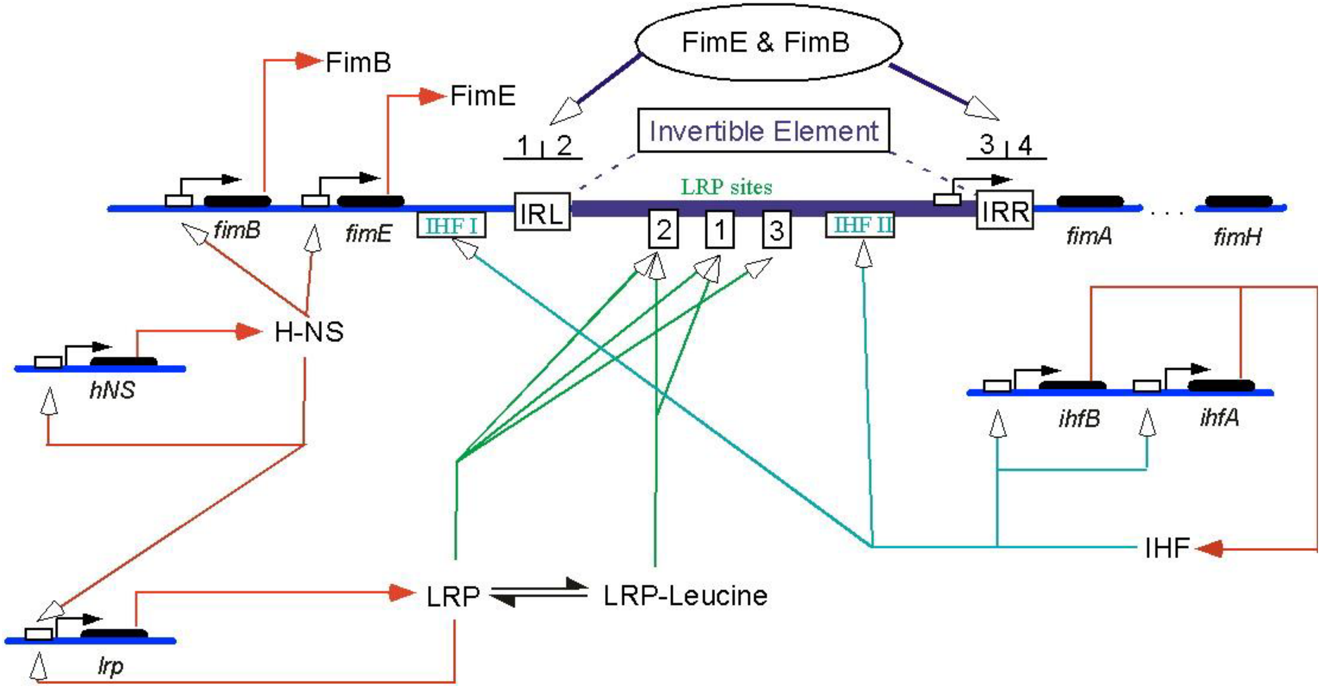
- One recombinase model



- Not sigmoidal -> *cannot act as small-signal switch*
- *Lost of environmental control*
- *Diminished, decoupling* of response speed and %ON

Evolutionary advantage to sensitive, **robust** control & **decoupling** of **response speed** and **%ON** control/robustness/parameter sensitivity?

Design questions from literature

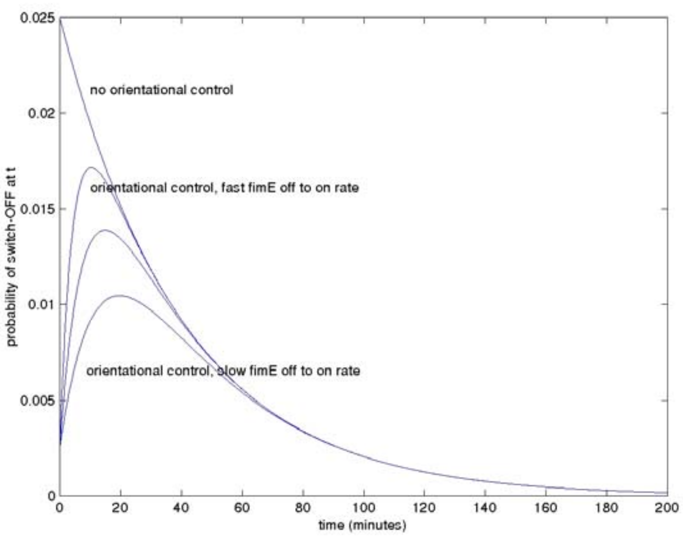


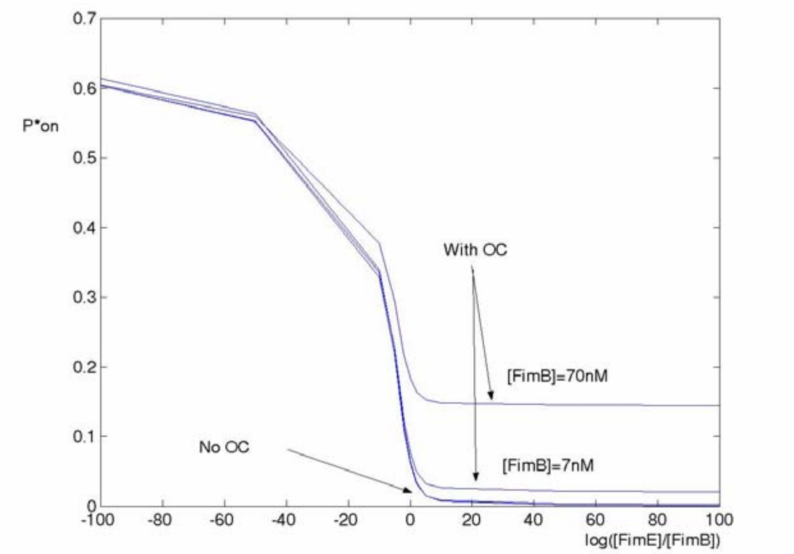
•Why *two* independent recombinases when one would suffice?

•Role of *orientational control* of *fimE*?

•How is temperature control achieved? (*tuned to mammalian body temperature*)

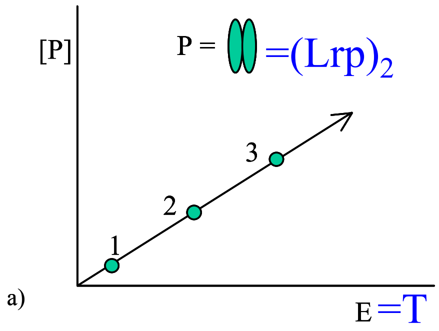
•Role of *orientational control* of *fimE*?

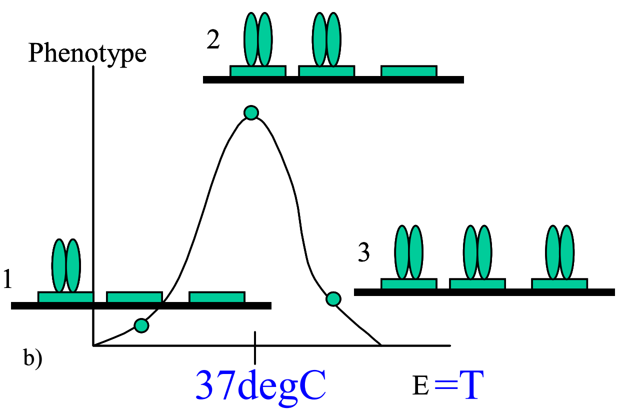




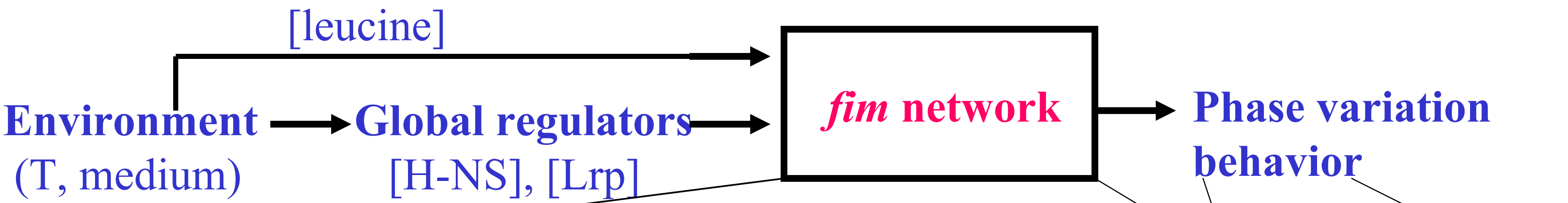
(1) Memory, (2) delay (keeps switch on long enough to build pili), (3) environmental sensitivity, (4) prevents chatter.

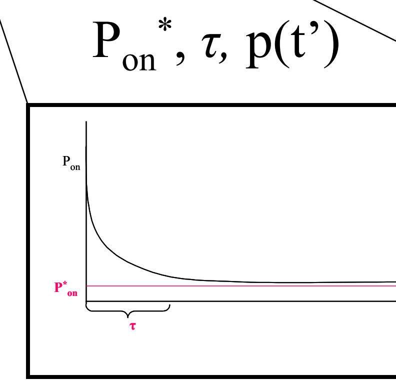
•How is temperature control achieved? (*tuned to mammalian host*)



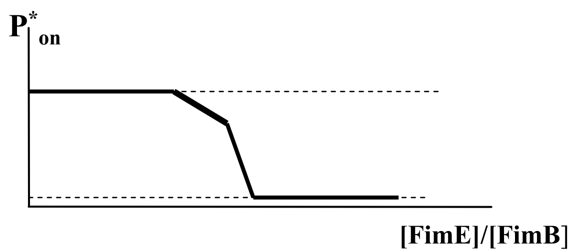


The *fim* network: an overview

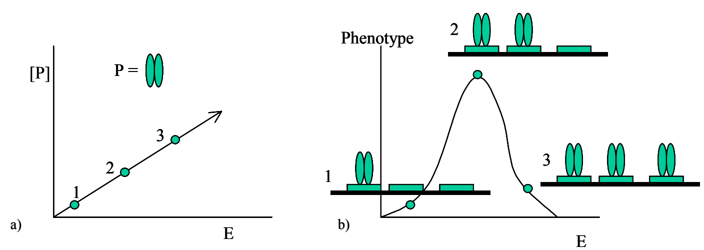




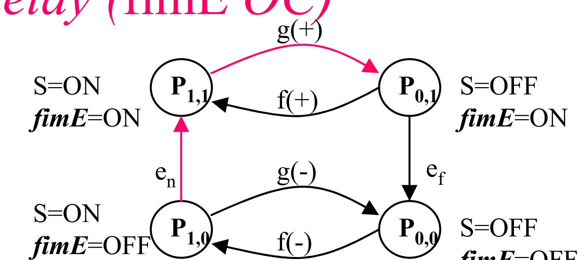
1. *A recombinase ratio controlled switch*



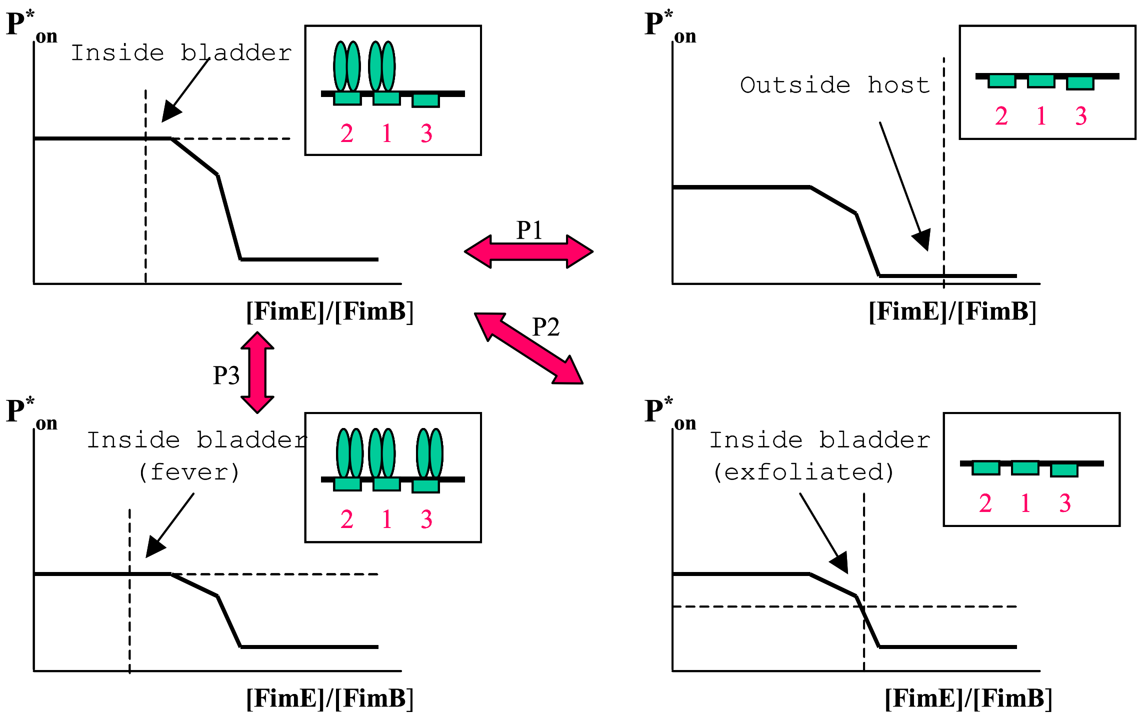
2. *A temperature tuning motif*



3. *A time delay (fimE OC)*



Pathways to infection



- P1: Outside of host (unpiliated) ⟹inside host (piliated)

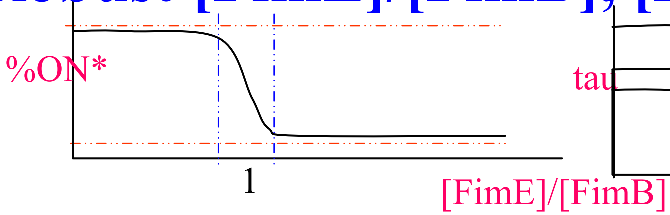
  T ↑ ⟹ H-NS derepression ⟹[FimE]/[FimB]↓, [Lrp]↑, Lrp-A occupied
- P2: Exfoliation in host

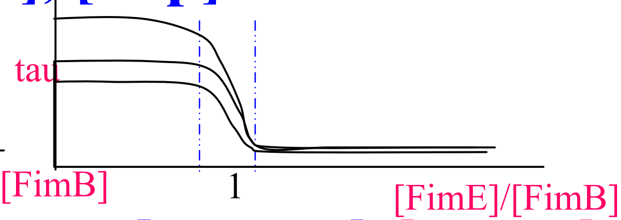
  Medium richness ↑ ⟹[FimE]/[FimB] ↑, [Lrp] ↓
- P3: Fever in host

  T>37degC ⟹[FimE]/[FimB]↓, [Lrp]↑, Lrp-A,3 occupied

Results Summary

•Robust [FimE]/[FimB], [Lrp] controlled switch:





•%ON* and response speed control decoupled

• *Two* vs. one recombinase …..sigmoid, decoupling, robustness

• ON-to-OFF *specificity of FimE* caused by switching rates,
not binding affinities

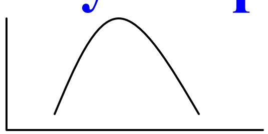
•Orientational control* of *fimE

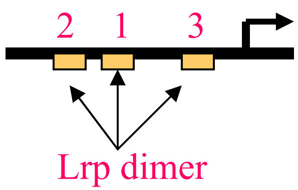
%ON(min), time ON greater, +redundant *fimE* spec.

Memory, increased sensitivity to environment

•Temperature tuning to mammalian body temperature

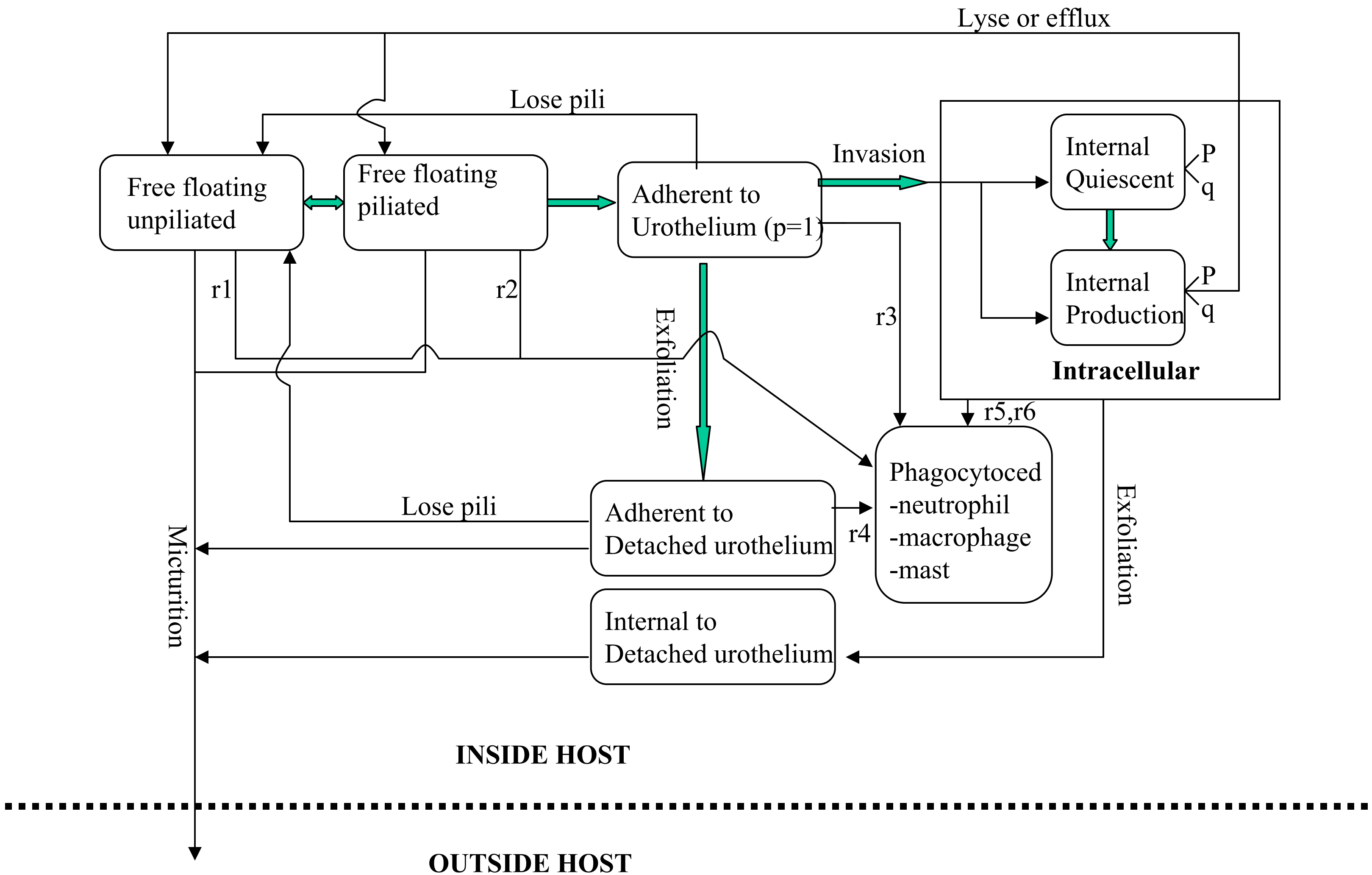
•Lrp site occupancy





•General mechanism for phenotype tuning to environment

Future work (?)



- **What game are we playing?**
- **3-D disease model.**

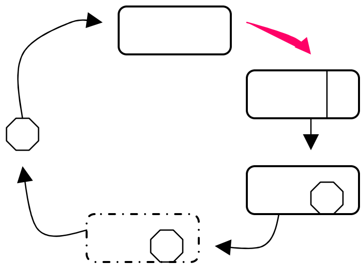
Coming attractions

***B. subtilis* sporulation initiation**

- 48 molecular species
- Devices: two-component, phosphorelay,
  agonist/antagonist pair operons,
  import/export timing loops.
- >8 feedback loops, 2 (-), 6 (+)
- Signal integration, amplification, noise filtering?

Representations/analyses

1. Connectivity (0,1)
2. Connectivity/Activity (0,1,-1,+/-1)
3. C/A/Inequality $(0, k_i, |k_n| < |k_{n-1}| < \ldots |k_1|)$
4. Piecewise linear
5. …
6. Biophysical nonlinear

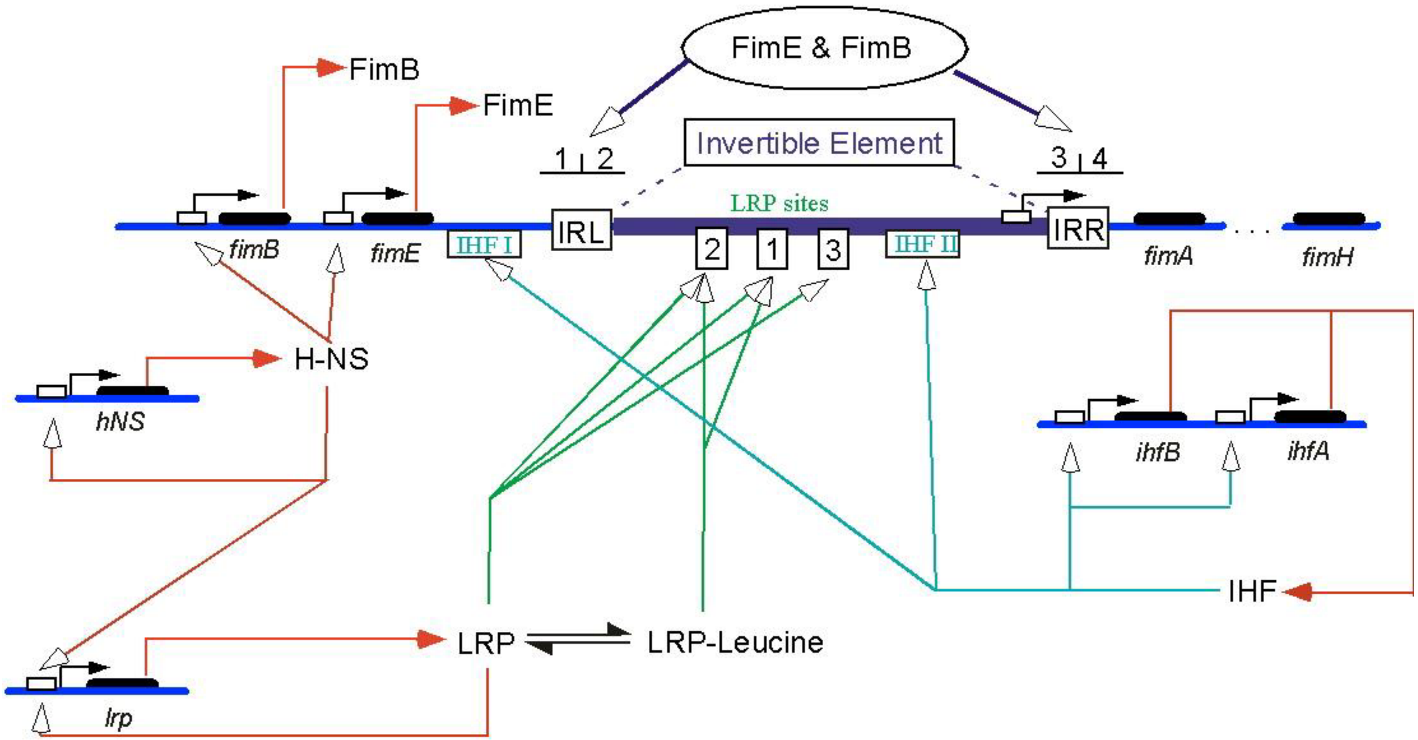


BioSPICE specs., algorithms

- Construct connectivity matrices
- Find optimal ordering for block diagonal, matrix pencil
- Identify and categorize destabilizing forces: catalytic elements, pairs,
  +/- feedback loops
- …and so on…

The End

Question Addressed



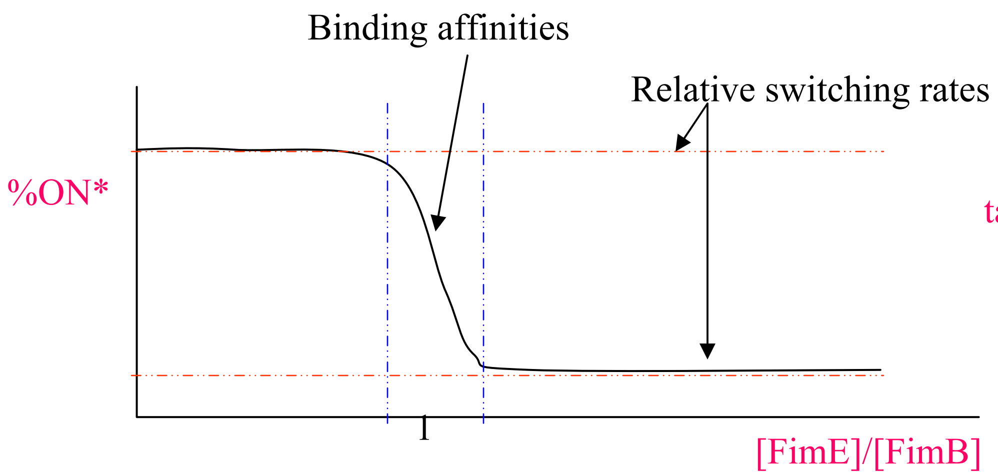
•**Source of ON-to-OFF *specificity of FimE*? .3/cell/gen vs 10^{-4}/cell/gen**

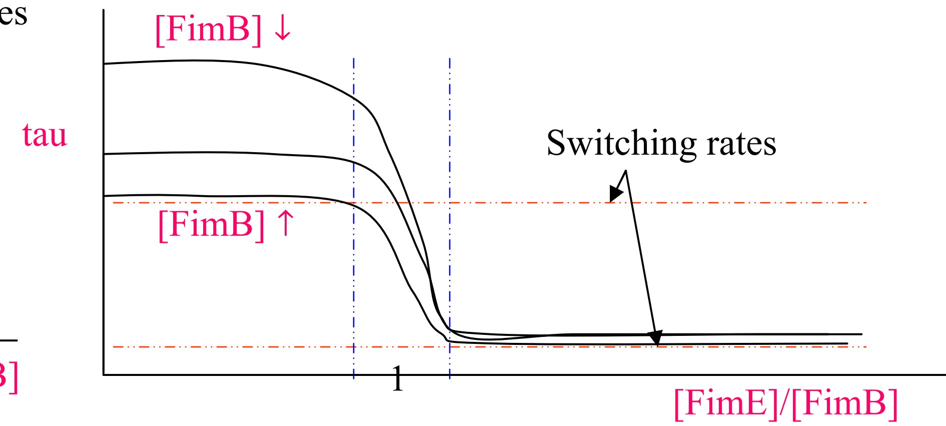
•**Why *two* independent recombinases when one would suffice?**

•**Role of *orientational control* of *fimE*?**

•**How is temperature control achieved? (*tuned to mammalian body temperature*)**

FimE ON-to-OFF specificity

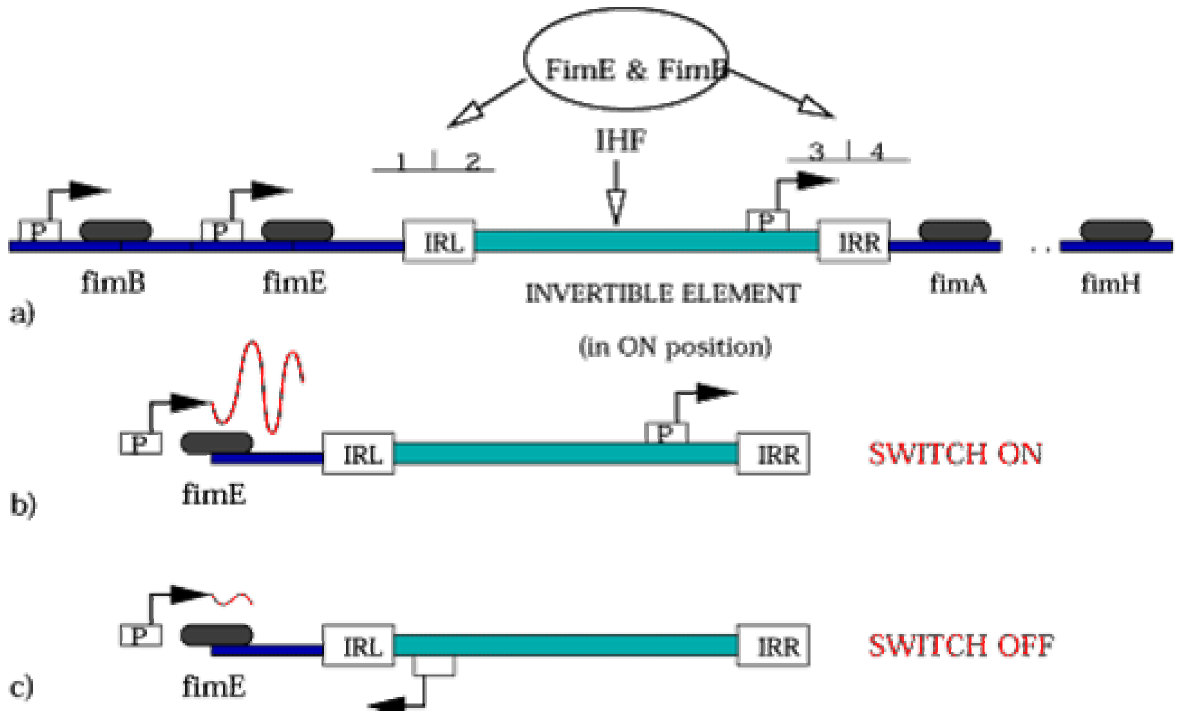




Depends on operating points.

For most, ***switching rates dominate, not binding affinities.***

System-level Questions



- **Basic switch operation:**

- **Source of ON-to-OFF *specificity of FimE*?**

- **Why *two* independent recombinases when one would suffice?**

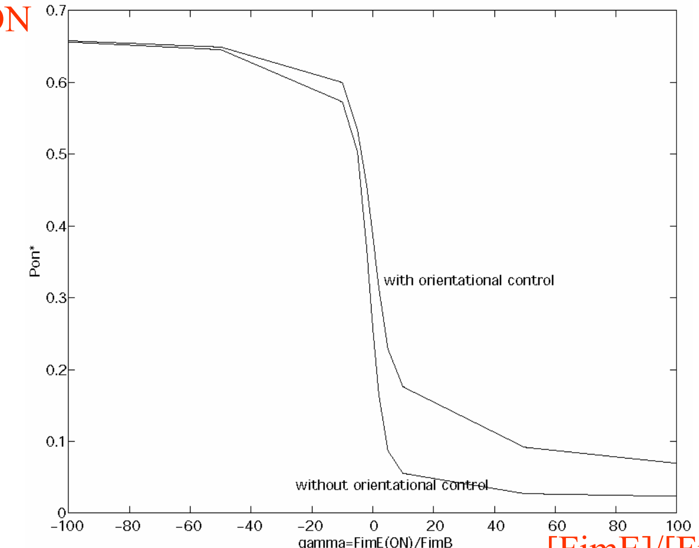
- **Role of *orientational control* of *fimE*?**

- **How is temperature control achieved? (*tuned to mammalian body temperature*)**

fimE Orientational Control (OC)

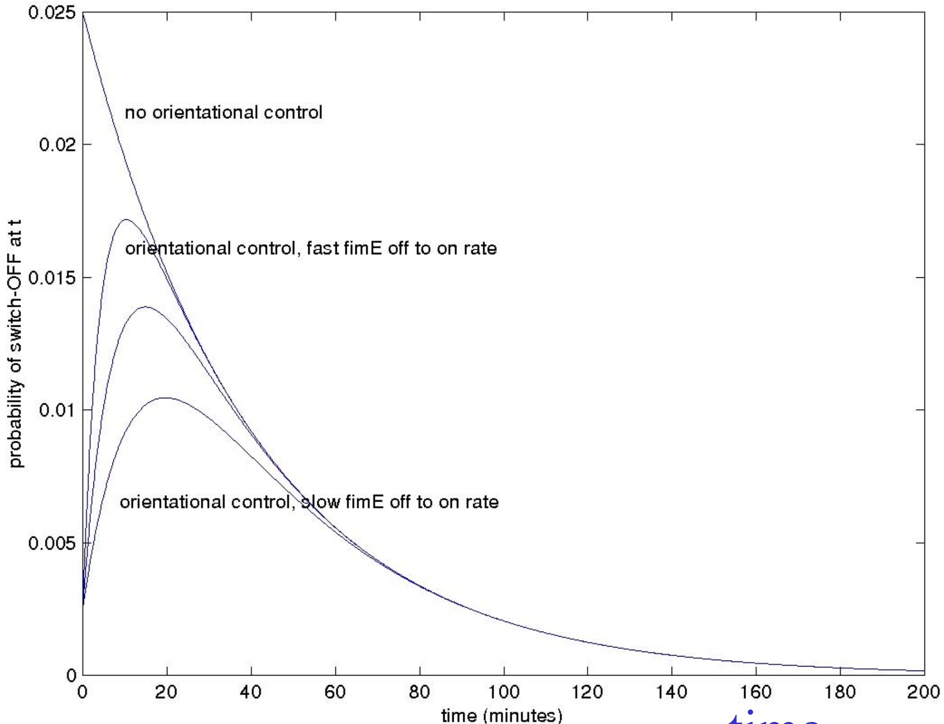
- Ensures switch ON long enough to build pili
- Increases **sensitivity** to growth medium, temp
- Adds **stochasticity** (increases turn OFF/detachment rate noise)

%ON



[FimE]/[FimB]

P(ON-to-OFF)



time

Q: Does *fimE* orientational control contribute to FimE specificity?

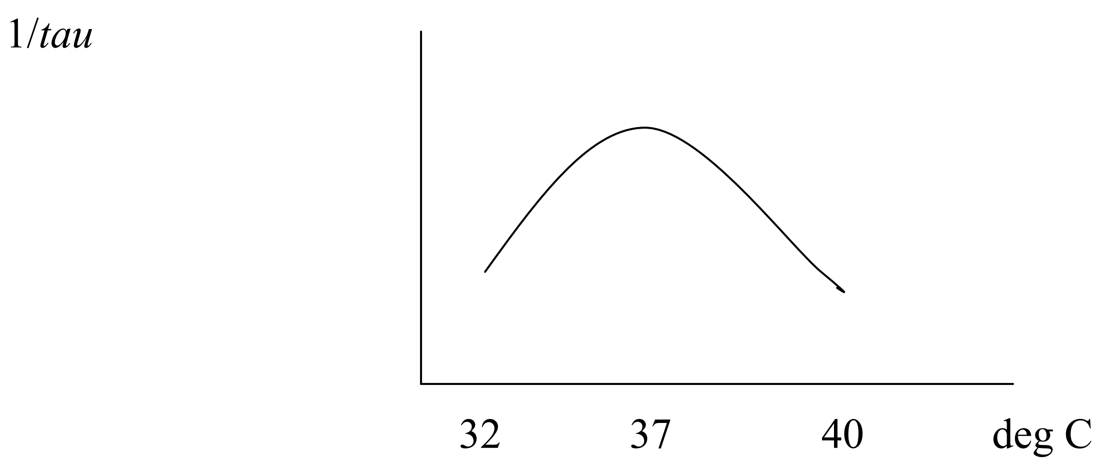
A: Yes and no. No, in that ..

(Time ON and minimum %ON greater than it would be without orientational control)

Yes, in that IF FimE unstable, OC maintains specificity even if high OFF-to-ON rate (acts as redundant mechanism)

System-level Questions

- **Basic switch operation:**
- **Source of ON-to-OFF *specificity of FimE*?**
- **Why *two* independent recombinases when one would suffice?**
- **Role of *orientational control* of *fimE*?**
- **How is temperature control achieved? (*tuned to mammalian body temperature*)**

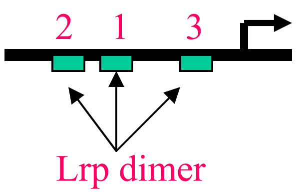


Local maximum in switching rate in fimE- mutant at 37 deg C

Hypothesis Development

•FimE out of picture…forget *alpha*=[FimE]/[FimB]
•know switching rate monotonic in [FimB]
•Simplest answer: [FimB] has local max w/Temp…..no, not the way H-NS works.

•Lrp?
•H-NS represses lrp, H-NS controlled by T, so T ↑ should imply Lrp ↑

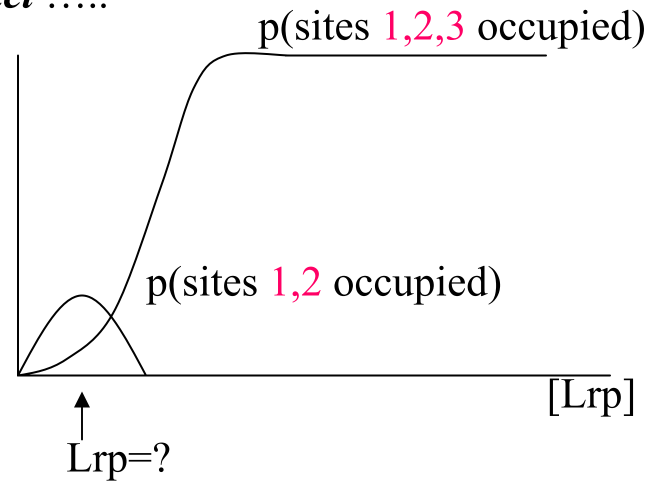


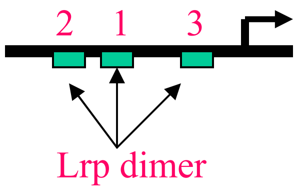
•How does Lrp bind to switch?
- •Three sites. Either (1), (2) bound with Lrp, or (1),(2),(3) bound with Lrp
- •If site (3) mutated out, **switching rate** ↑ (*leucine experiments*)

Hypothesis: ***local maximum in switch rate corresponds to local maximum in Lrp site-1,2 occupancy***

Hypothesis tested, confirmed, generalized

Model …..





Data ⇨ binding affinities ⇨ max occurs at physiologic [Lrp], T=37

General mechanism? Evolutionarily plausible for phenotype 'tuning'

(e.g. b-galactosidase operon, osmoregulatory porin regulation)

Maximum # extrema = (# energetically separated binding site clusters) - 1

Future Work Plans

Study virulence

•E. coli cell/population model + tissue model $\Longrightarrow$ ***disease analysis***

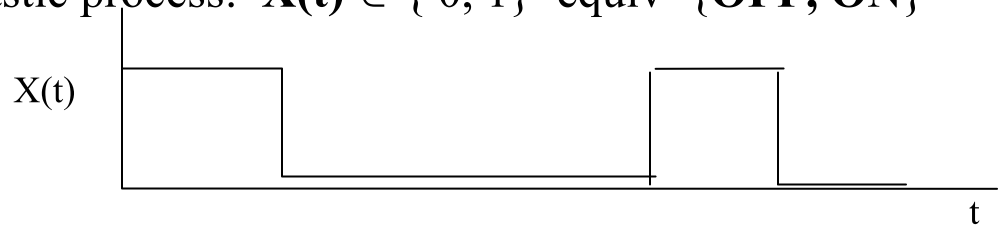
Genetic engineering

•Control motif library $\Longrightarrow$ genetic circuit CAD

design and build multi-dimensional gene expression/repression systems

Model invertible element

Stochastic process: $\mathbf{X(t)} \in \{ 0, 1\}$ equiv {**OFF, ON**}



Associated with ***probability distribution*** **P(t)**

P_{on} = Probability switch is in ON position

ASSUME: Markov process ➔ **Master Equation** formulation

$$\dot{P}_{on}(t)=W_{off\text{-}to\text{-}on}\,(1-P_{on}(t)) - W_{on\text{-}to\text{-}off}\,P_{on}(t)$$

Mathematical expressions of behavioral descriptors P_{on}^{*}, P(t'), τ

- Without *fimE* orientational control

$$P^{*}_{on} = f/(f+g) \text{ and time constant } \tau = 1/(f+g)$$

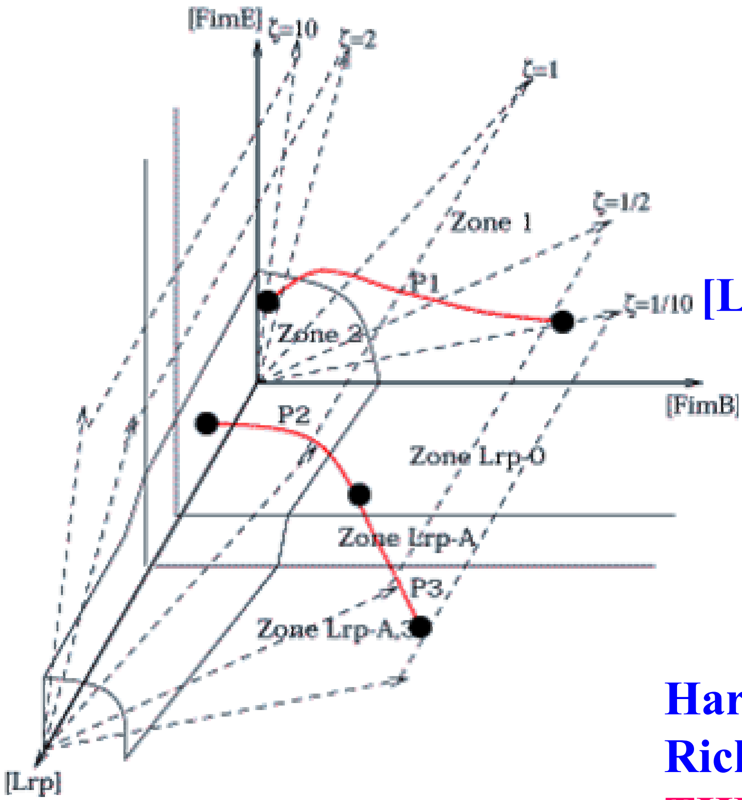
$$P(t')_{on \to off} = g \times e^{-gt'}$$

- With *fimE* orientational control

$$P^{*}_{on} = \frac{\dfrac{g(+)f(-)}{e_n} + \dfrac{f(+)f(-)}{e_f} + f(-)}{\dfrac{g(+)f(-)}{e_n} + \dfrac{f(+)f(-)}{e_f} + \dfrac{g(+)f(-)}{e_f} + \dfrac{g(+)g(-)}{e_n} + f(-) + g(+)}$$

$$P(t')_{on \to off} = g(-)e^{-(g(-)+e_n)t'} + g(+)e^{-g(+)t'}\left(1 - e^{-e_n t'}\right)$$

Environmental control



[FimE]/[FimB]: ⇓ TEMP, ⇑ nutrition (H-NS)

[Lrp]: ⇓ NUTRITION, ⇑ temp (growth rate, H-NS)

FimE,B: Zone 1- high T, poor nutrition

Zone 2- lower T, rich nutrition

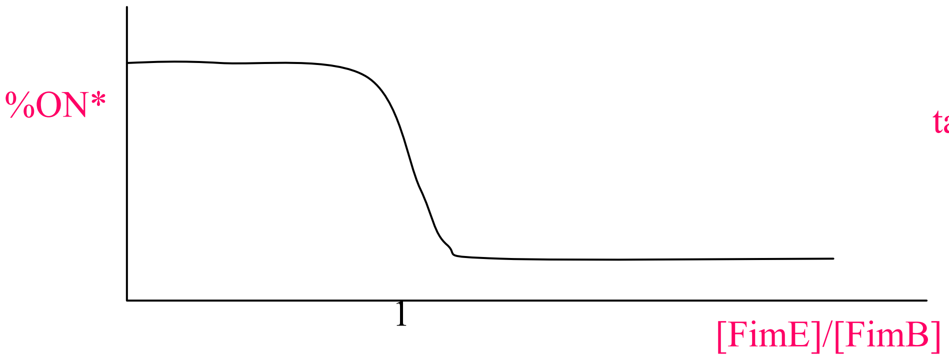
SURVIVAL:

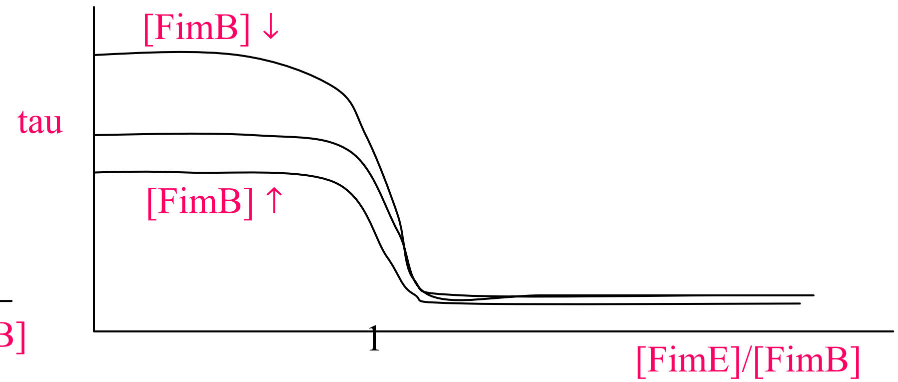
Harsh - more pili, slower turn-OFF

Rich - fewer pili, fast turn-OFF

THE BODY: interplay of both, temperature tuned

Decoupling of Equilibrium %ON, Response Speed





- Both **sigmoids** - sensitive, insensitive regions w/ respect to [FimE]/[FimB]
- Sigmoid **Max/Min asymptotes**
  - same parameters
  - different - **Pon*** controlled by **relative** differences, ***tau*** controlled by **absolute** values

- **Coupled** by *alpha*= [FimE]/[FimB]
- **Decoupled** by [FimE], [FimB], switching rates, binding affs

Consequence: *can control for changes in response time, detachment without changing equilibrium %ON, attachment*