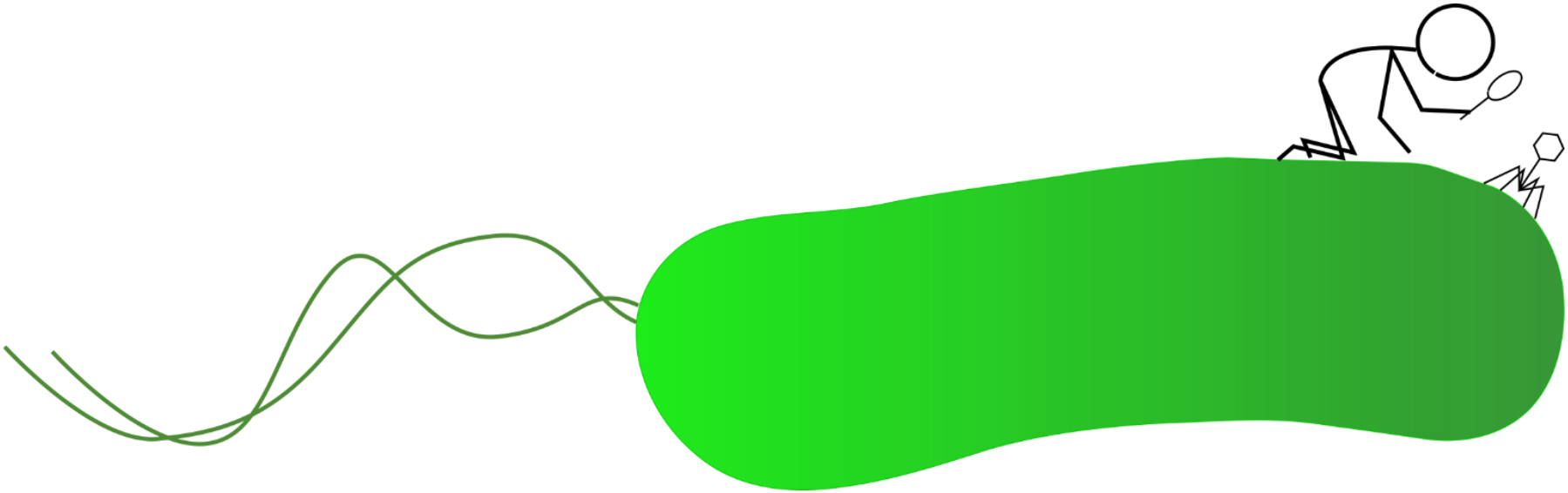
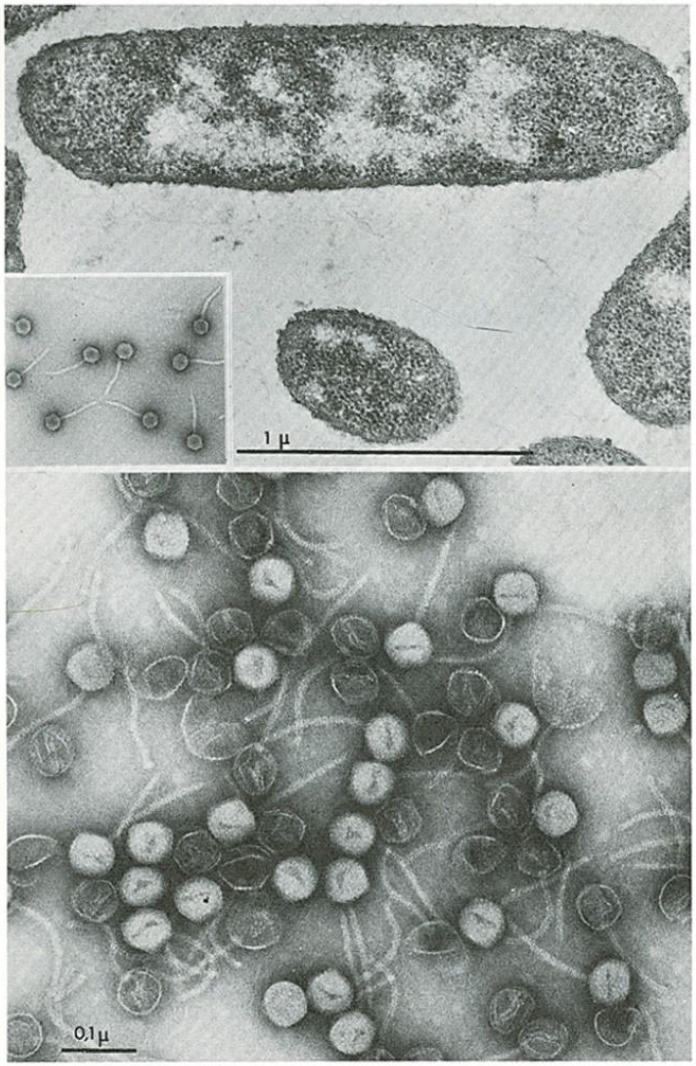


Optimising dormancy vs. virulence decisions in bacteriophage

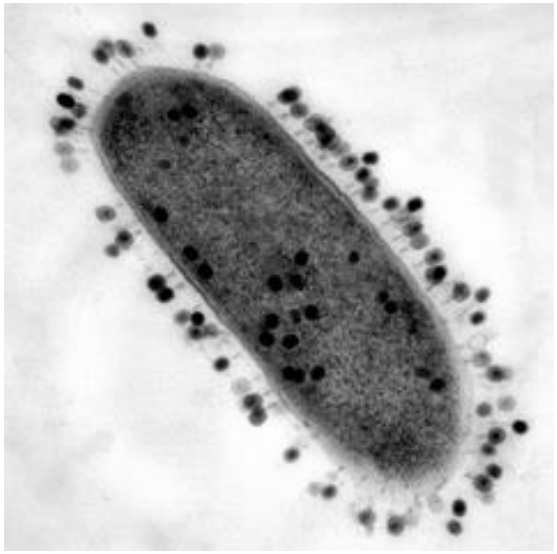
Sandeep Krishna
Simons Centre for the Study of Living Machines
National Centre for Biological Sciences TIFR, Bangalore
(Collaborators: Akshit Goyal, Aswin Seshasayee, Ian Dodd, Kim Sneppen, Mikkel Avlund,
Sine Svenningsen, Szabolcs Semsey, Vaibhhav Sinha; Funding: NCBS, Niels Bohr Institute, Simons Foundation)

IMSc Chennai Workshop on Spins, Games & Networks, Dec 2024

Bacteriophage: an unusual predator-prey system

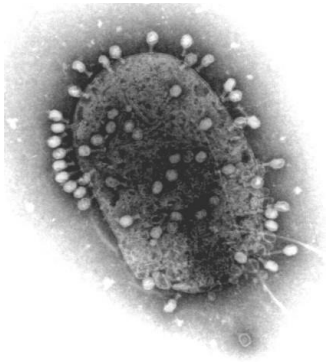


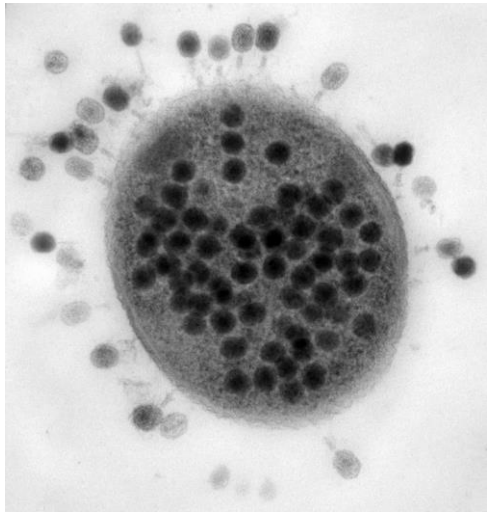
Escherichia coli (top) and λ phage particles.
[Courtesy of E. Boy de la Tour, F. Eiserling, and E. Kellenberger.]



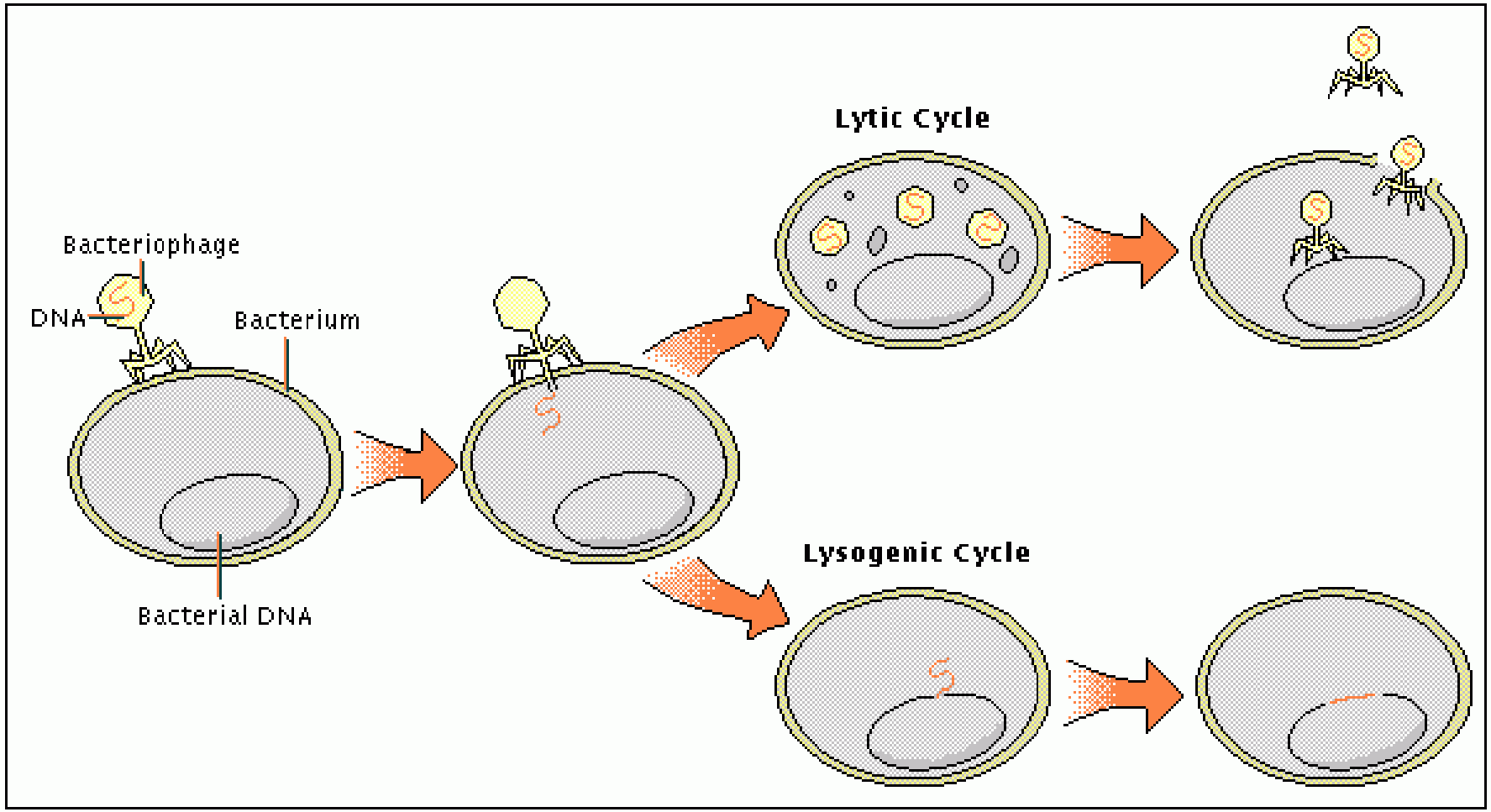
Ref: http://viromag.wordpress.com/2009/03/13/bacteriophages-viruses-of-bacteria/

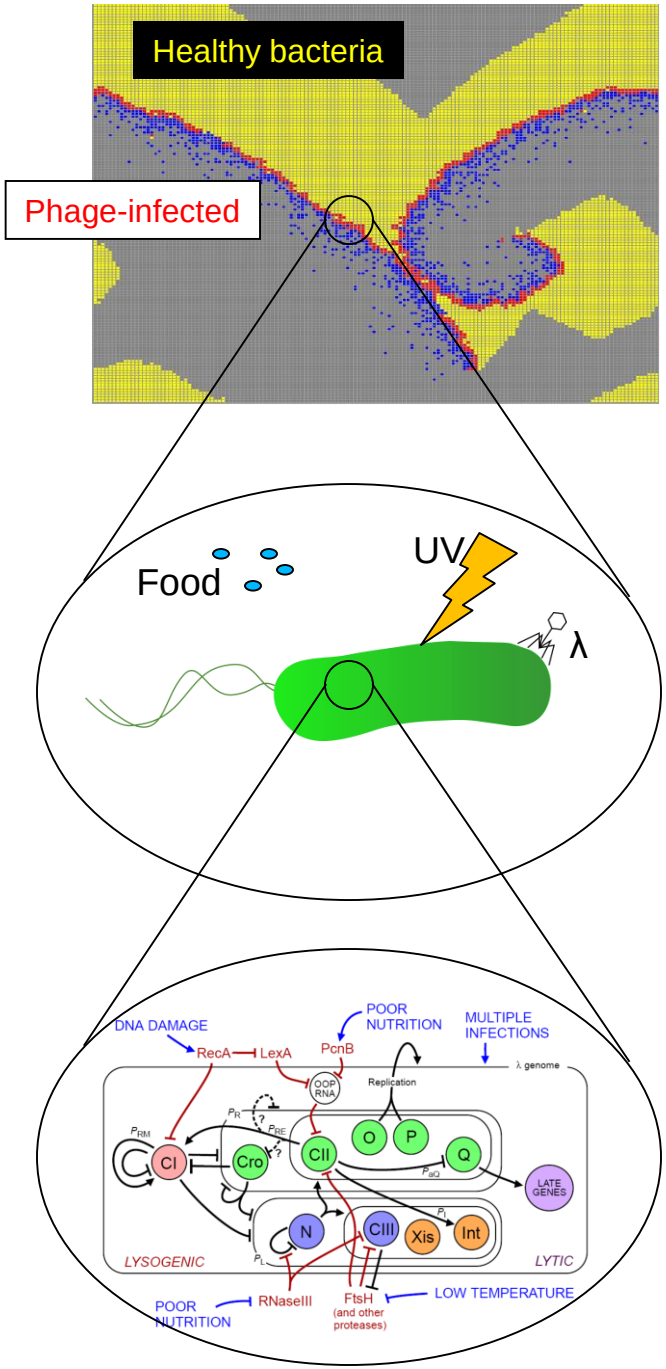
Ref: http://www.absoluteastronomy.com/topics/Bacteriophage





Temperate bacteriophage: Lysis-lysogeny decisions





Population/Ecosystem level

What are good lysis-lysogeny strategies for a phage when, say, it is competing with other phage species for a bacterial host?

How are the population (and evolutionary) dynamics of phage-bacteria ecosystems influenced by different bacterial defences against phage?

Cellular level

Why is only a narrow 5-15% lysogeny percentage observed in laboratory phage infections?

What conditions make a phage-infected bacterium go preferentially lytic, or lysogenic?

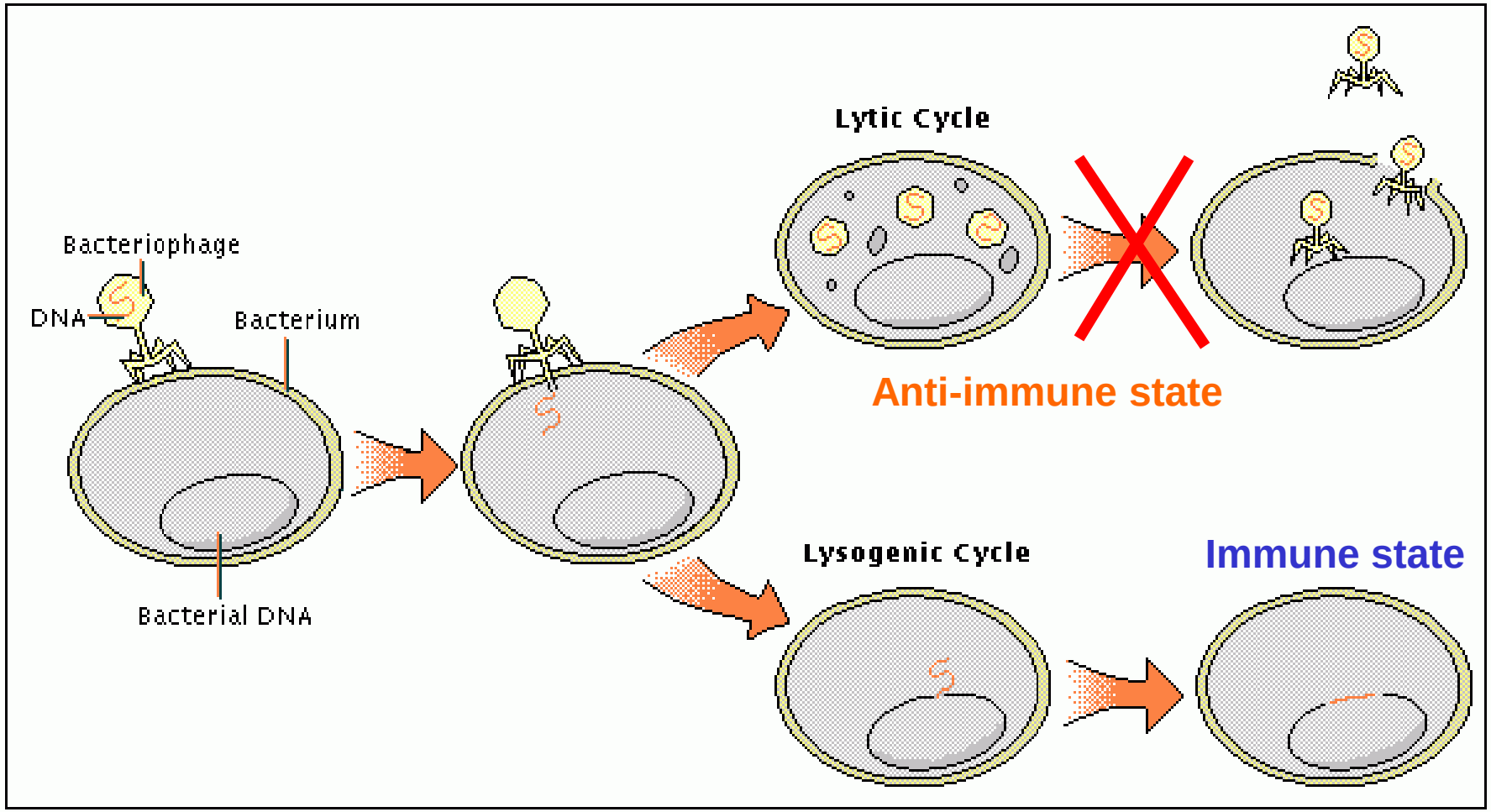
What aspects of the bacterial cell state bias the decision?

Subcellular level

How is the lysis-lysogeny decision regulated?
What produces bistability?
What makes the network robust to noise?

How does the phage network integrate information about the environment (e.g. does it use bacterial quorum sensing)?

Temperate bacteriophage: Lysis-lysogeny decisions

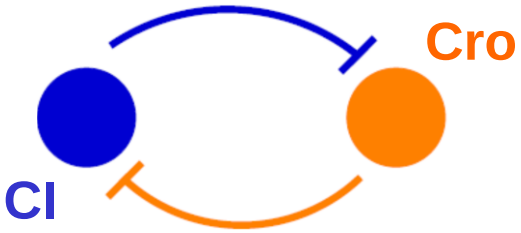


Two very stable states: probability of exiting ~O(10^{-5}-10^{-6}) per cell per generation
Stable even with a single copy of the genome left

“Standard model” of λ

Ptashne, A Genetic Switch: Phage Lambda Revisited

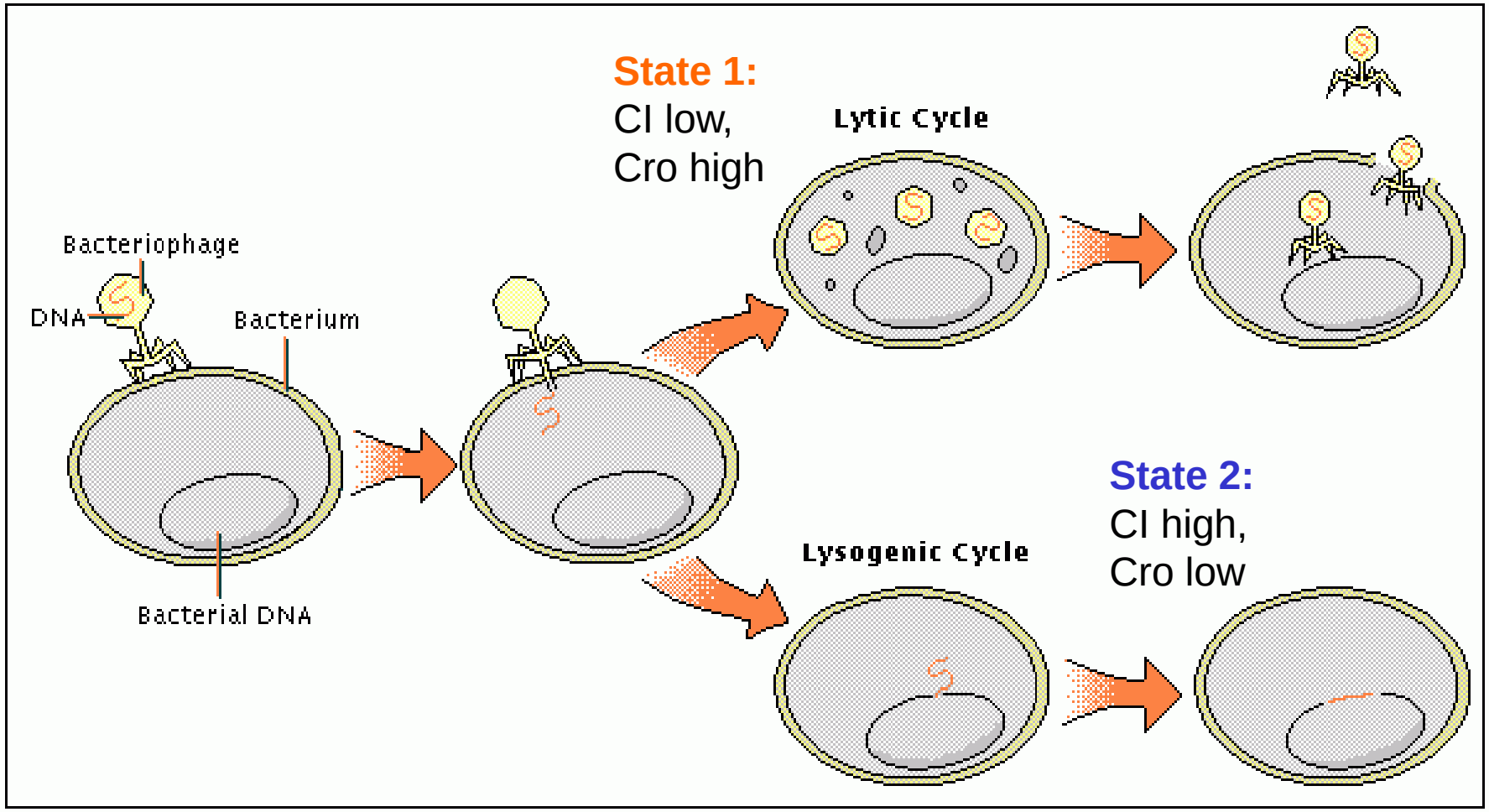
Ptashne & Gann, Genes and Signals



- Simple bistable switch (A represses B; B represses A)
- Two states:

1. Lytic (**CI** low, **Cro** high)
2. Lysogenic (**CI** high, **Cro** low)

Temperate bacteriophage: Lysis-lysogeny decisions



Genome of phage λ

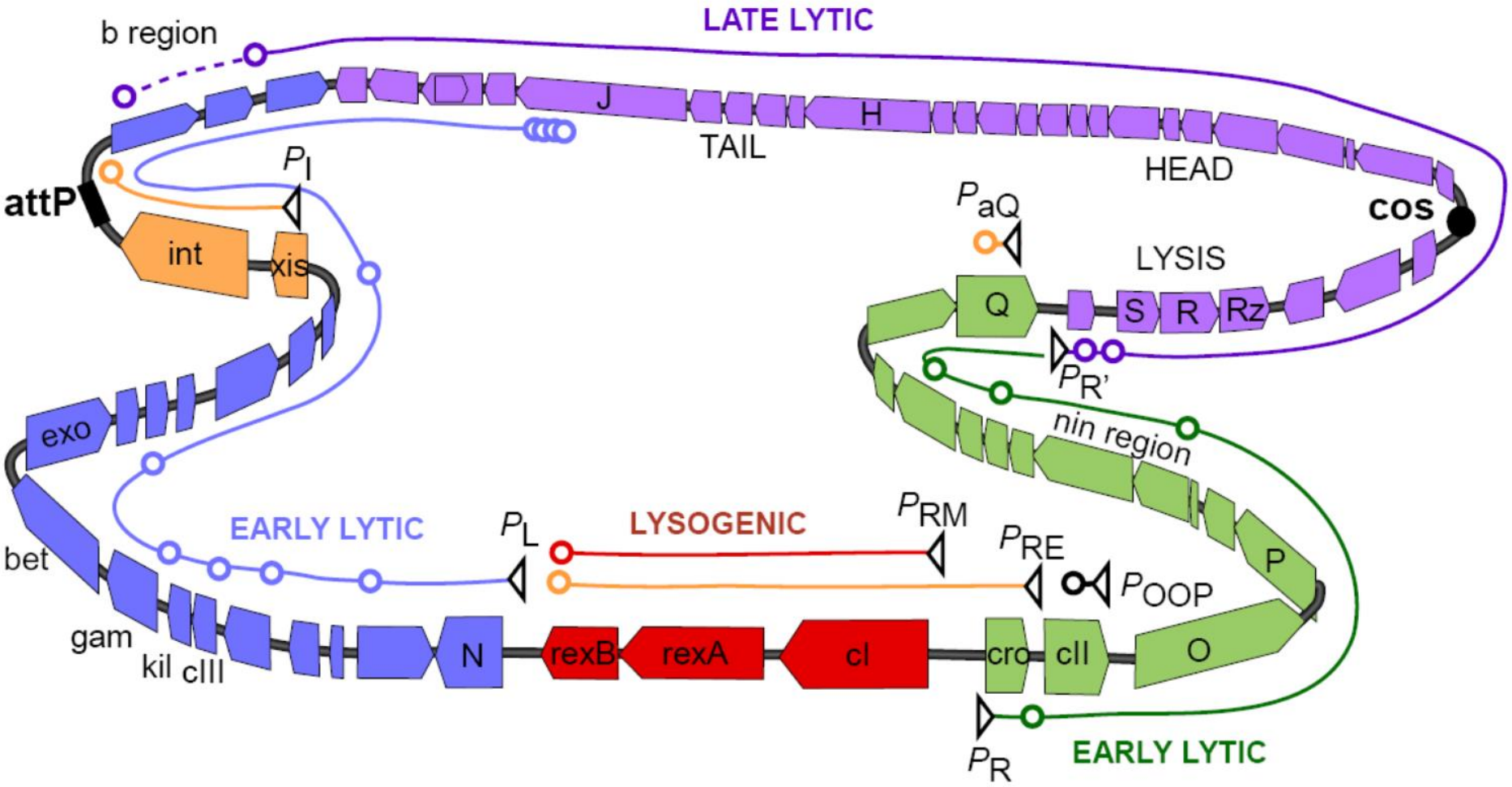
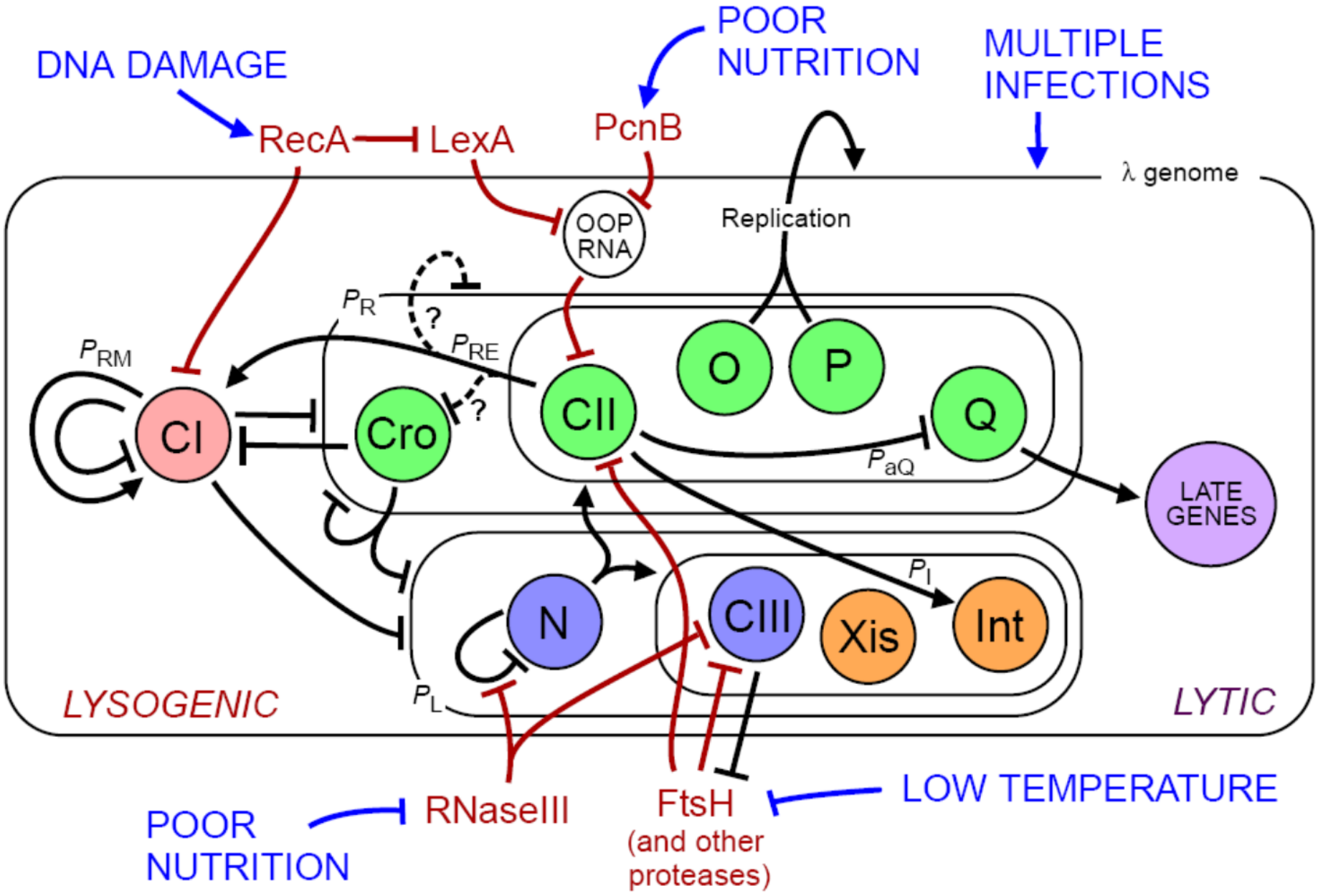
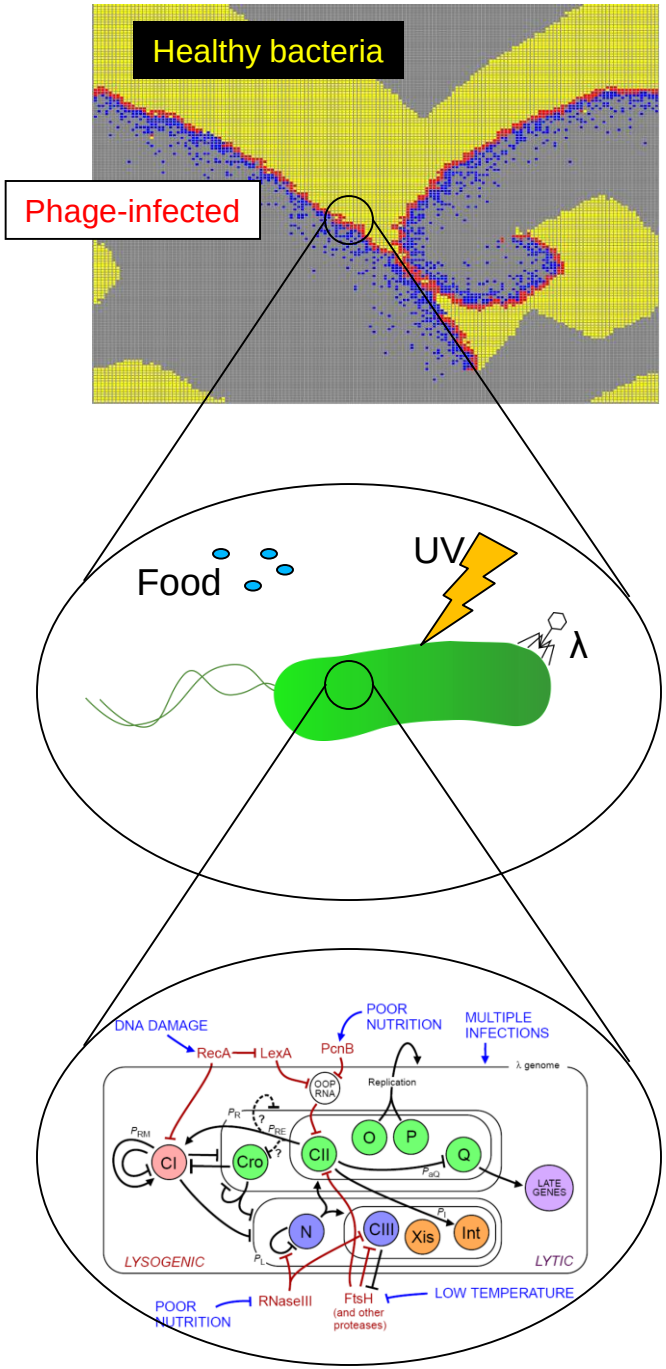


Image courtesy Keith Shearwin, Adelaide Univ.


DNA DAMAGE
RecA
LexA
POOR
NUTRITION
PcnB
MULTIPLE
INFECTIONS
λ genome
OOP
RNA
Replication
P_R
P_{RE}
P_{RM}
CI
Cro
CII
O
P
Q
P_{aQ}
LATE
GENES
N
CIII
Xis
Int
P_I
P_L
LYSOGENIC
LYTIC
POOR
NUTRITION
RNaseIII
FtsH
(and other
proteases)
LOW TEMPERATURE



Population/Ecosystem level

What are good lysis-lysogeny strategies for a phage when, say, it is competing with other phage species for a bacterial host?

How are the population (and evolutionary) dynamics of phage-bacteria ecosystems influenced by different bacterial defences against phage?

Cellular level

Why is only a narrow 5-15% lysogeny percentage observed in laboratory phage infections?

What conditions make a phage-infected bacterium go preferentially lytic, or lysogenic?

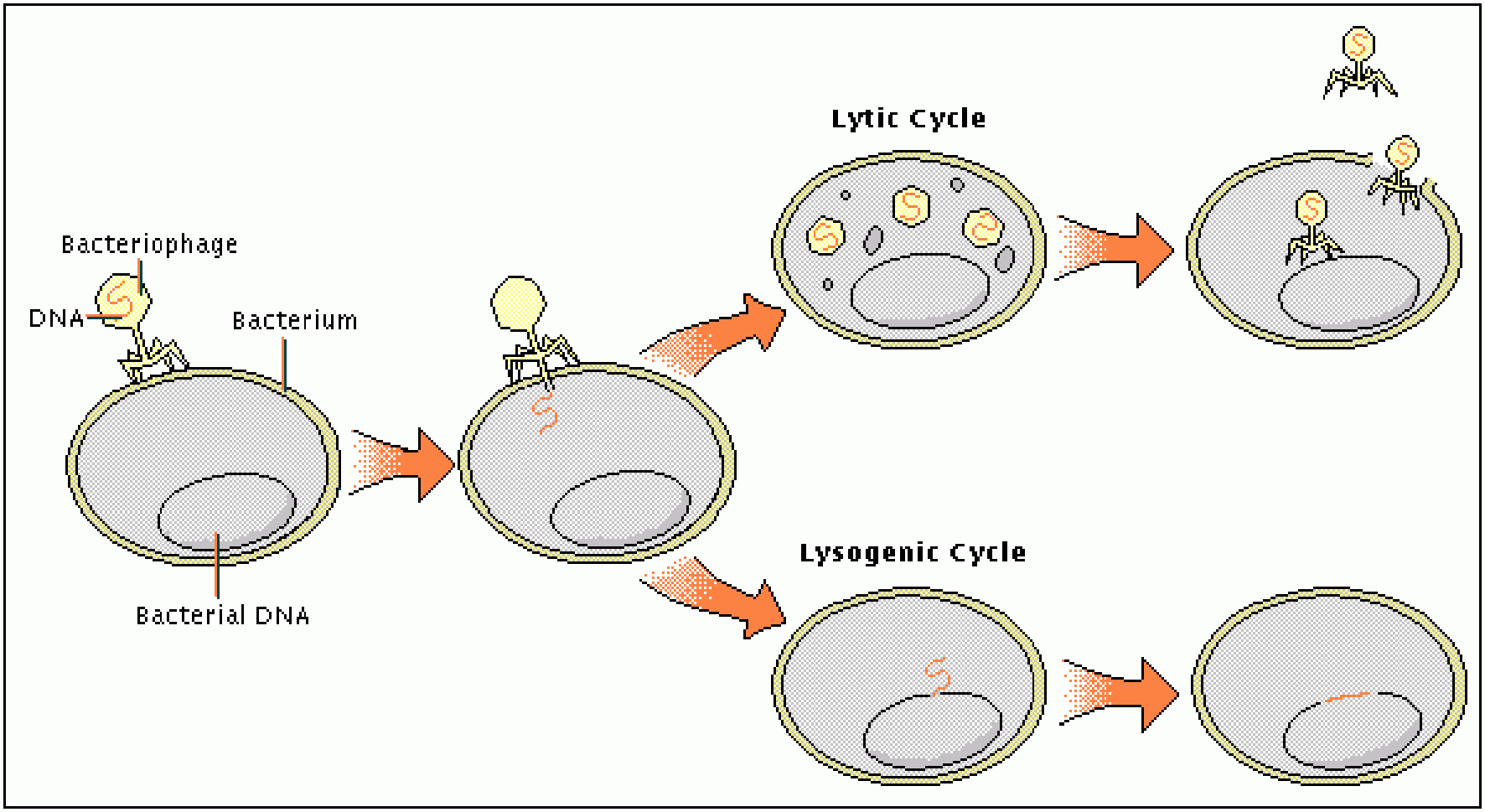
What aspects of the bacterial cell state bias the decision?

Subcellular level

How is the lysis-lysogeny decision regulated?
What produces bistability?
What makes the network robust to noise?

How does the phage network integrate information about the environment (e.g. does it use bacterial quorum sensing)?

Observed lysogeny propensity lies in a narrow range

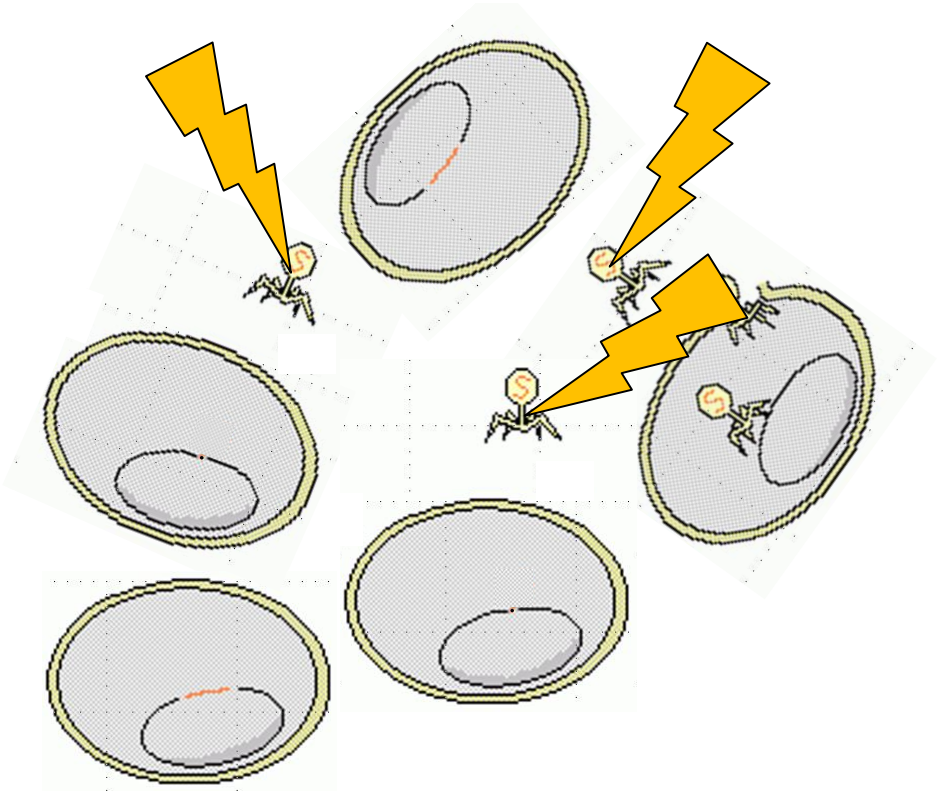


In bulk experiments, lysogeny percentage is observed to be **5-15%** for a wide variety of temperate phage

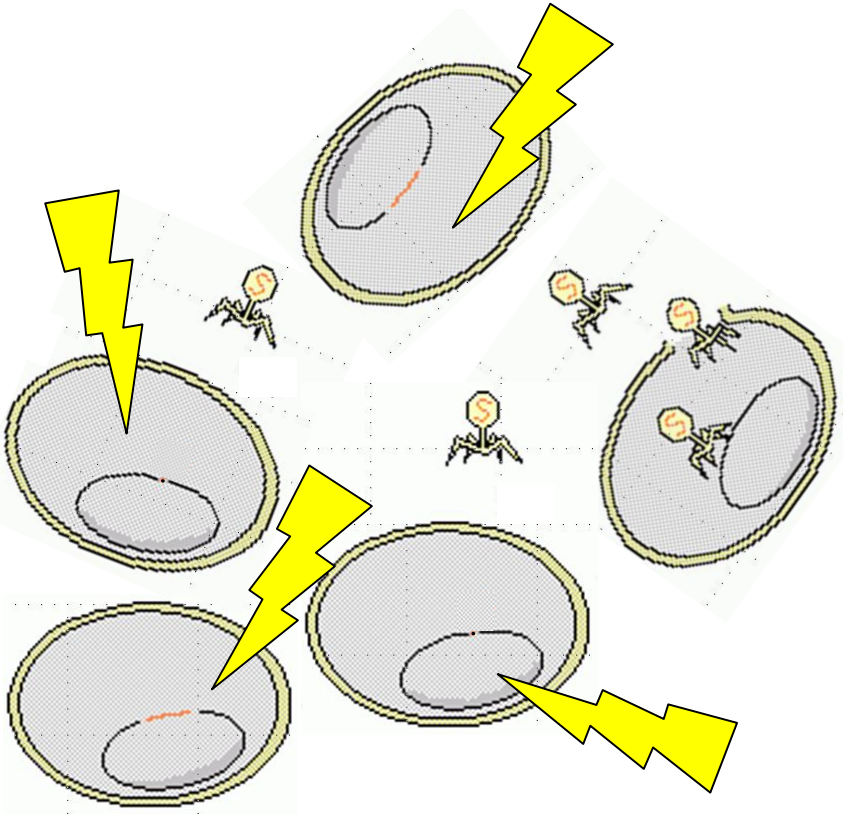
Ikeuchi, Kurahashi. 1978. J Bacteriol 134(2):440 – 445; Kourilsky. 1973. Mol Gen Genet 195; Maynard et al. 2010. PLoS Genet 6(7):e1001017; Broussard et al. 2013. Mol Cell 49(2):237–248; Schubert et al. 2007. Genes Dev 21:2461–2472; Hong et al. 1971. PNAS 68(9):2258-2262.

Bet hedging in an uncertain environment

Environmental conditions that are dangerous for free phage



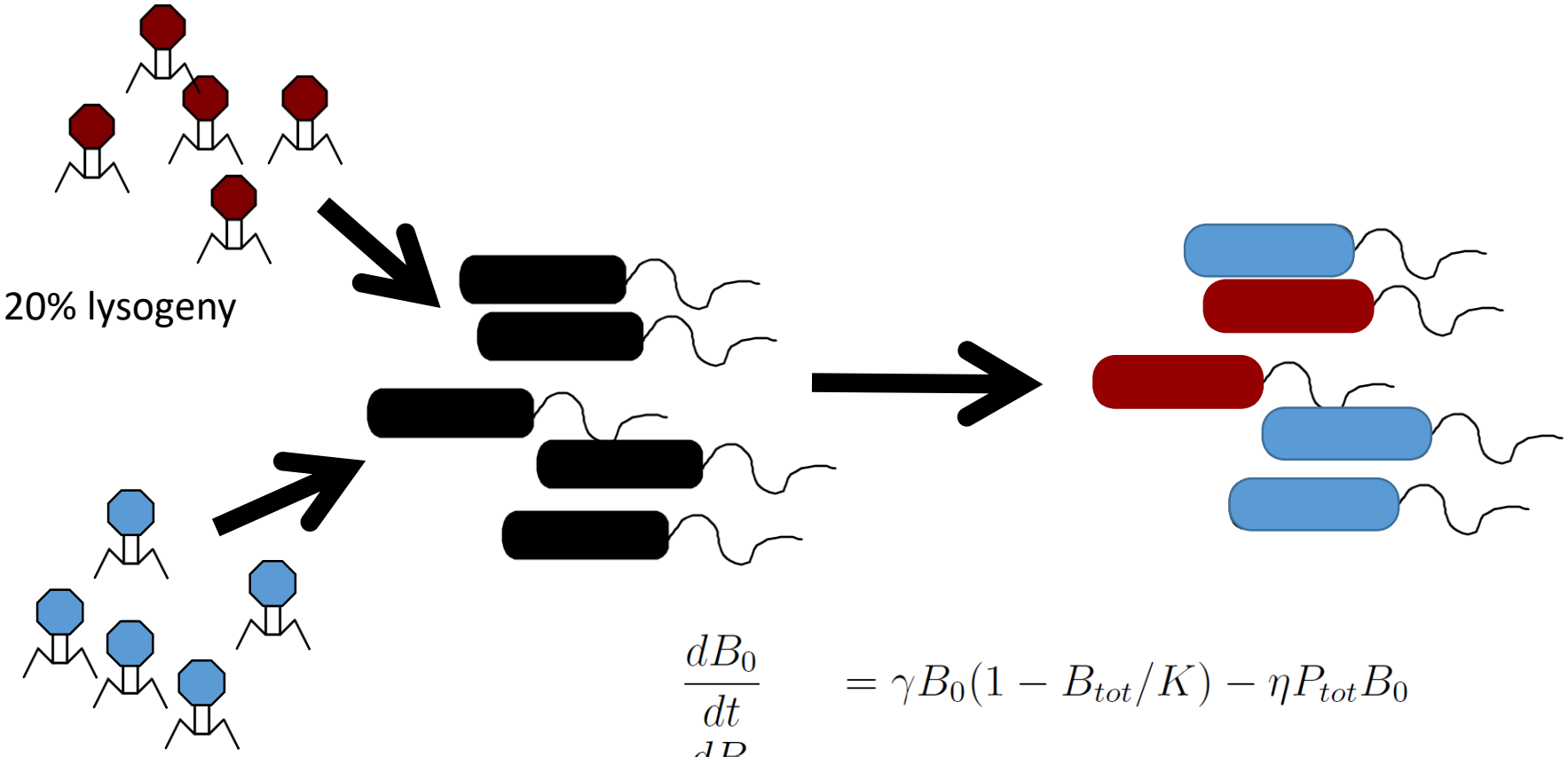
Environmental conditions that are dangerous for bacteria



Optimal bet hedging: lysogeny % is set by the relative frequencies and intensities of the different types of environmental catastrophes

Kelly, J. L., Jr. A new interpretation of information rate. Bell Syst. Tech. J. 35, 917–926 (1956); Avlund, Dodd, Semsey, Sneppen, Krishna (2009) Why do phage play dice? J. Virology 83, 11416; Maslov, Sneppen (2015) Well-temperate phage: optimal bet-hedging against local environmental collapses. Sci. Rep. 5:10523.

An alternative: Phage competition

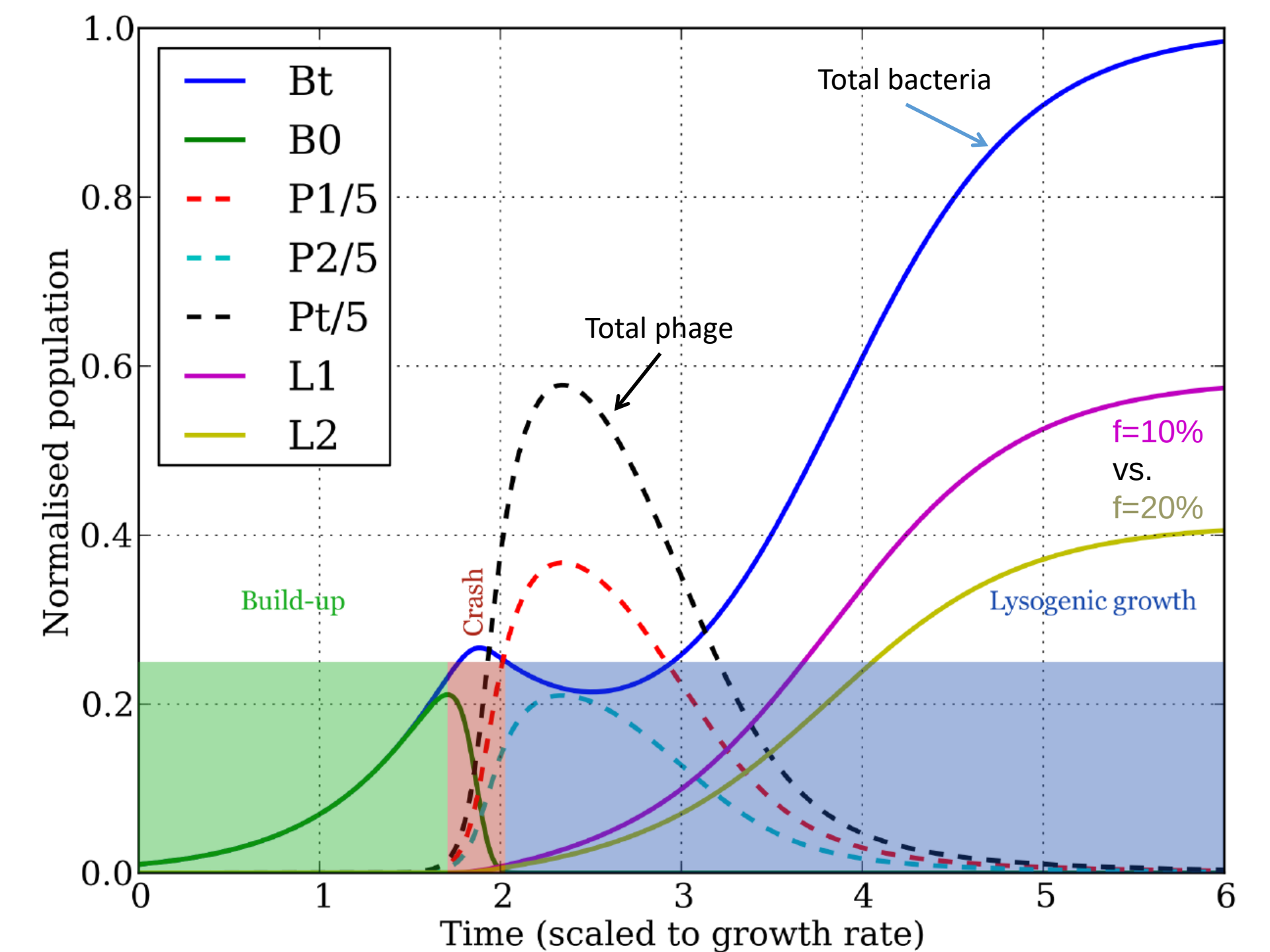


$$\frac{dB_0}{dt} = \gamma B_0(1 - B_{tot}/K) - \eta P_{tot} B_0$$

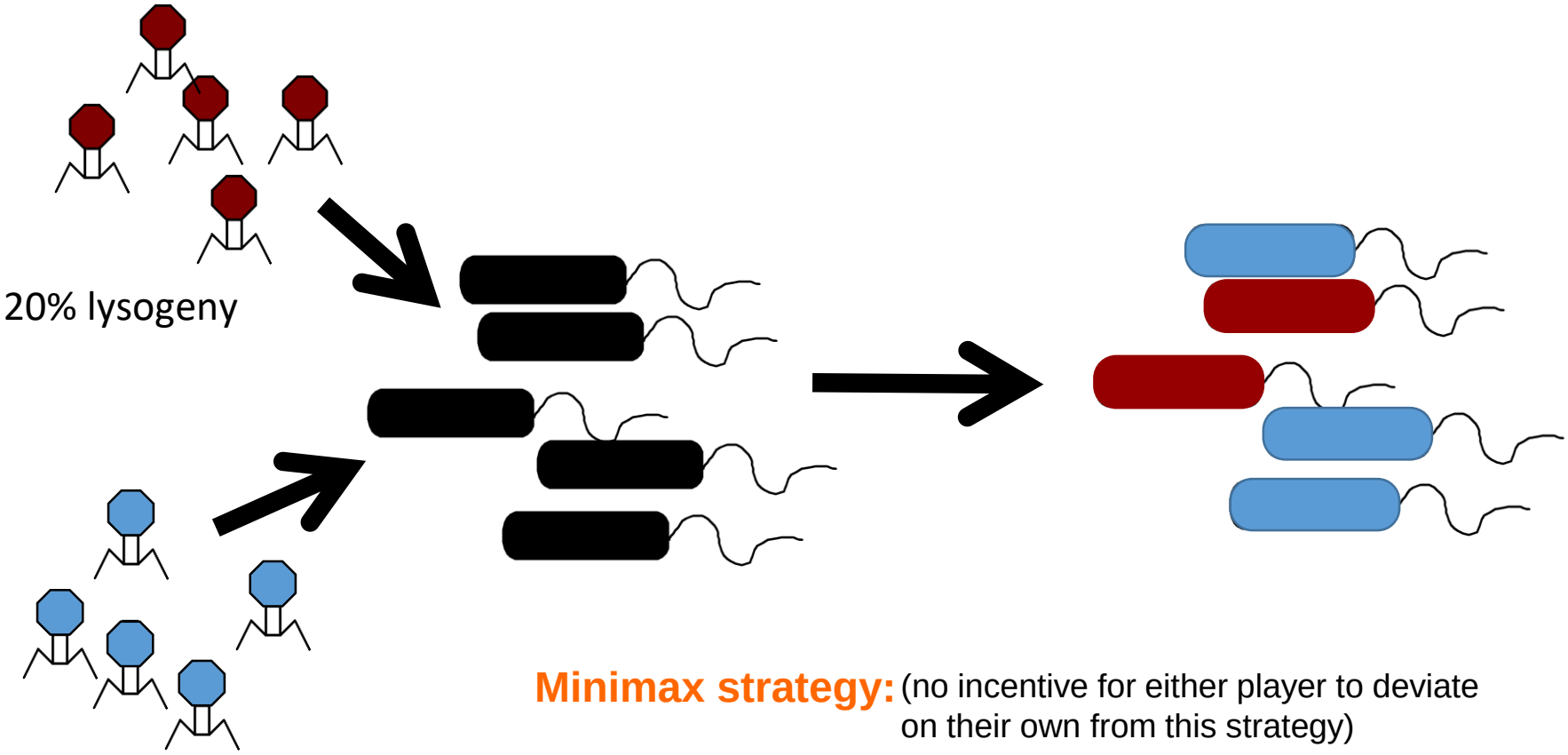
$$\frac{dB_i}{dt} = \gamma B_i(1 - B_{tot}/K) + \eta P_i B_0 - \delta B_i$$

$$\frac{dL_i}{dt} = \gamma L_i(1 - B_{tot}/K) + f_i \delta B_i$$

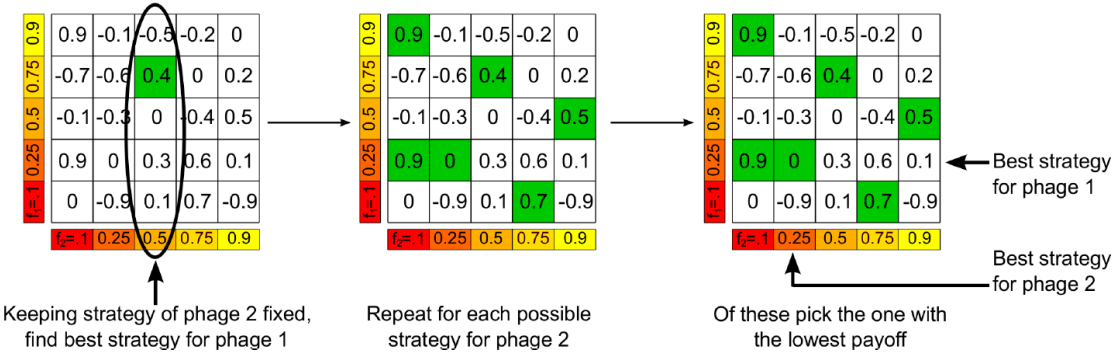
$$\frac{dP_i}{dt} = \beta(1 - f_i)\delta B_i - \eta P_i B_{tot}$$


Bt
B0
P1/5
P2/5
Pt/5
L1
L2
Total bacteria
Total phage
f=10%
vs.
f=20%
Build-up
Crash
Lysogenic growth
Normalised population
Time (scaled to growth rate)
0.0
0.2
0.4
0.6
0.8
1.0
0
1
2
3
4
5
6

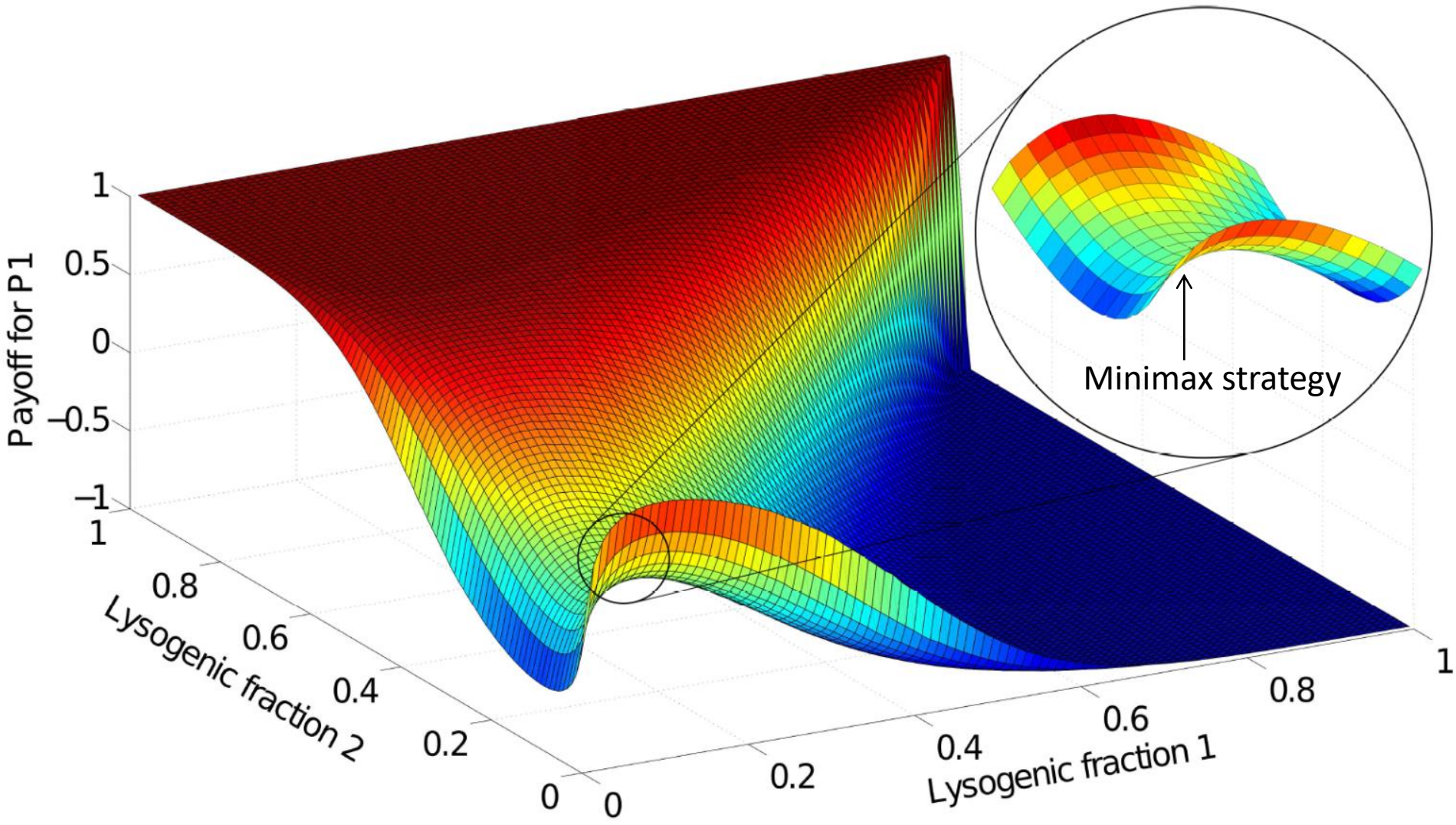
A game-theory perspective

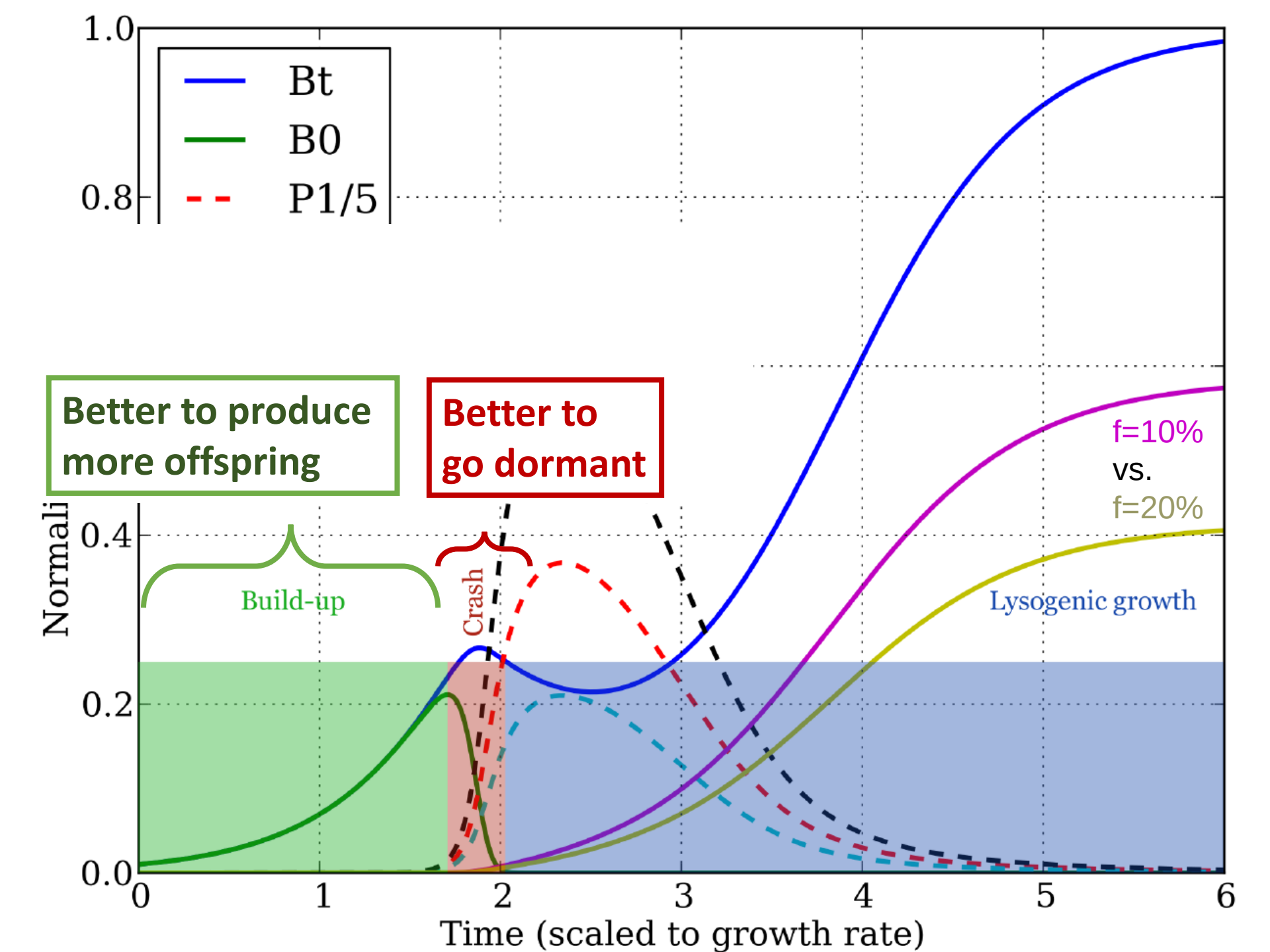


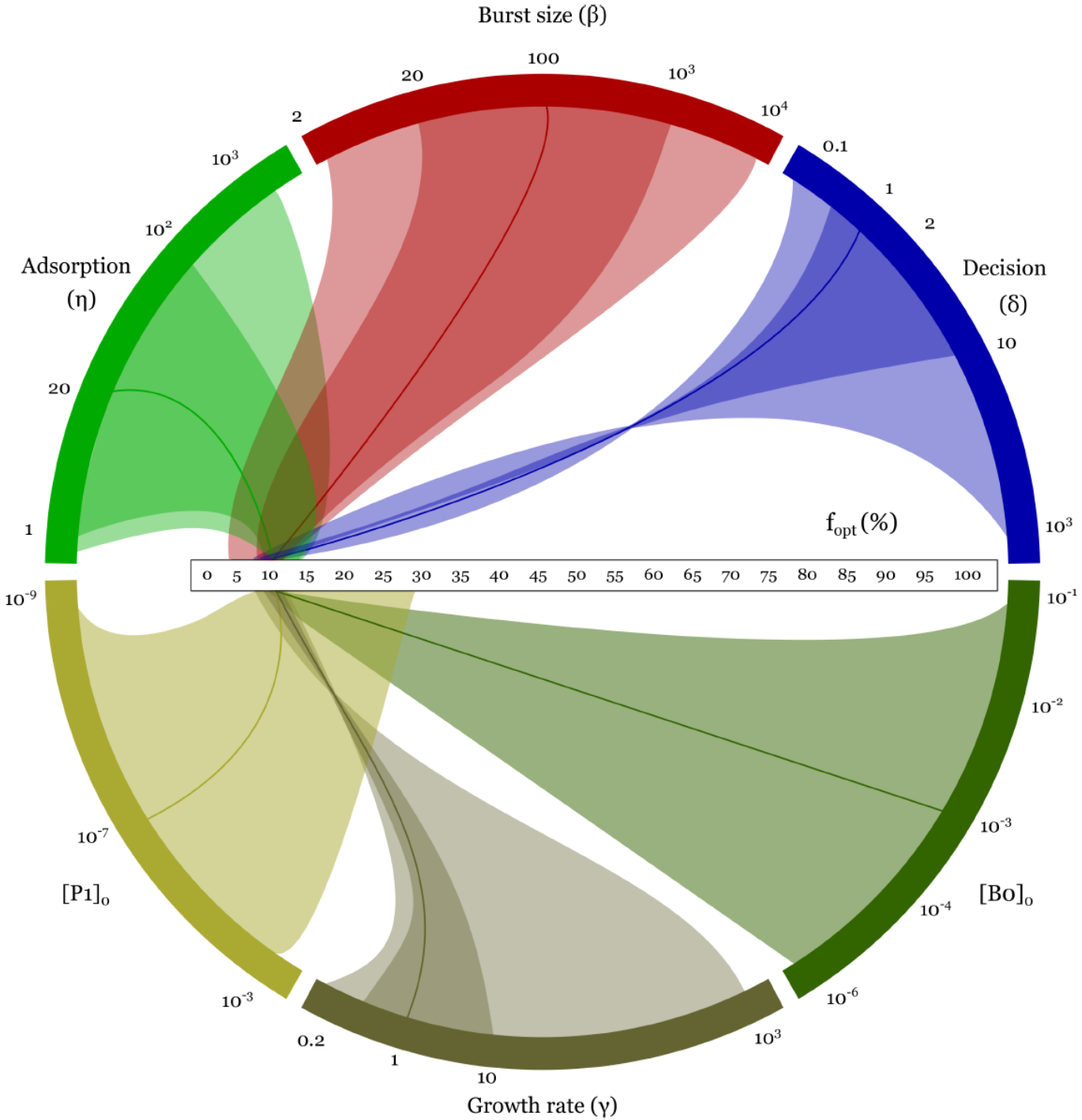
Minimax strategy: (no incentive for either player to deviate on their own from this strategy)

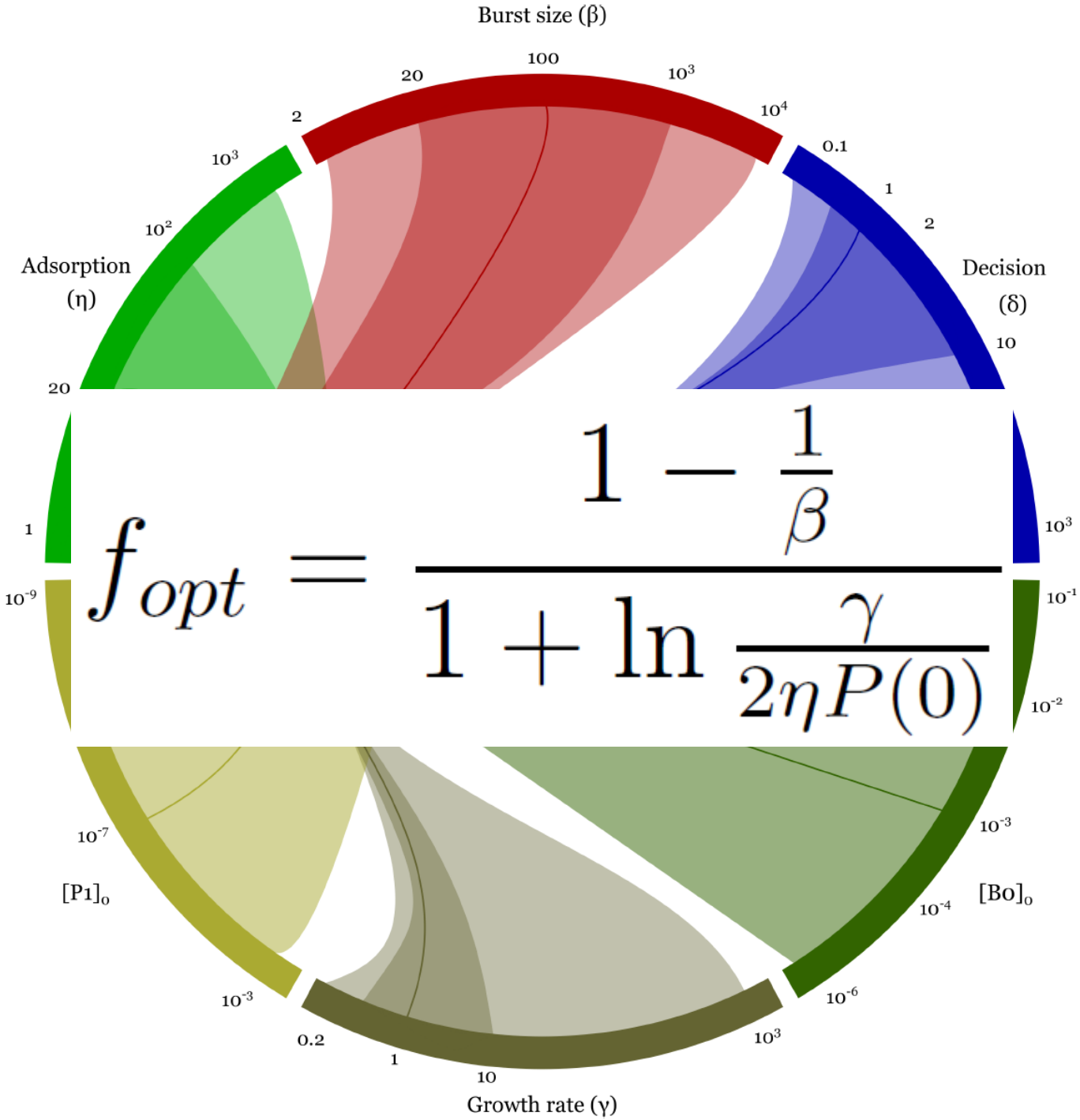


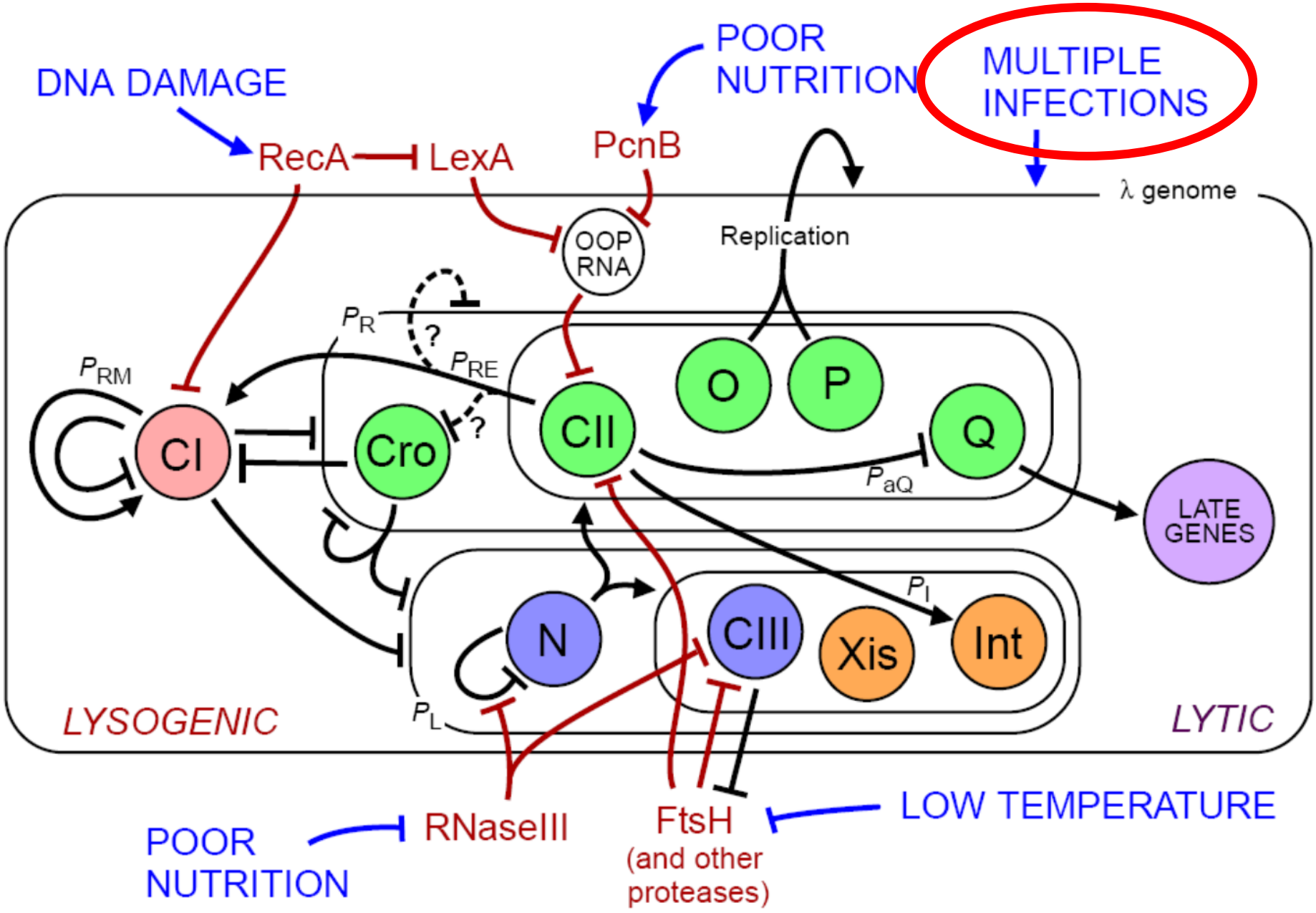
2-player symmetric zero-sum game



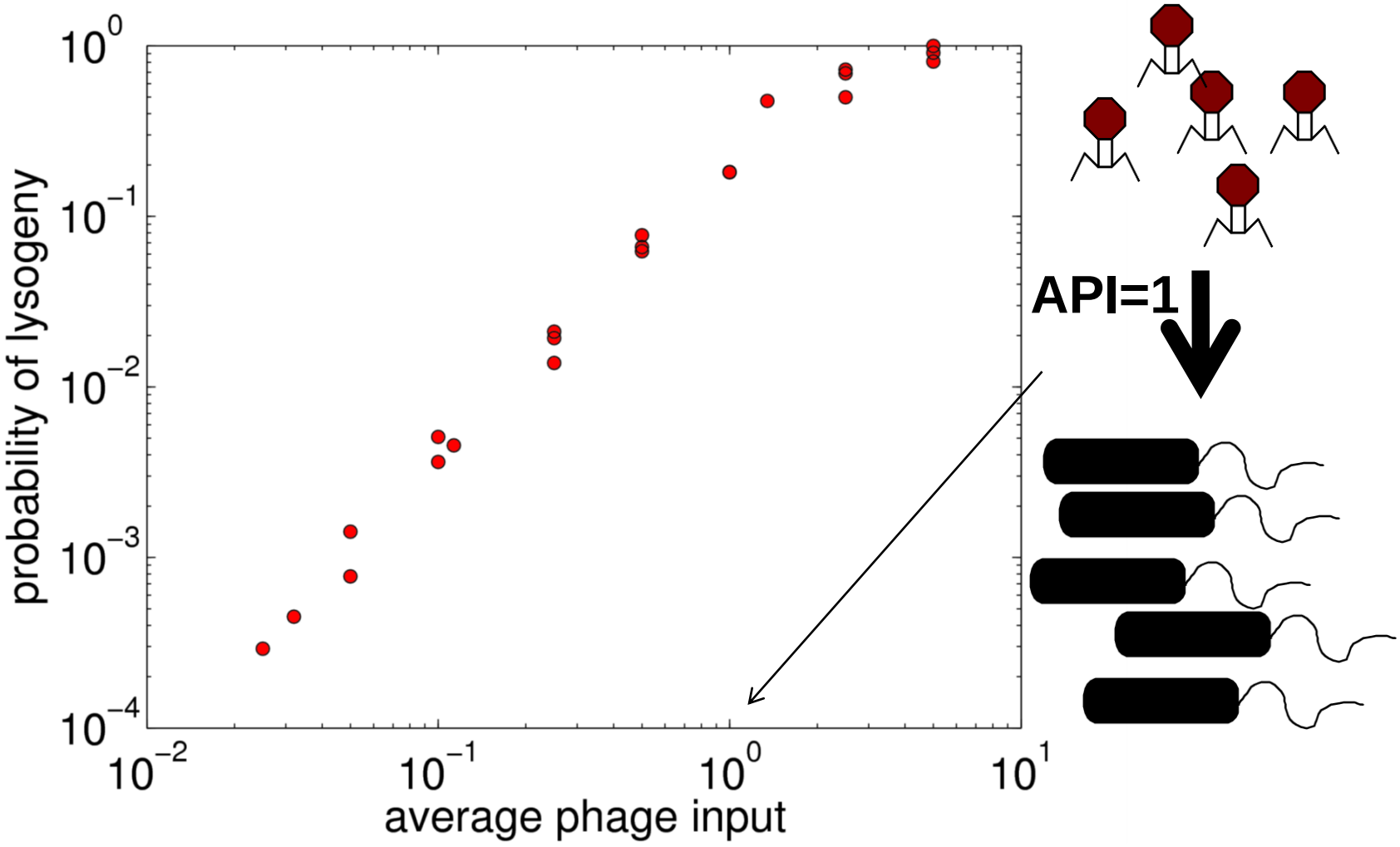

Bt
B0
P1/5
Better to produce more offspring
Better to go dormant
f=10%
vs.
f=20%
Build-up
Crash
Lysogenic growth
Normali
Time (scaled to growth rate)
1.0
0.8
0.4
0.2
0.0
0
1
2
3
4
5
6


Burst size (β)
100
20
2
10^3
10^4
Adsorption
(η)
10^3
10^2
20
1
10^-9
Decision
(δ)
0.1
1
2
10
10^3
f_opt (%)
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100
10^-1
10^-2
10^-3
10^-4
10^-6
[Bo]_0
10^-7
[P1]_0
10^-3
0.2
1
10
10^3
Growth rate (γ)


Burst size (β)
100
20
2
10^3
10^4
0.1
1
2
10
Adsorption
(η)
20
1
10^-9
Decision
(δ)
10^3
10^-1
10^-2
$f_{opt} = \frac{1 - \frac{1}{\beta}}{1 + \ln \frac{\gamma}{2\eta P(0)}}$
10^-7
[P1]0
10^-3
0.2
1
10
Growth rate (γ)
10^3
10^-6
10^-4
10^-3
[Bo]0


DNA DAMAGE
RecA
LexA
POOR
NUTRITION
PcnB
MULTIPLE
INFECTIONS
λ genome
OOP
RNA
Replication
P_R
?
P_{RE}
P_{RM}
CI
Cro
?
CII
O
P
Q
P_{aQ}
LATE
GENES
P_I
N
CIII
Xis
Int
P_L
LYSOGENIC
LYTIC
POOR
NUTRITION
RNaseIII
FtsH
(and other
proteases)
LOW TEMPERATURE

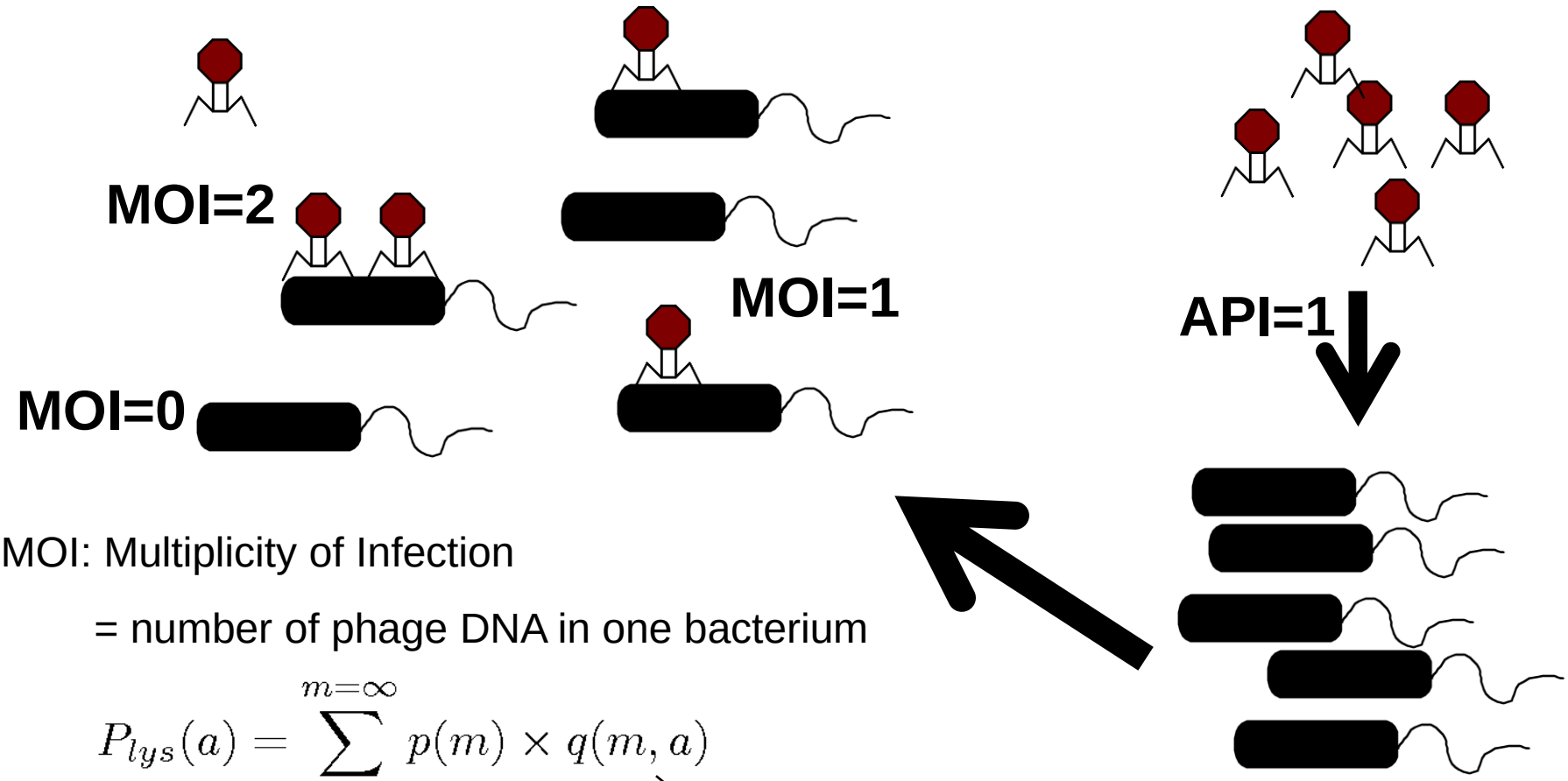
Kourilsky's experiment



(API: ratio of total phage to total bacteria)

P. Kourilsky: Molec. gen, Genet. 122, 183-195 (1973); Biochimie 56, 1517-1523 (1974).

Kourilsky's experiment



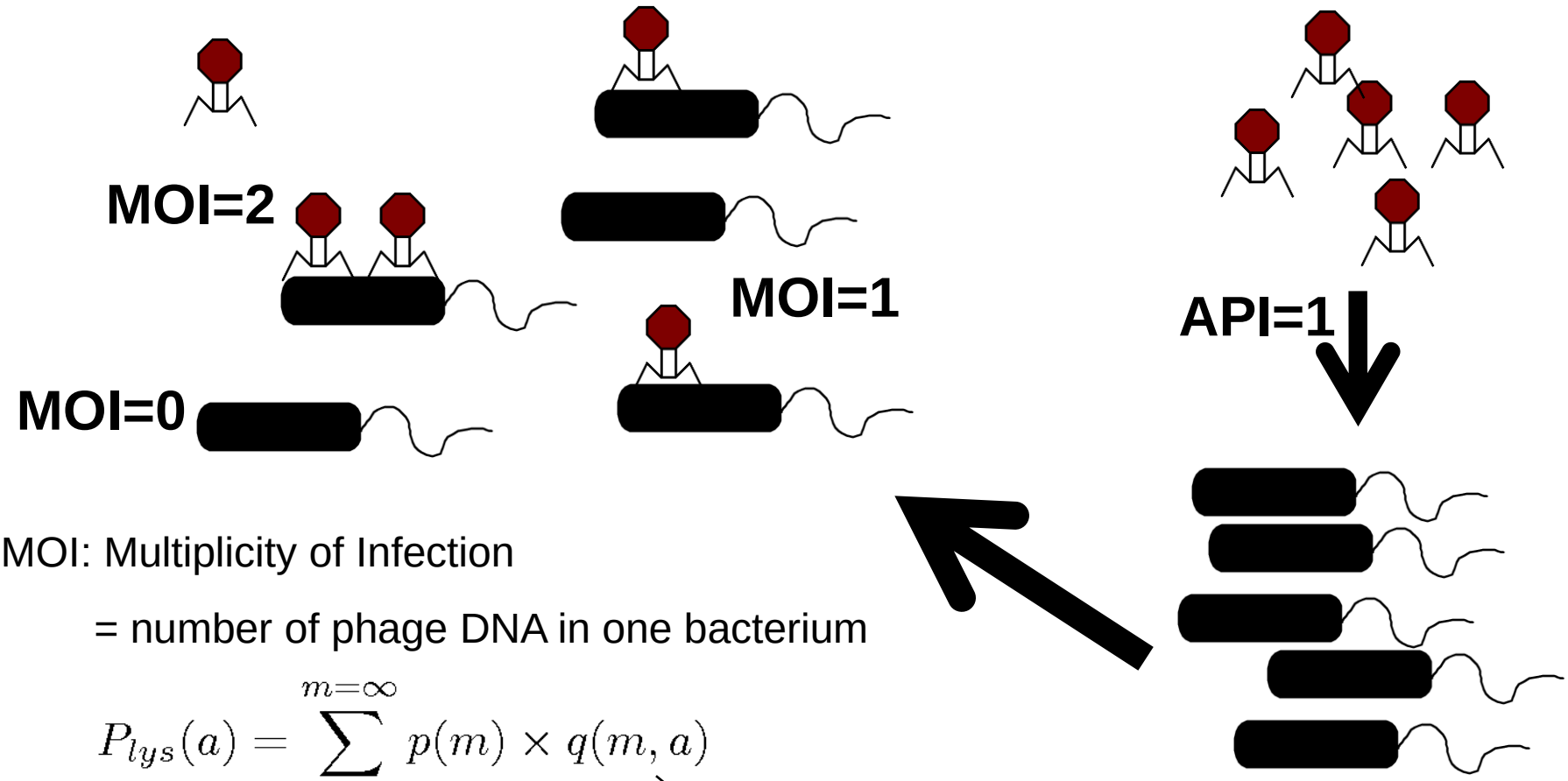
MOI: Multiplicity of Infection

= number of phage DNA in one bacterium

$$P_{lys}(a) = \sum_{m=0}^{m=\infty} p(m) \times q(m,a)$$

Prob. Lysogeny if API is *a* = **Prob. Lysogeny if MOI is *m*** X **Prob. of getting MOI *m* if API is *a***

Kourilsky's experiment



MOI: Multiplicity of Infection

= number of phage DNA in one bacterium

$$P_{lys}(a) = \sum_{m=0}^{m=\infty} p(m) \times q(m,a)$$

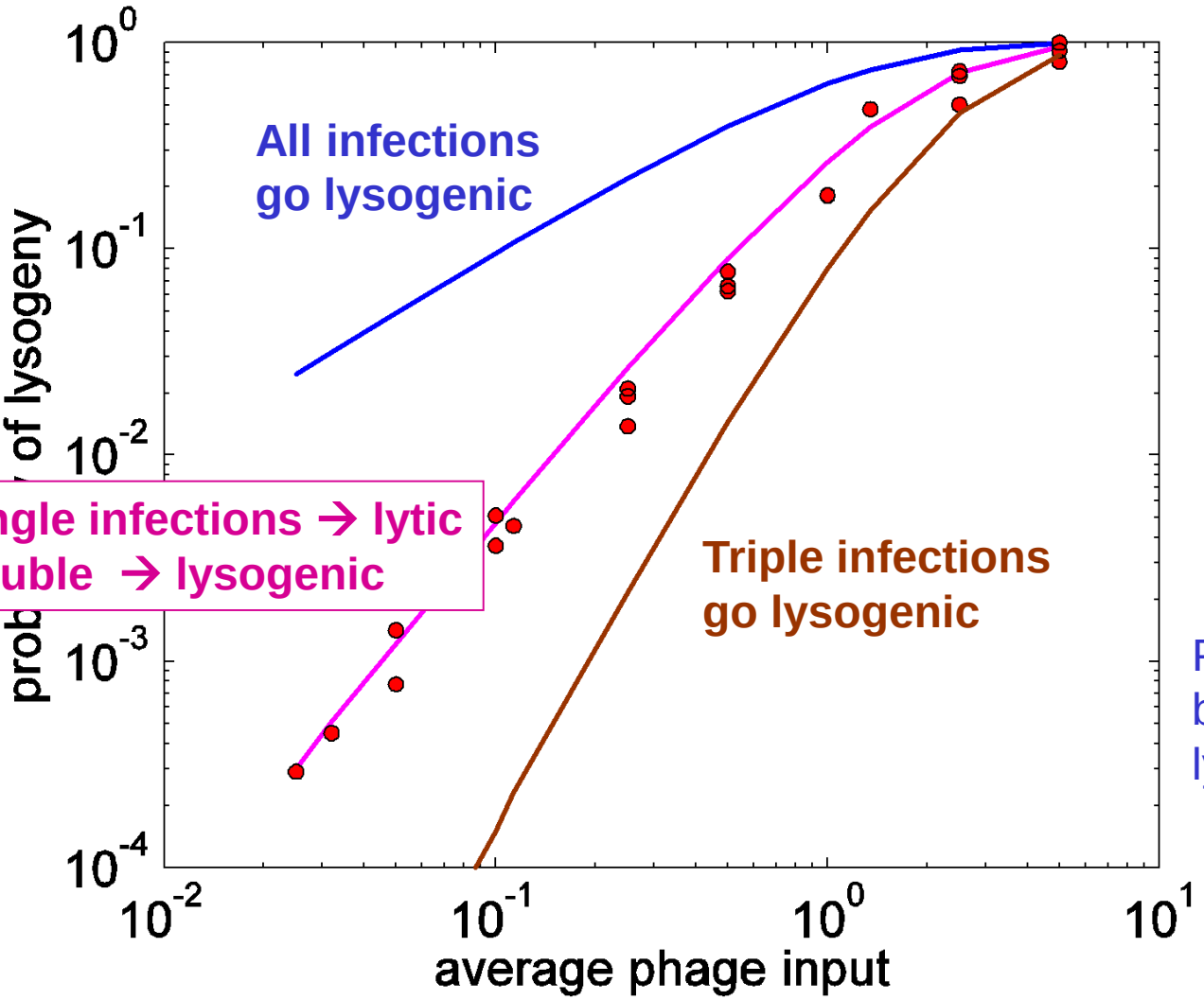
Prob. of getting MOI *m* if API is *a*

$$q(m,a) = \frac{a^m}{m!} e^{-a}$$

Poissonian assumption:

Each phage randomly & independently finds a bacterium to infect

Kourilsky's experiment



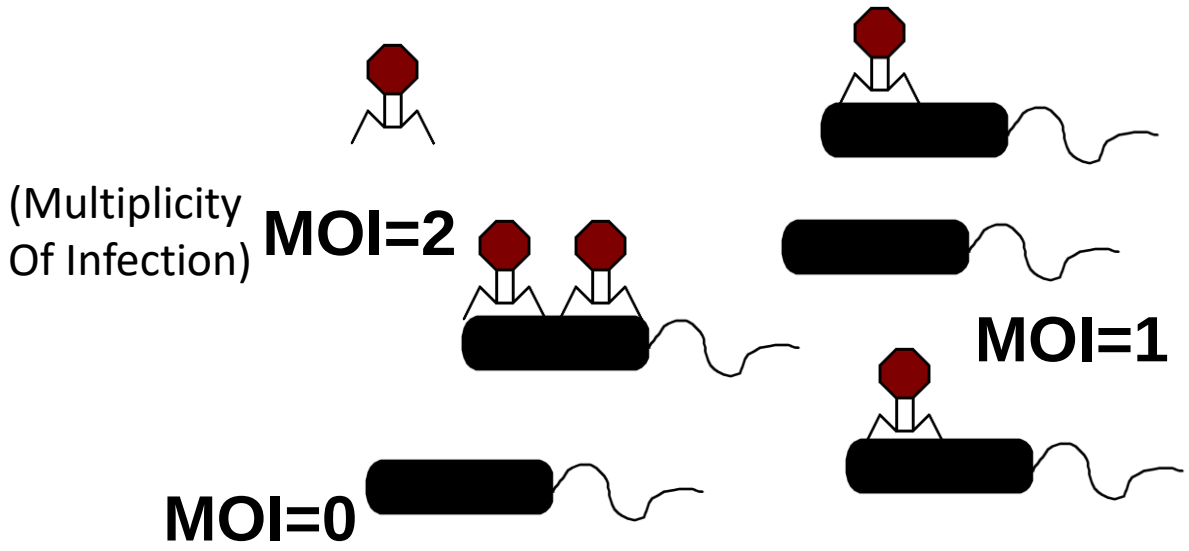
Number of phage DNA in one bacterium

m =	1	2	>2
	1	1	1
p(m)=	0	1	1
	0	0	1

Probability that bacterium will go lysogenic

M. Avlund, I. B. Dodd, S. Semsey, K. Sneppen, S. Krishna
Why do phage play dice? J. Virology 83, 11416 (2009).

Heterogeneity due to multiple infections



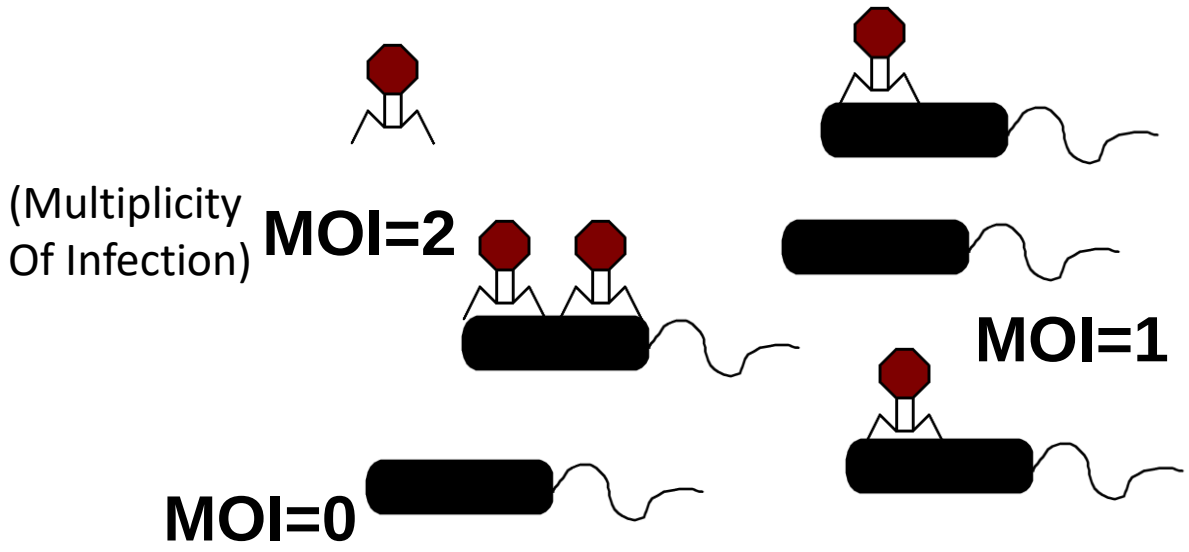
What is the most competitive strategy if lysogeny % is allowed to be different for different MOI?

$$\begin{aligned}
\frac{dB_0}{dt} &= \gamma B_0(1 - B_{tot}/K) - \eta P_{tot} B_0 \\
\frac{dB_i}{dt} &= \gamma B_i(1 - B_{tot}/K) + \eta P_i B_0 - \delta B_i \\
\frac{dL_i}{dt} &= \gamma L_i(1 - B_{tot}/K) + f_i \delta B_i \\
\frac{dP_i}{dt} &= \beta(1 - f_i)\delta B_i - \eta P_i B_{tot}
\end{aligned}$$

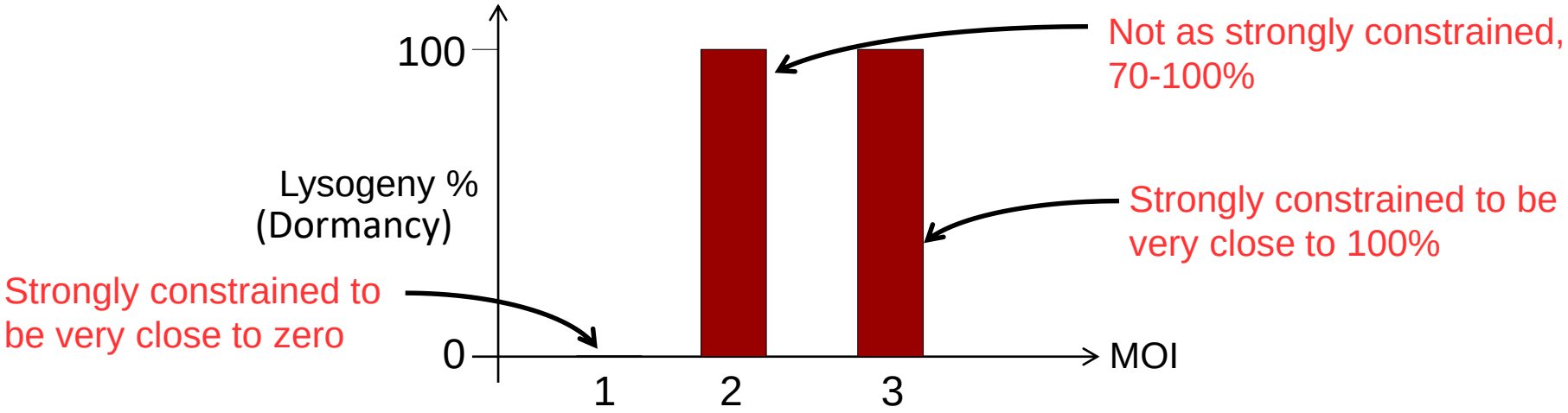
Modify previous equations to allow multiple infections

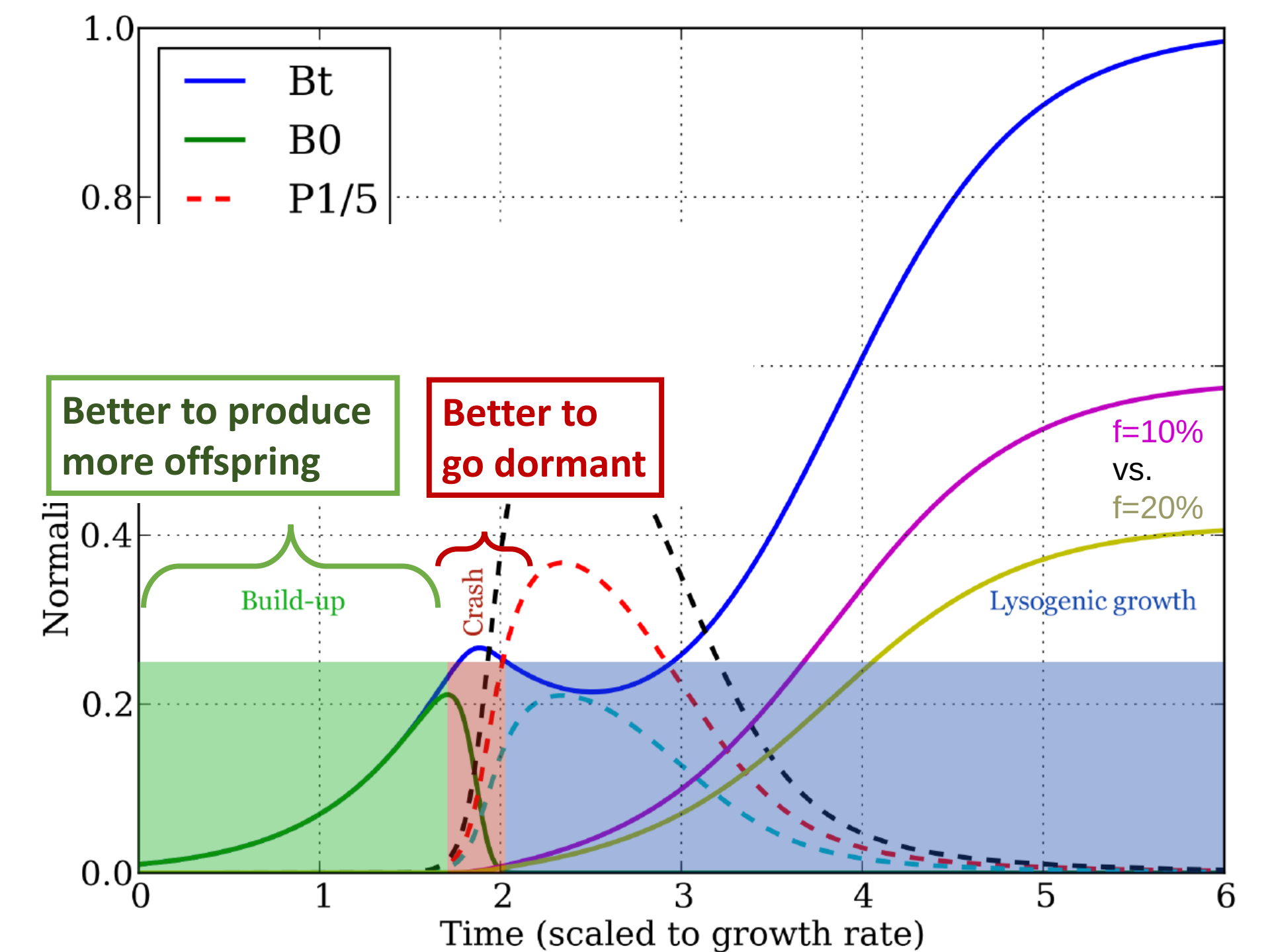
$$\begin{aligned}
\frac{dB_0}{dt} &= \gamma B_0(1 - B_{tot}/K) - \eta P_{tot} B_0 \\
\frac{dB_{i,1}}{dt} &= \eta P_i B_0 - \eta P_i B_{i,1} - \delta B_{i,1} \\
\frac{dB_{i,m}}{dt} &= \eta P_i B_{i,m-1} - \eta P_i B_{i,m} - \delta B_{i,m} \\
\frac{dL_i}{dt} &= \gamma L_i(1 - B_{tot}/K) + \delta \sum_{m=1}^{\infty} f_i(m) B_{i,m} \\
\frac{dP_i}{dt} &= \beta\delta \sum_{m=1}^{\infty} ((1 - f_i(m)) B_{i,m}) - \eta P_i B_{tot}.
\end{aligned}$$

Heterogeneity due to multiple infections



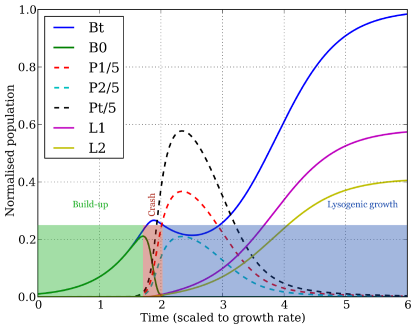
What is the most competitive strategy if lysogeny %
is allowed to be different for different MOI?

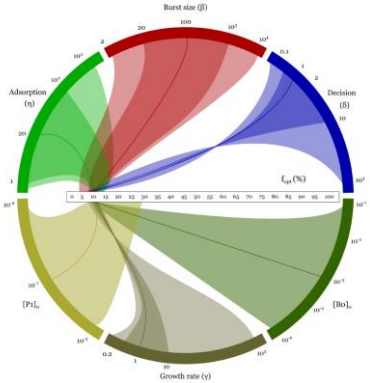



Bt
B0
P1/5
Better to produce more offspring
Better to go dormant
f=10%
vs.
f=20%
Build-up
Crash
Lysogenic growth
Normali
Time (scaled to growth rate)
1.0
0.8
0.4
0.2
0.0
0
1
2
3
4
5
6

Summary

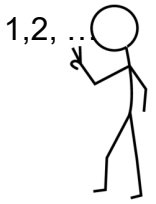
Phage competition puts an evolutionary pressure on the dormancy vs death decision





Optimal dormancy under competition is extremely robust to parameter variation, and matches experimental observations of 5-15%

We also predict that phage should learn to count!

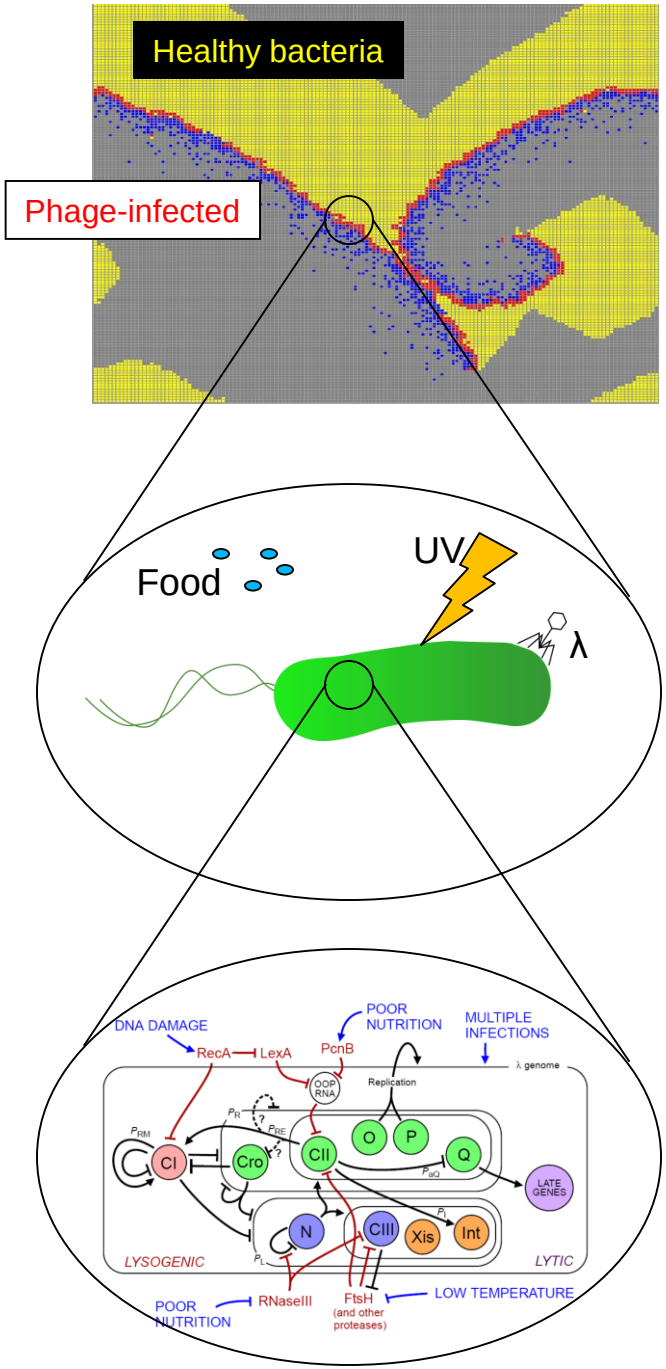


(Sinha V, Goyal A, Svenningsen SL, Semsey S and Krishna S (2017) Front. Microbiol. 8:1386.)

What other information is useful for a phage to make a “good” death vs dormancy decision? Density of bacteria? Bacterial growth rate? The microscopic state of an individual bacterium?

A phage can only receive information from inside a bacterium. So what bacterial information sensing systems does it hijack? Do bacteria actively manipulate the information a phage receives?

How do inevitable noise and uncertainty in information constrain the space of strategies for a phage?



Population/Ecosystem level

What are good lysis-lysogeny strategies for a phage when, say, it is competing with other phage species for a bacterial host?

How are the population (and evolutionary) dynamics of phage-bacteria ecosystems influenced by different bacterial defences against phage?

Cellular level

Why is only a narrow 5-15% lysogeny percentage observed in laboratory phage infections?

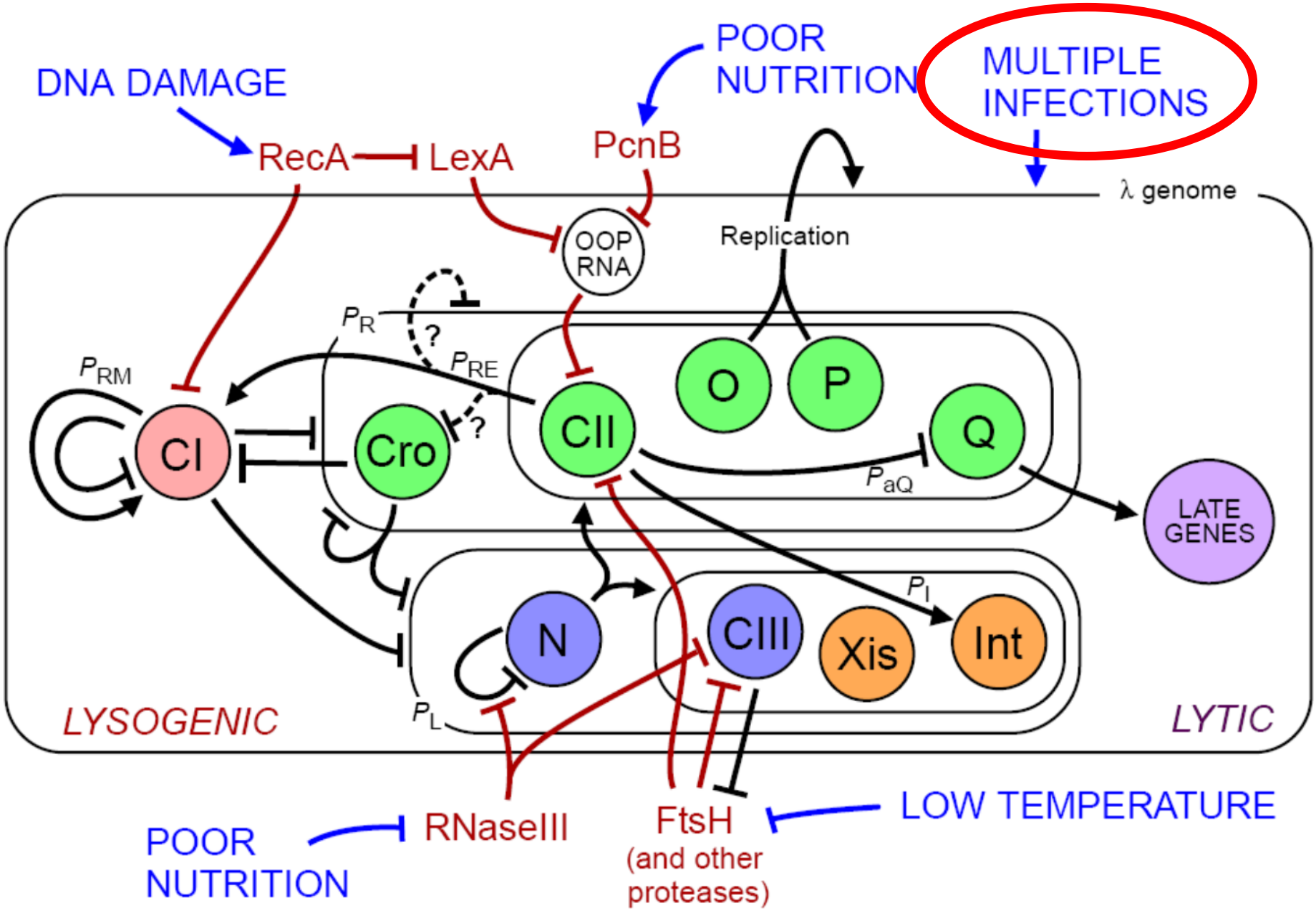
What conditions make a phage-infected bacterium go preferentially lytic, or lysogenic?

What aspects of the bacterial cell state bias the decision?

Subcellular level

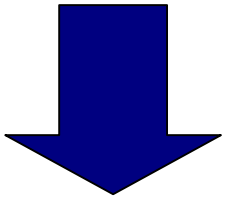
How is the lysis-lysogeny decision regulated?
What produces bistability?
What makes the network robust to noise?

How does the phage network integrate information about the environment (e.g. does it use bacterial quorum sensing)?


DNA DAMAGE
RecA
LexA
POOR
NUTRITION
PcnB
MULTIPLE
INFECTIONS
λ genome
OOP
RNA
Replication
P_R
?
P_{RE}
P_{RM}
CI
Cro
?
CII
O
P
Q
P_{aQ}
LATE
GENES
P_I
N
CIII
Xis
Int
P_L
LYSOGENIC
LYTIC
POOR
NUTRITION
RNaseIII
FtsH
(and other
proteases)
LOW TEMPERATURE

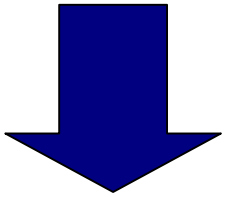
An alternate approach

Choose the building blocks



Build a class of dynamical systems

Subject them to some functional task



What range of behaviour is possible?

How would one construct a given behaviour?

Are there many ways of doing so?

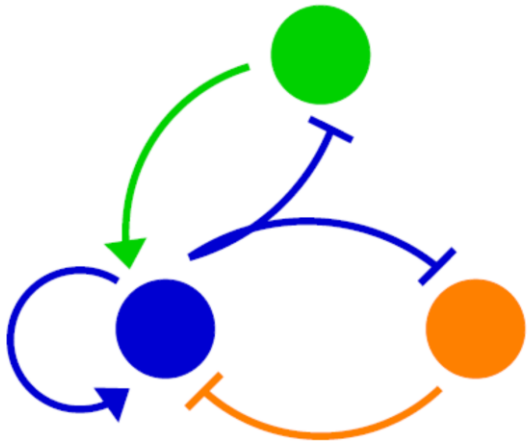
1-protein motifs

e.g. self-activator

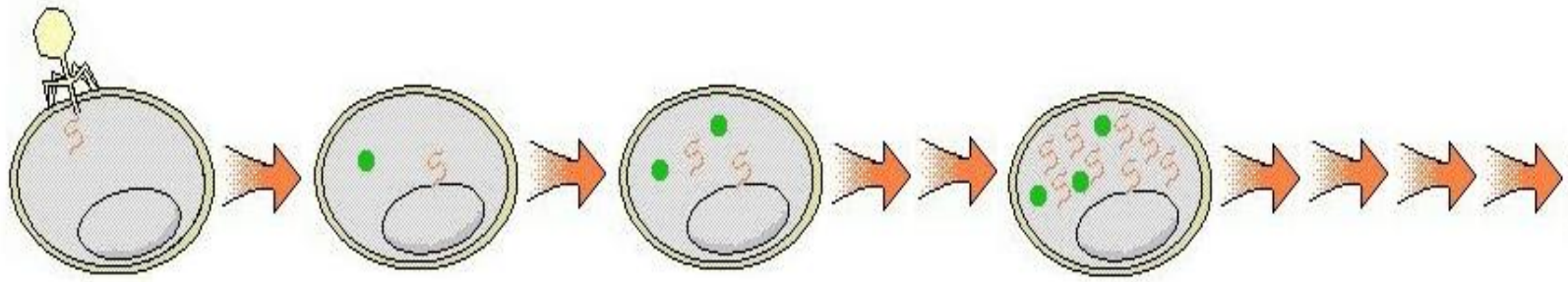
2-protein motifs

e.g. mutual repressors or mutual activators

3-protein motifs

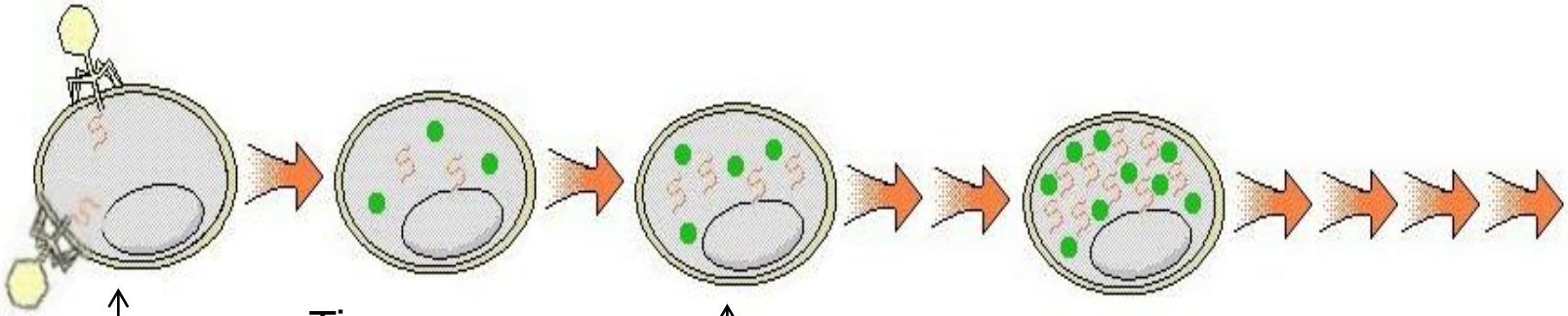


MOI=1



State 1

MOI=2



State 2

Initially all phage proteins are at zero

Time passes: proteins are produced

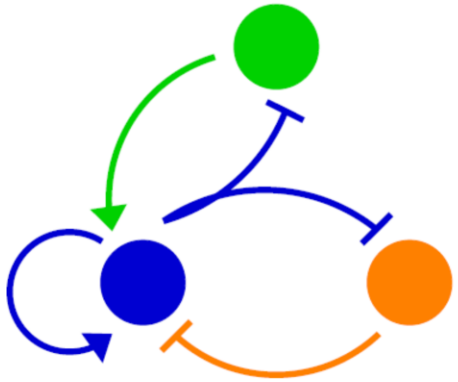
3 min

Phage genomes replicate

Task: find motifs that are bistable and can count genomes.

Network =

Motifs



x

Parameter sets

- Protein stabilities
- Production rates
- Repression, activation strengths and cooperativities

100 million ~ **100** **x** **1 million**

Example motif

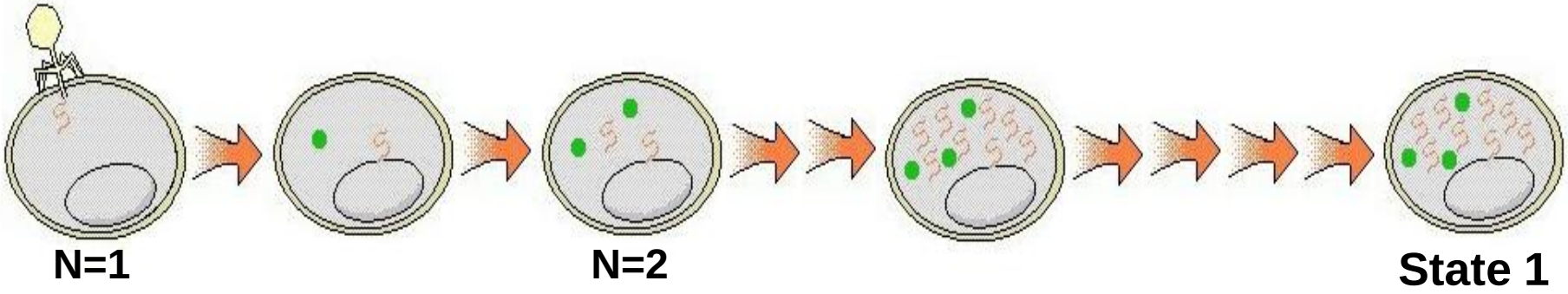
One self-activating protein

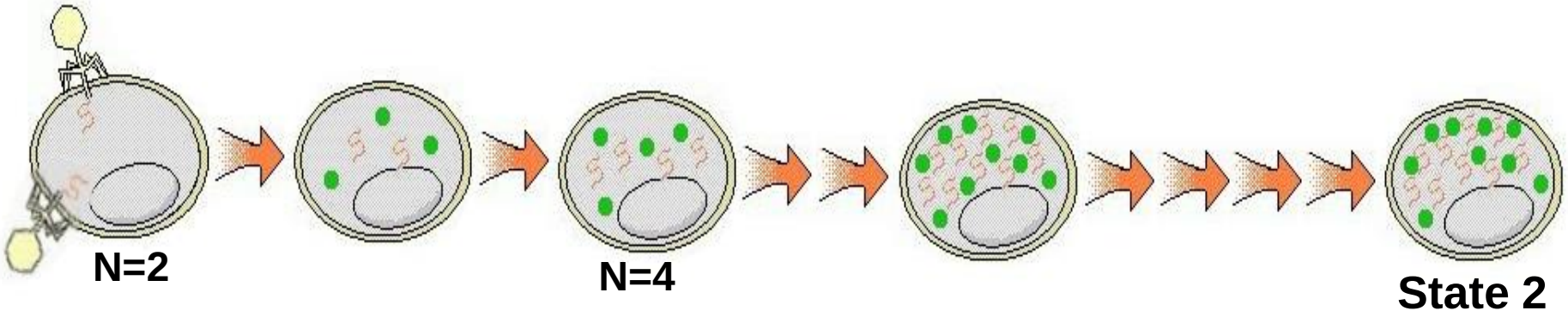
$$\frac{d(CI)}{dt} = N\left[\frac{(CI)^h}{(CI)^h + K^h}\right] - \gamma(CI)$$

Degradation rate of protein

Number of phage genomes

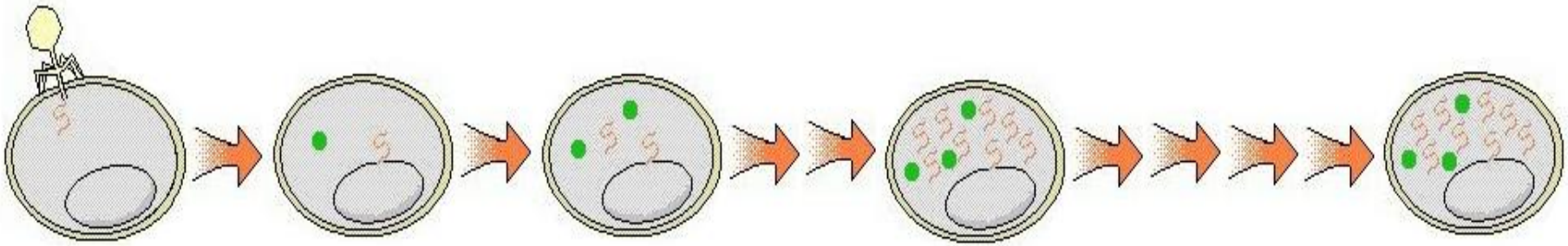
Concentration of protein





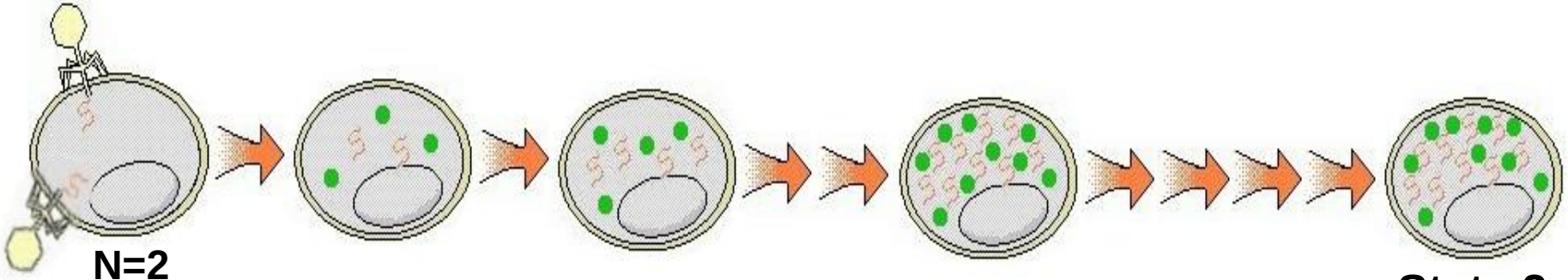
$$\frac{d(CI)}{dt} = N\left[\frac{(CI)^h}{(CI)^h + K^h}\right] - \gamma(CI)$$

Number of phage genomes



N=1

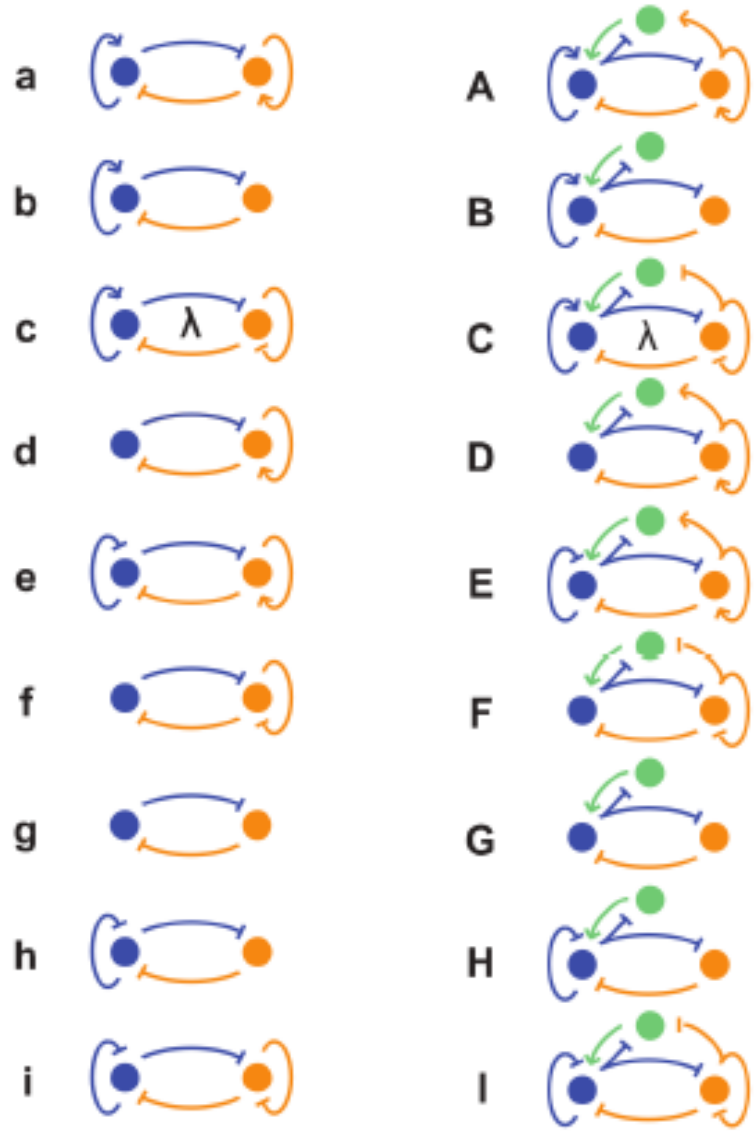
State 1



N=2

State 2

- Is state 1 sufficiently distinct from state 2?
- Are states 1 and 2 stable when N is brought down to 1?



There are many ways to make a bistable circuit that can also count.

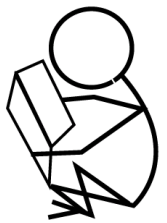
Motifs without positive feedback don't work

1 protein motifs don't work

2 protein mutual activators don't work

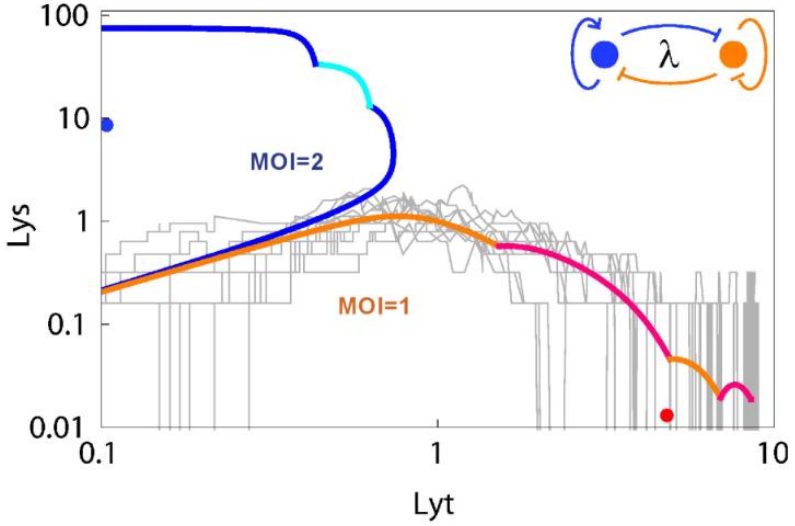
2 protein mutual repressors do work

Avlund, Dodd, Sneppen, Krishna (2009) J. Mol. Biol. 394, 681
Avlund, Krishna, Semsey, Dodd, Sneppen (2010) PLoS ONE 5(12): e15037



	Motif	Determ.	Stochastic
a		1112	0
b		1054	2
c		563	1
d		462	0
e		127	0
f		447	0
g		753	0
h		295	0
i		171	0
Total		5142	3

Two-protein motifs are very sensitive to noise



	Motif	Determ.	Stochastic
a		1112	0
b		1054	2
c		563	1
d		462	0
e		127	0
f		447	0
g		753	0
h		295	0
i		171	0
Total		5142	3

	Motif	Determ.	Stochastic *p.lys.cII*	Stochastic *p.lys/p.cII*	Stochastic *cII shut-off*
A		273	2	0	6
B		397	37	13	80
C		326	29		
D		78	1		
E		33	1		
F		186	12	3	16
G		267	21	7	36
H		89	8	2	13
I		67	4	3	6
Total		1808	117	39	211

Three-protein motifs are more robust to noise than two-protein networks

Why are three protein motifs more robust than two protein motifs?

Tasks:

1. Do N=1 and N=2 go to two distinct states?

2. Are the two states stable?

	Short half-lives	Long half-lives
	✓	✗
	✗	✓

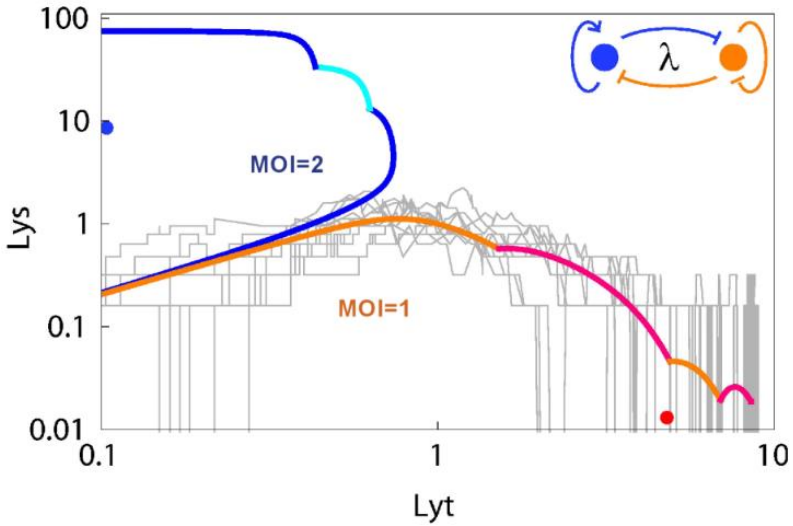
Why are three protein motifs more robust than two protein motifs?

Maintaining the decision

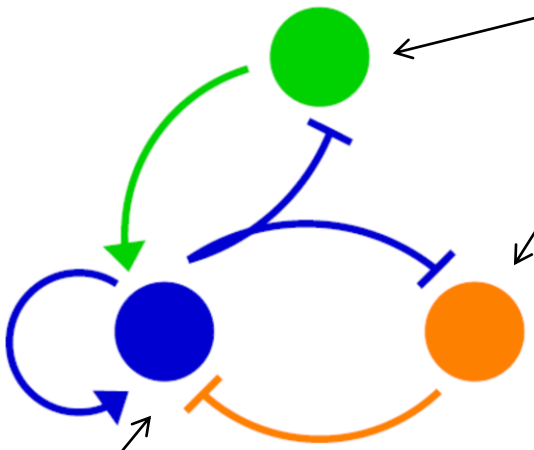
Longer half-lives provide more stability

Making the decision

Short half-lives help to rapidly trigger the positive feedback loop



Why are three protein motifs more robust than two protein motifs?



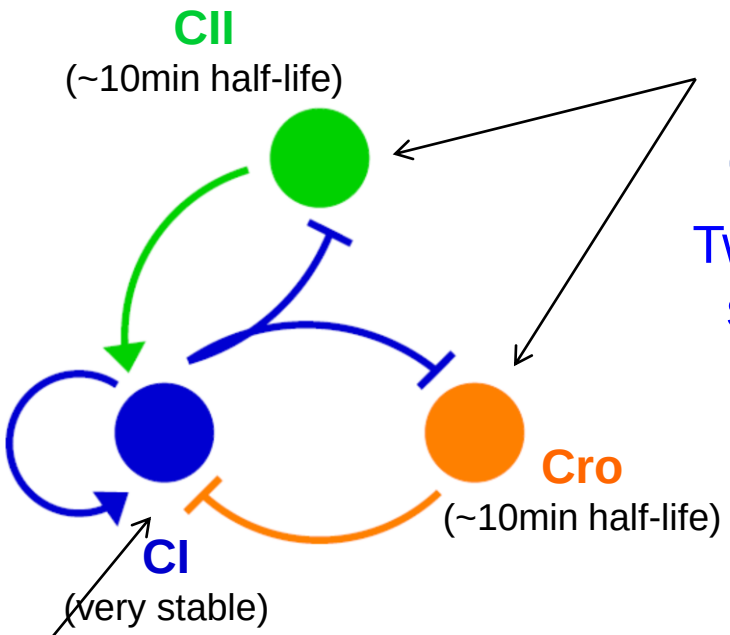
Making the decision

Two proteins with short half-lives

Maintaining the decision

Third protein with a long half-life

Why are three protein motifs more robust than two protein motifs?



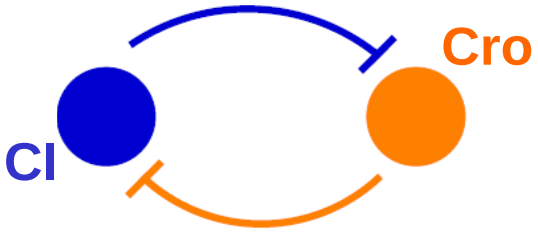
Making the decision

Two proteins with short half-lives

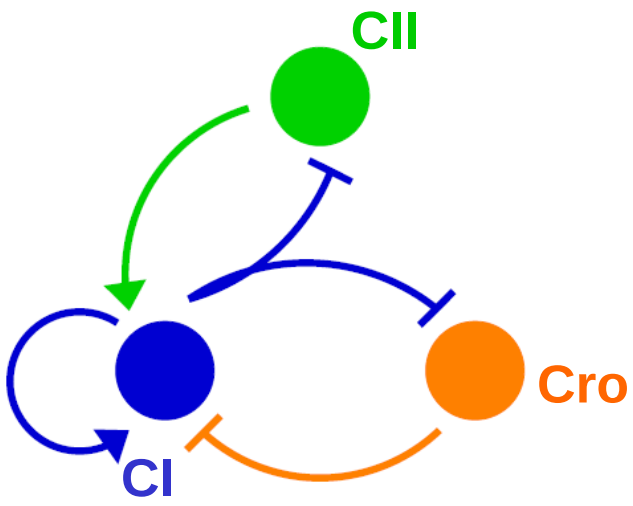
Maintaining the decision

Third protein with a long half-life

Summary



Standard model of lambda needs revision



Separating decision-making from decision-maintenance

In phage lambda:

- unstable CII and Cro may make the lysis-lysogeny decision
- while the stable CI maintains the decision later

In other kinds of developmental decisions? The immune system?