An Appealing Computational Mechanism Drawn from Bacterial Quorum Sensing

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Talk Content

- **Brief Introduction to QS**
- **Biological Significance**
- **Medical Relevance**
- **Biological Mechanism**
- **Wet Lab Experiments**
- **Mathematical Modelling**
- **Computational Modelling**
- **Computational Challenges & Conclusions**

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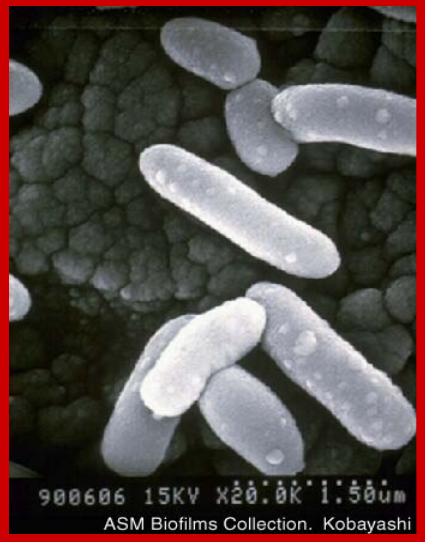
  http://www.cs.nott.ac.uk/~lxl/SIGSAR/index.html

- **Prof. Rozenberg**

- **MolCoNet Leiden Workshop Organizers**

Communication in the Unicellular Bacterial World

- Sexual Exchange – Conjugation
- Protecting Your Niche
- Combating Host Defences
- Population Migration

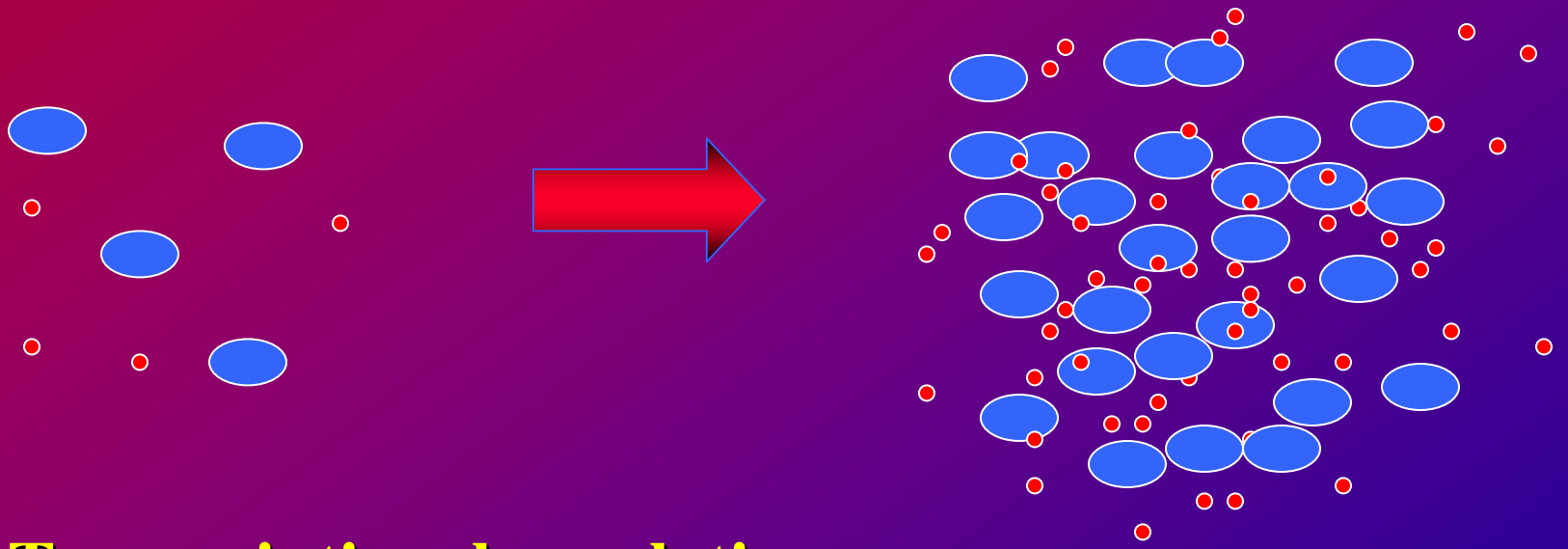


Quorum Sensing

Cell-to-cell communication via a diffusible signal

Low Cell Density

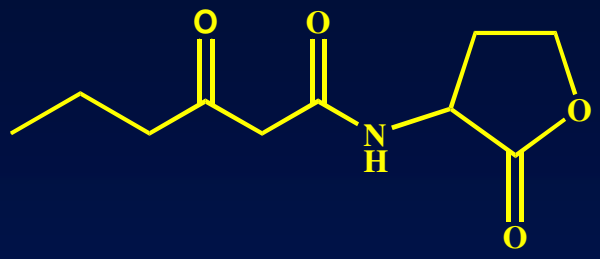
High Cell Density



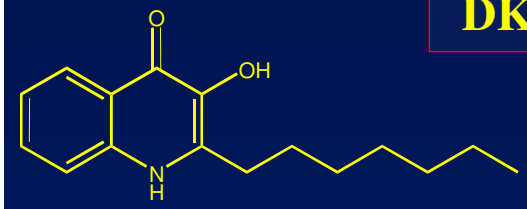
- Transcriptional regulation
- Phenotypic change

Quorum Sensing Signalling Molecules

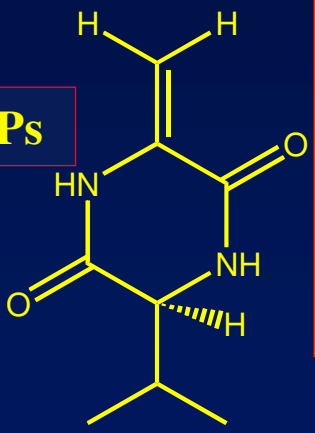
***N*-Acylhomoserine Lactones (AHLs)**



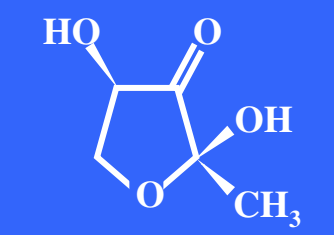
PQS

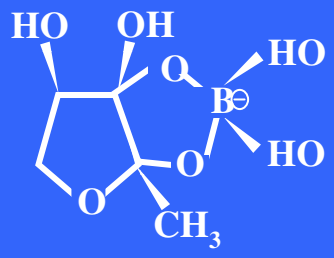


DKPs

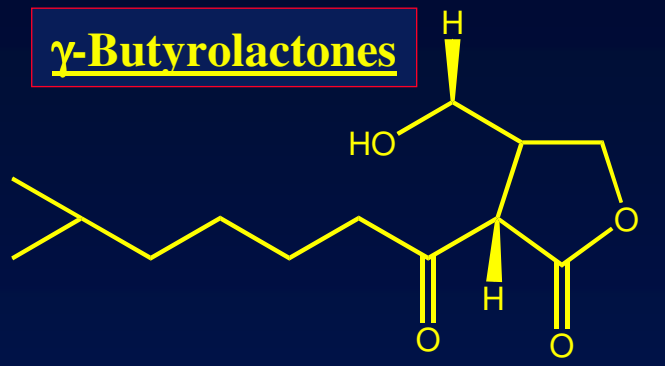


AI-2?



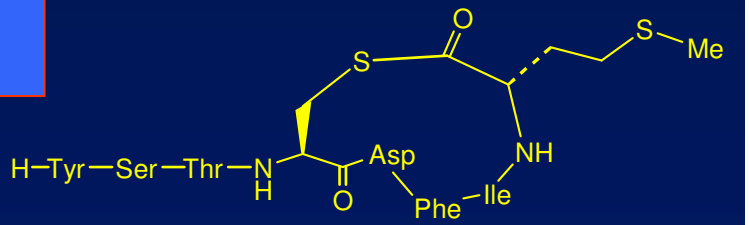


γ-Butyrolactones



Linear Peptides

Cyclic Peptide Thiolactones



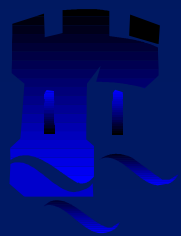
C18-FAME

Gram-negative

Gram-positive

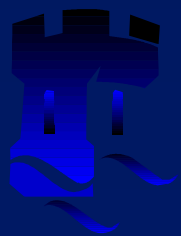
Bacteria co-ordinate gene expression in a population through communication

Why?

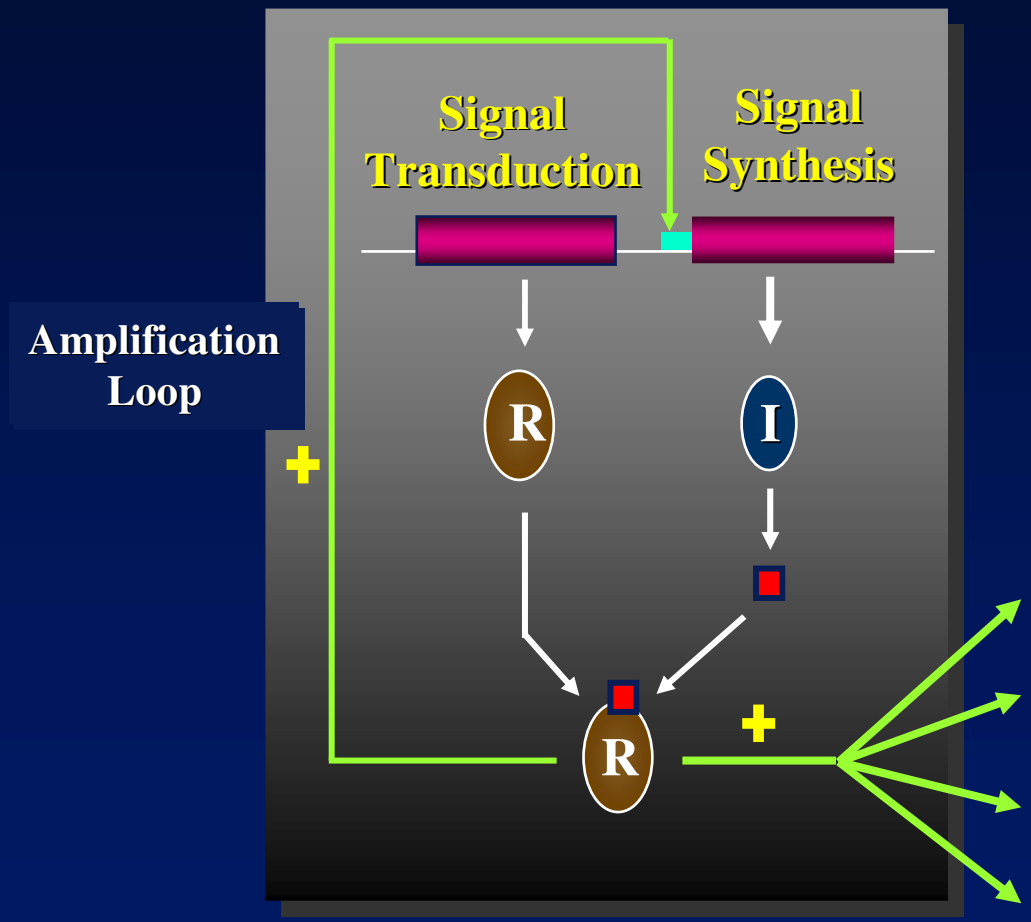


An Important Concept:

Only by pooling the activity of a quorum of cells can a bacterium be successful



Quorum Sensing Regulatory Circuits



Multiple Gene Expression

- Virulence
- Secondary Metabolites
- Motility and Swarming
- Conjugation
- Biofilm Development
- Growth Inhibition

Why study *Pseudomonas aeruginosa?*

Ubiquitous

- Isolated from soil, water, plants, animals

Clinical significance

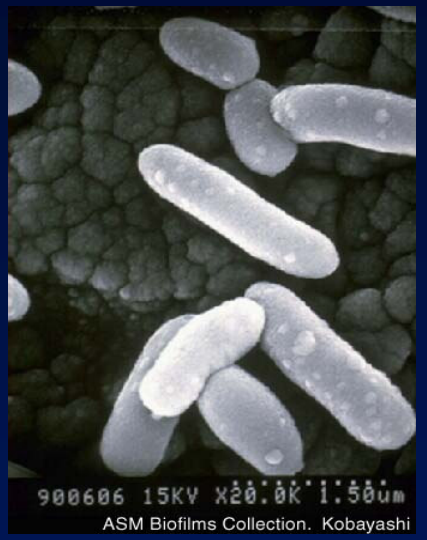
-Can infect almost every organ
(important as a nosocomial infection)

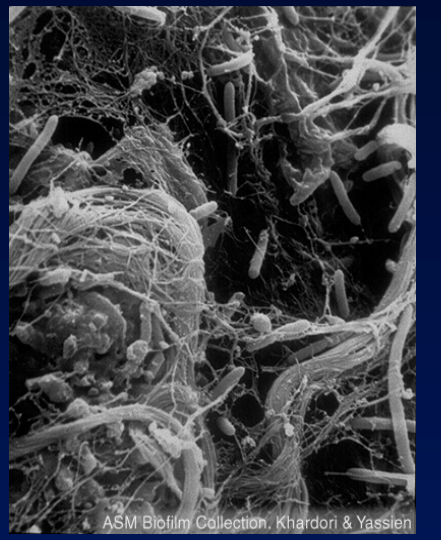
- Major cause of death in CF patients

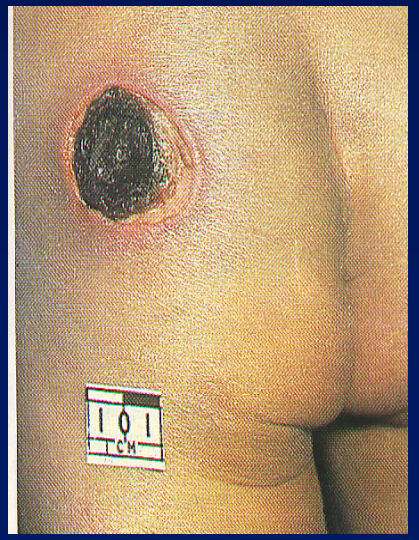
- Can colonise catheters, heart valves
  contact lenses

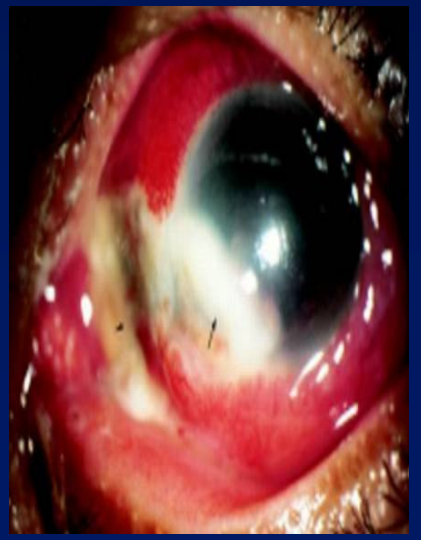
- Burn wound sepsis

- Immunodeficient patients

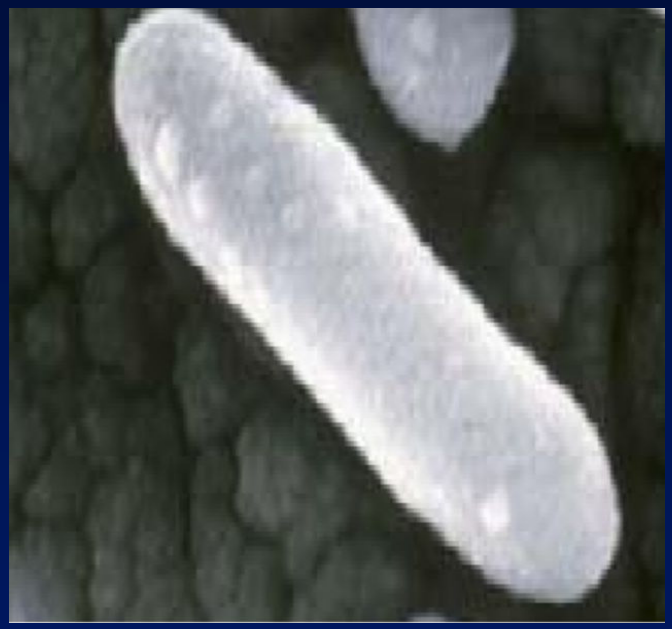








Virulence determinants in *Pseudomonas aeruginosa*



Pyocyanin

Staphylolytic enzymes

Neuraminidase

Lectins

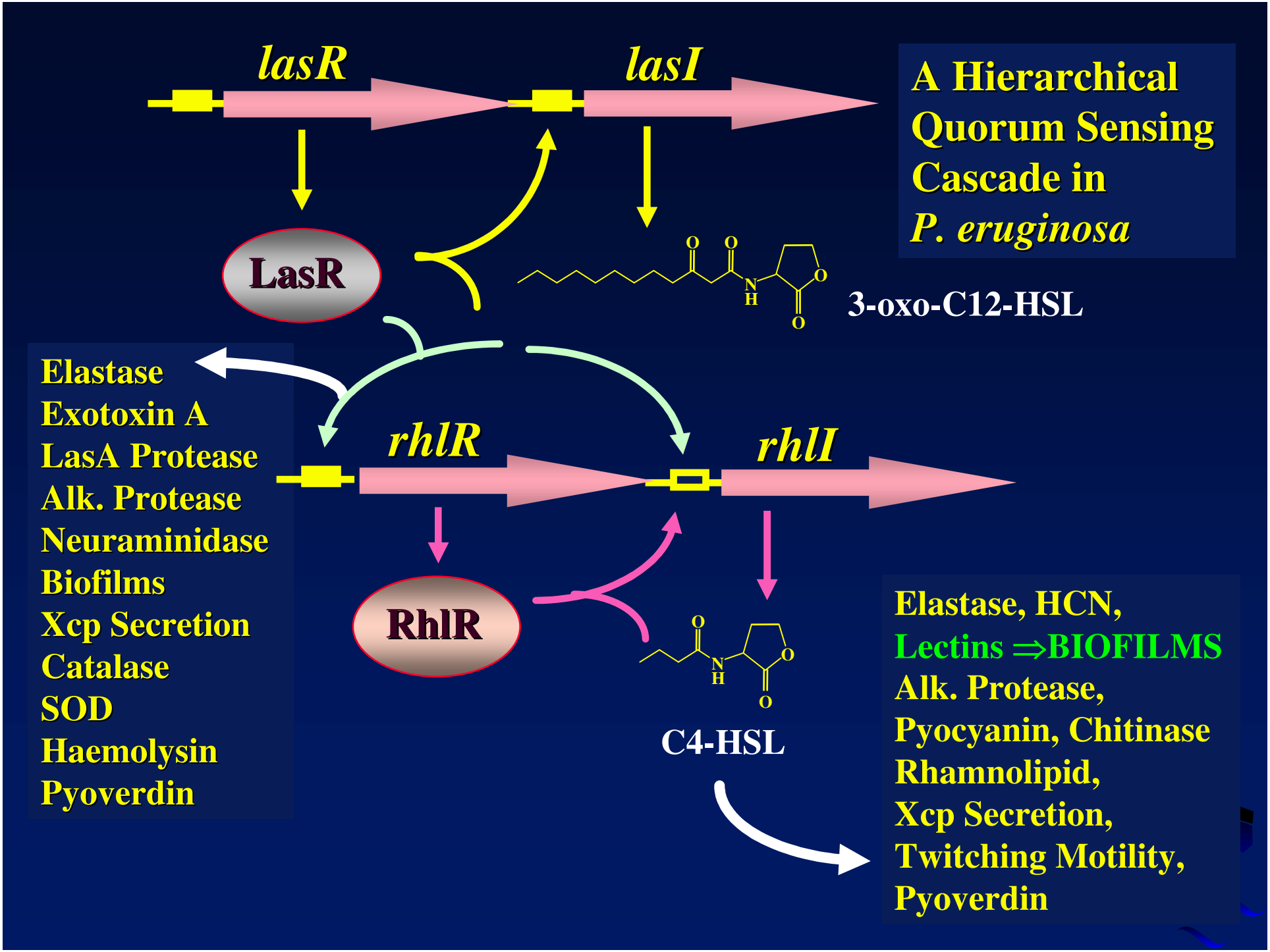
Alginate

Elastase

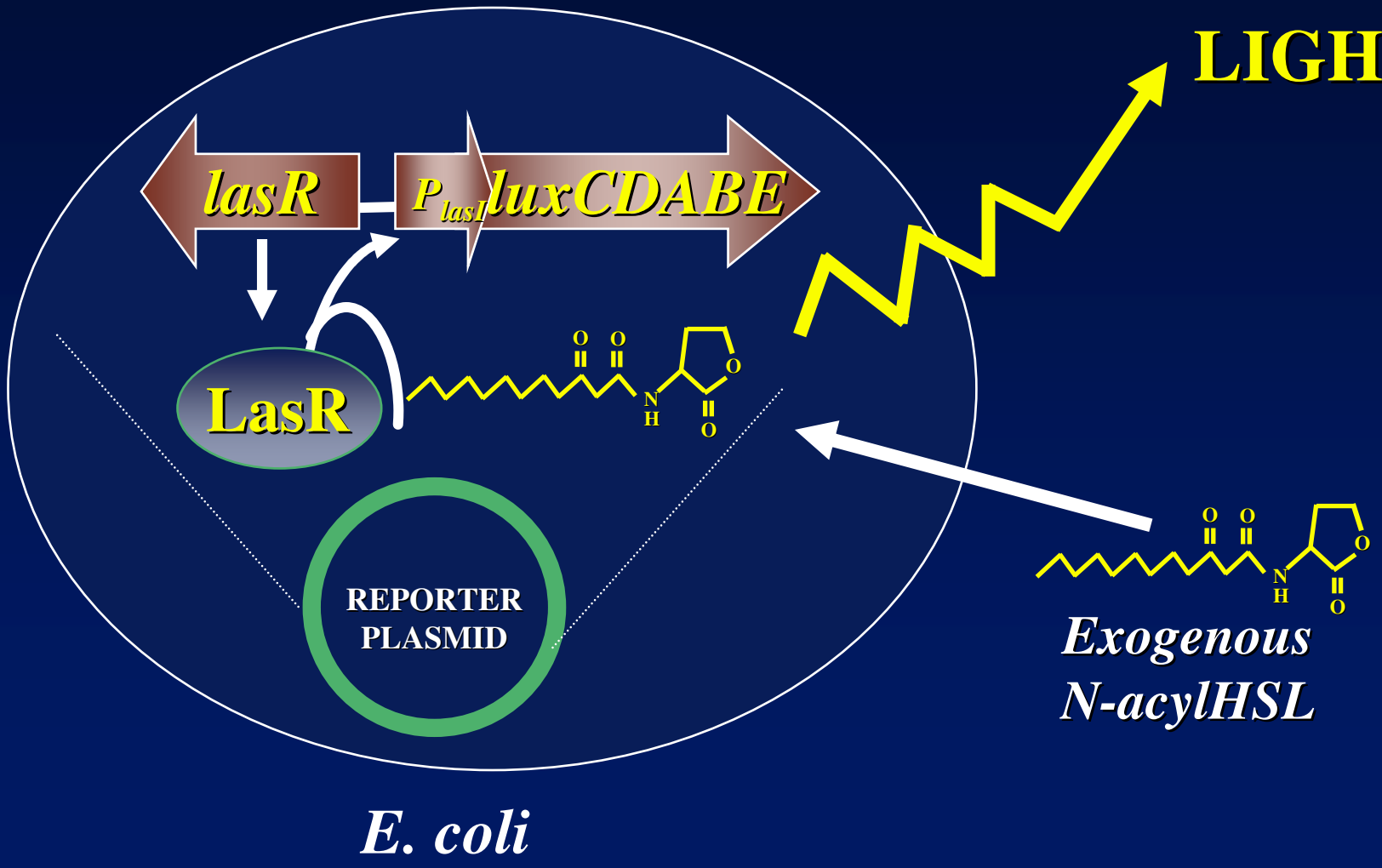
Proteases

Exotoxin A

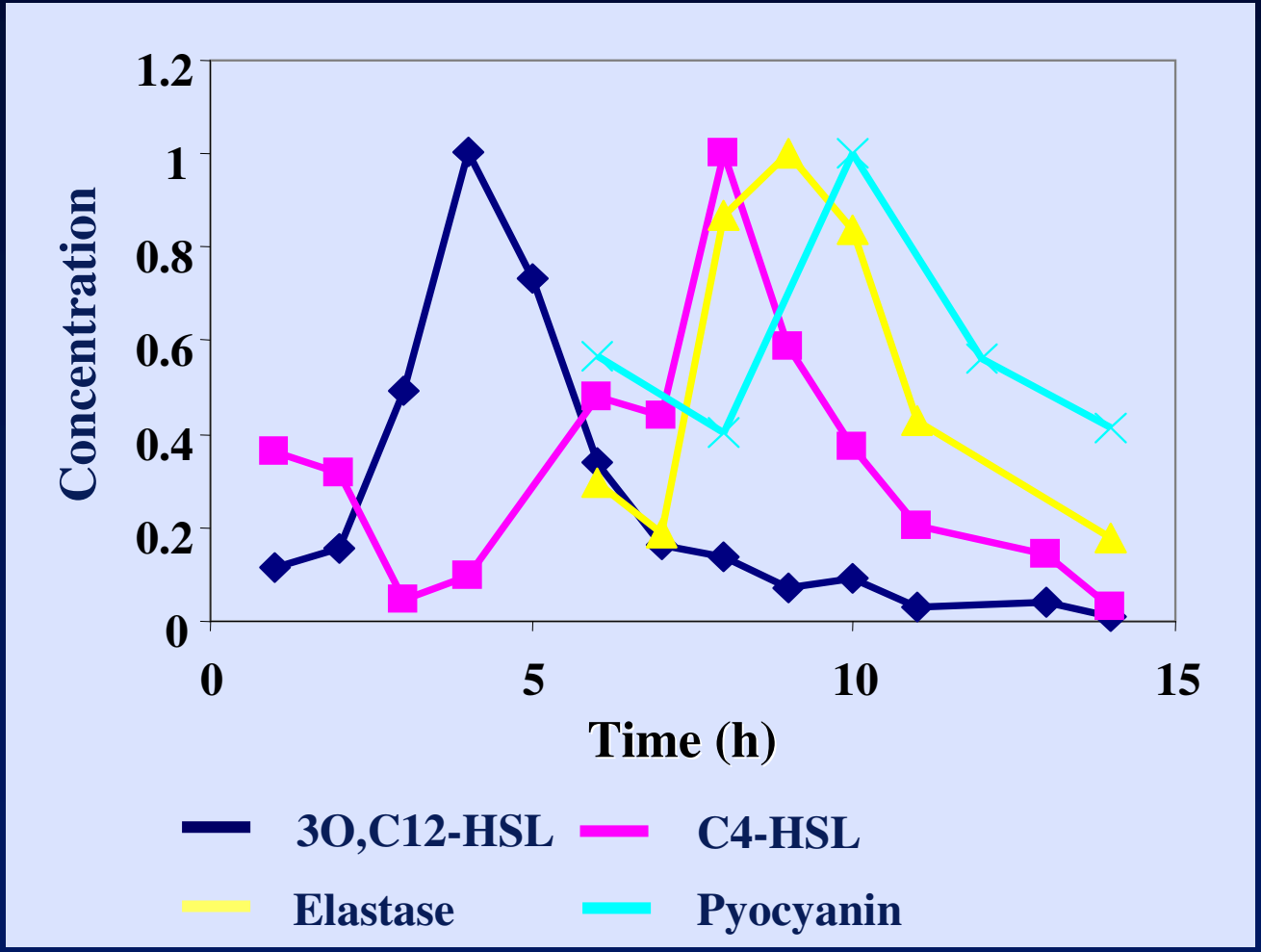
Haemolysin


lasR
lasI
A Hierarchical Quorum Sensing Cascade in P. eruginosa
LasR
3-oxo-C12-HSL
Elastase
Exotoxin A
LasA Protease
Alk. Protease
Neuraminidase
Biofilms
Xcp Secretion
Catalase
SOD
Haemolysin
Pyoverdin
rhlR
rhlI
RhlR
C4-HSL
Elastase, HCN,
Lectins ⇒BIOFILMS
Alk. Protease,
Pyocyanin, Chitinase
Rhamnolipid,
Xcp Secretion,
Twitching Motility,
Pyoverdin

Construction of a Sensitive Biosensor for AHLs produced by *P. aeruginosa*

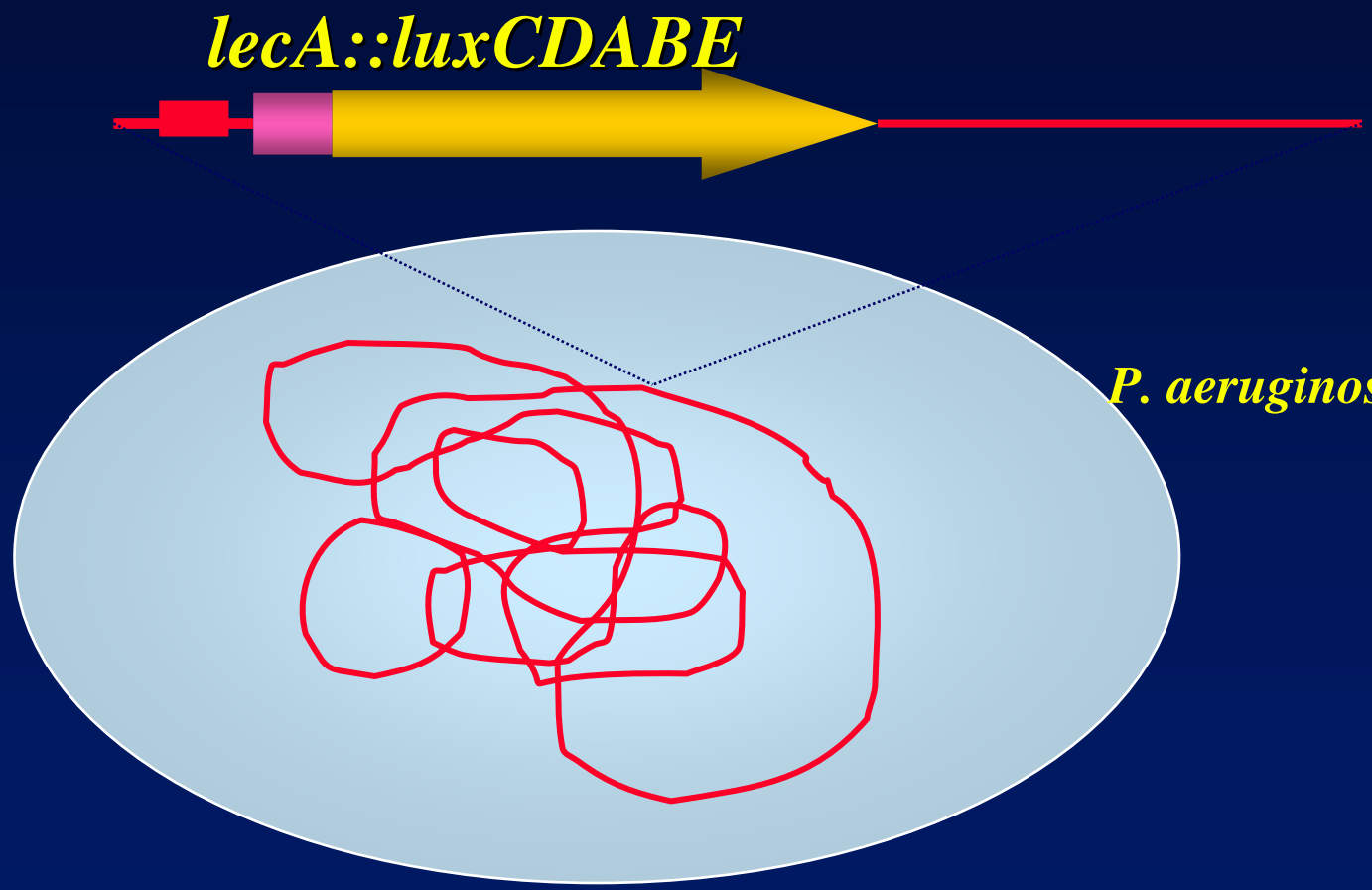


Kinetics of AHL Production in *P. aeruginosa*



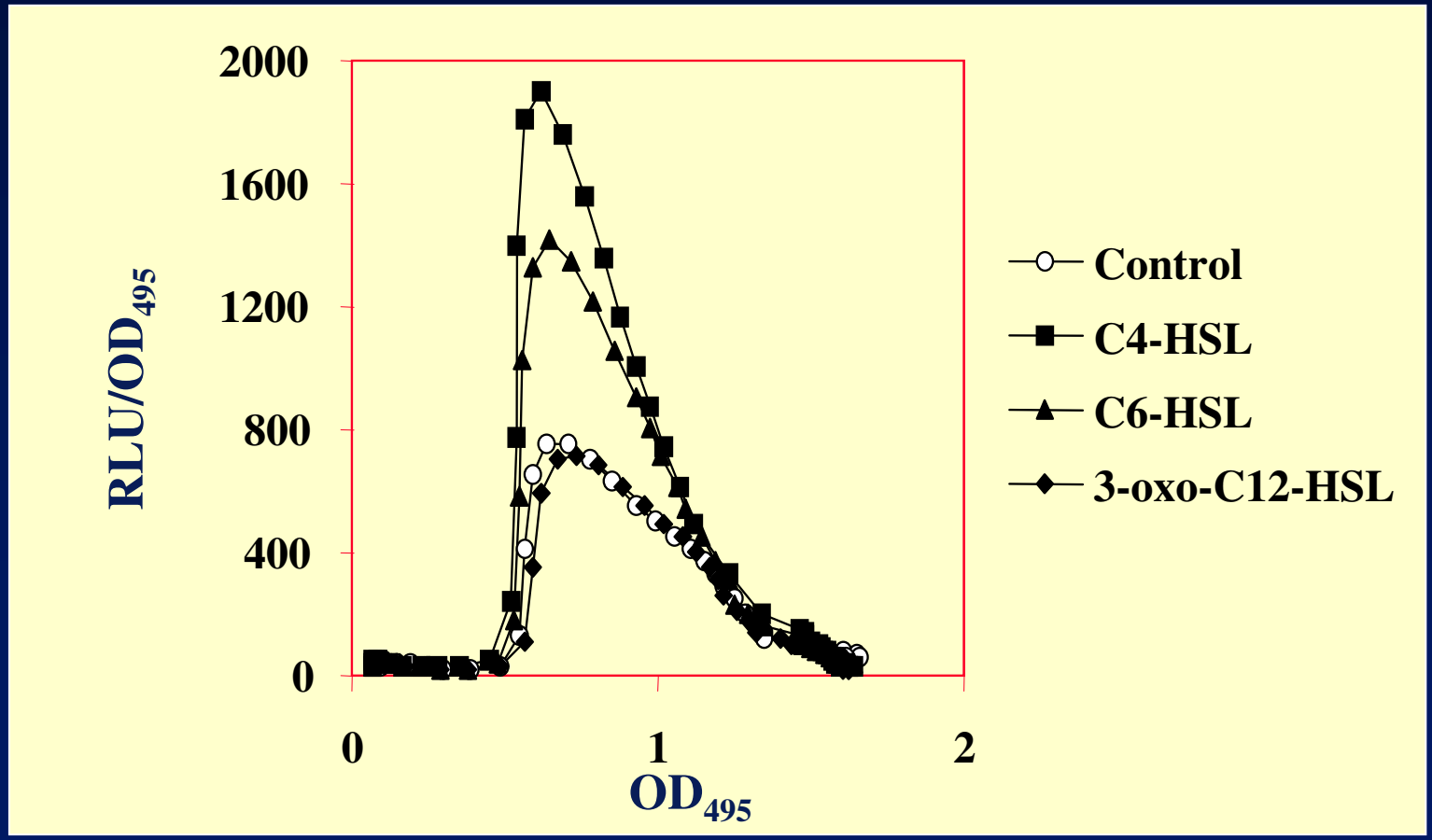
Supra-regulation of quorum sensing

Construction of *lux*-based *lecA reporter*



Over-riding the Quorum

Addition of *N*-acylhomoserine lactones to *P. aeruginosa* *lecA::luxCDABE* does not advance lecA expression



Swarming
in *P.aeruginosa lecA::lux* Tn5 mutants

PAO1 *lecA::lux*

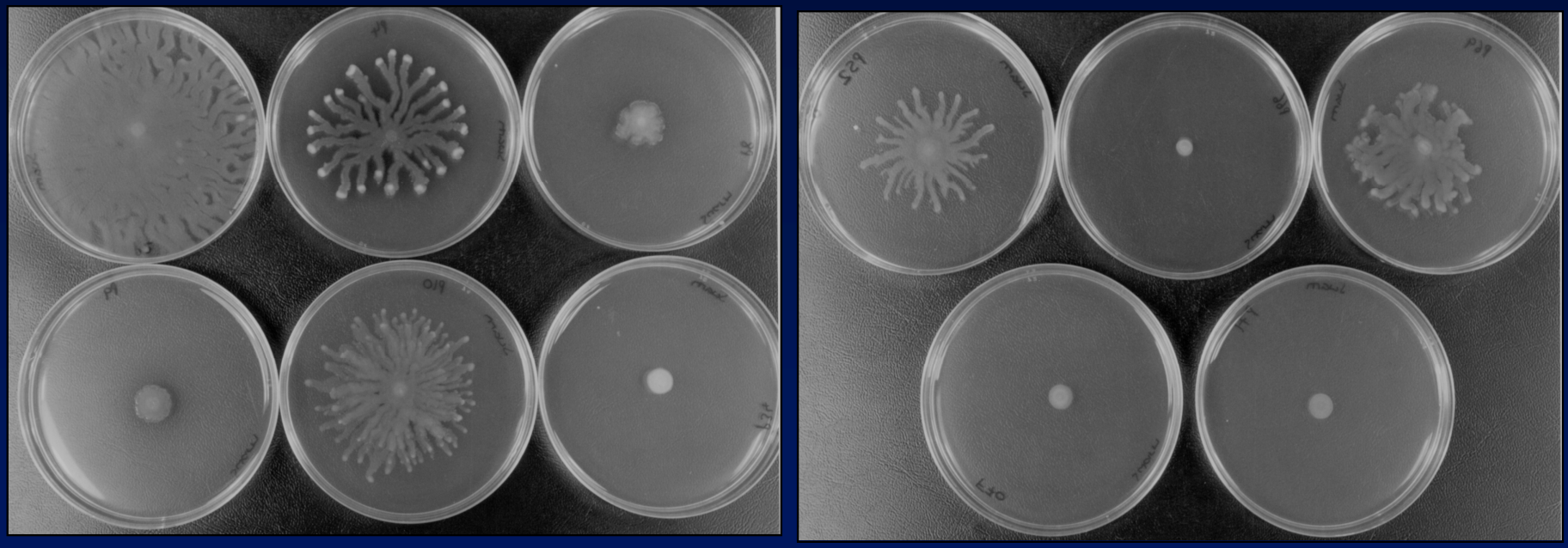
P4 (*lasR*$^{-}$)

P8

P52 (*clpA*$^{-}$)

P66 (*pyrF*$^{-}$)

P69



P9 (*rpoS-*)

P10 (*mvaT*$^{-}$)

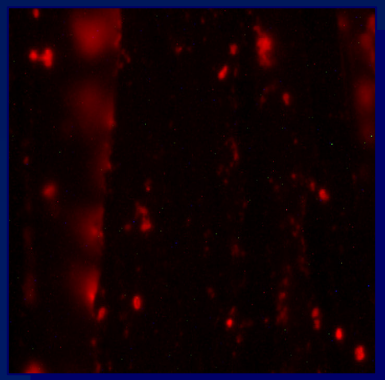
P34 (*rhlR*$^{-}$)

P70 (*pyrE*$^{-}$)

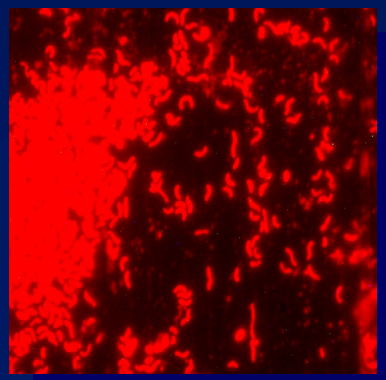
P71 (*carB*)

Increased biofilm surface coverage in a *P. aeruginosa mvaT* mutant

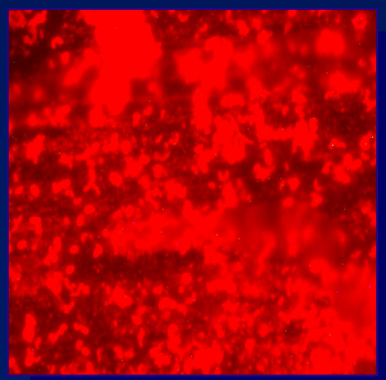
lecA^{-} PAO1 *mvaT*$^{-}$



lecA^{-}

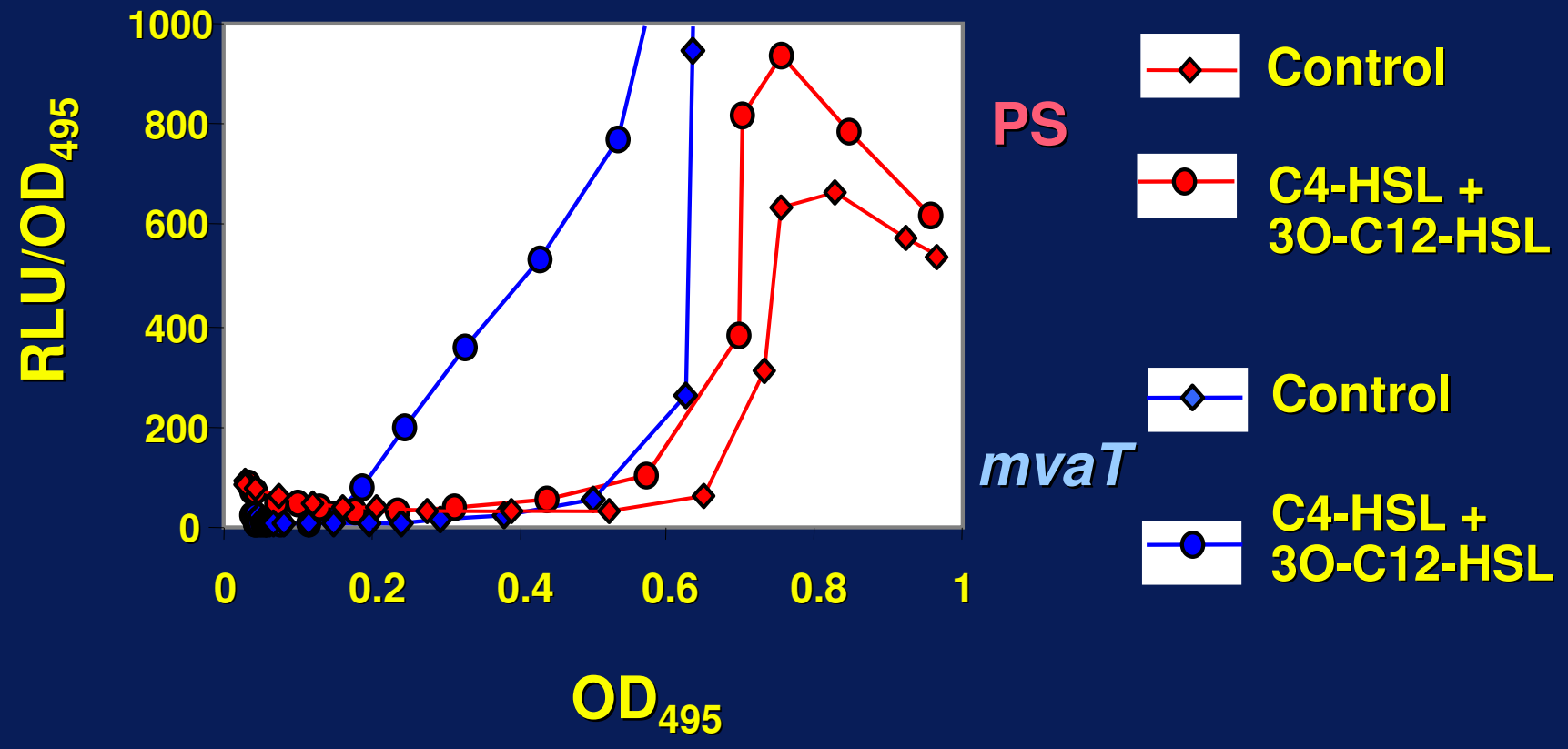


PAO1

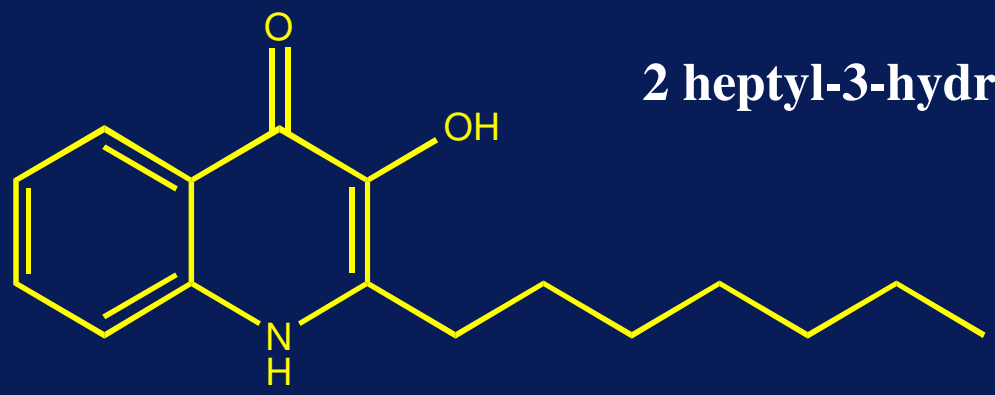


mvaT^{-}

Exogenous AHL advances *lecA::lux* expression in the *mvaT* mutant



The Pseudomonas Quinolone Signal (PQS)

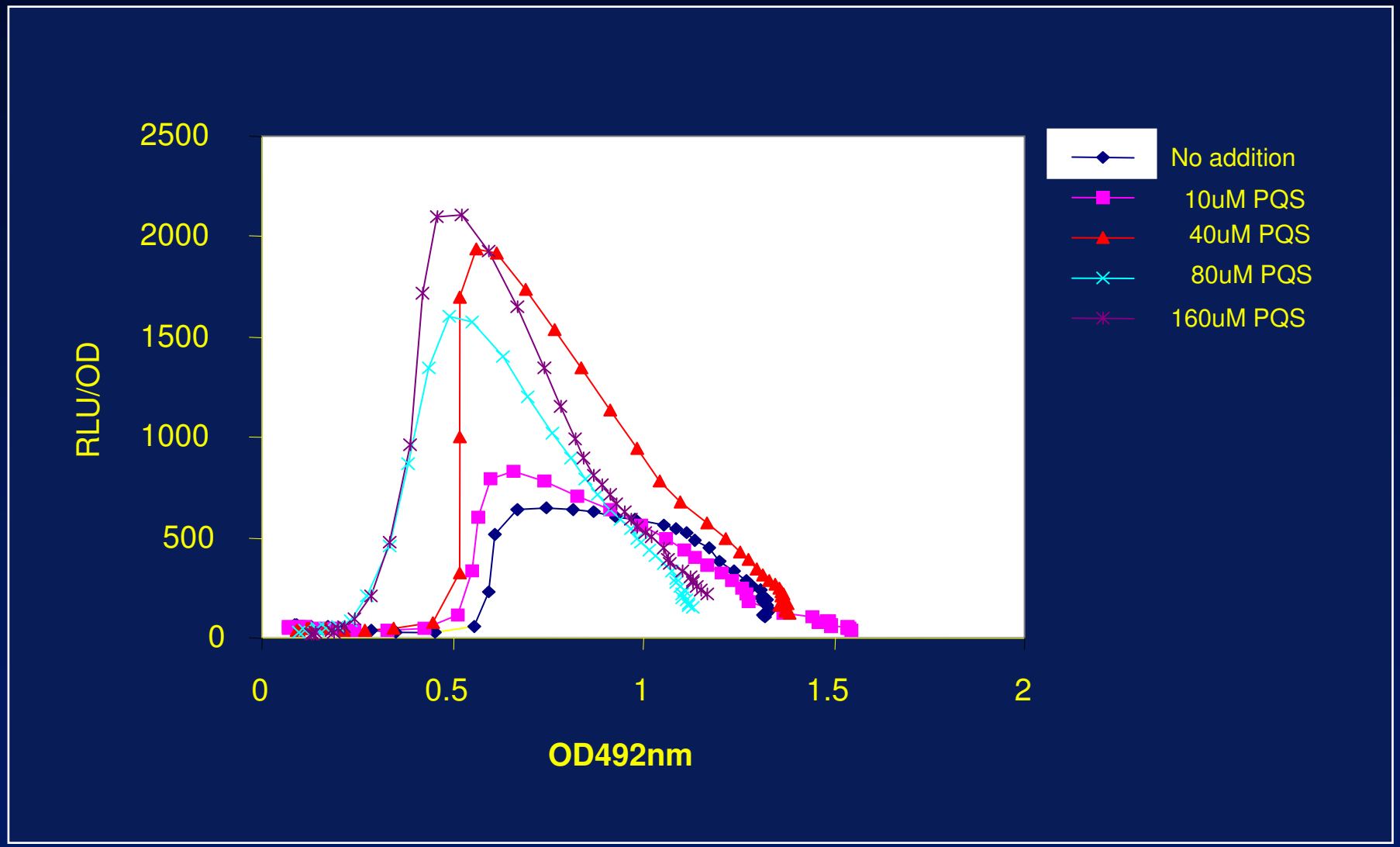


2 heptyl-3-hydroxy-4-quinolone

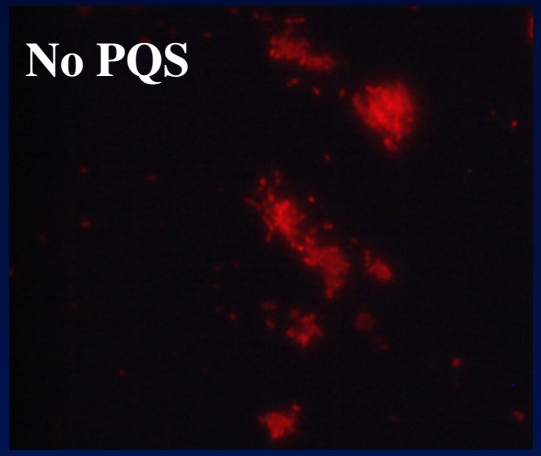
- PQS induces *lasB* (elastase) expression
- Synthesis depends on a functional LasRI
- PQS signal transduction depends on a functional RhlRI
- PQS is produced in late stationary phase (24-48 h)

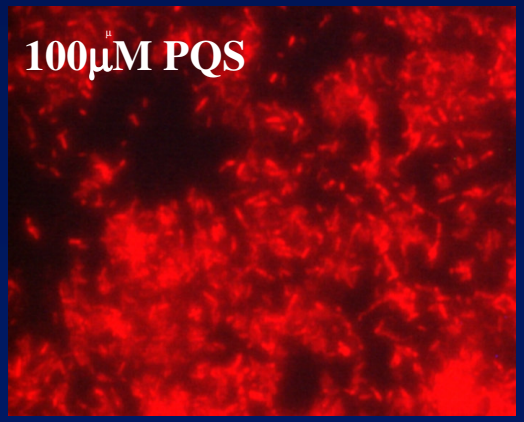
Pesci *et al*. (1999) PNAS 96:1129-11234
McKnight *et al*. J. Bact. 182:2702-2708

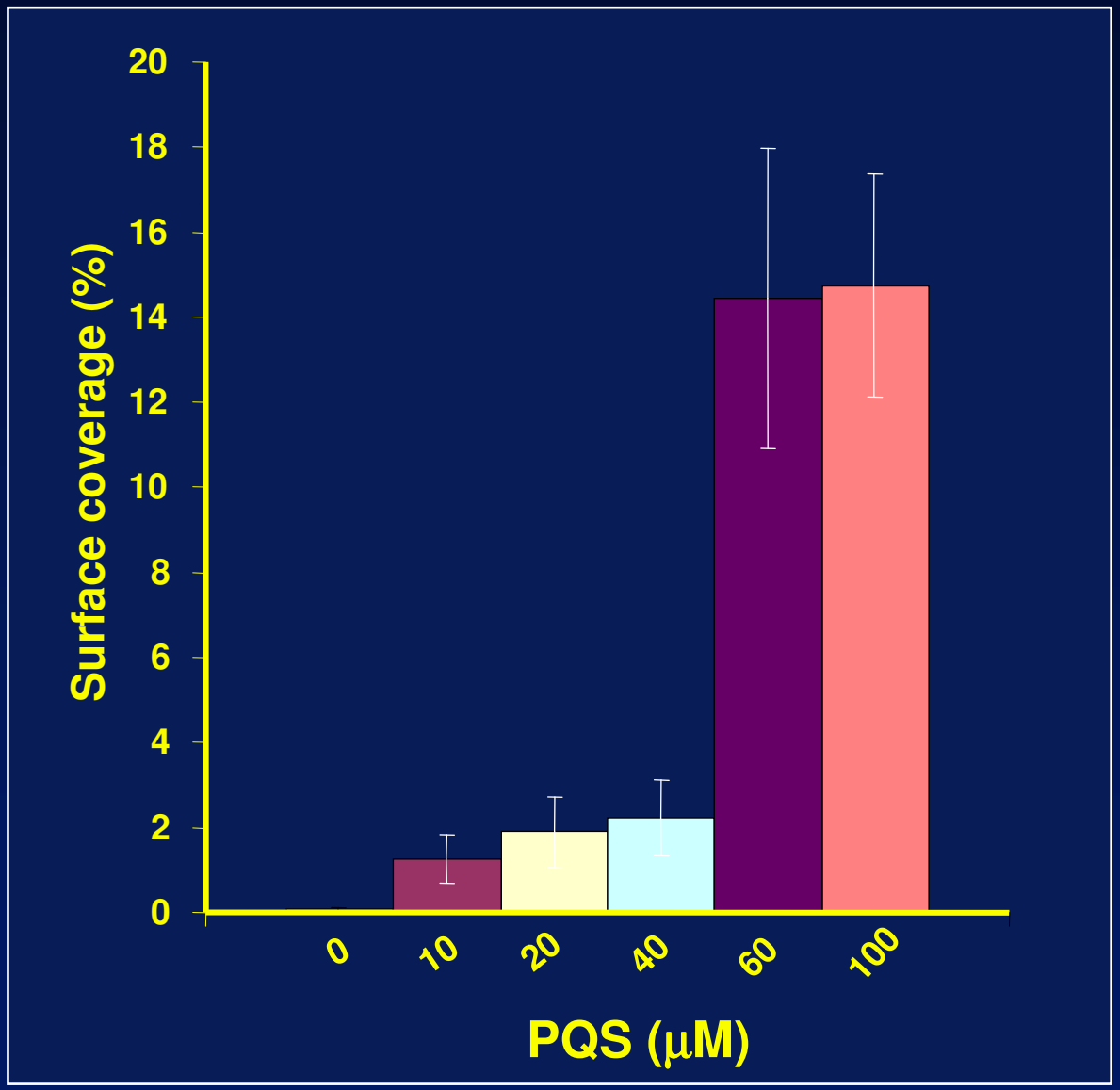
PQS overcomes the "Quorum" in *P. aeruginosa*



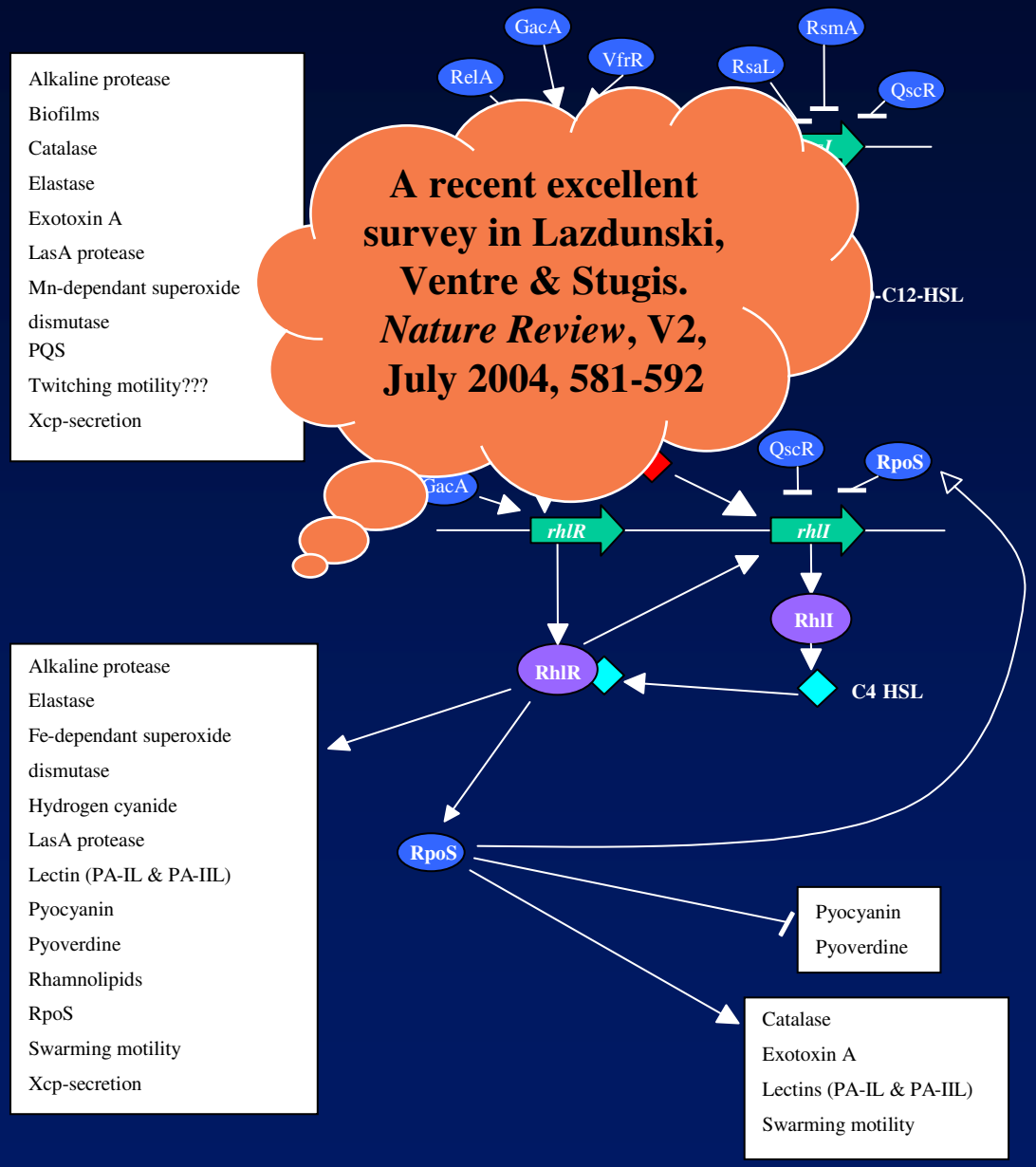
PQS enhances biofilm formation in *P. aeruginosa*







Quorum sensing in *P. aeruginosa*



Mathematical Modelling of QS

If parameters (e.g. kinetics, degradation rates, etc) are well know:

- **Differential Equations (e.g. Borysuk & Tison 1998)**
- **Mode Switch Differential Equations (e.g. Belta et al 2001)**
- **Stochastic Simulation (e.g. Gibson & Bruck 2000)**

If parameters are not (or poorly) known:

- **Qualitative Modelling (pioneer by S. Kauffman 1969)**

An Example
(by Dockery & Keener, 2000)

Molecules		Concentrations		Formation/Degradation Rates
1. LasR	⟺	1. R	⟺	1. k_R
2. LasI		2. I		2. k_2
3. 3O-C12-HSL		3. S		3. k_S
4. LasR-3O-C12-HSL		4. C		4. k_C, k_{RS}
5. mRNA LasR		5. mR		5. k_1
6. mRNA LasI		6. mI		6. k_{mI}

In what follows X' = dX/dt

Molecules R and S are dimerised at rate k_{RS} and degraded at rate k_C which gives the following equation:

$$C' = k_{RS} * R * S - k_C * C \quad (1)$$

Receptor protein R is consumed during the binding with S, degrades with a rate k_R and is produced by the break-up of C and by the translation of mRNA R at rate k1:

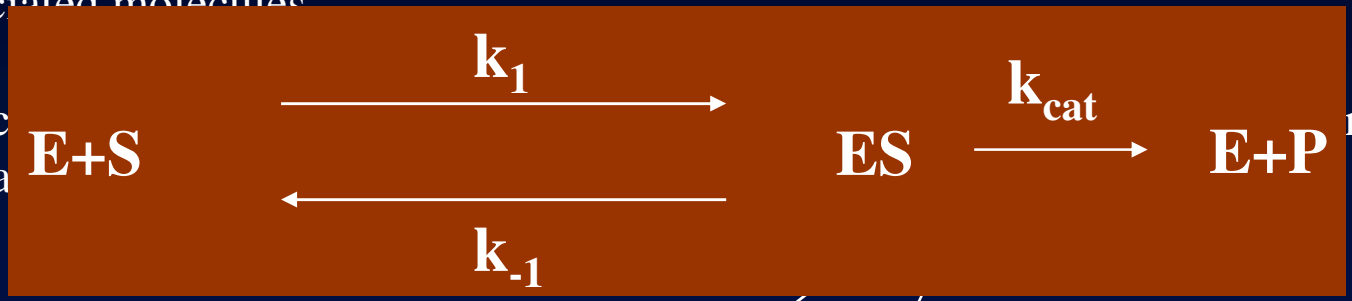
$$R' = -K_{RS} * R*S - K_R * R + k_C * C + mR * K_1 \quad (2)$$

the messenger degrades at rate K_S, is consumed when complex C is formed but it builds up by the degradation of C and by the enzyme I at rate K_2,

$$S' = -K_S * S - k_{RS} * R * S + k_c * C + I * K_2 \quad (3)$$

The last terms in Eqs. 2 and 3 depend on the rate of genes transcriptions and then translation for the associated molecules

Genes transc rate.
After some a



$$C' = k_{RS} * R * S - k_C * C \qquad (4)$$

$$R' = -K_{RS} * R*S - K_R * R + k_C * C + V_R \frac{C}{K_R + C} + R_0 \qquad (5)$$

$$S' = -K_S * S - k_{RS} * R * S + k_c * C + V_S \frac{C}{K_I + C} + S_0 \qquad (6)$$

Basal rates

Note that binding between R and S (to give C) is usually faster than the mRNA transcription, this is modelled by assuming $k_c * C = k_{RS} * R * S$ which put into Eqs 4,5 and 6 gives:

(Q)Steady-state

But… all of these ignores the media

$$C' = \frac{k_{RS}}{k_C} R * S$$

$$R' = -K_R * R + V_R \frac{C}{K_R + C} + R_0 \qquad (8)$$

$$S' = -K_S * S + V_S \frac{C}{K_I + C} + S_0 \qquad (9)$$

We need to include terms to account for the fact that S migrates across the cellular wall (with conductance δ) into the extra cellular space that has a (uniform) local cellular density of ρ. The concentration of S in the extra cellular space (E') is:

$$(1-\rho)(E' + k_E E) = \delta(S-E) \qquad (10)$$

and Eq. 9 needs to accommodate the conductance across the cellular wall of molecules S:

$$\rho^* \left(S' + K_S * S - V_S \frac{C}{K_I + C} - S_0\right) = -\delta (S-E) \qquad (11)$$

Degradation rate of S outside de wall

It is possible to manipulate Eqs. 7,8, 10 and 11 and simplify the system's description as follows:

$$R' = -K_R * R + V_R \frac{C}{K_R + C} + R_0 \qquad (12)$$

$$S' = V_S \frac{C}{K_I + C} + S_0 + d(\rho) * S \qquad (13)$$

With $C = \frac{k_{RS}}{k_C} * R * S$ and $d(\rho) = K_S + \frac{\delta}{\rho} * \frac{k_E * (1-\rho)}{\delta + k_E * (1-\rho)}$

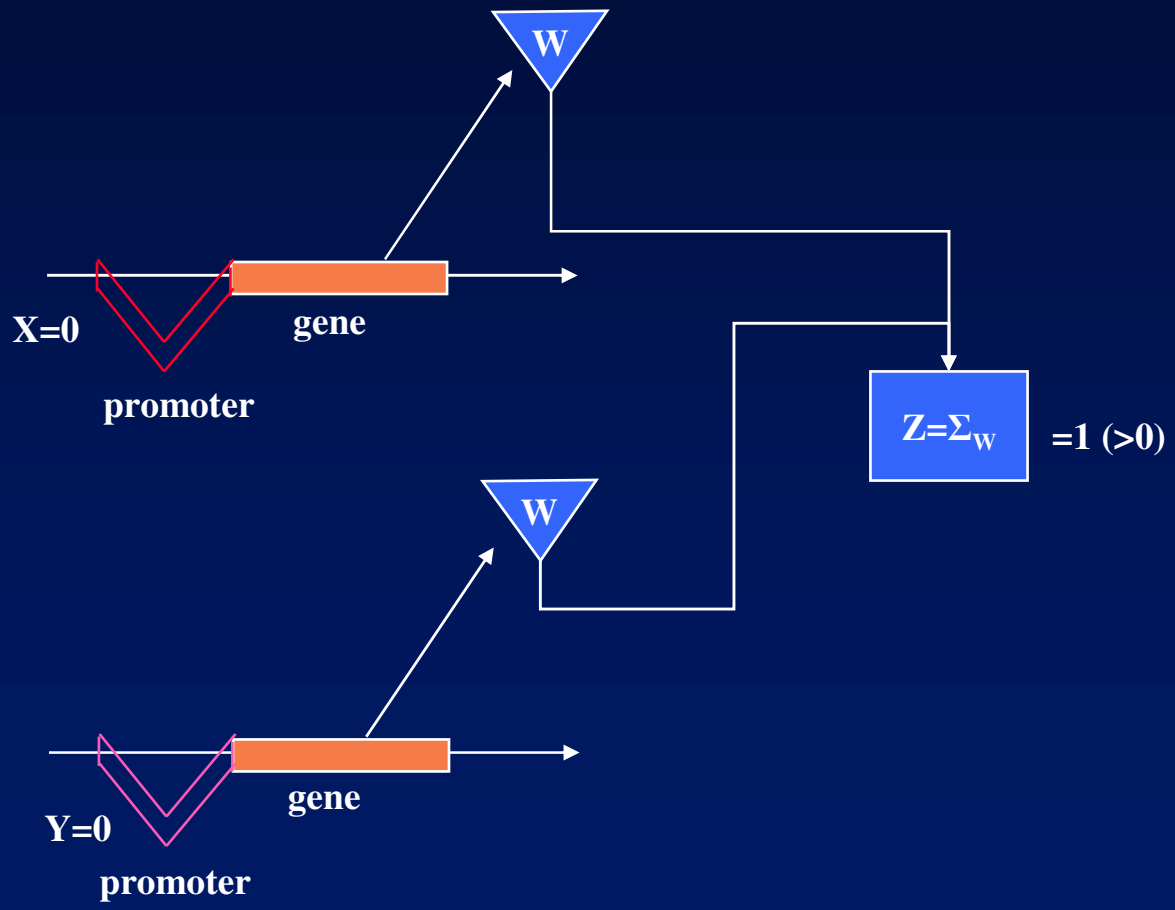
The system described in the last two equations shows two steady state regimes for the concentrations of R and S, one for when the cellular concentration is low (in which case R and S are low) and a different regime when cellular concentration is high (in which case R and S have high concentrations). This switching mechanism is characteristic of QS.

Computational Modelling of QS

R. Weiss and colleagues have shown (Weiss et al, 2003) that various activities *within* the cell can be understood as performing computations

A NAND gate:

X	Y	Z
0	0	1
1	0	1
0	1	1
1	1	0



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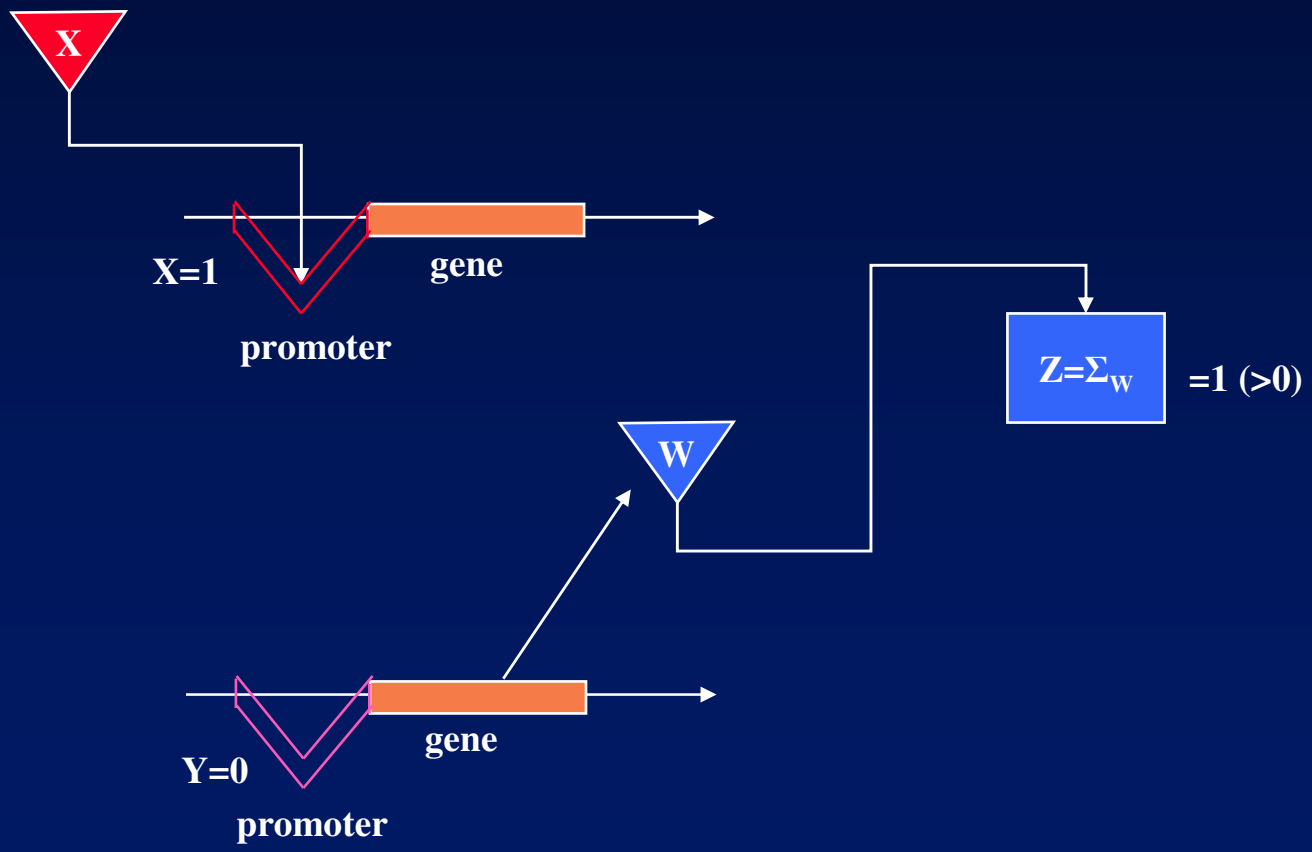

A NAND gate:

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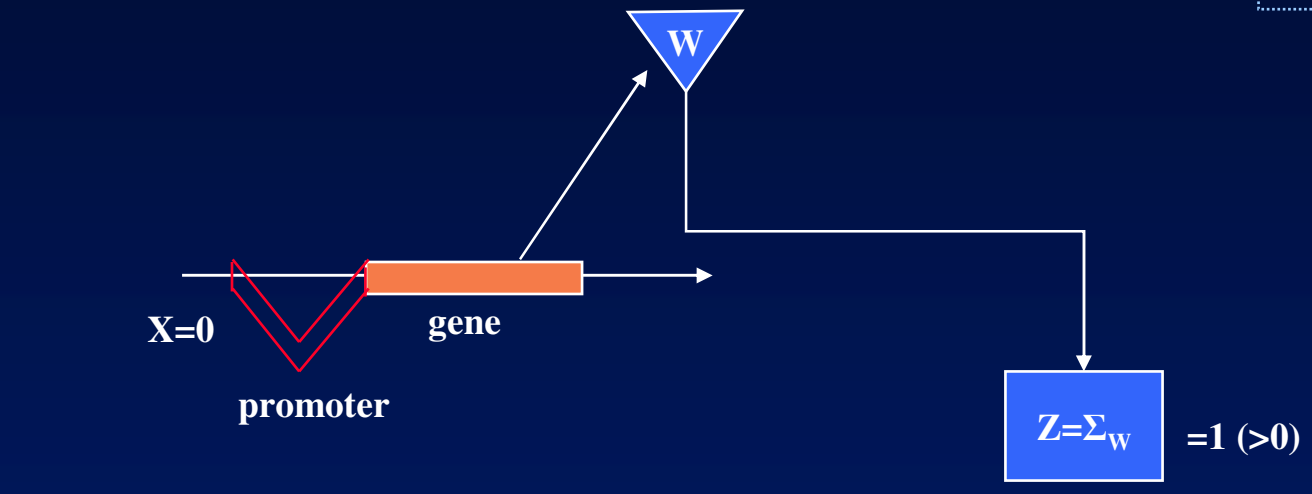
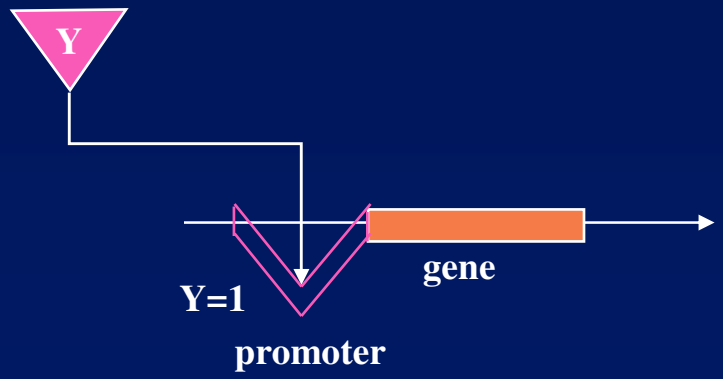
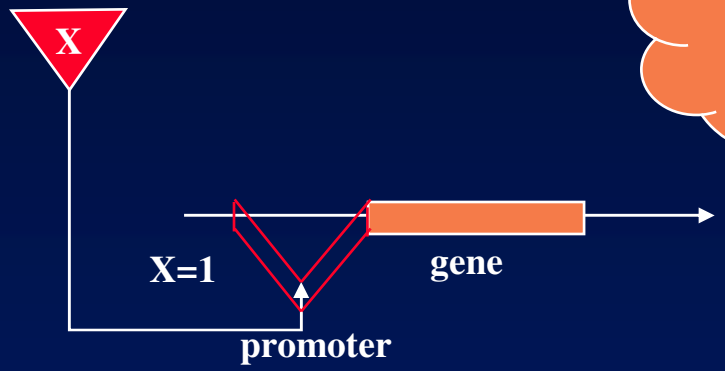




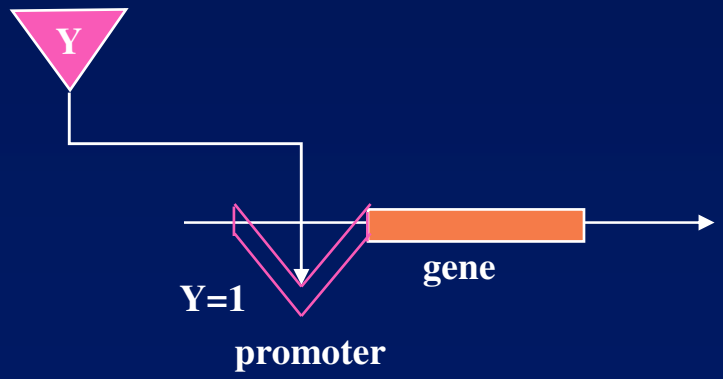
A NAND gate:

X	Y	Z
0	0	1
1	0	1
0	1	1
1	1	0

an of course NAND is a universal logic gate

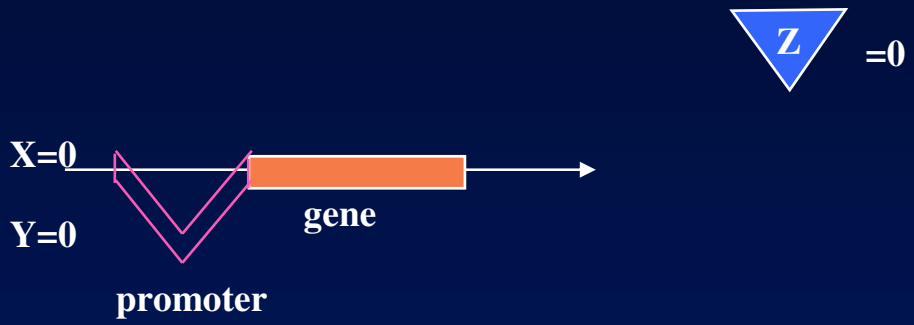


$Z=\Sigma_W$ =0

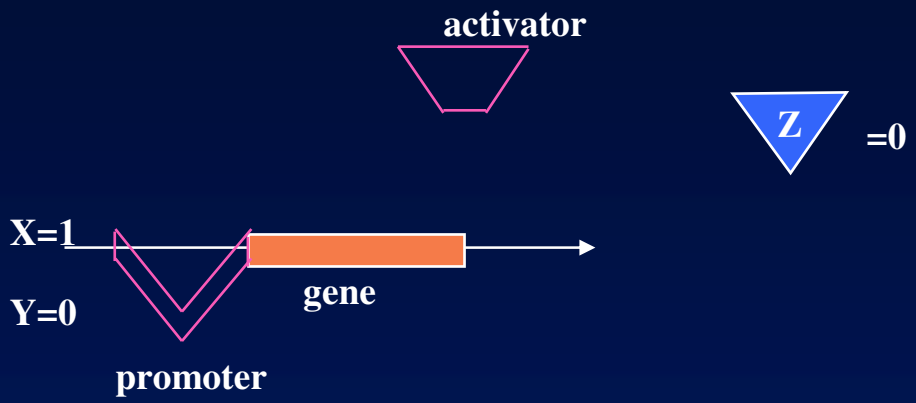


Weiss et al write:

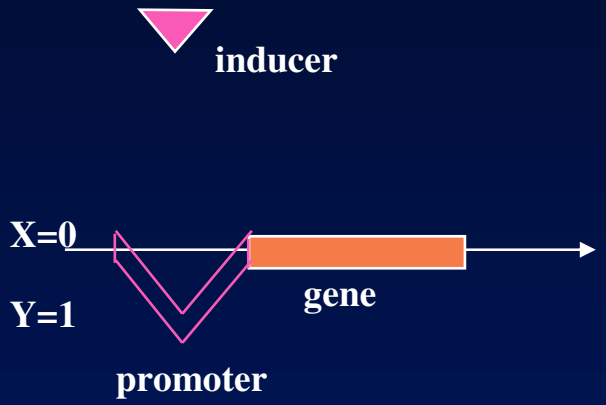
“The AND gate is utilized by cells to detect incoming messages sent by other neighbouring cells….”



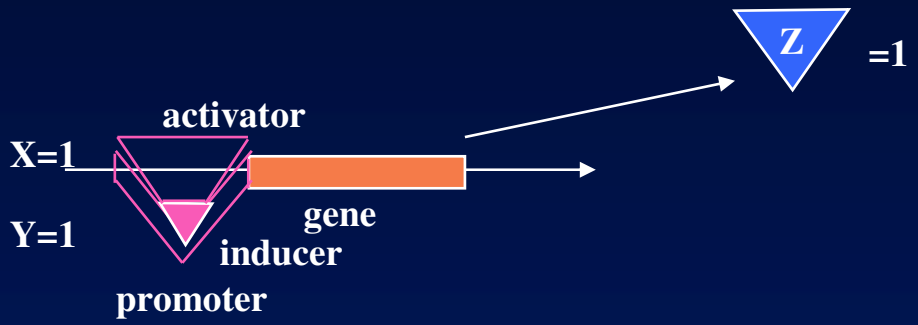
X	Y	Z
0	0	0
1	0	0
0	1	0
1	1	1



X	Y	Z
0	0	0
1	0	0
0	1	0
1	1	1



X	Y	Z
0	0	0
1	0	0
0	1	0
1	1	1



X	Y	Z
0	0	0
1	0	0
0	1	0
1	1	1

That is: *a cell* detects and incoming signal (inducer) and changes behaviour accordingly BUT this leaves out both the role of other cells and the medium in which they live

Moreover, there is still what Weiss et al call the "Kinetic Mistmatch problem"

The use of “wet” logic so far has only achieved the processing of a few bits at a time

Thus, the state of the art is at the same level of current quantum computing devices

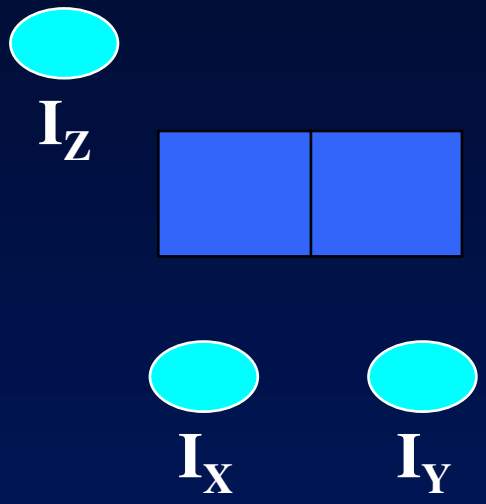
Moreover, there is still what Weiss et al call the “Kinetic Mistmatch problem”

which hampers the scalability of these systems to larger *within* the cell computations (let alone multicellular systems)

A Computational & population/medium view of Quorum Sensing

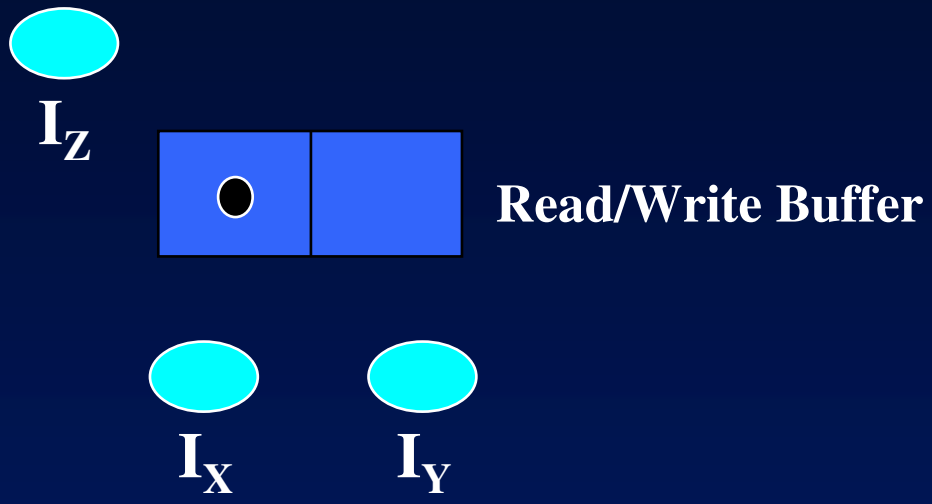
- **Individuals are producers** (They try to get rid of what they produce)
- **The buffer has a limited capacity:**
  - **buffer is said to be on if it can be written**
  - **buffer is said to be off if it cannot be written(i.e. its full)**
- **Individuals write to the buffer if it is on**
- **(some individuals could be consumers)**

What's I_Z's interpretation of the *buffer's* computation????



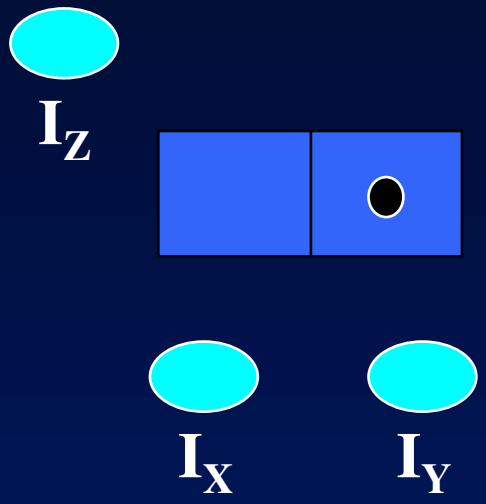
X	Y	B	⟷	Z
0	0	1		QS-

What's I_Z's interpretation of the buffer's computation would be????



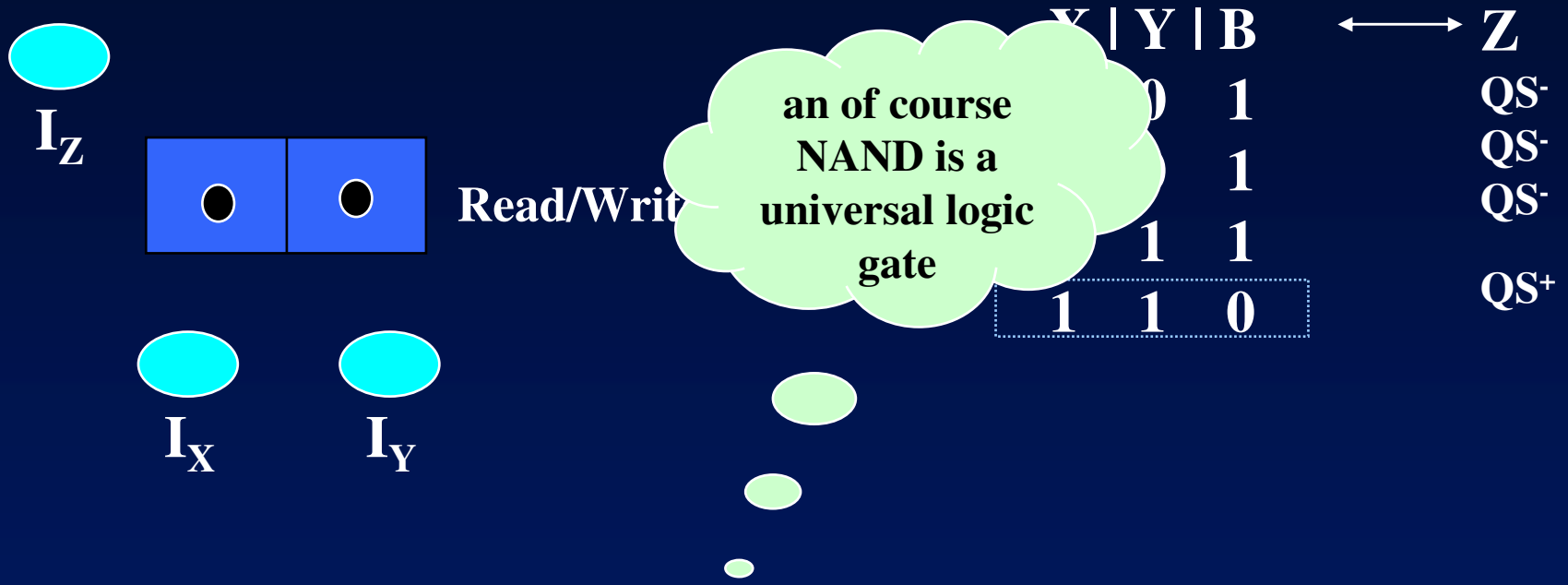
X	Y	B	⟷	Z
0	0	1		QS-
1	0	1		QS-

What's I_Z's interpretation of the buffer's computation would be????



X	Y	B	⟷	Z
0	0	1		QS-
1	0	1		QS-
0	1	1		QS-

What's I_Z's interpretation of the buffer's computation would be????



The buffer implements a NAND function

QS_Z = NOT (NAND (X,Y)) = AND (X,Y)

Cells

Medium

Some Simulations With CQS

Very low cells density (50)

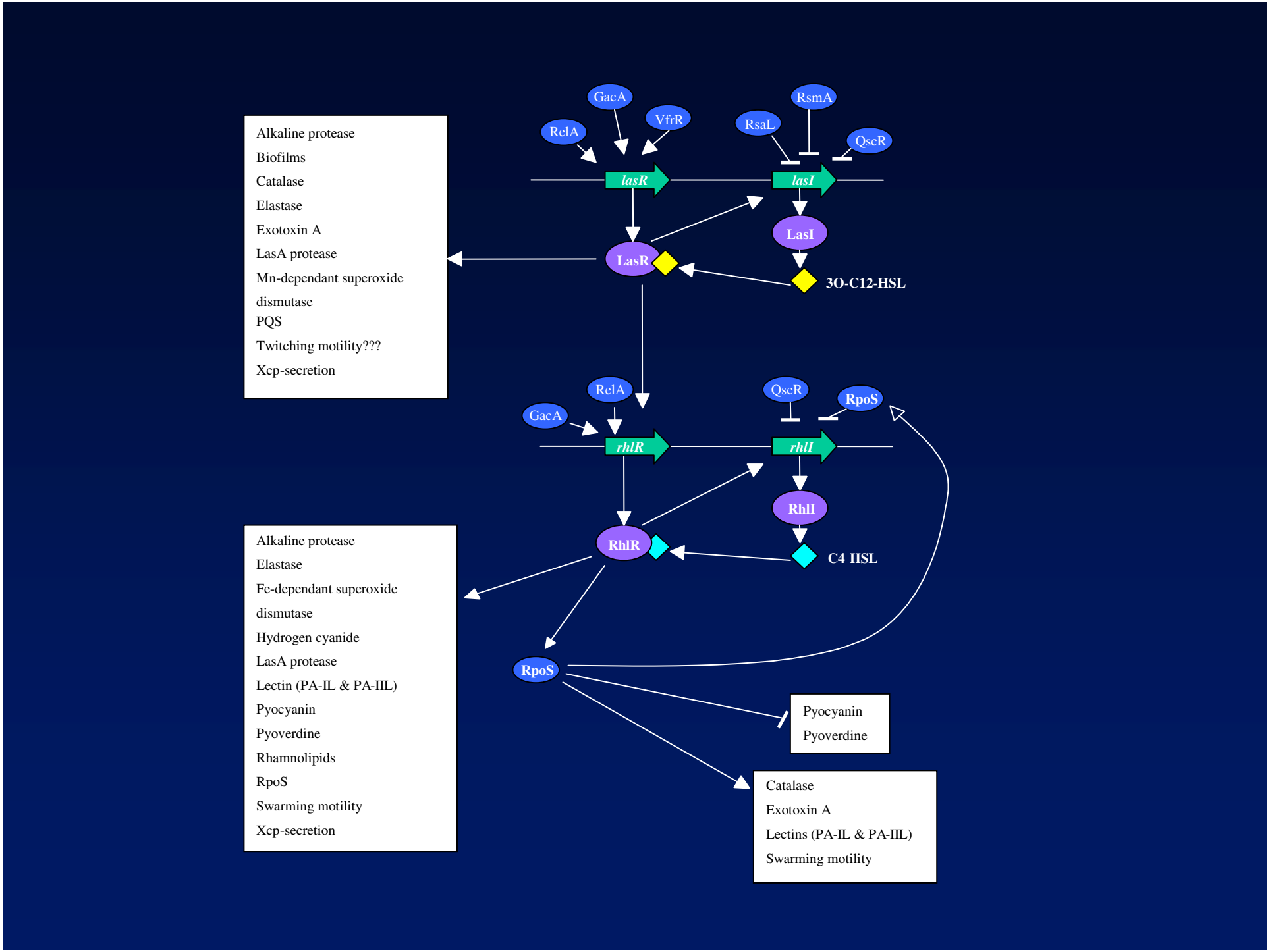
Medium cells density (100)

High cell density (300)

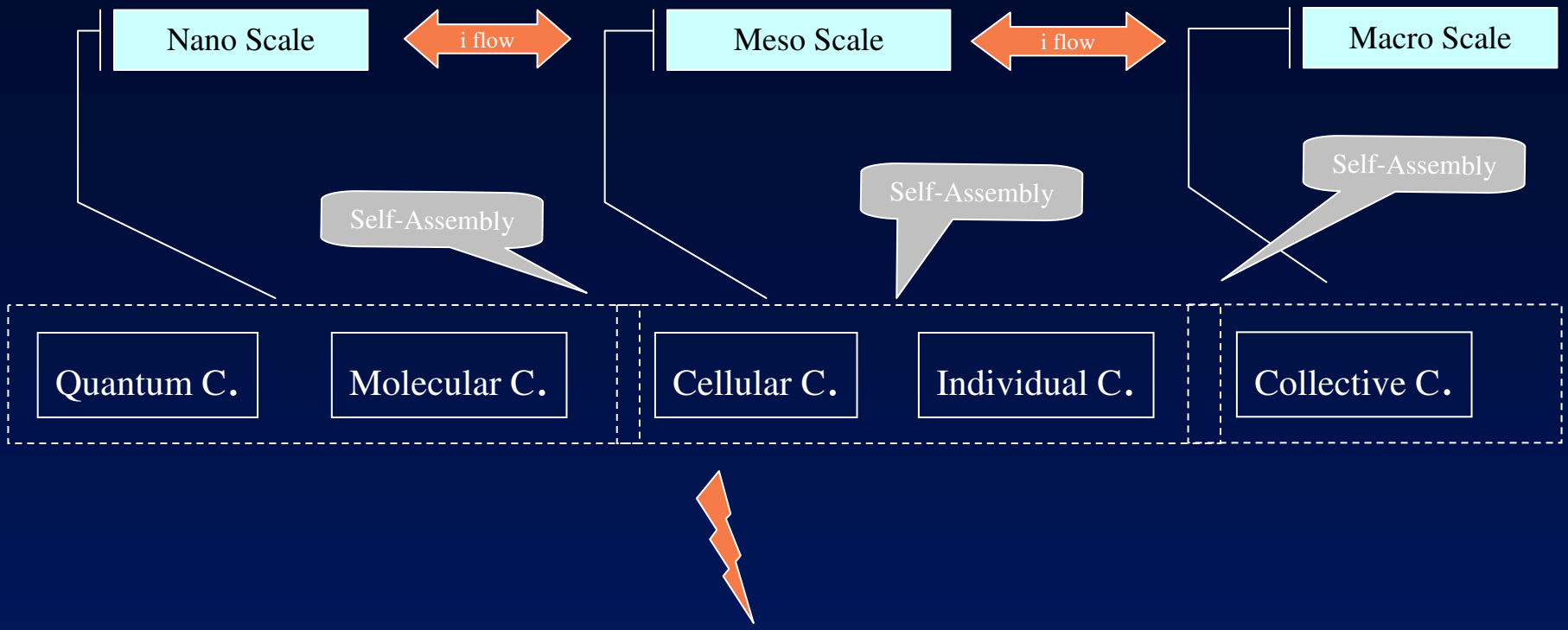
A colony and the medium (in which it lives) can be thought of as:

- **a (loose) network of computational devices**
- **the data and information carriers are shared**
- **the shared data is used in various distinct computations simultaneously**
- **computation occurs at various scales simultaneously**
- **partial computations belong to more than one larger computations**

when poorly understood causes the KMP


GacA
VfrR
RelA
RsmA
RsaL
QscR
lasR
lasI
LasI
LasR
3O-C12-HSL
Alkaline protease
Biofilms
Catalase
Elastase
Exotoxin A
LasA protease
Mn-dependant superoxide dismutase
PQS
Twitching motility???
Xcp-secretion
RelA
GacA
QscR
RpoS
rhlR
rhlI
RhlI
RhlR
C4 HSL
Alkaline protease
Elastase
Fe-dependant superoxide dismutase
Hydrogen cyanide
LasA protease
Lectin (PA-IL & PA-IIL)
Pyocyanin
Pyoverdine
Rhamnolipids
RpoS
Swarming motility
Xcp-secretion
RpoS
Pyocyanin
Pyoverdine
Catalase
Exotoxin A
Lectins (PA-IL & PA-IIL)
Swarming motility

Trans-Scale Computing:



Trans-Scale Computing

Trans-Scale Computing

The use of computational substrates at various scales simultaneously

In MC *processors* at one level become *data* at the next level up and become *systems* at the next level down.

E.g.:

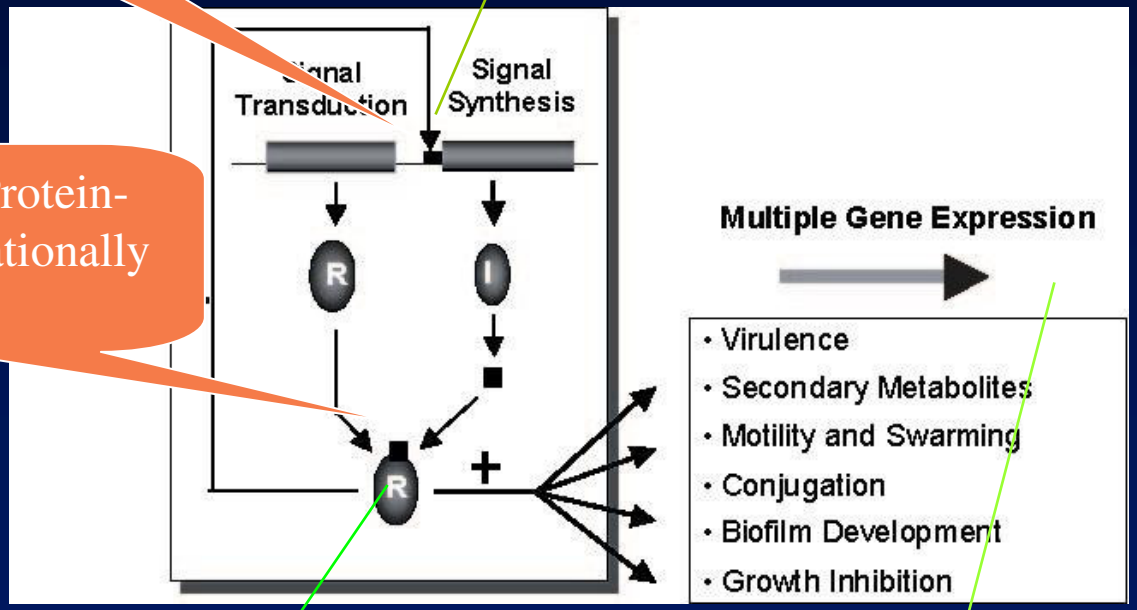
Cellular Computing and the Quorum Sensing Example

Bacterial Quorum Sensing

DNA, RNA are computationally complete

Protein-Protein, Protein-ligand are computationally complete

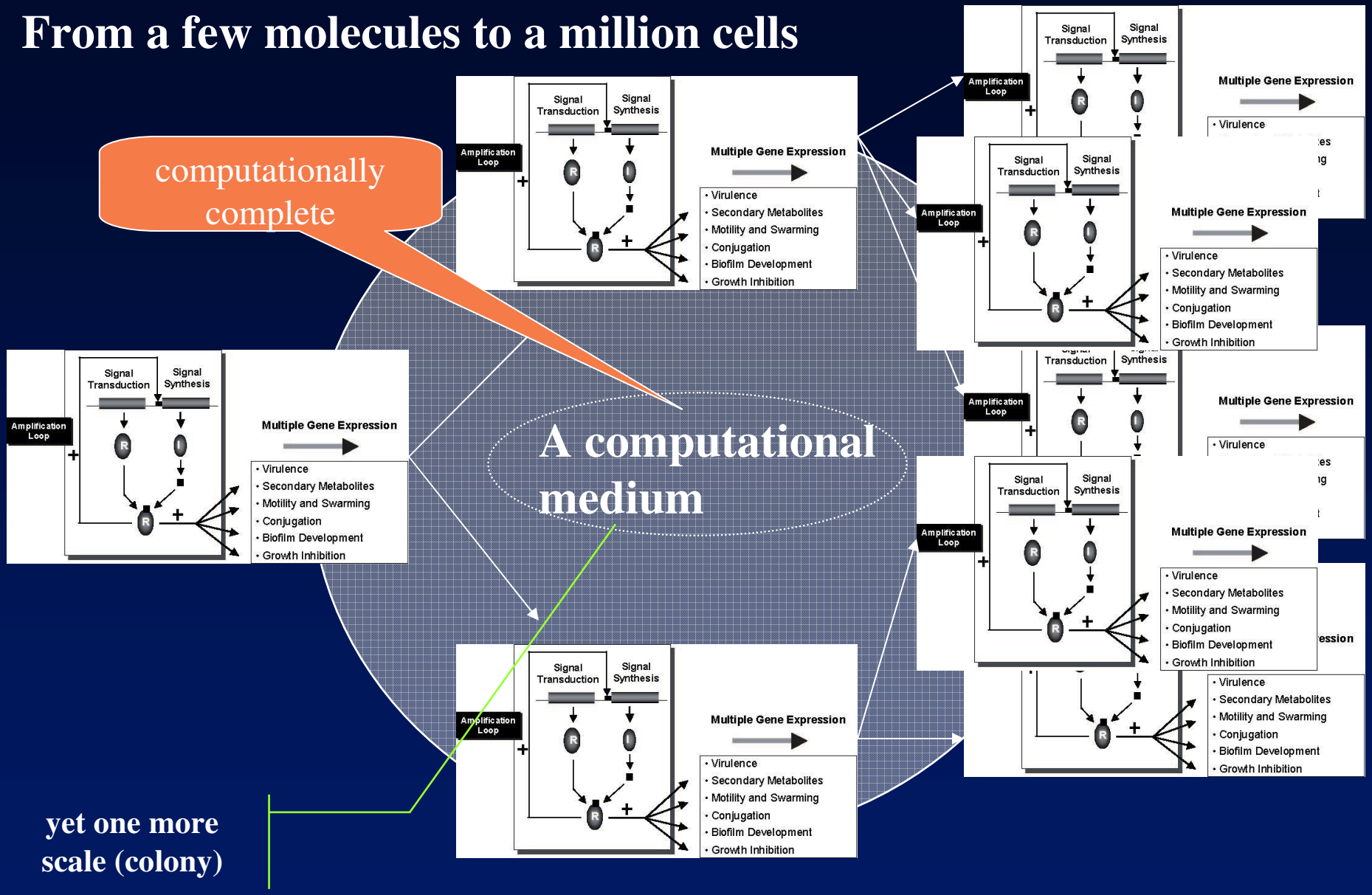
one scale



another scale

yet another scale (cell)

From a few molecules to a million cells



- It has been recognised that, although differential equation based models have a very definite role to play in modelling biological systems they *are not* a panacea that covers the whole spectrum of what an integrative approach across multiple scales needs.
- There are many cases indeed where differential equations if not completely inadequate are at least the wrong tool to use (e.g. cumbersome, intractable, etc).

- The following scheme classifies the “type” of systems one may want to model and will help illustrate this point

	I	II	III
a	Time Dependent	Continuous: Approx by Finite element methods or Finite differenc e methods	Determinis tic
b	Spatially Structured: • Steady state • Spatio/Te mporal	Discrete	Stochastic

Every system can be modelled by a variety of tools but in general we can say that:

- Systems that fit into categories Ia, IIa or IIIa could be modelled well by Ordinary Differential Equations (ODEs)
- Systems that fit into categories Ia, IIb or IIa could be modelled well by Difference (delay) equations (DE).
- Systems that fit into categories Ib, IIa or IIIa could be modelled well by Partial Differential Equations (PDEs)
- Systems that fit into categories Ib, IIb, or IIIa could be modelled well by cellular automata or gas lattice models (CA/GL)
- Systems that fit into categories IIIb could be modelled well by probability distribution functions (PDFs), Maester Eq and/or Monte Carlo simulations
- Systems that fit into categories Ib, IIb, IIIa, IIIb could be modelled well by Multi-Agent systems (MAs)
- It is *clear* that there is overlap between the type of tools to use under different situations/systems of interest and one of the goals for scientists working on systems biology should be to defines appropriate metrics and protocols to assess the suitability of each tool vis-à-vis its “competitor” tools.

Conclusions & Challenges (I)

To model QS (and other biological computations) various strategies could be used:

- **Boolean networks a la Kauffman:**
  - **scalable**
  - **but not too accurate**
- **Non-linear ordinary differential equations:**
  - **accurate for small systems of MANY components**
  - **with the caveat of assuming continuous and deterministic system**
- **Stochastic systems:**
  - **expensive + lack of details on random distributions**
- **Algebraic/Formal Languages methods (P-Systems, Pi-calculus, etc)**
- **Computational Bottom-Up (agent based) Simulations:**
  - **oversimplified but scale well**
  - **interactive design tool for the discovery of spatio-temporal patterns**

Conclusions & Challenges (II)

But is it realistic to expect to be able to build exact stochastic models, or DE based models for large systems with multiple species???

The evolutionary algorithms analogy:

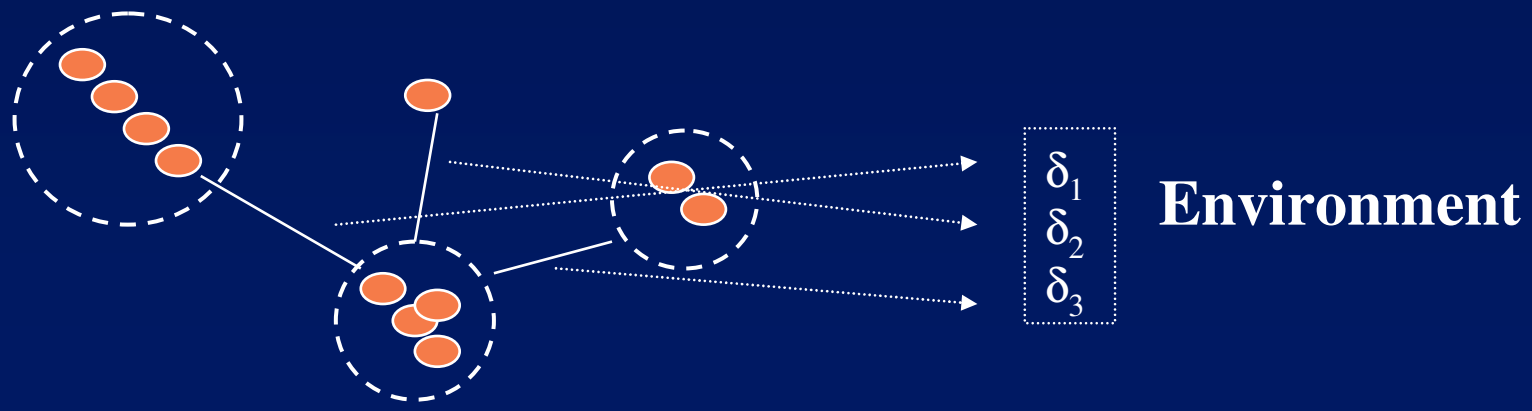
- **EAs are used every day to solve critical task**
- **EAs human-made complex systems**
- **There is no unified theory for EAs yet they are well built and used**
- **They are used because we have proven heuristic design principles**

Conclusions & Challenges (III)

P Systems are particularly appealing to model and understand QS

A Population P systems with the inclusion of the environment,PE, (Bernardini & Marian) seems to be a good compromise between formal theory, expressive modelling and potential for robust configurational search by, e.g., EAs

The modified PE system, PE', would have a hypergraph G where vertices will represent cells (with their own production / consumption rules) and links (shared media) connecting sets of cells:



Check out

- An Environment Aware P-Systems model of Quorum Sensing. By G. Terrazas, N. Krasnogor, M. Georghe, F. Bernardini, and S. Diggle, M. Camara. Proceedings of Computability in Europe (CiE), June 8-12, 2005, Amsterdam, The Netherlands, Lecture Notes in Computer Science, Springer-Verlag, 2005.
- An Appealing Computational Mechanism Drawn from Bacterial Quorum Sensing. By N. Krasnogor, M. Gheorghe, G. Terrazas, S. Diggle, P. Williams, M. Camara. In Bulletin of the European Association for Theoretical Computer Science, Num. 85, pp 135-148, February 2005.
- Membrane Computing - current results and future problems. By F. Bernardini, M. Gheorghe, N. Krasnogor, G. Terrazas. Proceedings of Computability in Europe (CiE), June 8-12, 2005, Amsterdam, The Netherlands, Lecture Notes in Computer Science, Springer-Verlag, 2005.
- Quorum Sensing P Systems. By F. Bernardini, M. Gheorghe and N. Krasnogor. *Journal of Theoretical Computer Science*, 2006 (to appear)

At www.cs.nott.ac.uk/~nxk

Conclusions & Challenges (IV)

Under which conditions PE' is sufficiently expressive?

Under which conditions a part(PE') is sufficiently expressive?

Can we create transition rules that demote expressiveness in a sub-hypergraph and promotes it elsewhere?

What sort of computational wave phenomena can we engineer?

Because of the "declarative" nature of P-systems for both the structure and rules they can be evolved (and simulated) to solve specific tasks…

Conclusions & Challenges (V)

QS is but one mechanism that allows us to coordinate behaviour across scales (from a few molecules to a million cells).

QS-like mechanisms could be useful also in other settings where the self-assembly of large systems is required.

Self-assembly is understood here in both a static and dynamic sense.

My suggestion is that to build usable Trans-Scale Computing Systems we will need to:

- **content ourselves with collections of partial theories**
- **robust simulation, optimisation and search methodologies to explore the space of configurations (e.g. *in silico* and *in vitro* evolution)**

Conclusions & Challenges (VI)

Even the design of VLSI has gone beyond the point where humans are the best designers and where humans apply only rational design

The ultimate goal of VLSI for defence and space exploration is Evolvable Hardware (i.e. an EA)

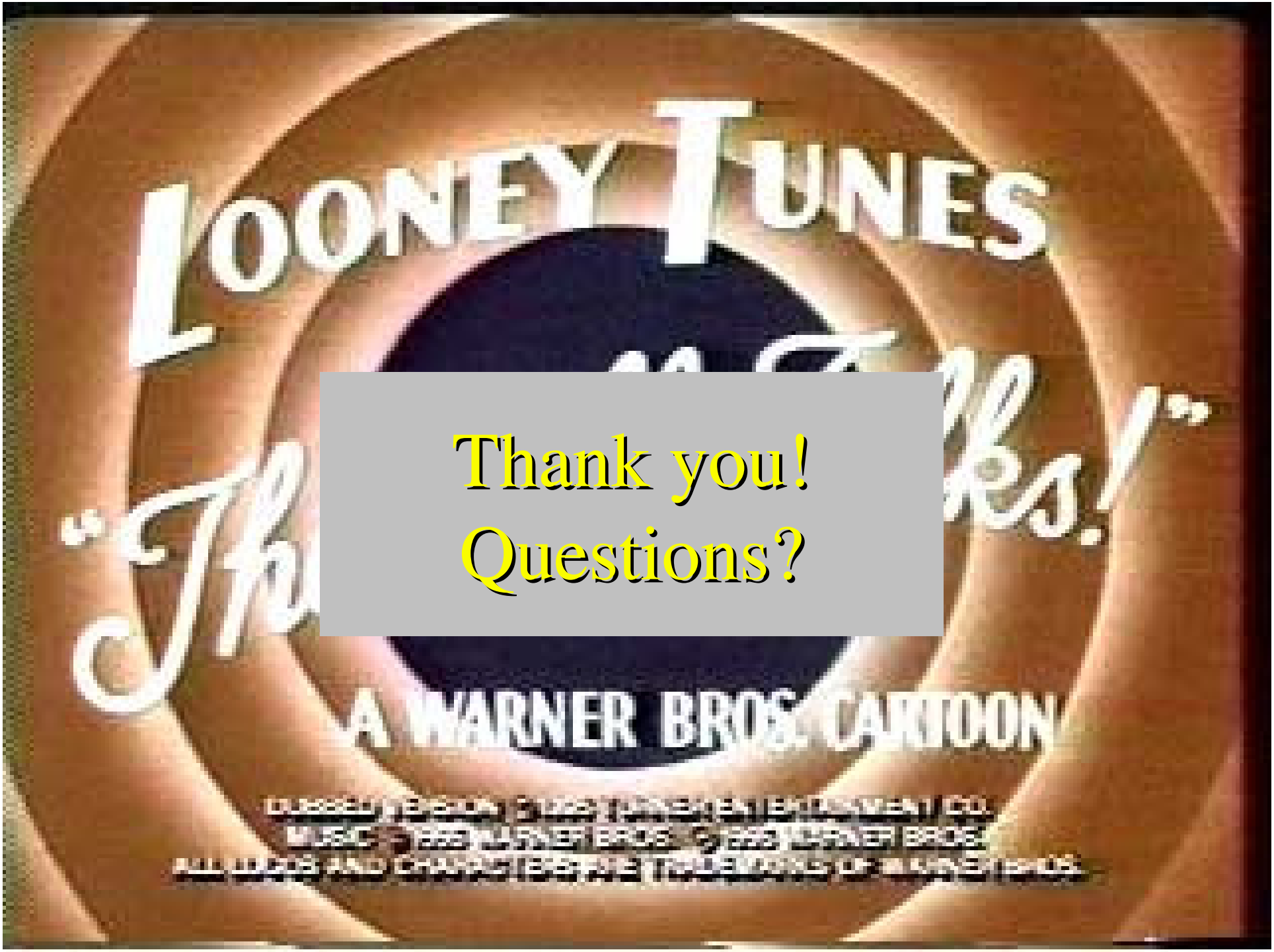
I very much doubt that the design of scalable Trans-Scale Computing systems in general (and cellular computing in particular) will, at the end of the day, be done by rational design alone but rather (as Jorge Luis Borges would say) I think it will be by a combination of healthy (partial) theories and (d)efficient practices!

Conclusions & Challenges (VII)

The engineering of large complex systems (e.g. multi-cellular communicating systems) has enormous challenges:

• redefinition of computation : do we *really* want to stick to definitions of computations which were formulated for non-Trans-scale systems?

• redefinition of program correctness: shouldn't we relax our concepts of correctness as to harness the intrinsic KMP making our programming endeavours less crisp?



Thank you!
Questions?