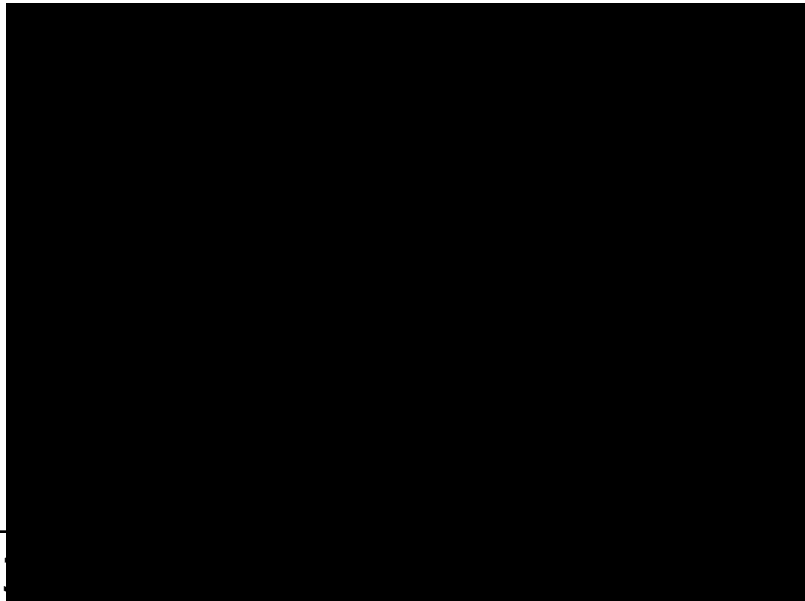
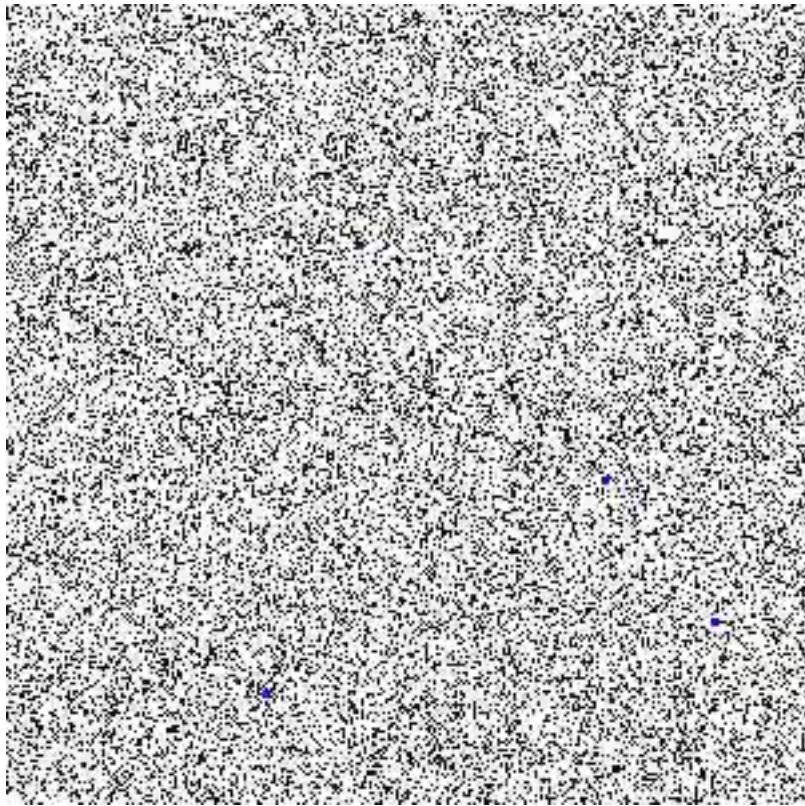
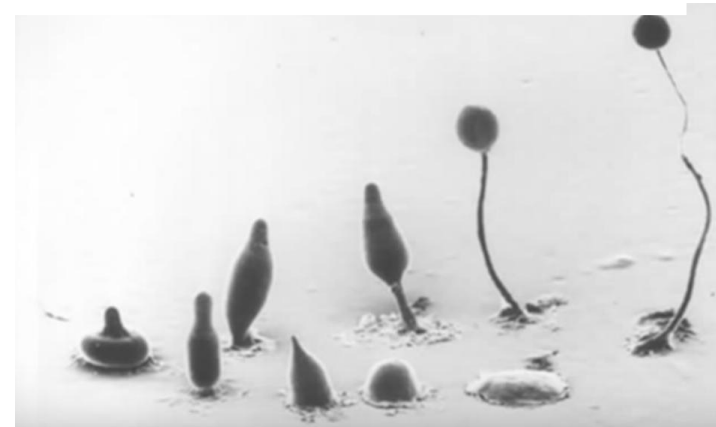


Quorum



Dictyostelium,
a slime mold



Quorum

Quorum signalling & sensing

- Introduction - Initial observations

- The chemicals of communication

- The receptors

- The intracellular pathways

 - Gram negative

 - Gram positive

- Intra- vs inter-species communication

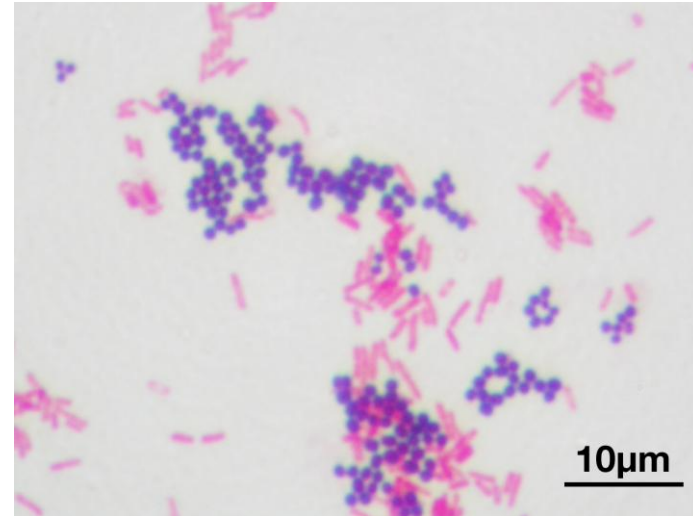
Quorum signalling & dealing with bacteria

Gram-negative vs Gram-positive bacteria

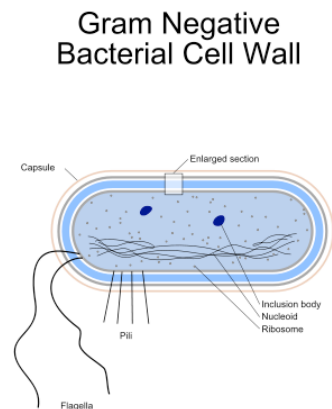
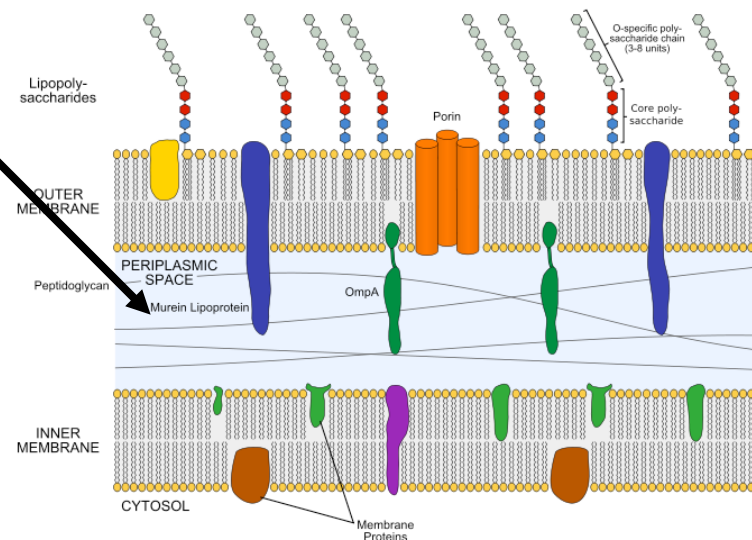
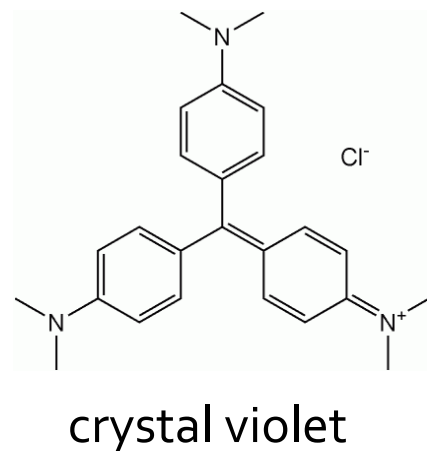
Gram's coloration of bacteria with crystal violet which labels peptidoglycan :

Gram-negative, e.g. *E. coli* => pink

Gram-positive e.g. *S. aureus* => purple

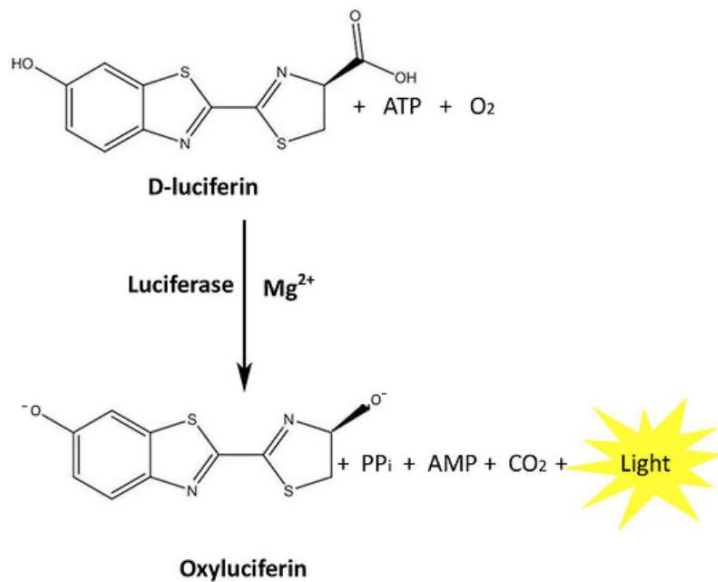


Gram-negative bacteria : peptidoglycan is occluded by outer membrane



Initial observations - the 1960's

The Gram negative bacterium *Vibrio fischeri* emits bioluminescence



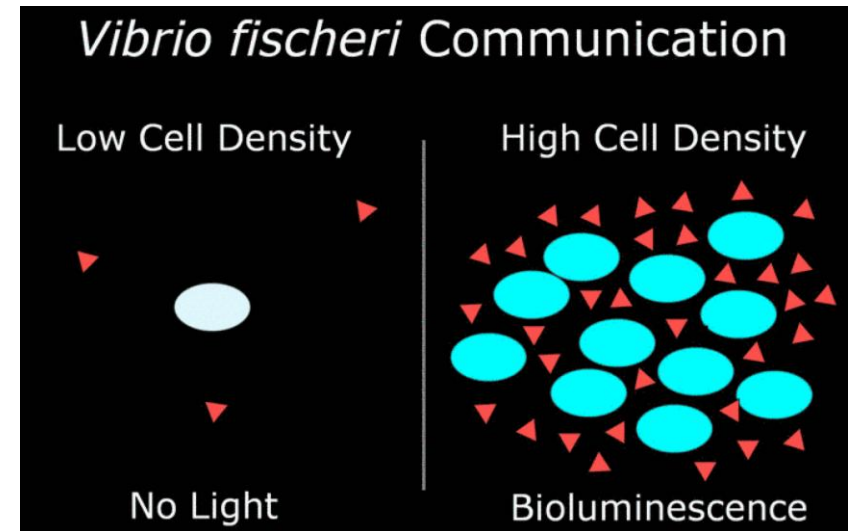
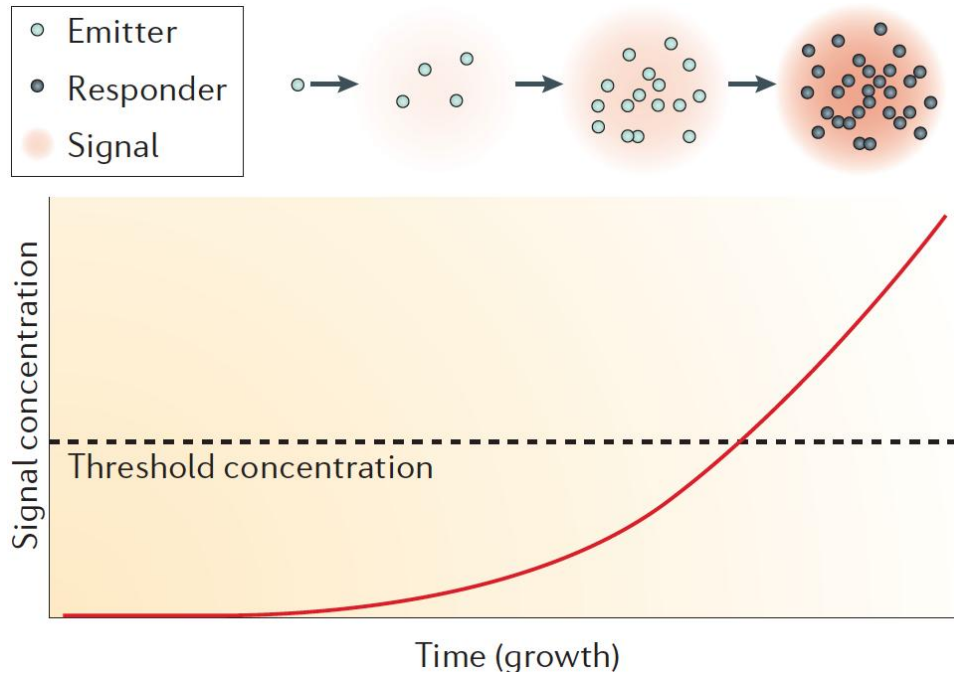
Only when i) the culture is very dense, and
ii) the cellular content of luciferase is high

Initial observations - since the 1960's

- We all carry a large variety of bacteria
- In general do not cause any problem

Chemical communication

- Initial postulate :
- Bacteria produce & secrete signalling molecules
 - When the concentration reaches a threshold,
the cells change behaviour
=> auto-stimulation



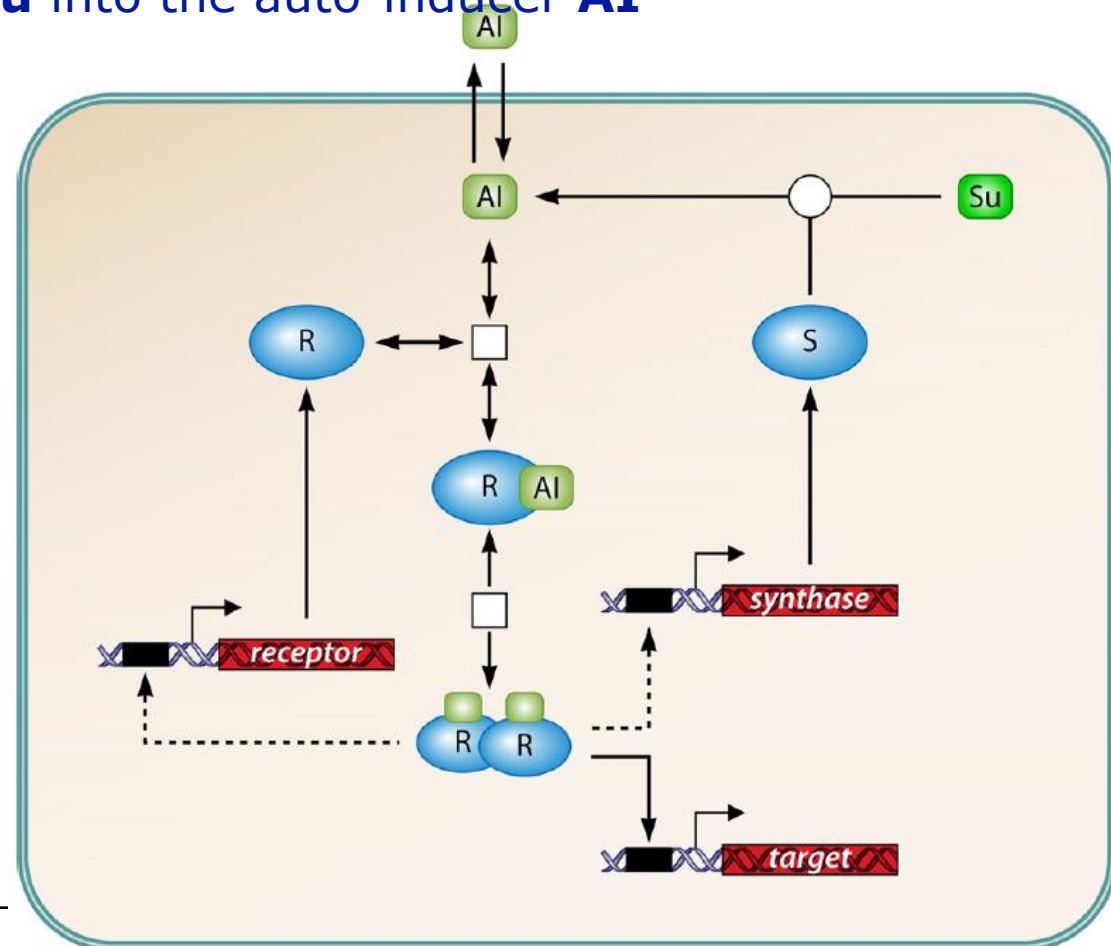
The hypothesis of chemical communication

Initial minimal hypothesis : Auto-induction

- 1) bacteria secrete a molecule : the auto-inducer **AI**
- 2) this molecule can activate a transcription factor **R**, directly or via-via
- 3) gene transcription and translation to produce enzyme **S**
- 4) enzyme **S** converts a substrate **Su** into the auto-inducer **AI**

=> a strong amplification !!

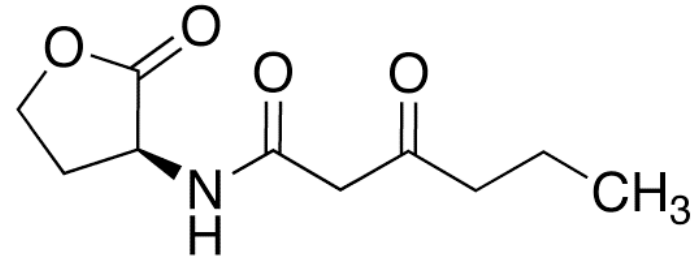
Communication among the same kind !



The chemicals of communication

Identification of the 1st auto-inducer **AI** molecule :

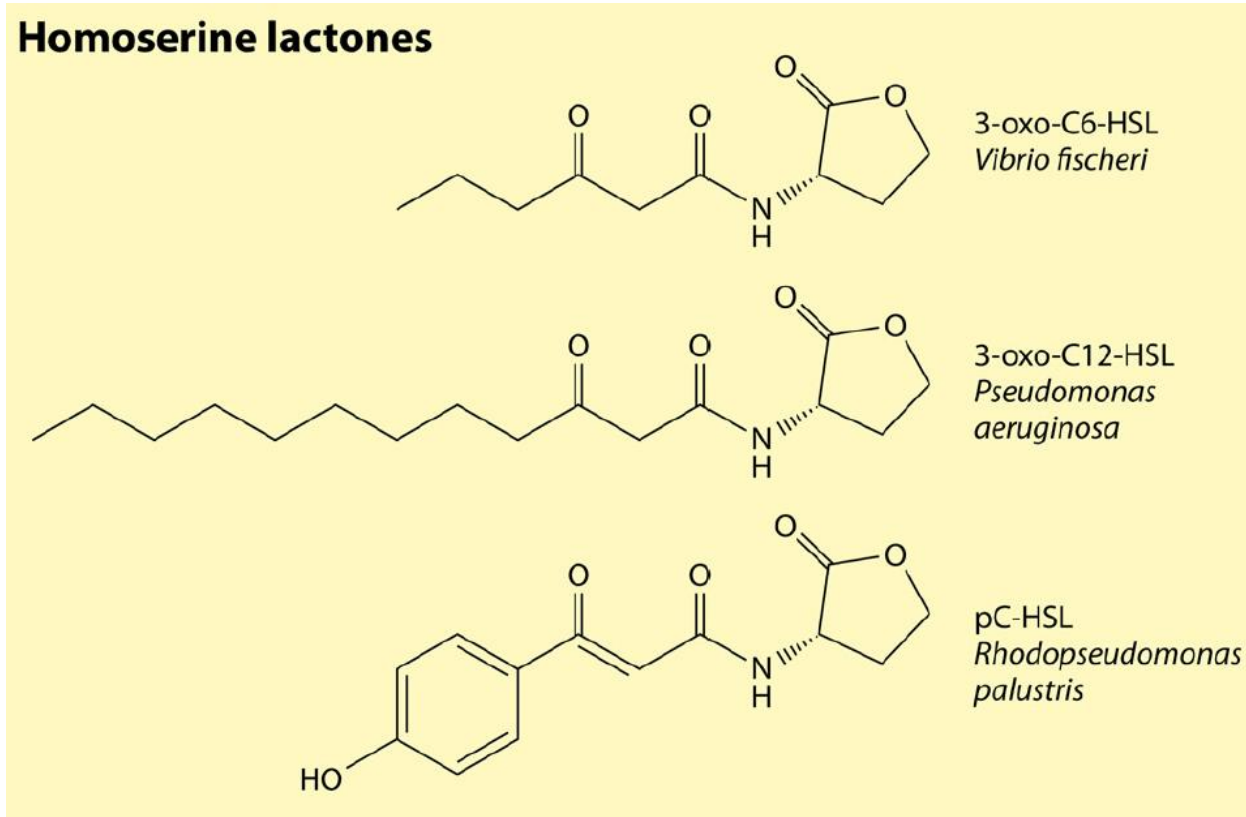
N-3-(oxohexanoyl) homoserine lactone



The chemicals of communication

- ÷ Species specific AI-molecules
- ÷ Different behaviour's induced.

=> i.e. talking with your now kind !!



Bioluminescence, motility,
host colonisation

Virulence, biofilm formation,
motility, iron acquisition

Migration towards food source

The transcription factors that binds the chemicals

Lux_R proteins : ÷ cytosolic protein that bind **AHL** compounds
produced by the same species !!

÷ transcription factors of the Lux operon LuxICDABE

Lux_I => synthesis of AHL compounds

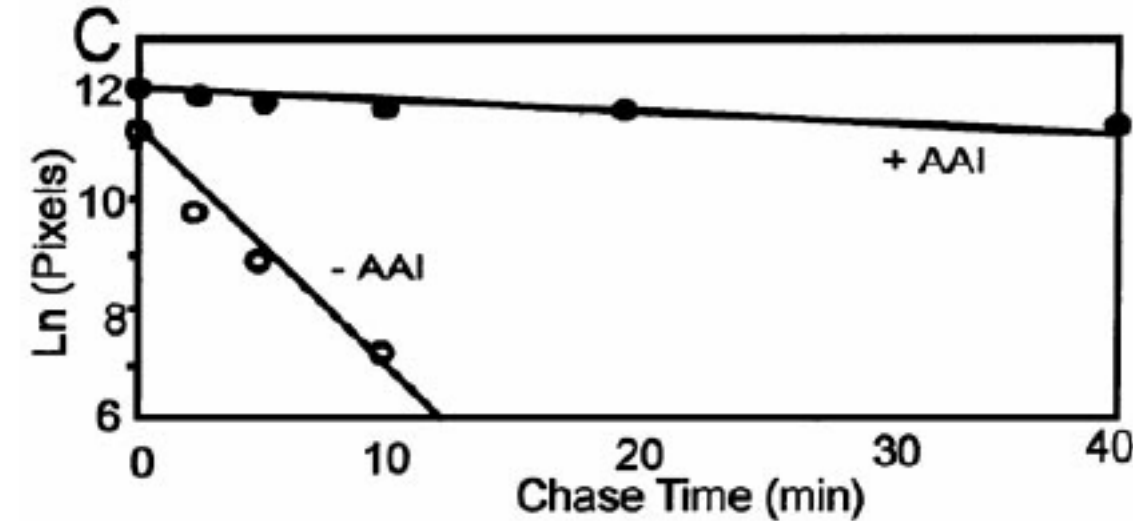
Lux_AB => luciferase

Lux_CDE => substrate for luciferase

Lux_R type proteins and ligand binding

The case of Tra_R from *Agrobacterium tumefaciens* :

- Presence of auto-inducer **AI** dramatically prolongs life-time of Tra_R



÷ AI binding seems to stabilise

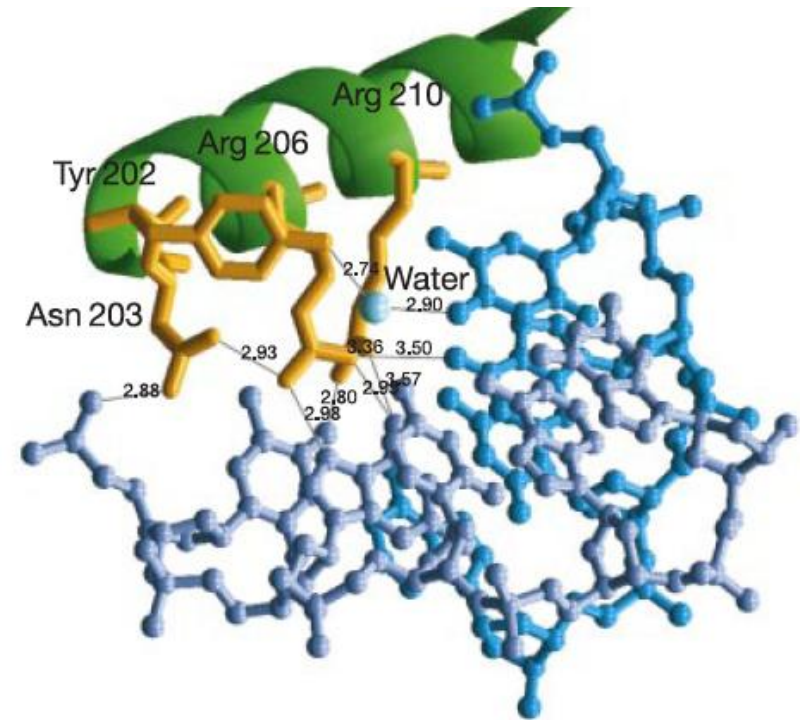
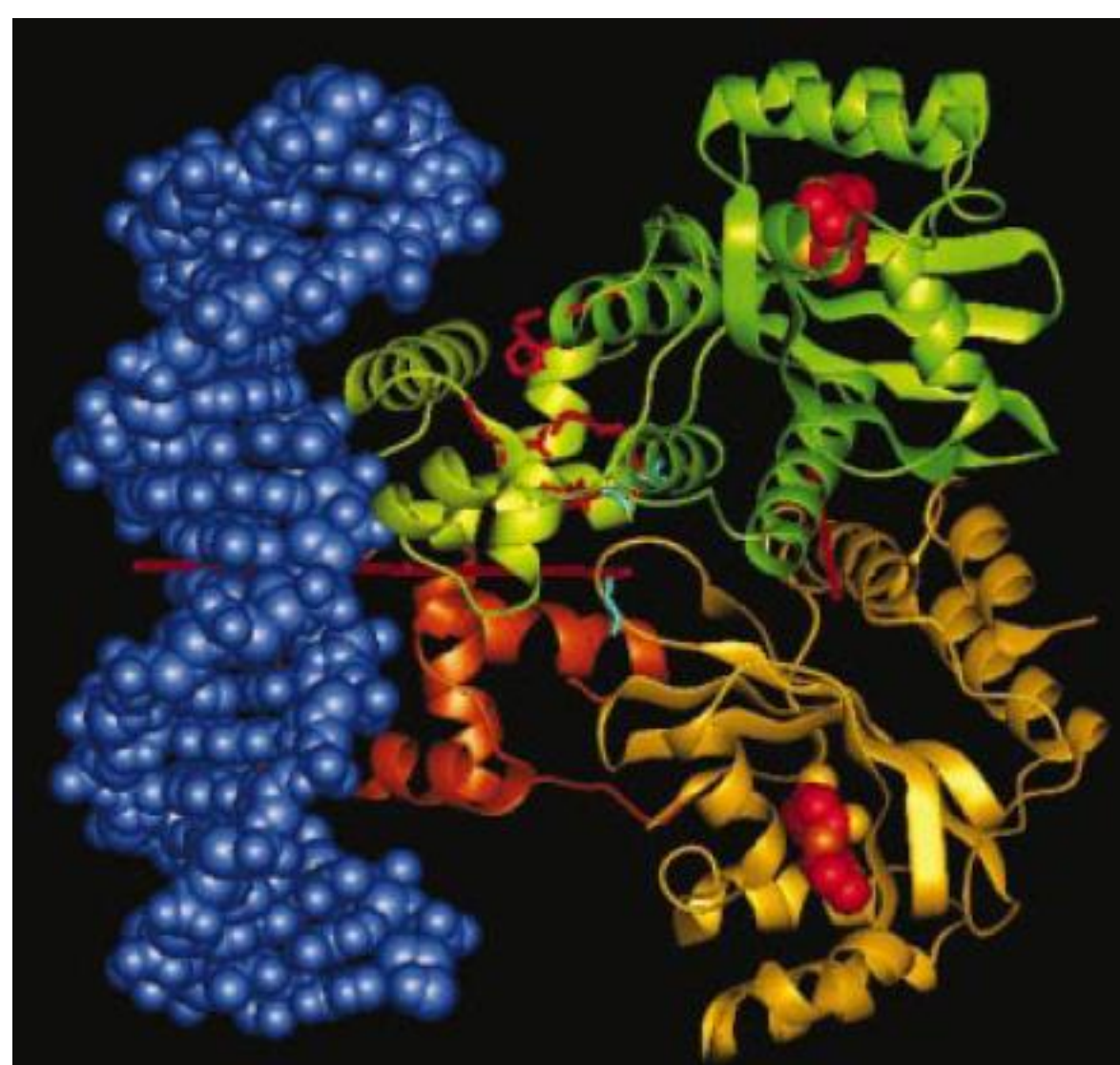
Proc. Natl. Acad. Sci. USA
Vol. 96, pp. 4832–4837, April 1999

=> without its cognate ligand, Tra_R is degraded

=> present only at low concentrations

Lux_R type proteins and DNA binding

Ligand-bound dimeric Lux_R proteins bind to their DNA-recognition elements
E.g. Tra_R with N-3-oxooctanoyl-L-homoserine lactone (OOHL)



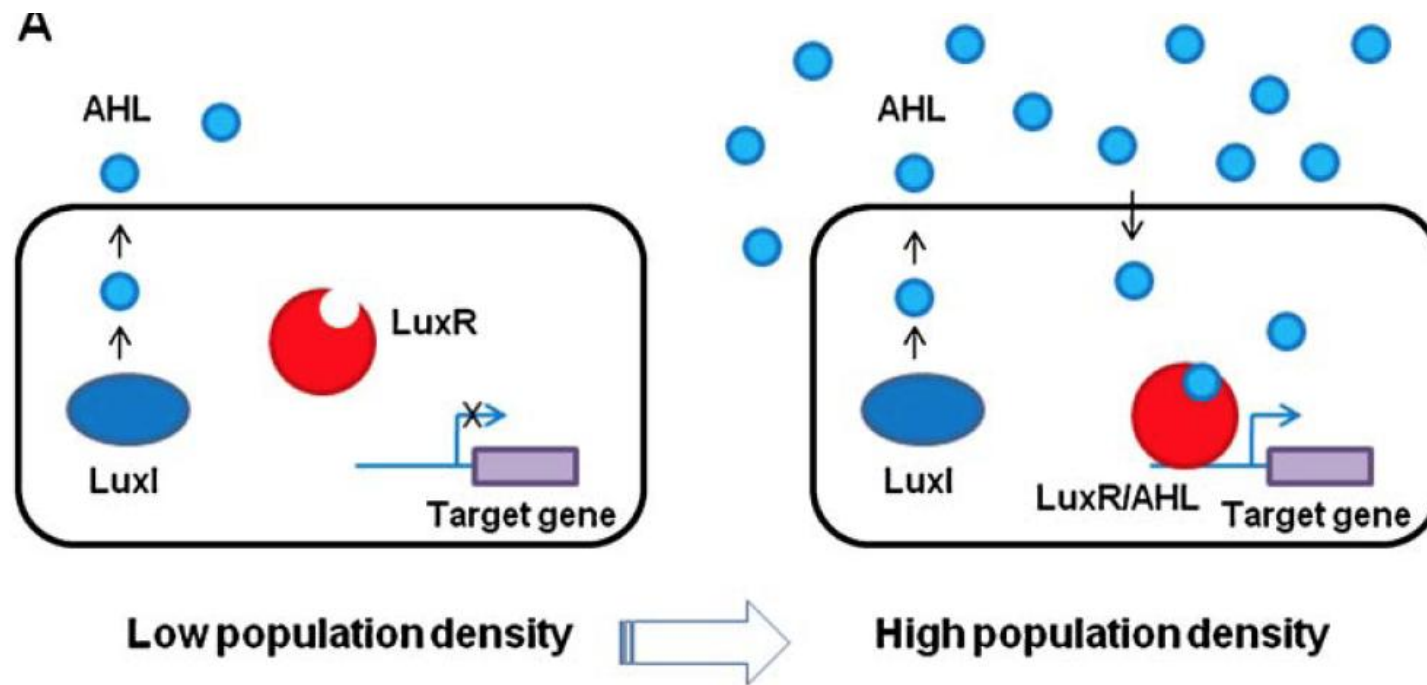
Helical recognition domain
binds to Tra-box on DNA

Nature **417**, 971–974 (2002)

The canonical model of species specific chemical communication

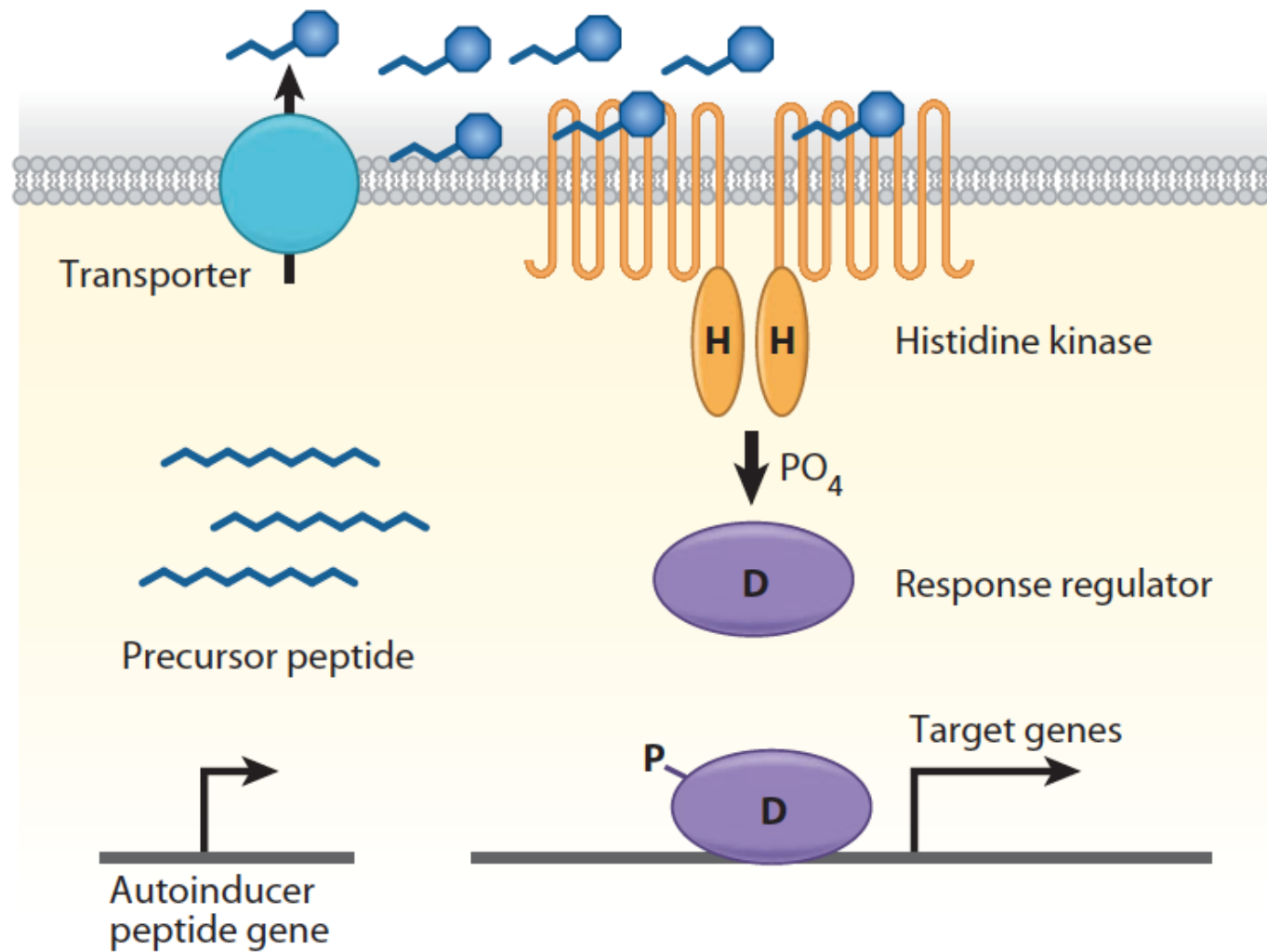
Initial minimal hypothesis : Auto-induction

- 1) bacteria secrete a molecule : the auto-inducer **AI** \Rightarrow AHL
- 2) this molecule can activate a transcription factor **R**, \Rightarrow Lux_R
- 3) gene transcription and translation to produce enzyme **S** \Rightarrow Lux_I
- 4) enzyme **S** converts a substrate **Su** into the auto-inducer **AI**



Quorum sensing in Gram-positive bacteria : Auto-Inducer Peptides

The canonical **Gram-positive** quorum-sensing scheme for intra-species using auto-inducer peptides **AIP**



Differences vs

Gram-negative system:

- ÷ AI's are larger, peptidic molecules "**AIP's**"
- ÷ Transporter
- ÷ Membrane receptor
- ÷ Intracellular signal relays

• Specific "peptide - transporter - receptor - response regulator" combinations

Auto-inducers of Gram-positive bacteria intra species

The signalling molecules are peptides, often modified

- precursors are often modified and then translocated over the plasma membrane by a pump

ADPITRQWGD

B. subtilis (ComX)

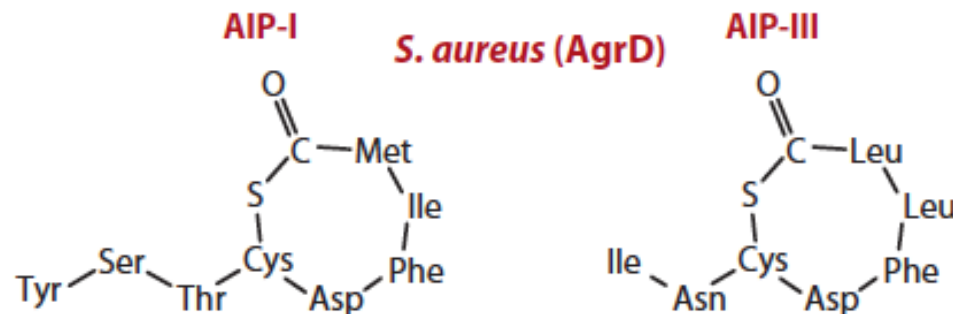
W is prenylated

ERGMT

B. subtilis (CSF)

EMRLSKFFRDFILQRKK

S. pneumoniae (CSP)

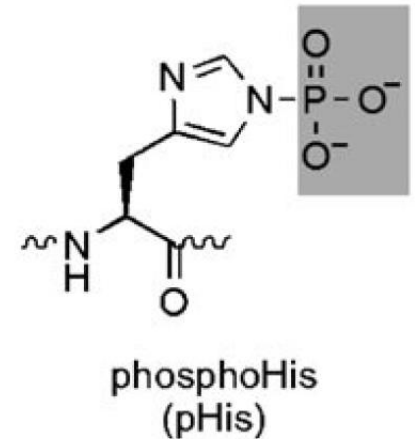


cyclised

Two-component detection system of Gram-positives

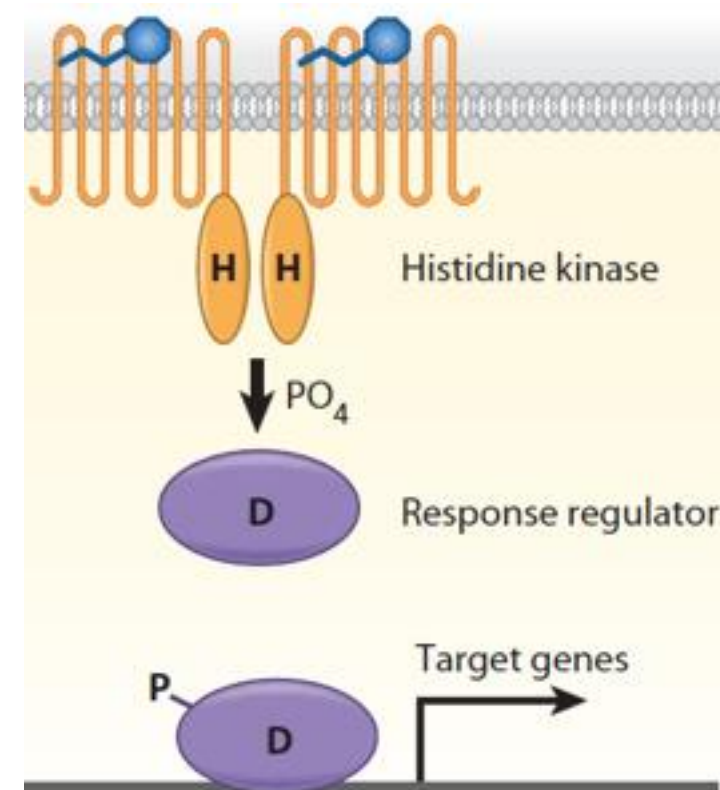
Two components :

- “sensor histidine kinase” (**SHK**): a plasma membrane receptor functionally similar to RTK
 - ligand bound receptors cross-phosphorylate each other on a **His** residue

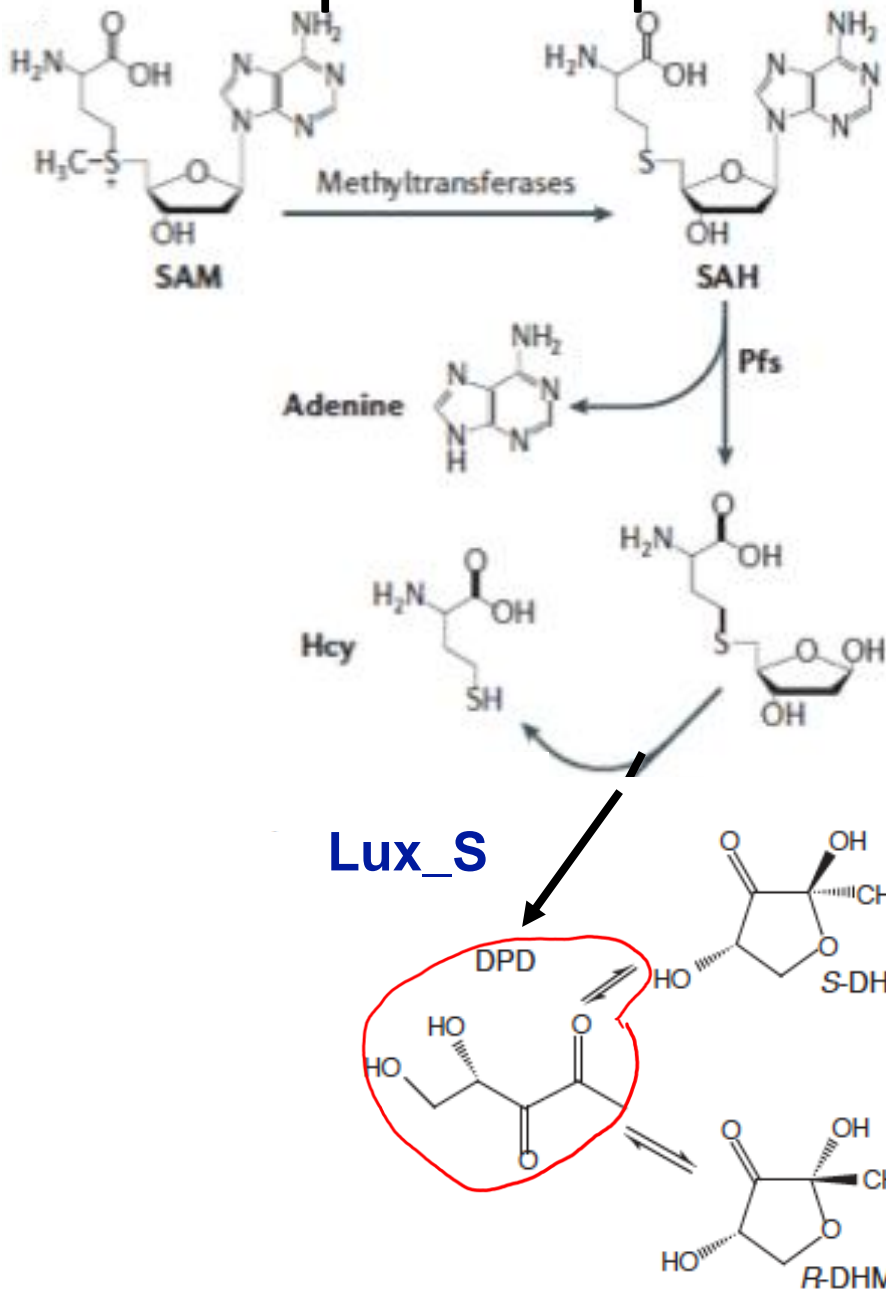


NB :SHK's also called “two component system”

- “response regulator” (**D**):
 - the **P_i** is transferred from the **SHK** to an **Asp** residue on **D** to render it active
 - => transcription of target operon
 - with often genes for producing enzyme, transporter, **SHK** and **D**

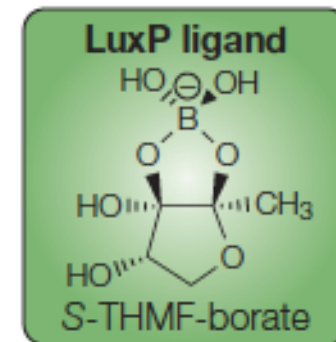


Inter-species quorum signalling - Lux_S-AI2 system



All based on a degradation product of S-adenosine-methionine (SAM), the

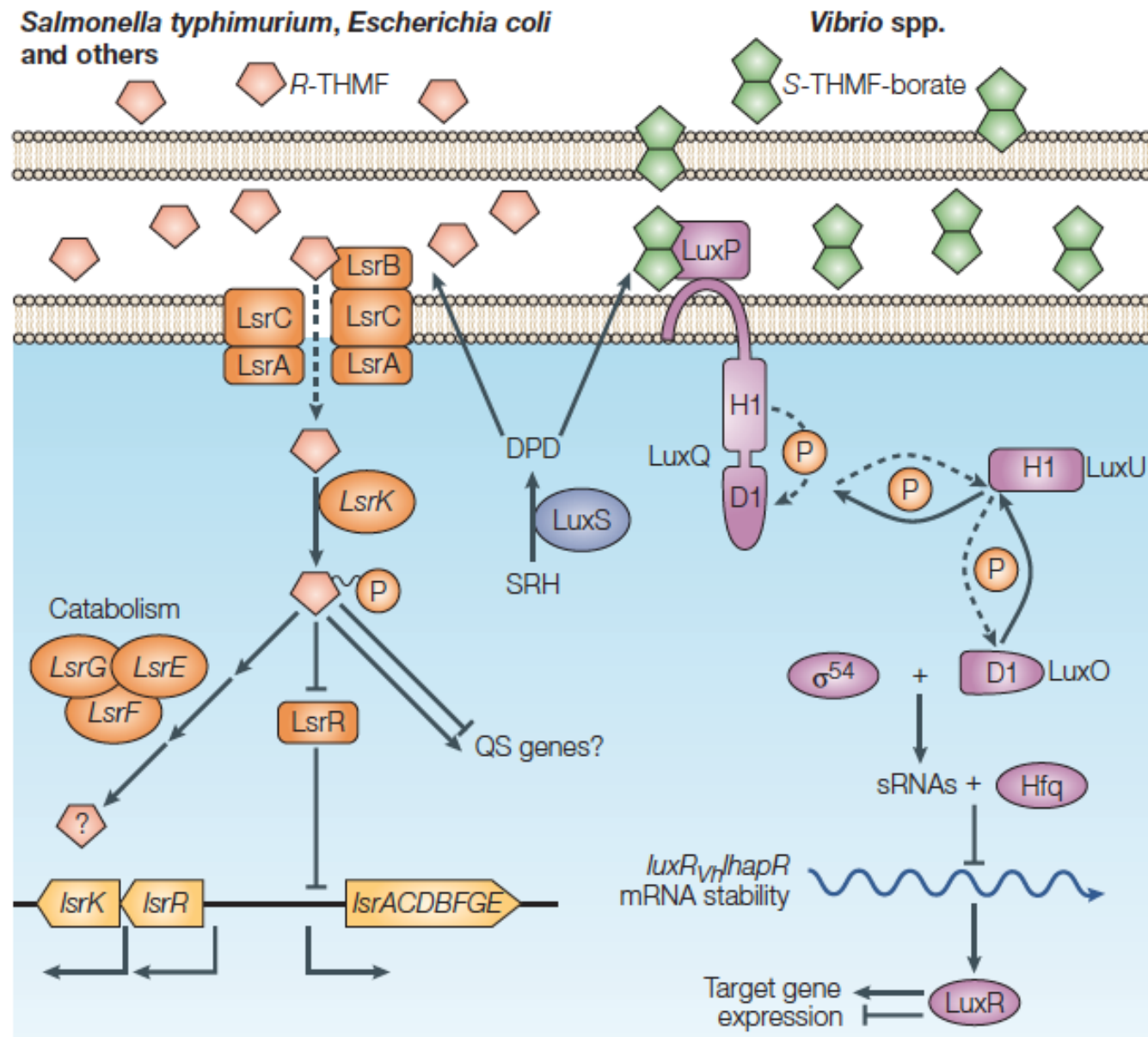
4,5-dihydroxy-2,3-pentadione **DPD** produced by the enzyme **Lux_S**



"AI-2"
canonical

Quorum AI-2 interspecies signalling

Detection and response schemes are



doi:10.1038/nrmicro1146

Quorum signalling methods - A comparison

Three types of autoinducer

① **Acyl-homoserine lactone (AHL)**

- Autoinducer used by Gram (-) bacteria for intraspecies communication

② **Autoinducer peptide (AIP)**

- Autoinducer used by Gram (+) bacteria for intraspecies communication

③ **Autoinducer-2 (AI-2)**

- Autoinducer used by many QS bacteria for interspecies communication

Table 1 | **Metabolic costs of signal production in bacterial cell-cell-signalling systems**

Signal	Metabolic Cost*	Example
Oligopeptides	High	184 ATP (for the AgrD protein of <i>Staphylococcus aureus</i> , 46-amino-acid preprotein) [‡]
N-acyl homoserine lactones	Intermediate	8 ATP (for butyryl-homoserine lactone, C4-HSL, produced by RhII in <i>Pseudomonas aeruginosa</i>) [§]
Autoinducer-2	Low/none	0–1 ATP

Quorum signalling to manipulate bacteria

“There must be of all in the world”

Cervantes 1615

Quorum-signalling induced behaviour

	Gram-negative: LuxI/R	Gram-positive: oligopeptide	Hybrid
Regulated behaviors	<p><i>P. aeruginosa</i>: Elastase production, rhamnolipid production (virulence factors)</p> <p><i>V. fischeri</i>: Bioluminescence</p> <p><i>Erwinia carotovora</i>: Exoenzyme production (virulence), antibiotic production</p> <p><i>A. tumefaciens</i>: Conjugation of the Ti plasmid</p> <p><i>Serratia liquefaciens</i>: Pigment production, antibiotic production</p> <p><i>Sinorhizobium meliloti</i>: Exopolysaccharide synthesis (symbiosis)</p>	<p><i>B. subtilis</i>: Competence, sporulation</p> <p><i>S. aureus</i>: Virulence, biofilms</p> <p><i>Lactococcus lactis</i>: Nisin production</p> <p><i>Streptococcus pneumoniae</i>: Competence</p>	<p><i>V. harveyi</i>: Luminescence, type III secretion</p> <p><i>Vibrio cholerae</i>: Virulence, biofilm production</p> <p><i>Vibrio anguillarum</i>: Protease production</p> <p><i>S. typhimurium</i>: Lsr transporter</p> <p>** <i>Photobacterium luminescens</i>: Antibiotic production</p> <p>** <i>Clostridium perfringens</i>: Toxin production</p> <p>** <i>Streptococcus pyogenes</i>: Hemolysin production</p>

TRENDS in Cell Biology

Quorum sensing to manipulate bacteria

÷ Control of bacteria:

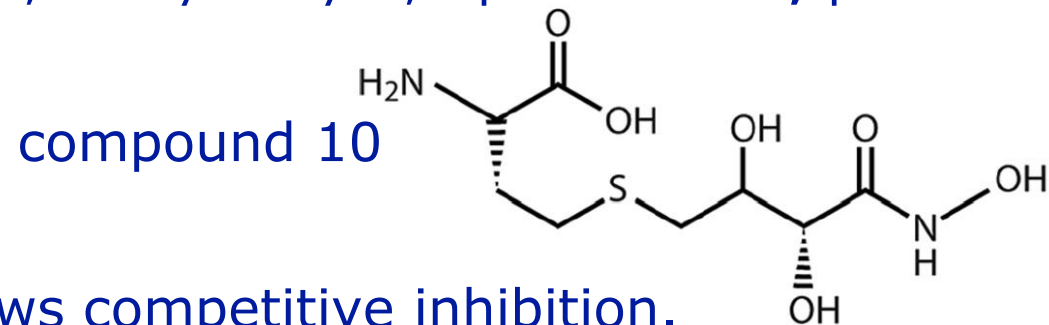
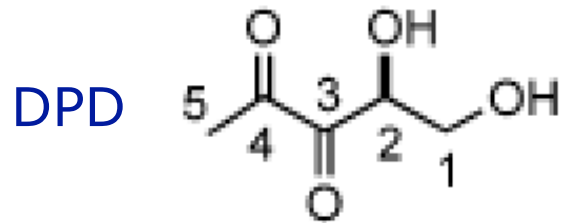
- Traditionally by antibiotics :: “let’s kill them !”
- Inhibition of quorum signalling :: “keep them calm !”

÷ Essential steps in the quorum procedure :

- signal production enzymes
- signal detection receptor systems
- signal amplification response regulators

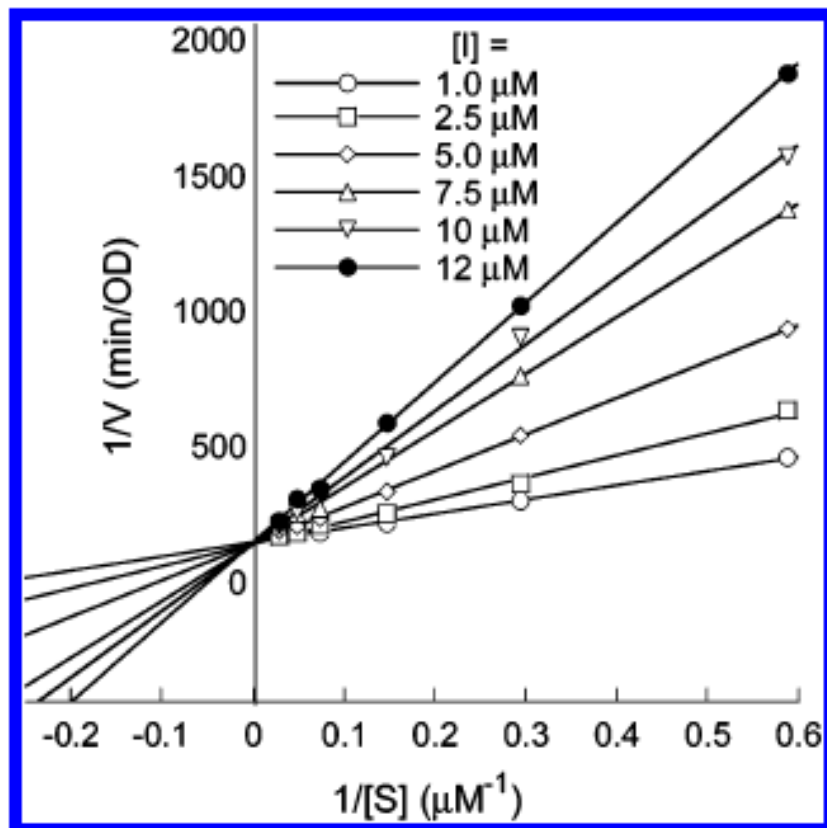
Inhibition of quorum signal production

- Inhibitor of Lux_S to produces 4,5-dihydroxy-2,3-petanedione, precursor of AI-2



Line-Weaver Burke plot shows competitive inhibition.

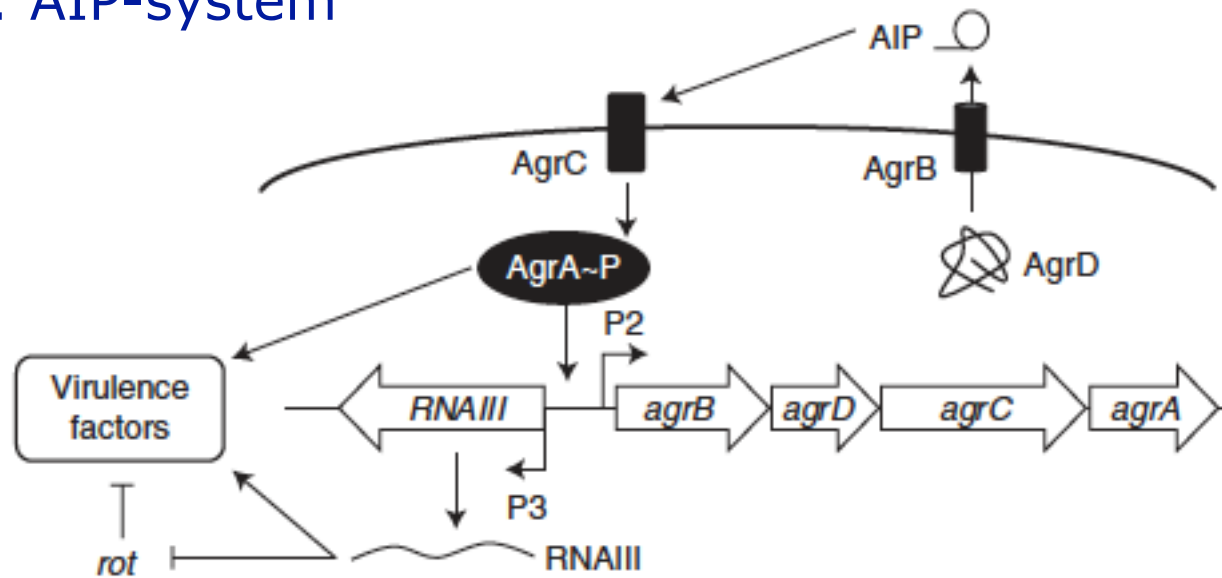
K_i ranges from 0.7 to 10 μM for Lux_S
from various species



Shen 2006 J Med Chem 49,3003

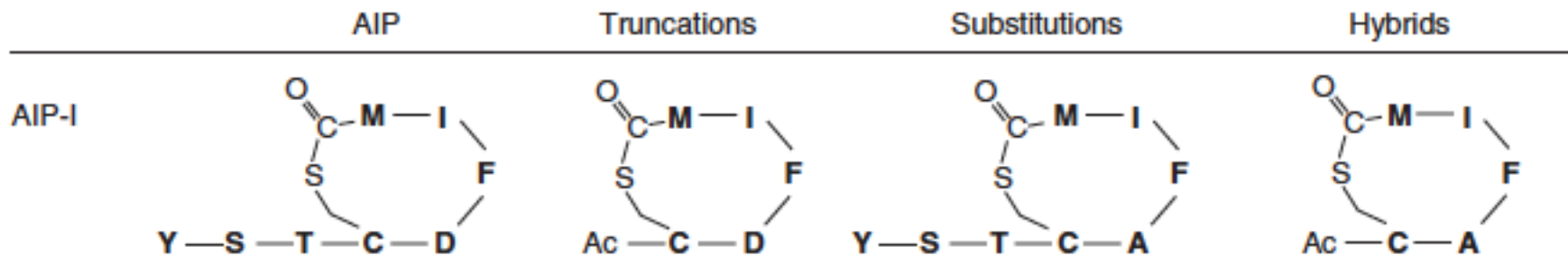
Inhibition of quorum signal detection

S. Aureus : AIP-system



NB: strains lacking ArgC are non-virulent

Developed inhibitors for AIP-1 with K_i 's ranging from nM to μ M.



Quorum

Quorum signalling & sensing

The density of bacteria influence their behaviour

Specific chemicals are used for communication
within & among species

Signalling pathways involve
specific recognition, and
amplification

Quorum sensing can be exploited to manage
bacteria

Further reading

Henke & Bassler "Bacterial social engagements"
TiCB 2004

Zhu & Kaufmann "Quo vadis quorum quenching?"
Curr Op Pharm 2013

Mion ea 2019

Duddy & Bassler 2021

Bonnie Bassler : TED talk 2009

