Quorum sensing

Microbial cell-to-cell communication

Contact: fiona.walter.nz@gmail.com

Website: https://fionastreasurechest.com/

Image source: https://www.pngegg.com/

Learning objectives

By the end of this lecture, you should be able to:

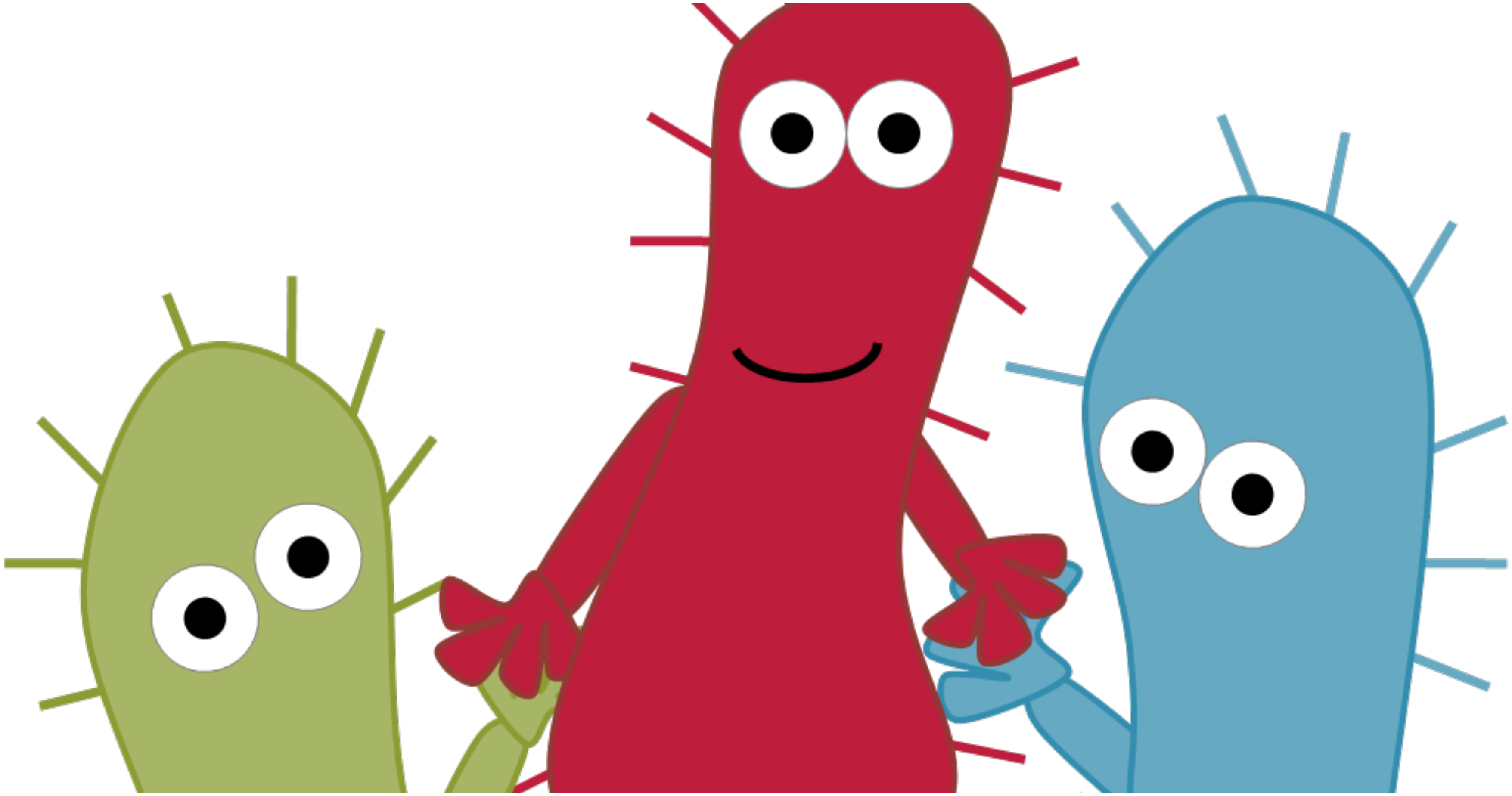
- Describe the process of quorum sensing by bacteria — Objective 1
- Explain, using detailed example(s), how quorum sensing can be manipulated to control microbial populations — Objective 2
- Describe applications for manipulation of quorum sensing — Objective 3

Hint! Previous exam questions:

- 2019: Describe the process of bacterial quorum sensing, and explain applications for the manipulation of quorum sensing.
- 2018: Describe the process of bacterial quorum sensing, and explain how it can be manipulated to control microbial populations.
- 2017: Describe the process of quorum sensing and explain how it can be manipulated to control microbial populations.

- Worth 20% of your overall grade!

What do **you** know about quorum sensing?



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Objective 1

What is quorum sensing?

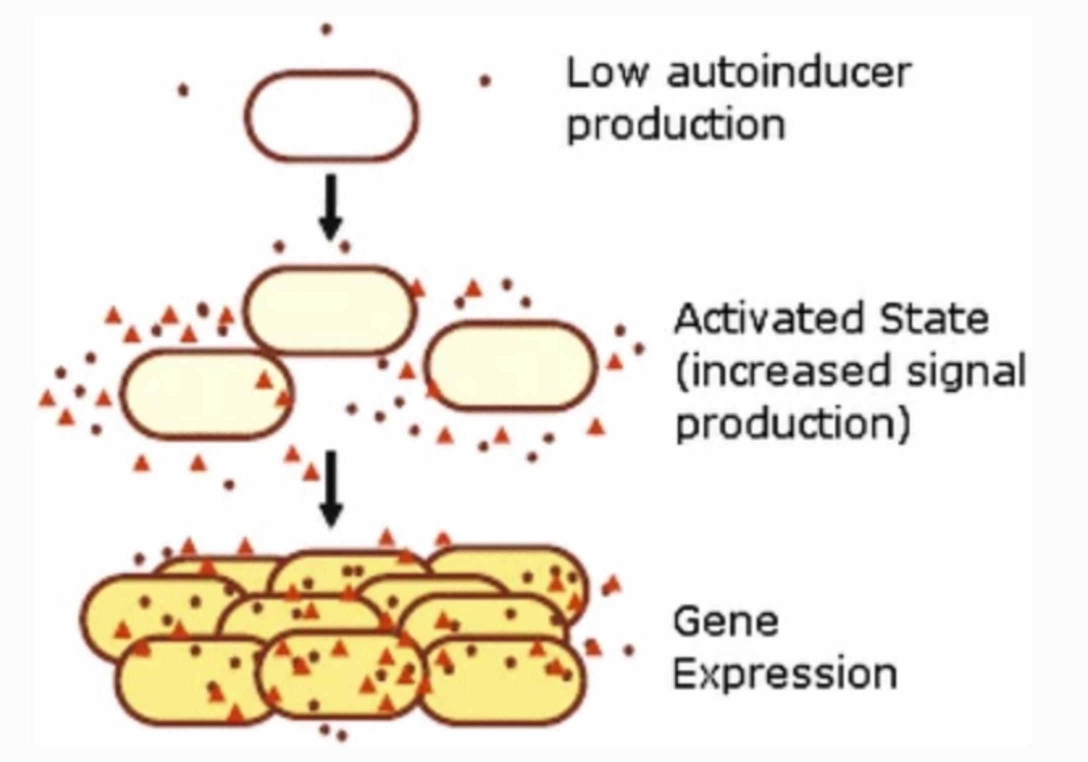


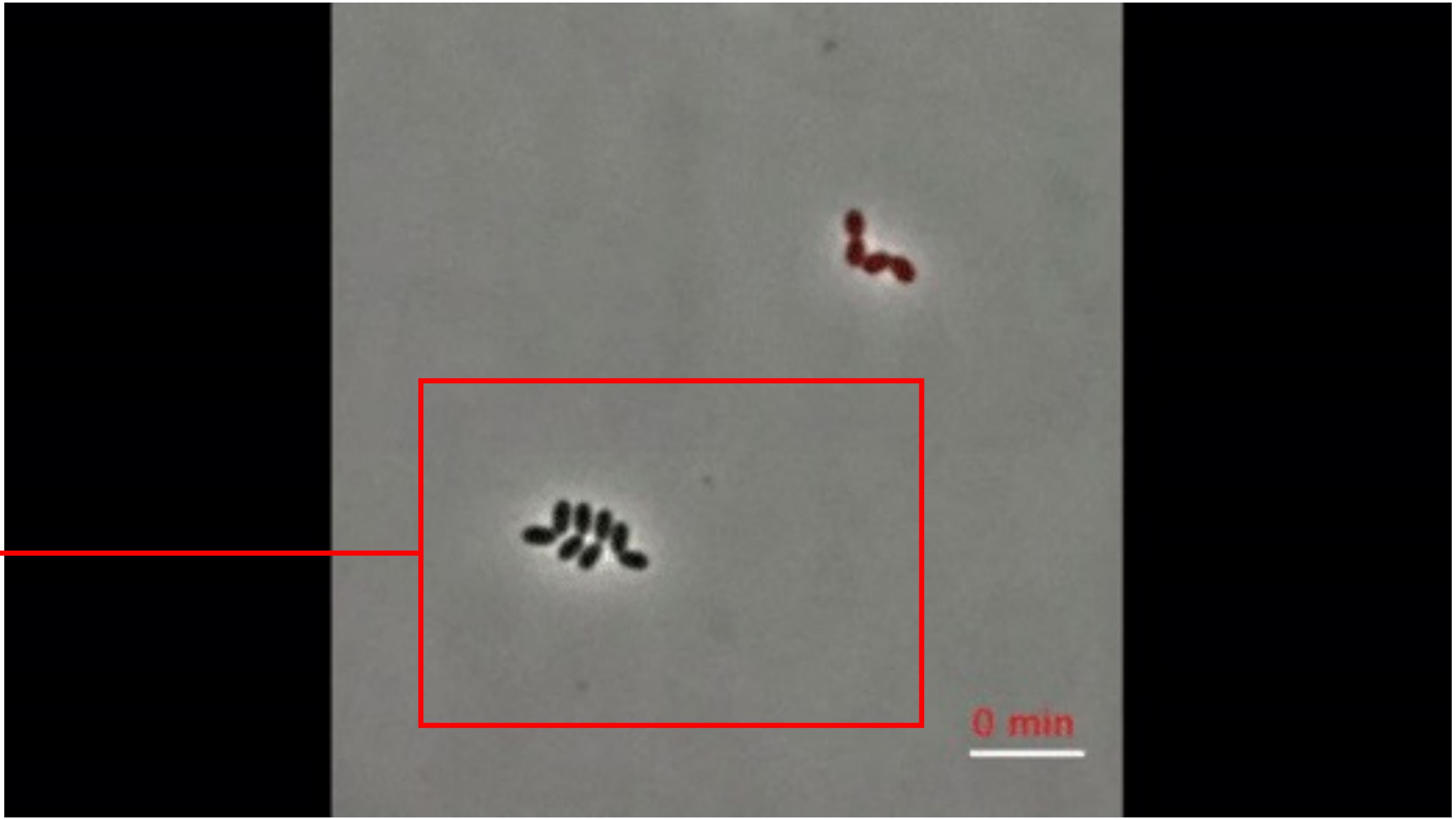
Image source: Péréz-Velázquez, Gölgeli & García (2016): Mathematical Modelling of Bacterial Quorum Sensing: A Review

- Bacterial communication
  → Within & between species
- Division of labour for difficult tasks
- Regulation of gene expression as a response to changes in population density
  → more cells = more **autoinducers** = gene expression

Objective 1

What is quorum sensing?

Gene expression happens very suddenly when **critical cell density** is reached!



Source: Morena-Gamez *et al*. (2017): Quorum sensing integrates environmental cues, cell density and cell history to control bacterial competence https://www.youtube.com/watch?v=XMgMhF3Sayo

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Objective 1

Quorum sensing example: *Vibrio harveyi*

- Gram-negative marine bacterium
- Opportunistic pathogen of marine life
  - Causes "luminescent vibriosis" amongst other illnesses
- Quorum sensing (QS) controls >750 genes
  - E.g. virulence, biofilm formation, bioluminescence, flagellar motility

- Research interest:
  - Loss of 8bn $ in revenue
  - Emerging pathogen due to climate change

Objective 1

V. harveyi: three-channel quorum sensing system

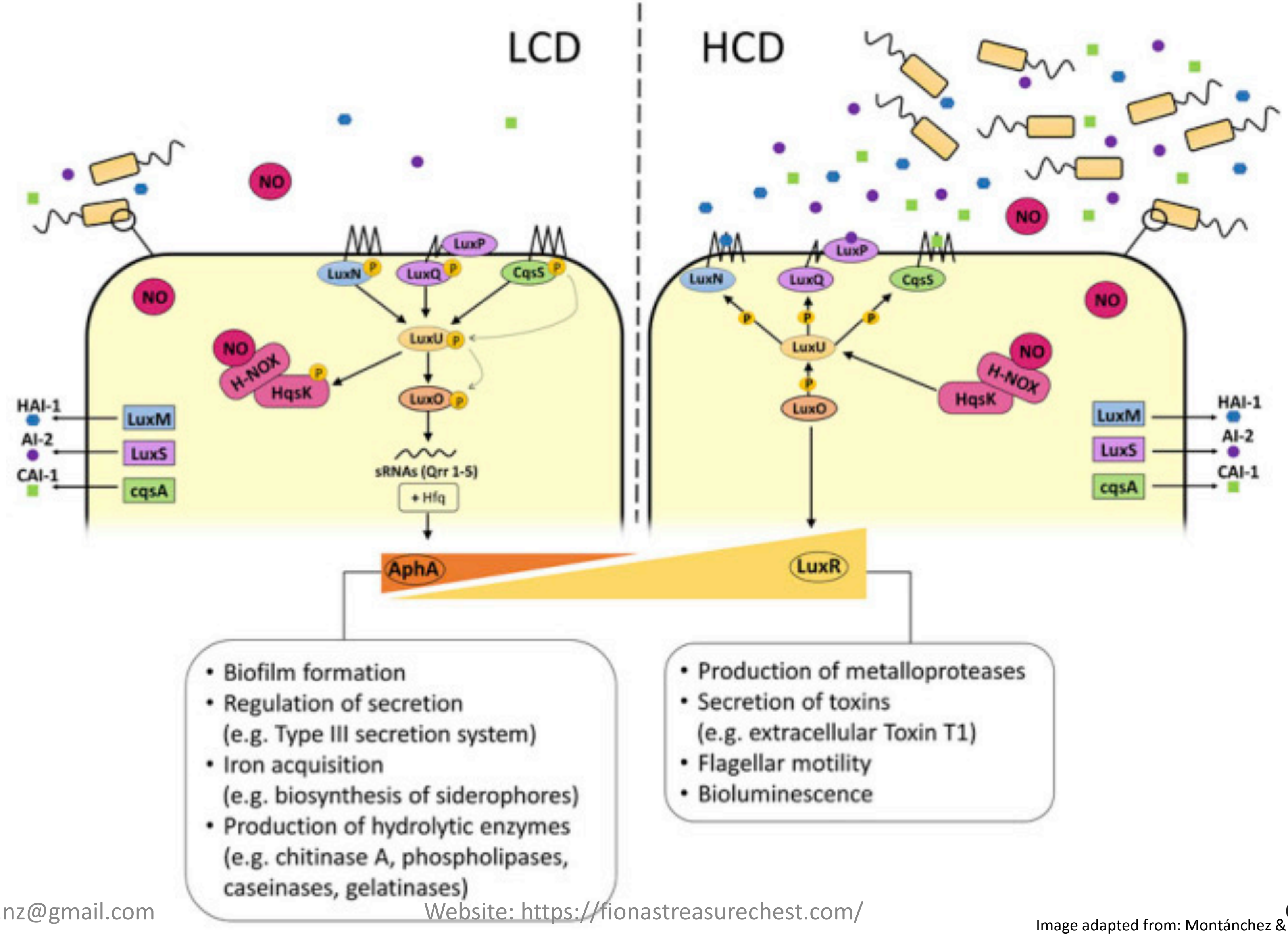


Image adapted from: Montánchez & Kaberdin (2020): *Vibrio harveyi*: A brief survey of general characteristics and recent epidemiological traits associated with climate change

Objective 1

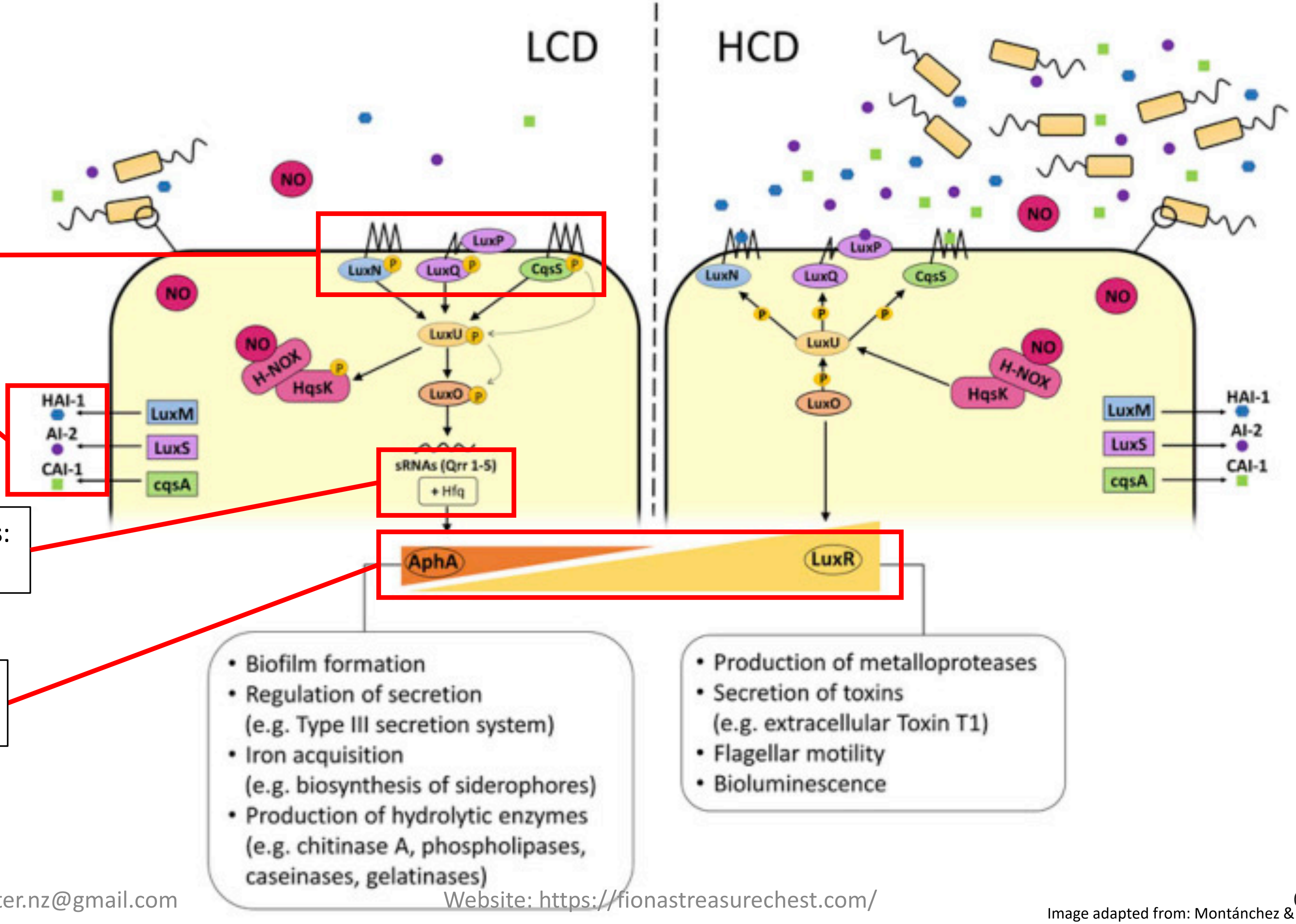
V. harveyi: three-channel quorum sensing system

Kinases at LCD:
LuxN, LuxP, CqsS

Auto-inducers:
HAI-1, CAI-1, AI-2

quorum regulatory sRNAs:
Qrr1-5

Master regulator at LCD:
AphA



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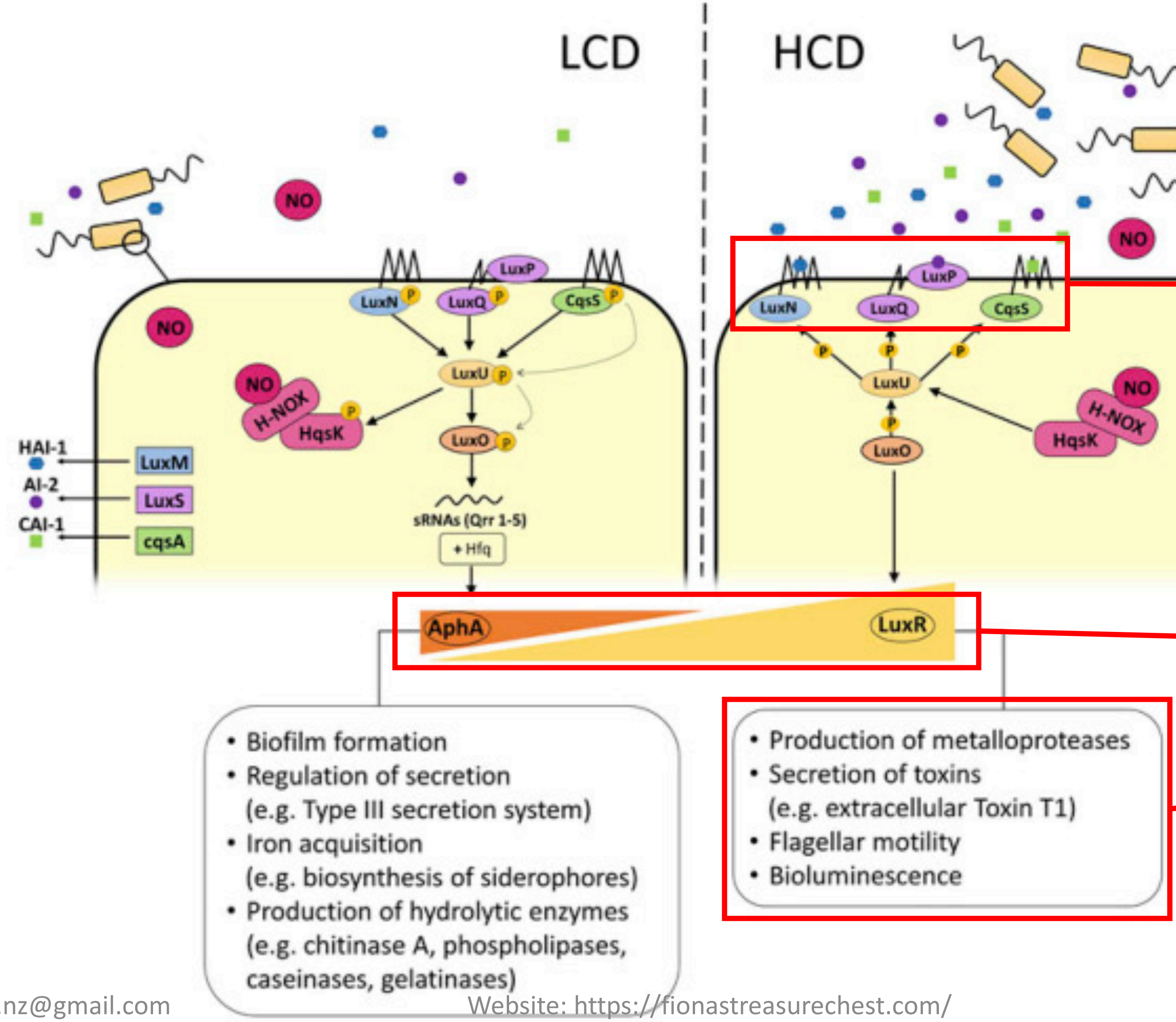
Objective 1

V. harveyi: three-channel quorum sensing system



Phosphatases at HCD: LuxN, LuxP, CqsS

Auto-inducers: HAI-1, CAI-1, AI-2

Master regulator at HCD: LuxR

HCD QS genes: *lux* operon

There are many different quorum sensing mechanisms, this one is only an example!

Objective 1

Why a **three-channel** quorum sensing system?

LuxN + LuxM + HAI-1:

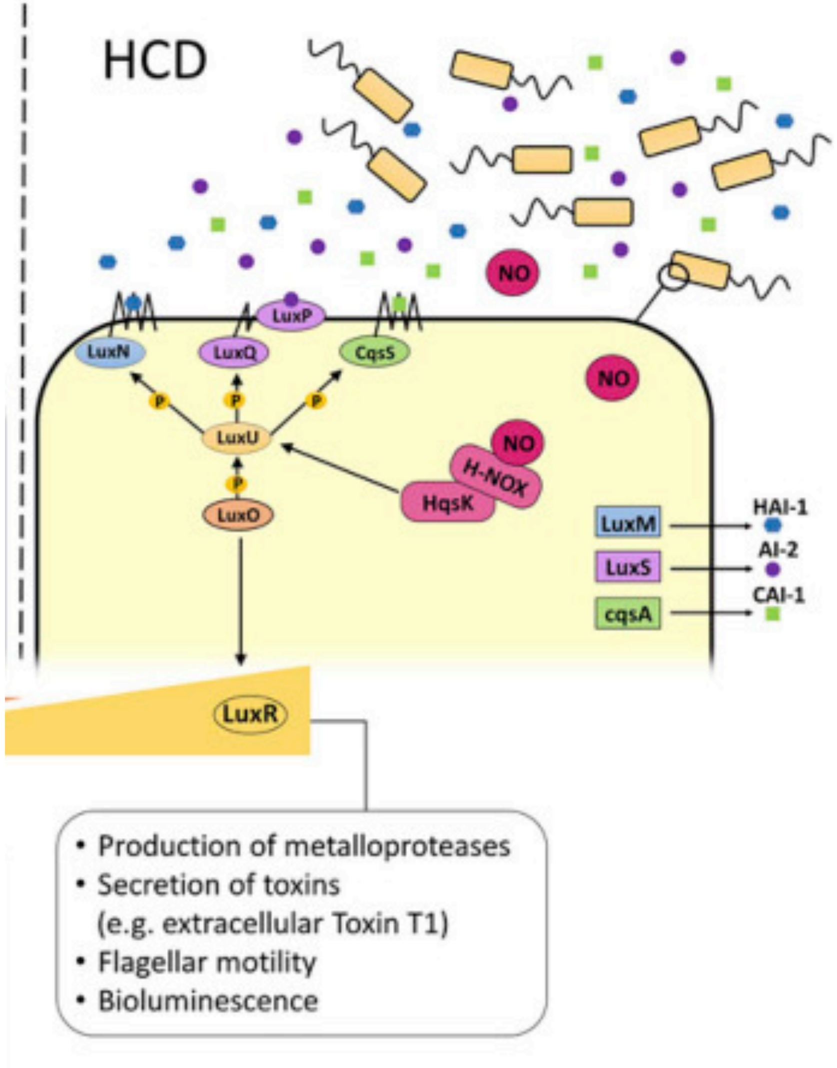
V. harveyi ↔ *V. harveyi*

CqsS + cqsA + CAI-1:

V. harveyi ↔ *Vibrios*

LuxQ/LuxP + LuxS + AI-2:

V. harveyi ↔ other species



Objective 1

V. harveyi: pathogenicity

- LuxR master regulator **on** at high cell density
  - regulates 115 promoters
  - → regulates 625 genes

Penaeid shrimp

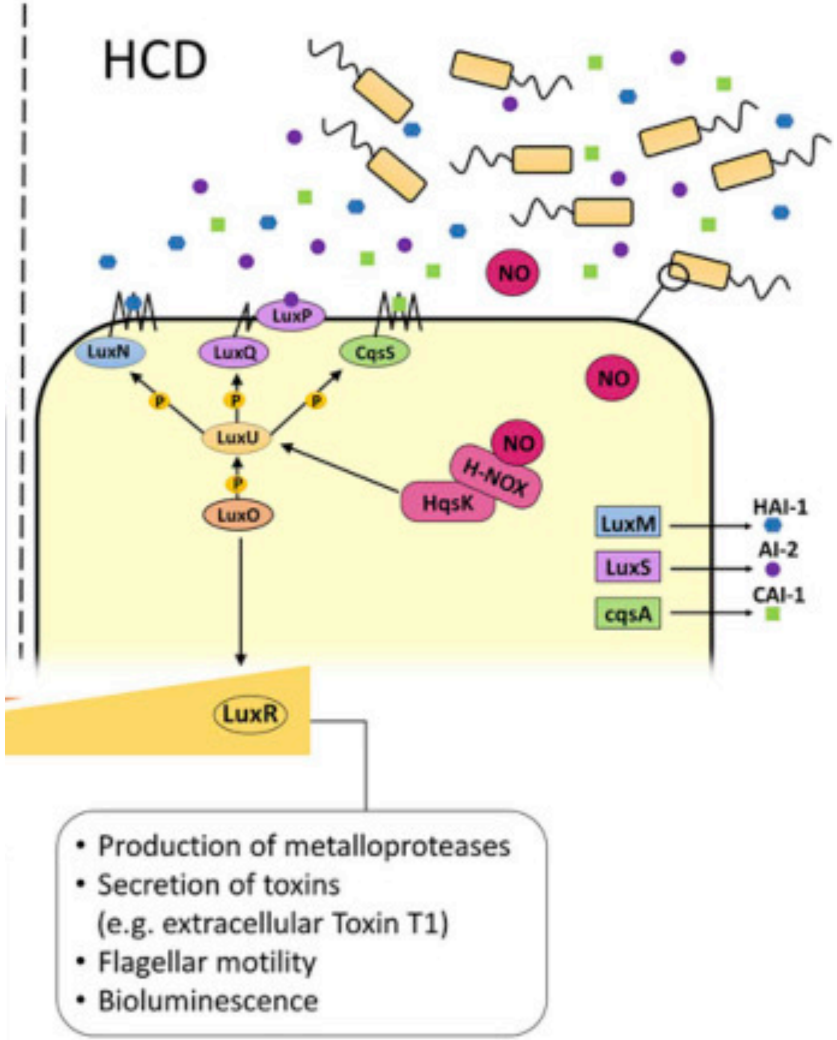
- LuxR **on** induces expression of genes for:
  - endotoxin lipopolysaccharide
  - extracellular proteases
  - interaction with bacteriophages

→ luminescent vibrosis

Flounders

- LuxR **on** induces expression of genes for:
  - extracellular haemolysin

→ flounder infectious necrotizing enteritis (FINE)



→ Expressed genes are environment & host dependent!

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Background

V. harveyi: pathogenicity & climate change

↑ temperature
↓ pH (ocean acidification)
↓ salinity

=

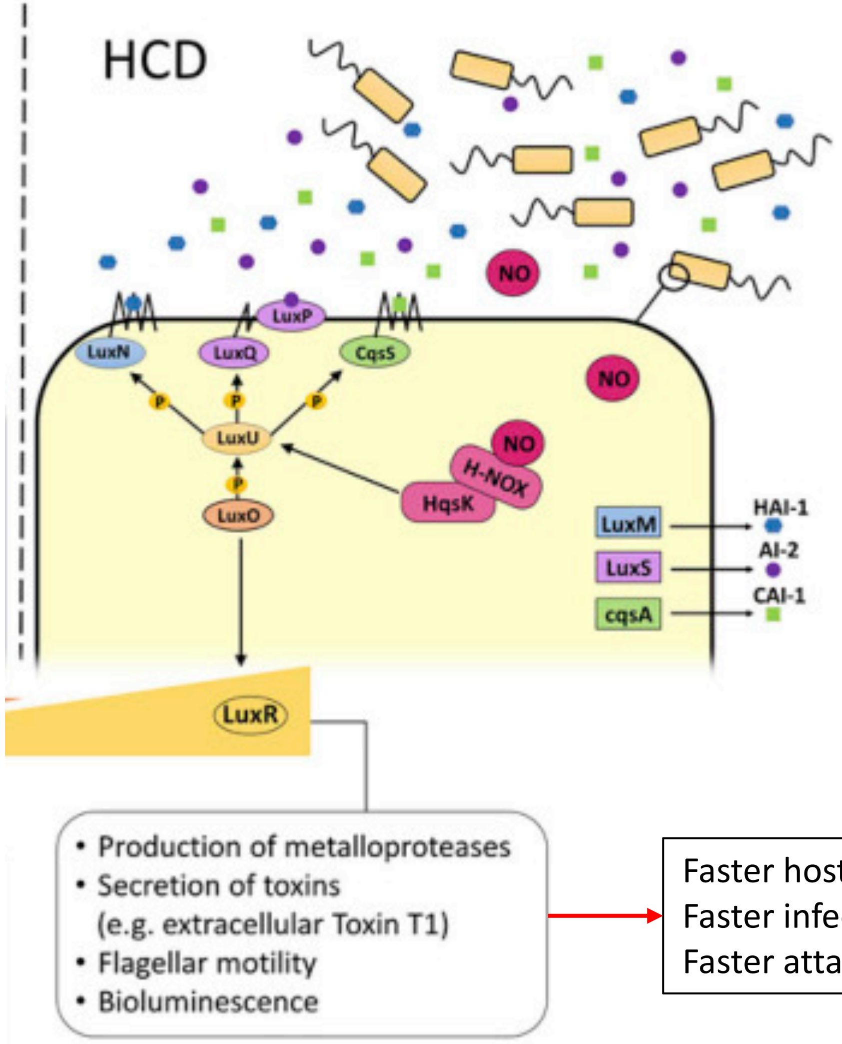
↑ autoinducer stability
↓ host immunity
↑ virulence

=

less *V. harveyi* required to activate QS genes

=

↑ infections in marine animals

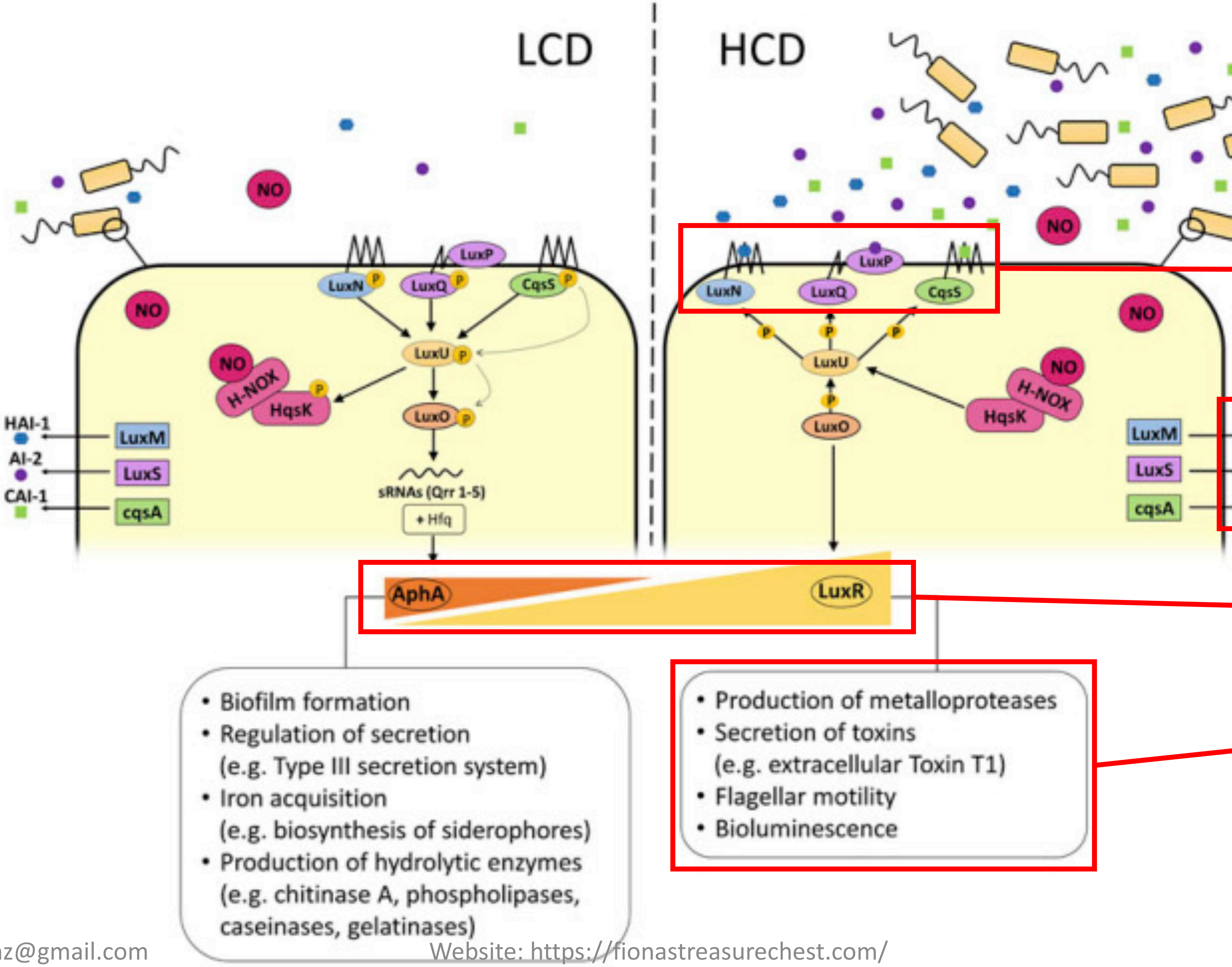


Faster host tissue degradation
Faster infection
Faster attachment to host surfaces

→ Climate change amplifies *V. harveyi* pathogenicity (QS) in aquaculture!

Objective 2

Where/how could we disrupt quorum sensing?



Phosphatases at HCD: LuxN, LuxP, CqsS

Auto-inducers: HAI-1, CAI-1, AI-2

Master regulator at HCD: LuxR

HCD QS genes: *lux* operon

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Disrupting quorum sensing

Competitive inhibition:
Distracting auto-inducer receptors

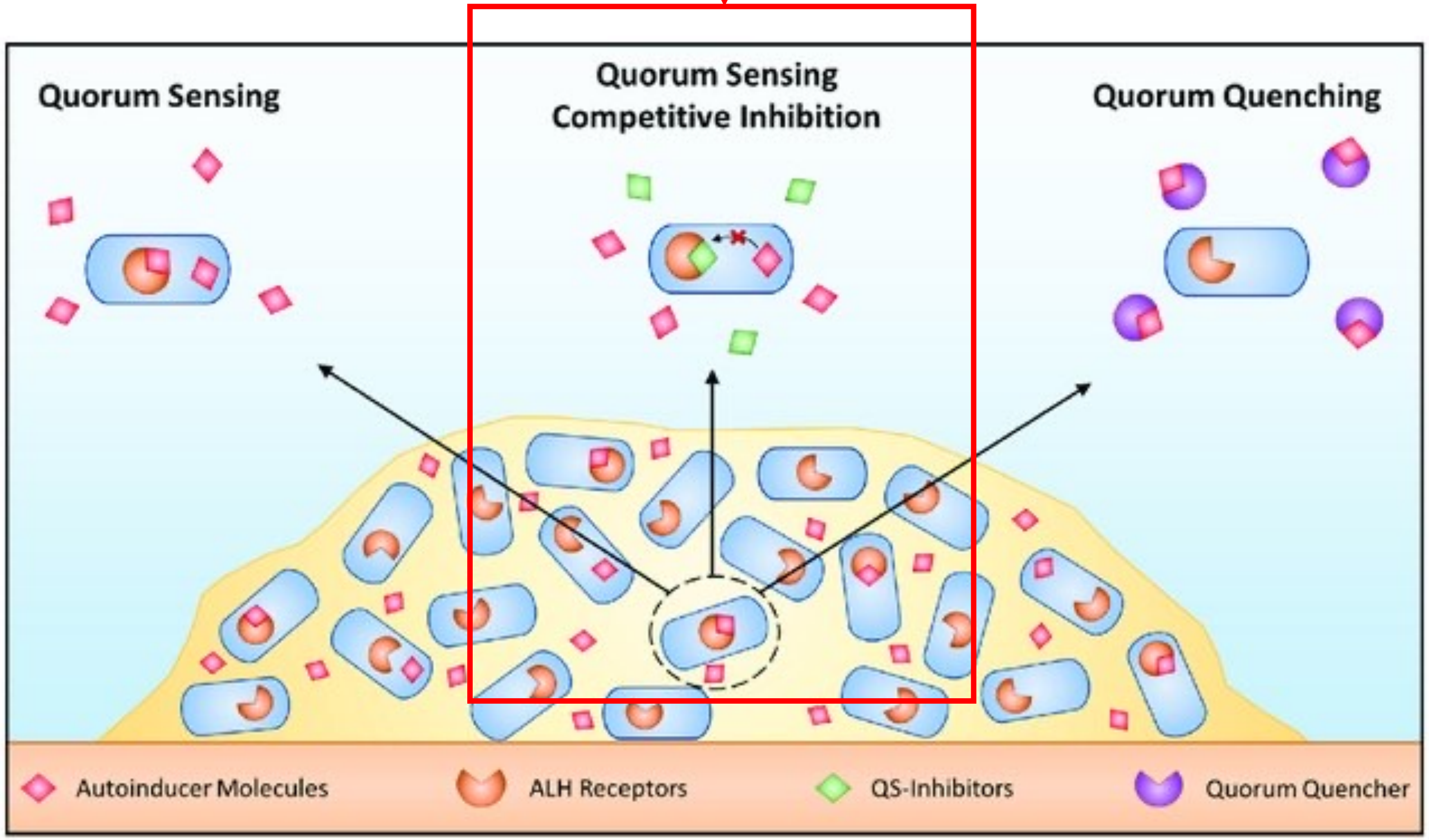


Image source: Beitelshees *et al*. (2018): Phenotypic Variation during Biofilm Formation: Implications for Anti-Biofilm Therapeutic Design

Objective 2

Disrupting quorum sensing

Quorum quenching: Distracting auto-inducers & other QS signals

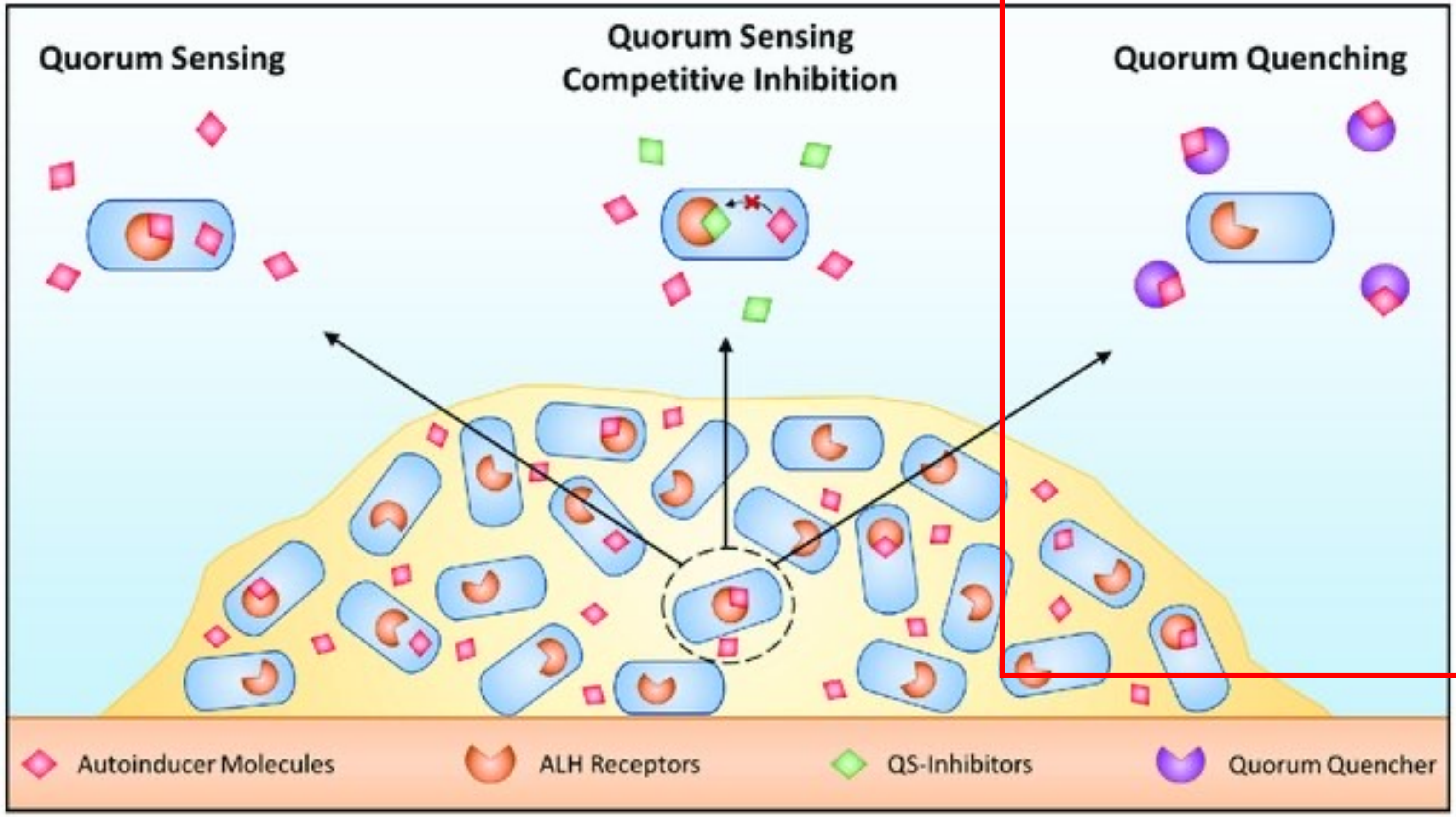
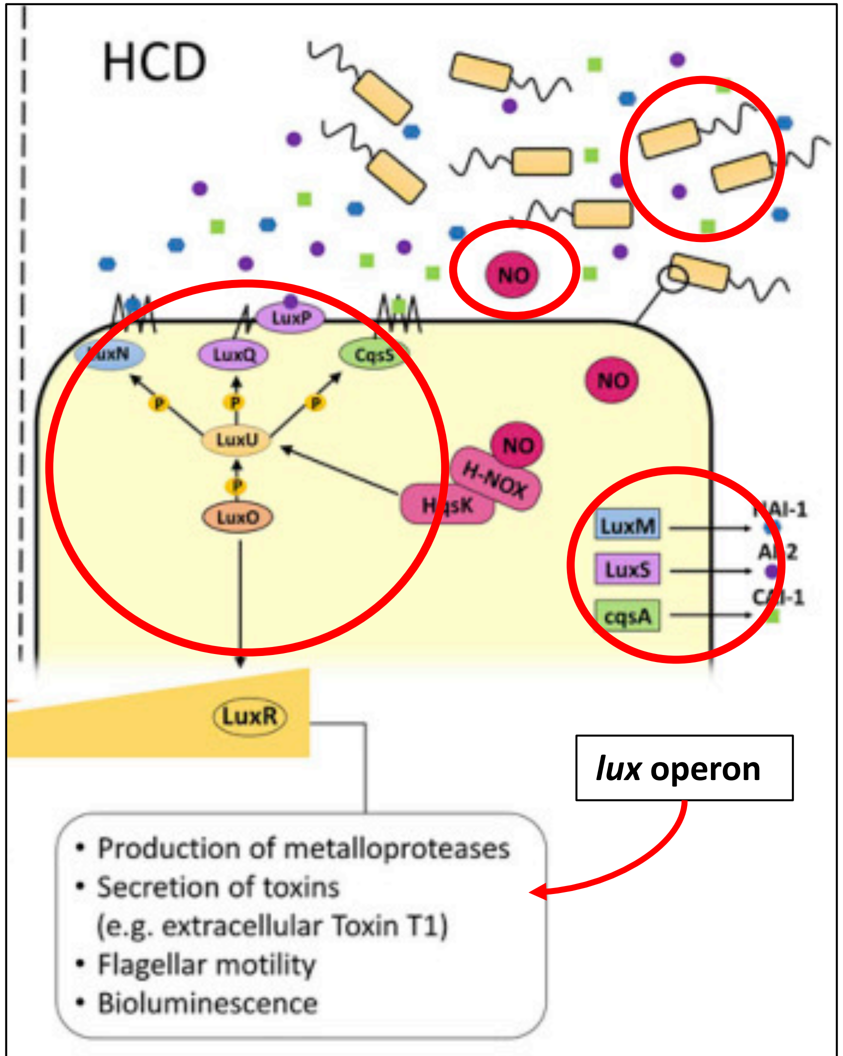


Image source: Beitelshees *et al*. (2018): Phenotypic Variation during Biofilm Formation: Implications for Anti-Biofilm Therapeutic Design

Objective 2

Disrupting quorum sensing: quorum quenching

- Enzymatic activity to interrupt QS
- Acquired or innate skill found in all kingdoms
  - In bacteria vs bacteria = competition for niche
  - In other kingdoms = immune defence
- Disrupts:
  - Synthesis of auto-inducers
  - QS-related cell-to-cell exchange
    - Nutrients
    - Information
  - Transport of QS signal
  - QS signal and response



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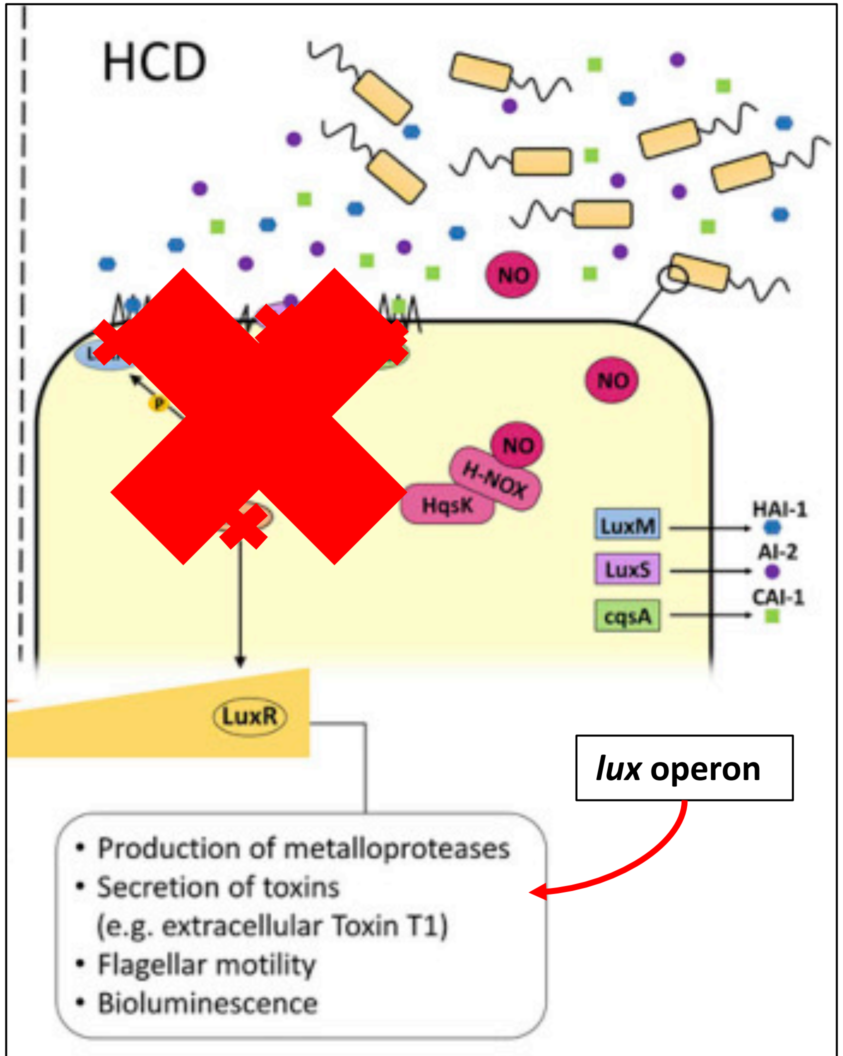
Disrupting quorum sensing in *V. harveyi*

- Defoirdt *et al.* (2007) studied how furanones can disrupt QS in *V. harveyi*
  - Furanones are compounds that regulate bacterial colonization on surfaces of algae
- Method:
  - Generated *V. harveyi* mutants
    - k/o *luxN*/*luxP* = only CqsS active
    - k/o *luxP*/*cqsS* = only LuxN active
    - k/o *luxN*/*cqsS* = only LuxP active
    - k/o *luxU* = no phosphorelay to LuxO
    - k/o *luxO* = no phosphorelay to LuxR
  - Furanones added to each mutant
  - Luminescence measured
- Result: Luminescence blocked in all mutants



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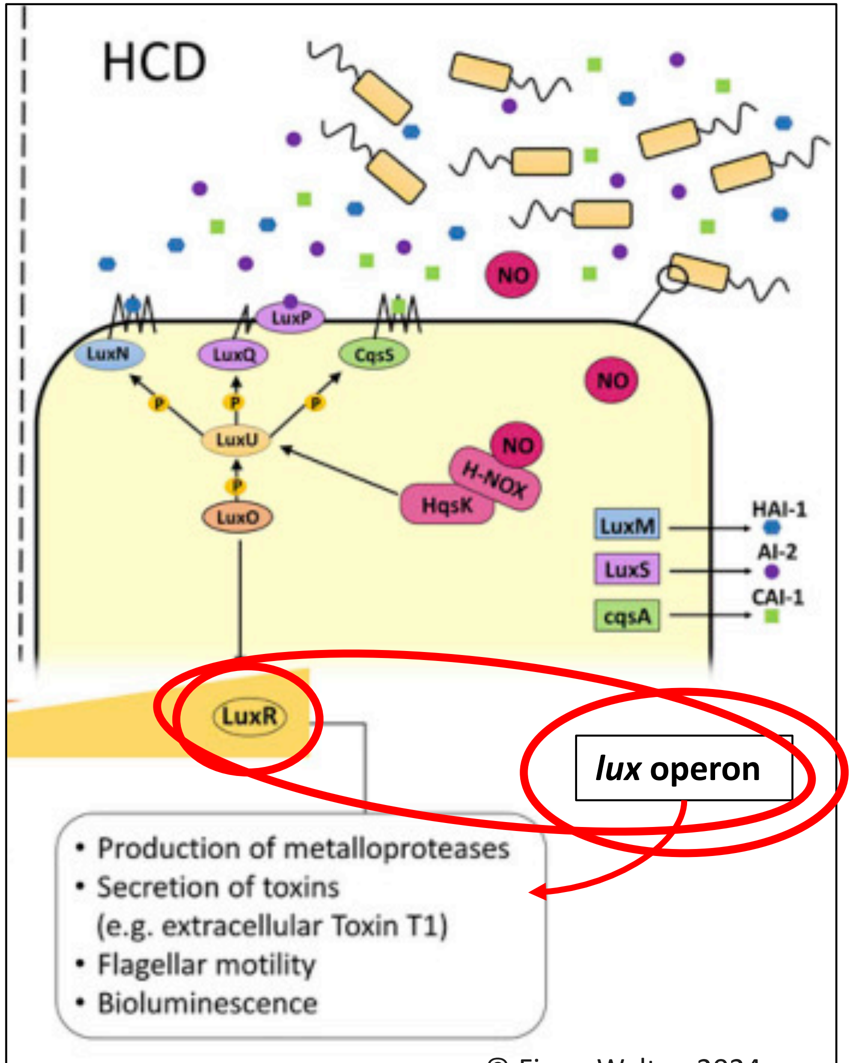
Disrupting quorum sensing in *V. harveyi*

- Method 2:
  - Wild-type *V. harveyi* + furanone
  - RT real-time PCR measured mRNA of *luxR*
- Result 2: *luxR* was normally expressed

- Method 3:
  - Radiolabeling LuxR protein & *lux* operon promoters
  - Autoradiograph and gel electrophoresis to visualize binding
- Result 3: LuxR protein not bound to *lux* operon promoters

- Conclusion: Furanones disrupt QS in *V. harveyi* by blocking LuxR promoter protein binding to *lux* operon



Applications for QS manipulation in ecology

- Aquaculture
  - Phytoplankton (e.g. *Chroococcus turgidus*) compound DTBMP reduces expression of *V. harveyi* master-regulator LuxR
  - Prevents biofilm formation and expression of virulence factors
  - Now commercial produced and commonly used to clean surfaces in shrimp farms
- Agriculture
  - *Bacillus* enzyme AiiA inactivates *Erwinia carotovora* auto-inducer AH2
  - *E. carotovora* = common vegetable pathogen causing soft rot
  - Enzyme disrupts QS cascade for virulent activity
  - Genes for this enzyme have been transformed into Chinese cabbage
- Antibacterial therapy, Wastewater treatment systems, etc.!

Summary

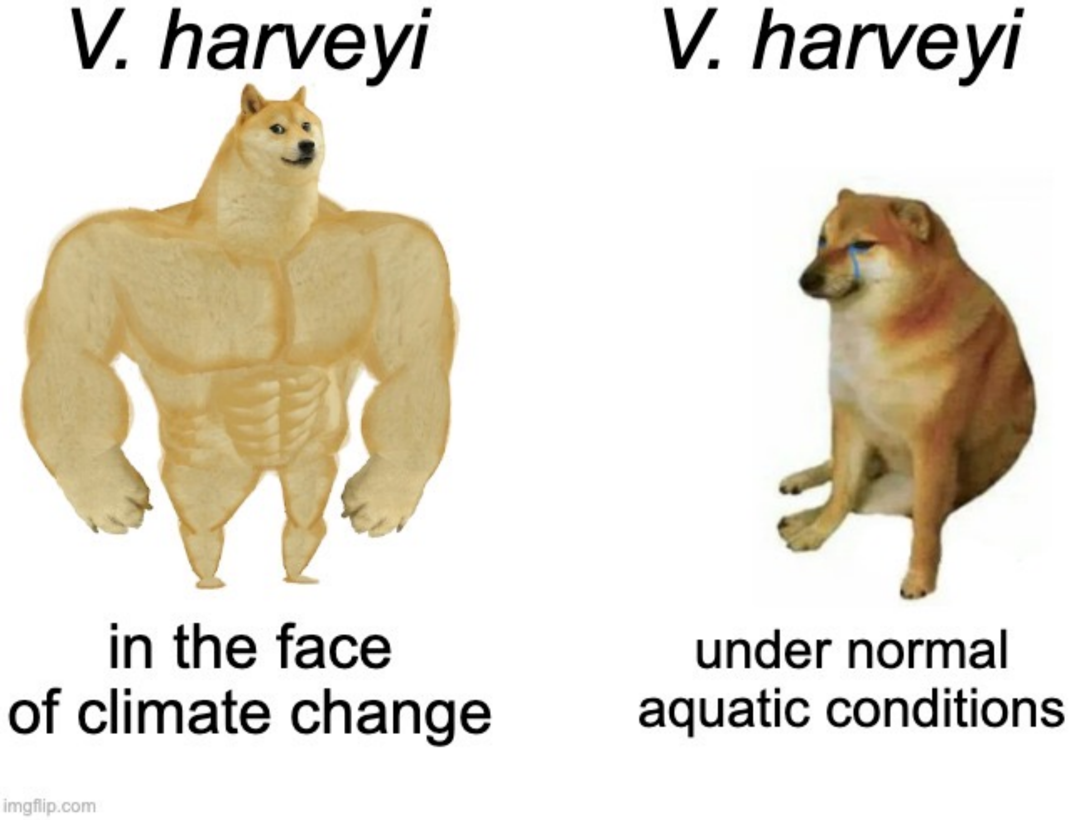
- QS allows communication within and between bacterial species
- QS regulates gene expression by responding to changes in population density
- *V. harveyi* uses a three-channel QS system to express virulence genes and is an emerging marine pathogen due to the effects of climate change
- *V. harveyi* QS can be disrupted by “quorum quenching” which is a process in which naturally occurring or artificially made enzymes disrupt the QS cascade
- Quorum quenching has many applications in ecology to reduce the spread of pathogens that use QS

V. harveyi: pathogenicity & climate change

+ more acidic water
= weaker hosts

+ warmer water
= quicker growth

+ becomes pathogen in mutualistic relationships
= a bit greedy



+ Da boi next door

+ Is torch for squids

+ Supplies nutrients to corals

Key papers

Review on Quorum sensing in Gram-negative bacteria:

Papenfort, K., & Bassler, B. L. (2016). Quorum sensing signal–response systems in gram-negative bacteria. *Nature Reviews Microbiology*, *14*(9), 576–588. https://doi.org/10.1038/nrmicro.2016.89

Penaeid shrimp & flounders:

Zhang, X.-H., He, X., & Austin, B. (2020). Vibrio harveyi: A serious pathogen of fish and invertebrates in mariculture. *Marine Life Science & Technology*, *2*(3), 231–245. https://doi.org/10.1007/s42995-020-00037-z

***V. harveyi* and climate change (this is my favorite paper!):**

Montánchez, I., & Kaberdin, V. R. (2020). Vibrio harveyi: A brief survey of general characteristics and recent epidemiological traits associated with climate change. *Marine Environmental Research*, *154*, 104850. https://doi.org/10.1016/j.marenvres.2019.104850

Quorum quenching:

Zhang, W., & Li, C. (2016). Exploiting quorum sensing interfering strategies in gram-negative bacteria for the enhancement of environmental applications. *Frontiers in Microbiology*, *6*. https://doi.org/10.3389/fmicb.2015.01535

Disrupting QS in *V. harveyi*:

Defoirdt, T., Miyamoto, C. M., Wood, T. K., Meighen, E. A., Sorgeloos, P., Verstraete, W., & Bossier, P. (2007). The natural furanone (5Z)-4-bromo-5-(bromomethylene)-3-butyl-2(5H)-furanone disrupts quorum sensing-regulated gene expression in Vibrio harveyi by decreasing the DNA-binding activity of the transcriptional regulator protein luxR. *Environmental Microbiology*, *9*(10), 2486–2495. https://doi.org/10.1111/j.1462-2920.2007.01367.x

Applications (Aquaculture):

Santhakumari, S., Jayakumar, R., Logalakshmi, R., Prabhu, N. M., Abdul Nazar, A. K., Karutha Pandian, S., & Veera Ravi, A. (2018). In vitro and in vivo effect of 2,6-di-tert-butyl-4-methylphenol as an antibiofilm agent against quorum sensing mediated biofilm formation of Vibrio spp. *International Journal of Food Microbiology*, *281*, 60–71. https://doi.org/10.1016/j.ijfoodmicro.2018.05.024

Applications (Agriculture):

Dong, Y.-H., Xu, J.-L., Li, X.-Z., & Zhang, L.-H. (2000). AiiA, an enzyme that inactivates the acylhomoserine lactone quorum-sensing signal and attenuates the virulence of Erwinia carotovora. *Proceedings of the National Academy of Sciences*, *97*(7), 3526–3531. https://doi.org/10.1073/pnas.97.7.3526

