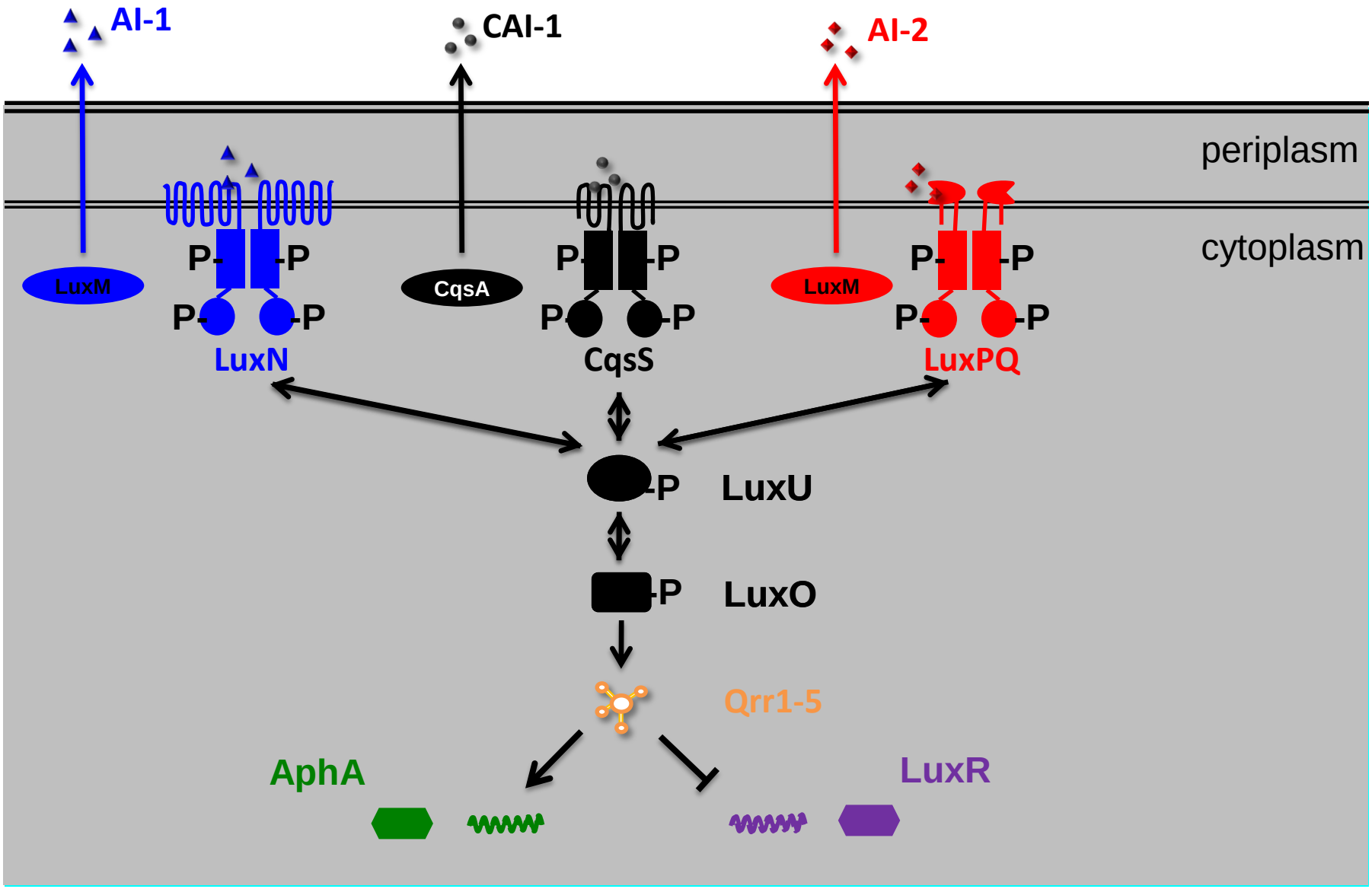
Modeling quorum sensing - why so many feedbacks?

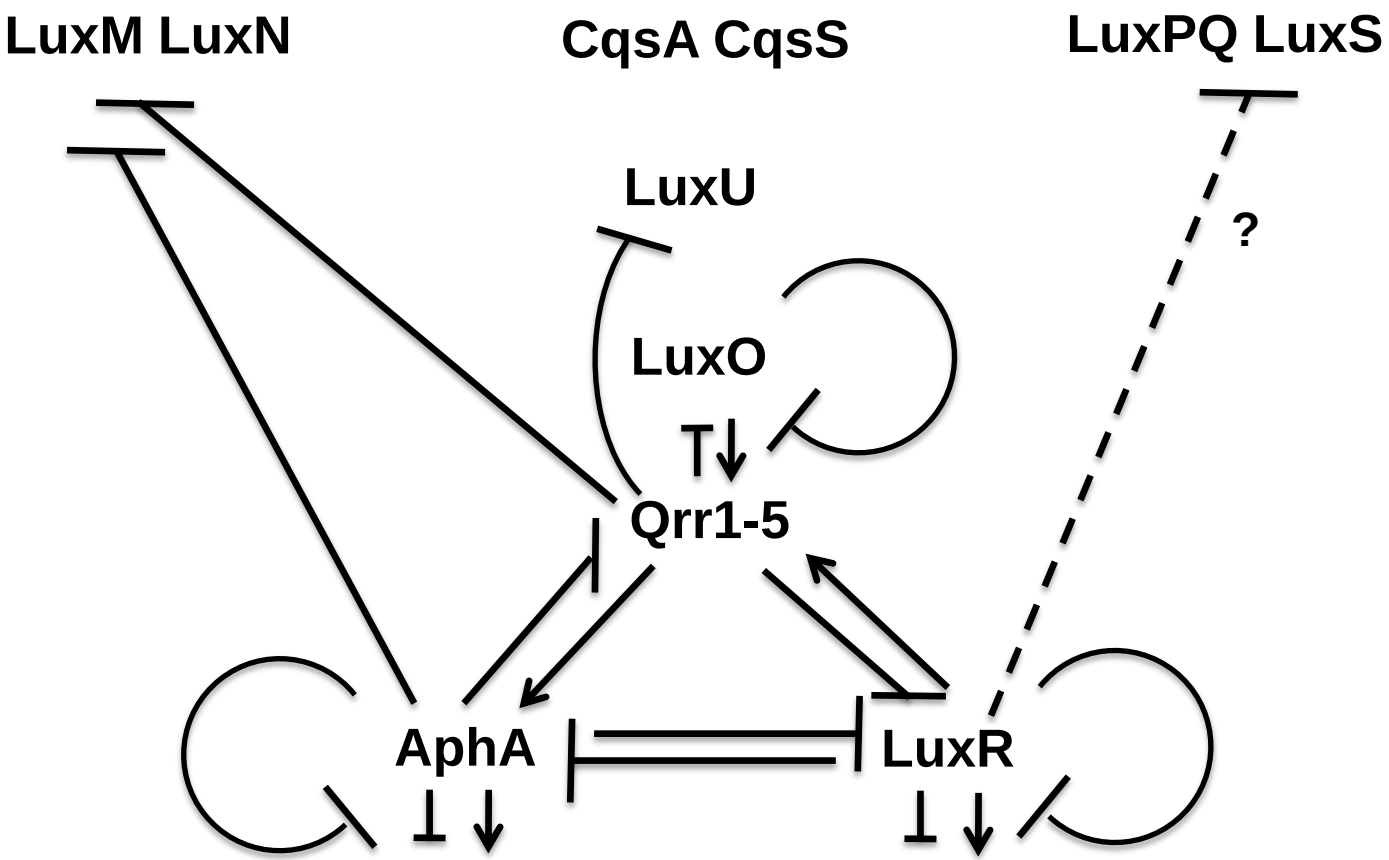
Ned Wingreen, Princeton

KITP August 6, 2014

Quorum sensing network in *V. harveyi*

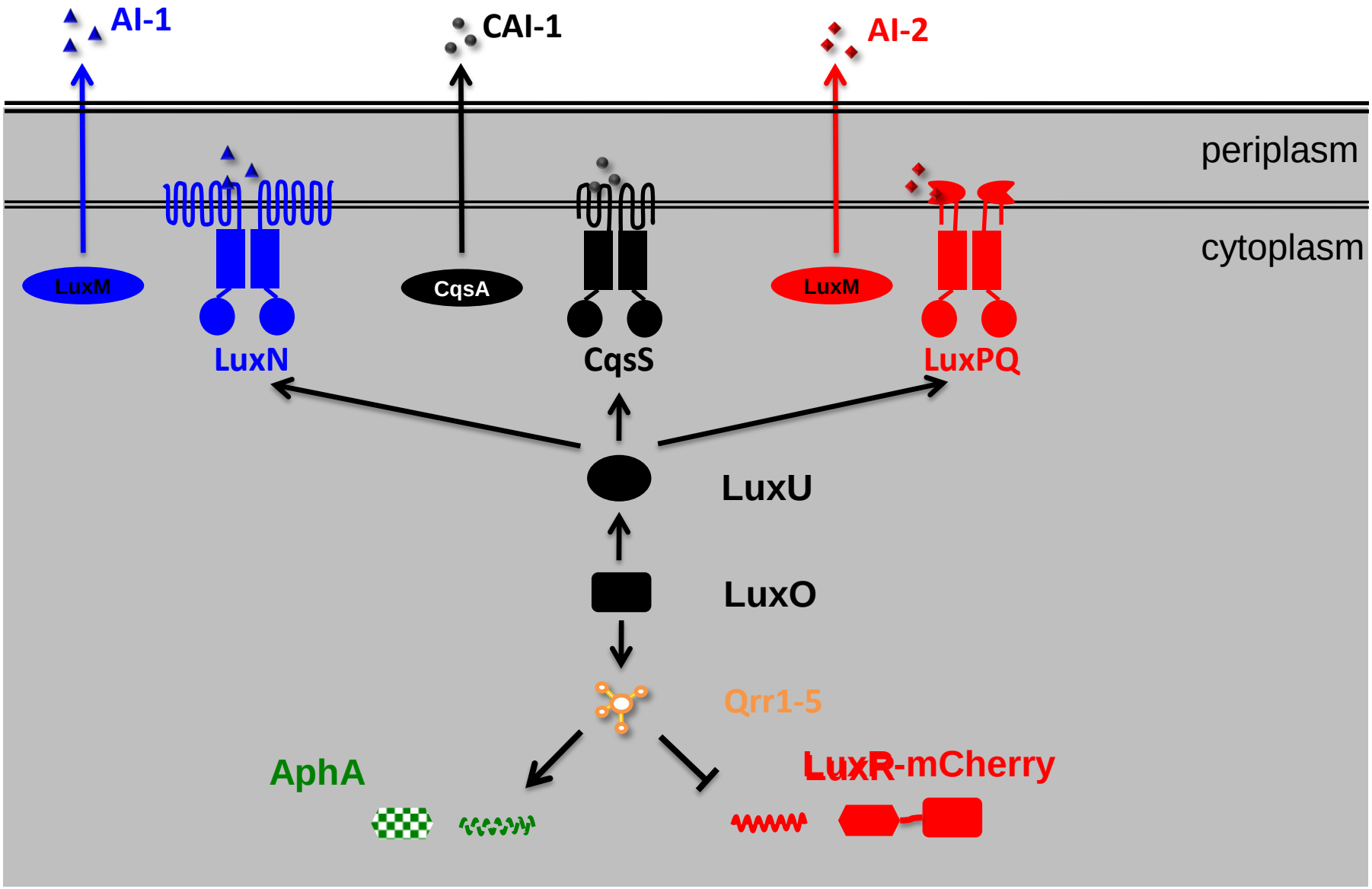


QS network has many internal feedbacks

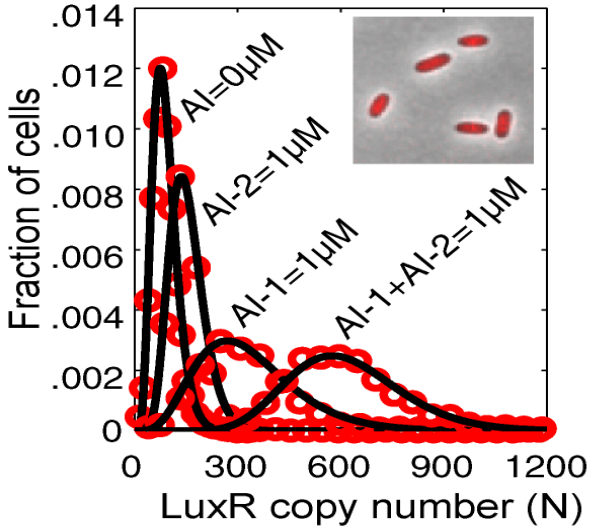


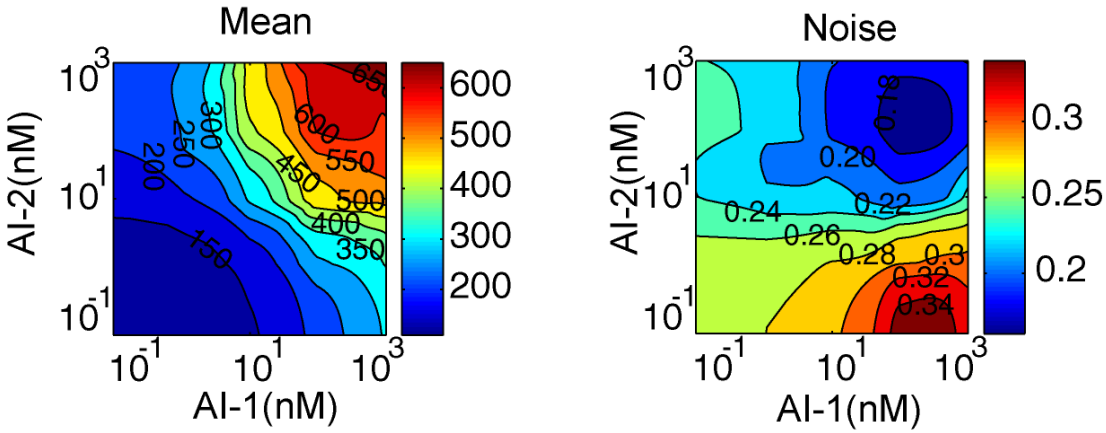
Rutherford *et al., Genes & Dev* (2011)
Shao & Bassler, *Mol Micro* (2012)

Engineered reporter strains



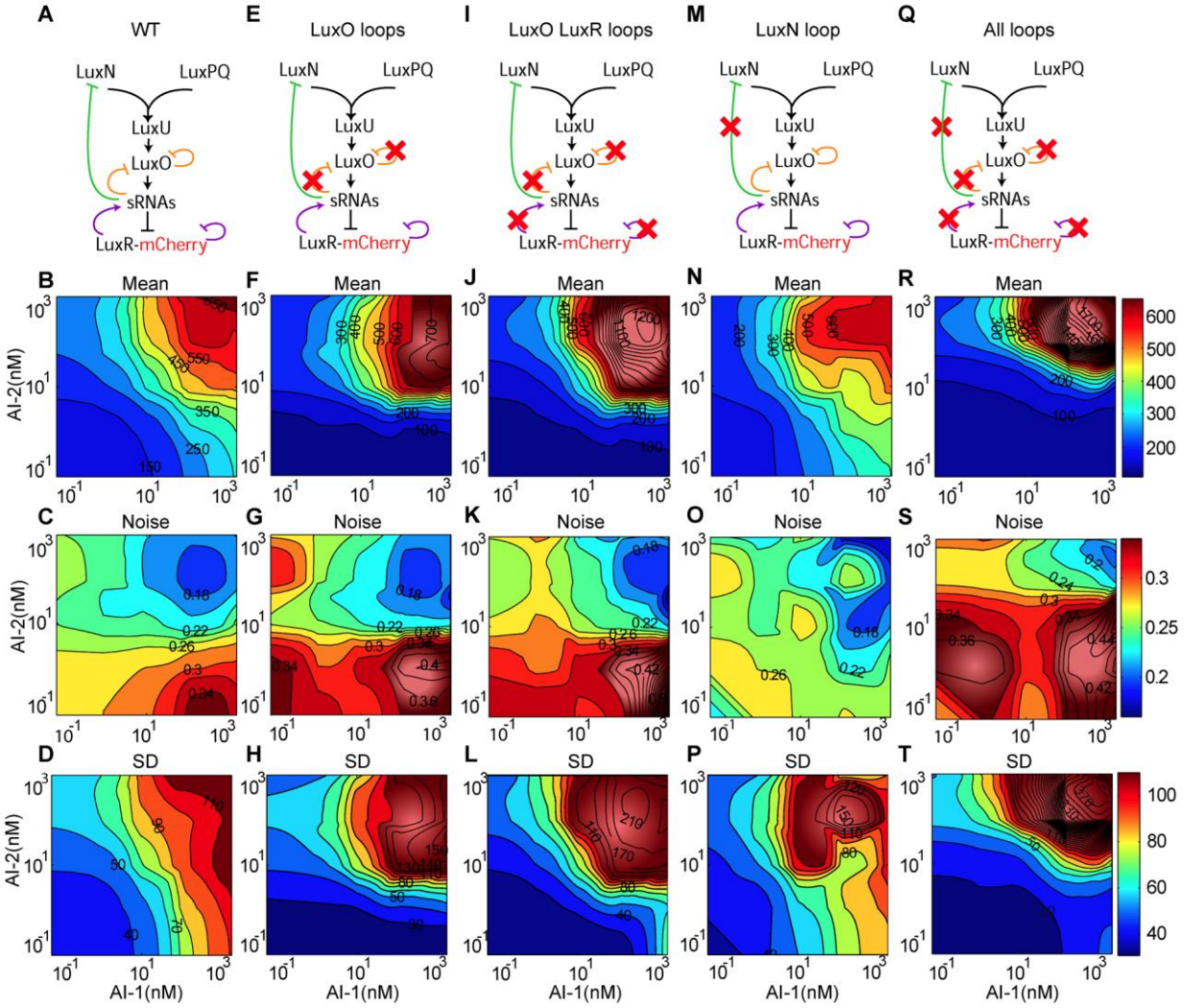
Single-cell measurements





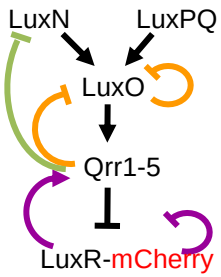
Teng *et al. Mol Sys Biol* (2011)

Each feedback does something…

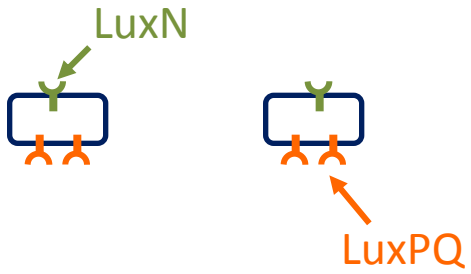


LuxN feedback regulates receptor ratio

WT



Low Cell Density



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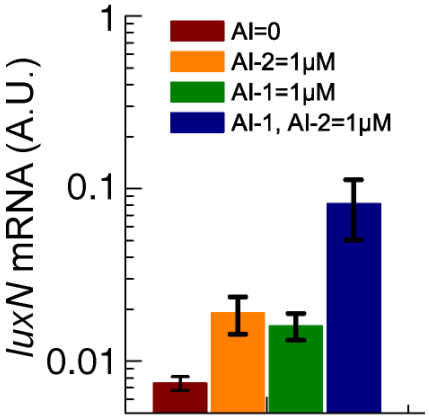
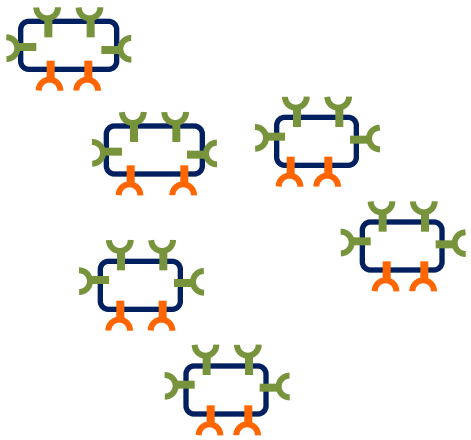
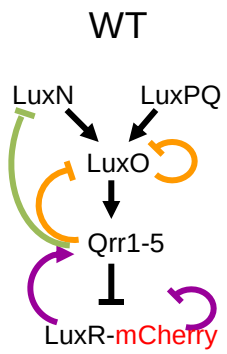


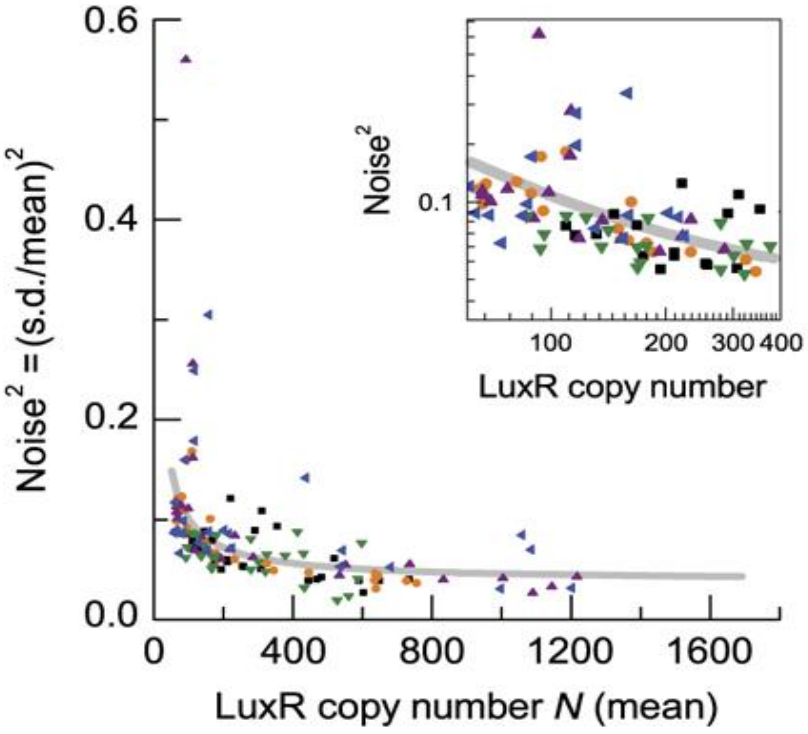
luxN expression

High Cell Density

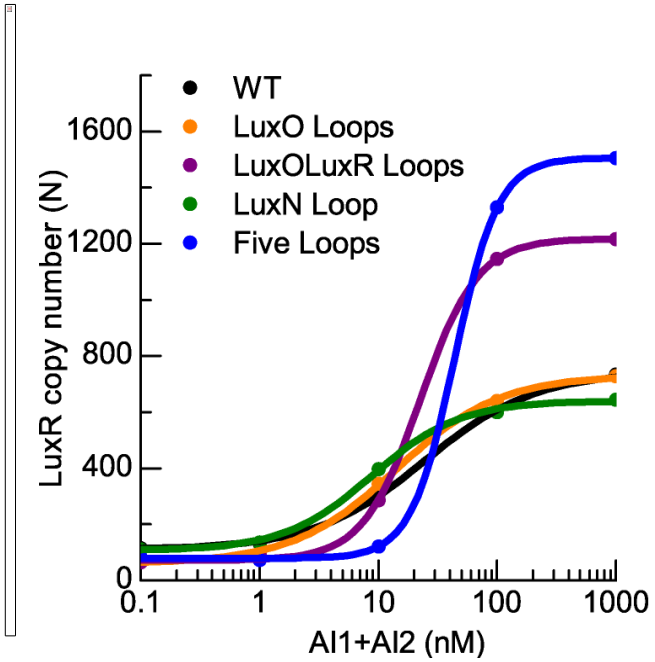


Core feedbacks have little effect on noise



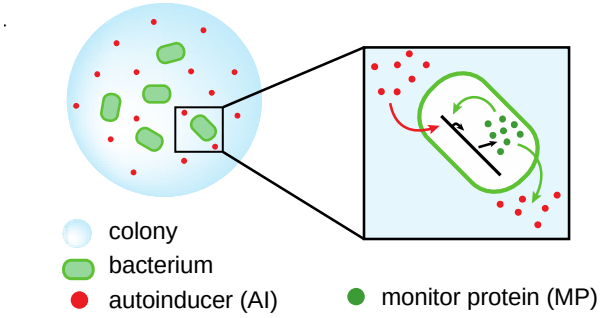


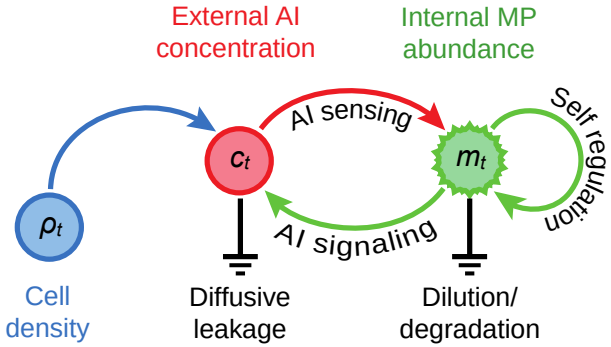
Feedback by LuxR controls input-output relation

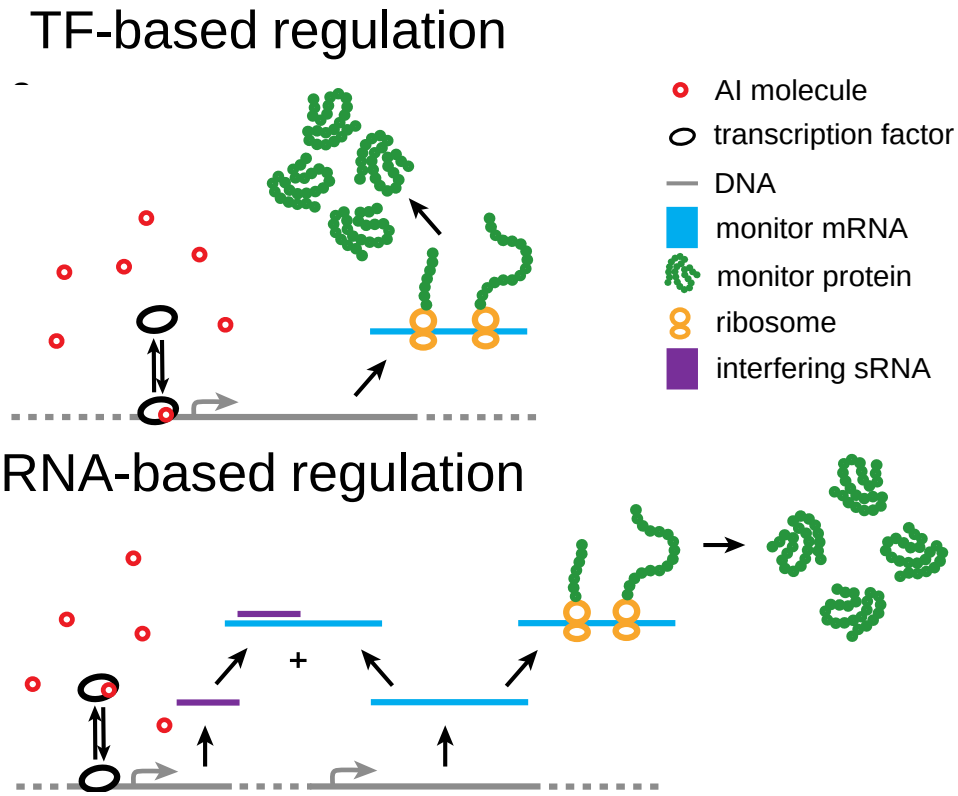


LuxR feedback increases AI input dynamic range and decreases LuxR output dynamic range.

Quorum-sensing feedbacks and mutual information





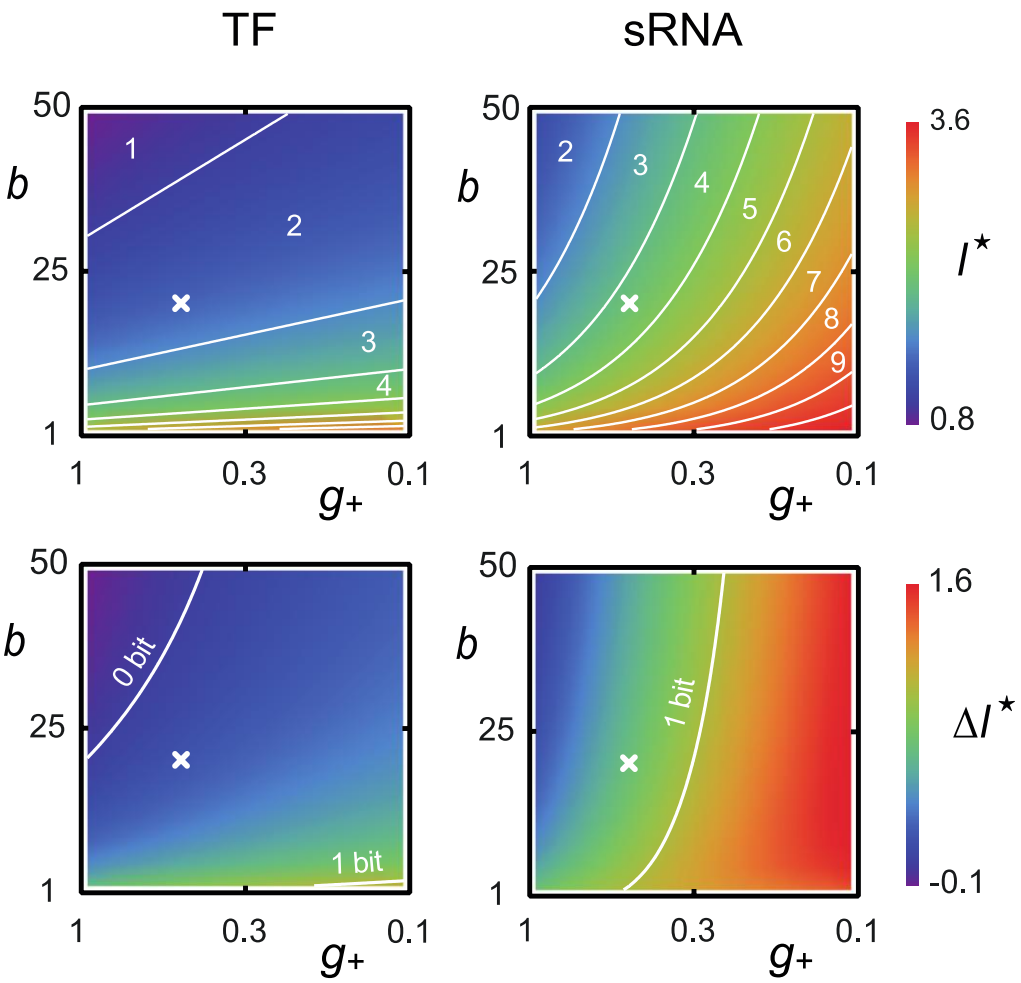


Feedbacks can optimize available information about cell density

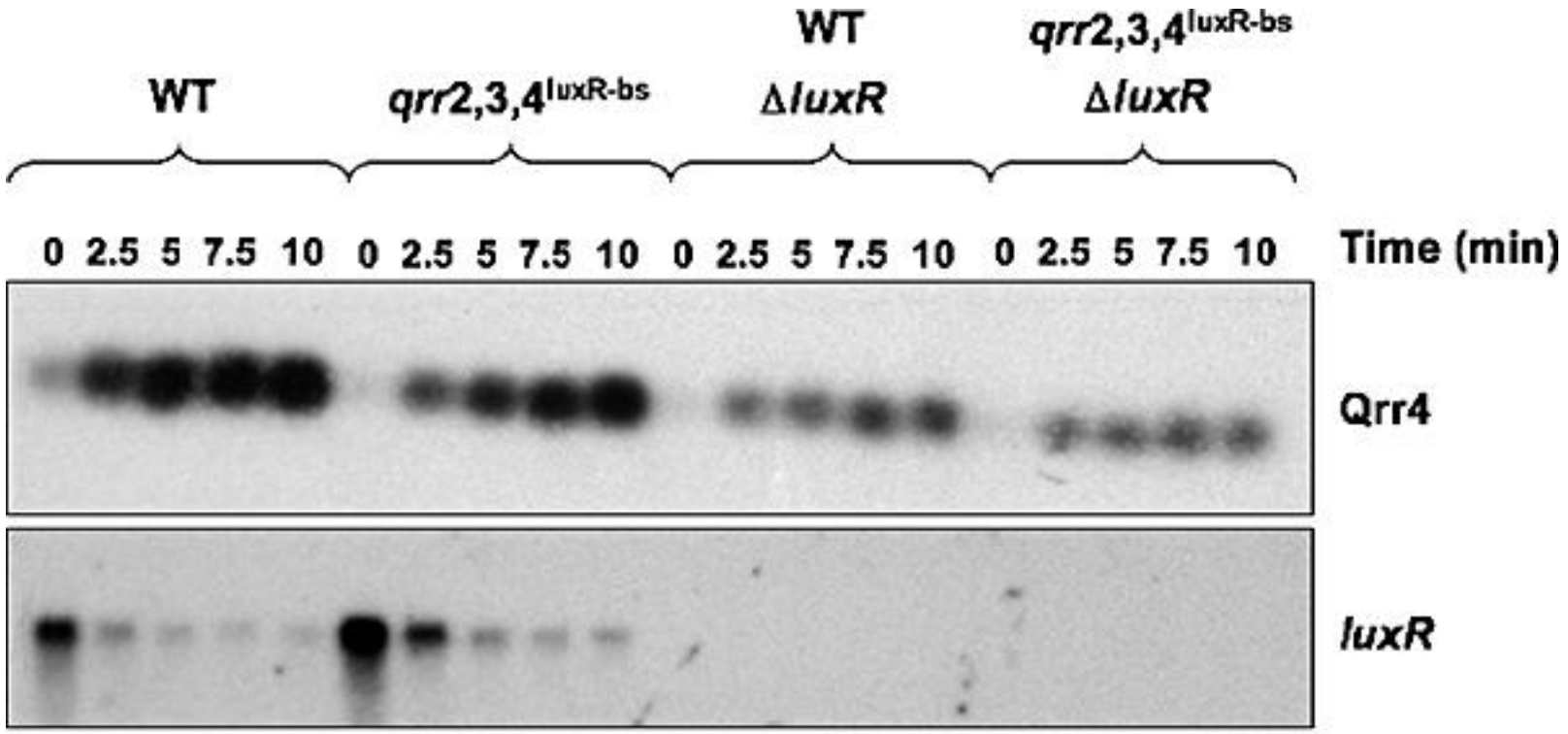
Mutual information

MI increase due to feedbacks

b = protein “burst size”
g+ = feedback inhibition



Feedback from LuxR speeds Qrr production at HCD ➔LCD transition



Tu *et al., Mol Micro* (2008)

Simple model for network dynamics

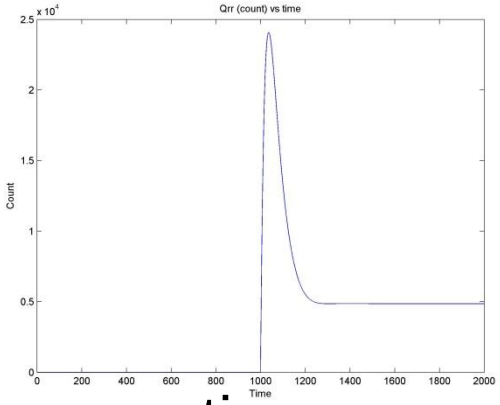
E.g. equations for Qrrs and *luxR* / LuxR:

$$\frac{d[Qrr]}{dt} = Q * V_{tscr}\left(\frac{[\text{LuxO}\sim\text{P}]}{K_M^{OP} + [\text{LuxO}\sim\text{P}]}\right)\left(\frac{K_I^A}{K_I^A + [\text{AphA}]}\right)\left(\frac{K_M^R + A_R^Q[\text{LuxR}]}{K_M^R + [\text{LuxR}]}\right)$$
$$-k_{qn}[Qrr][luxN]$$
$$-k_{qo}[Qrr][luxO]$$
$$-k_{qr}[Qrr][luxR]$$
$$-k_{qa}[Qrr][aphA]$$
$$\frac{d[luxR]}{dt} = V_{tsla}\left(\frac{K_M^A + A_A^R[\text{AphA}]}{K_M^A + [\text{AphA}]}\right)\left(\frac{K_I^R}{K_I^R + [\text{LuxR}]}\right) - k_{qr}[Qrr][luxR] - D_{mRNA}[luxR]$$
$$\frac{d[\text{LuxR}]}{dt} = V_{prot}[luxR] - D_{prot}[\text{LuxR}]$$

Simulate transitions:
LCD ➔HCD and HCD➔ LCD.

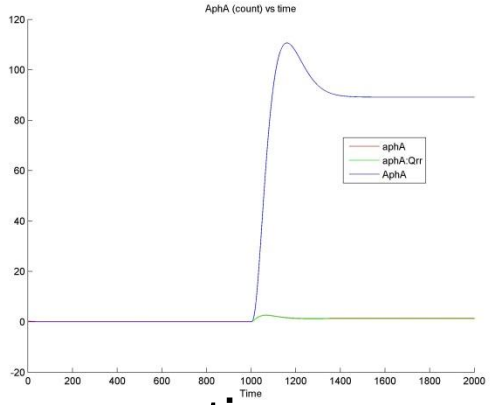
Model results for HCD➔LCD transition

Qrrs



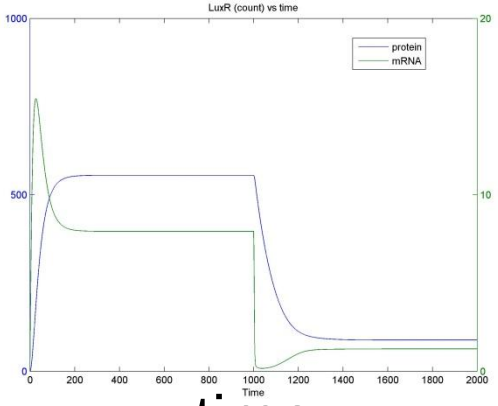
time

AphA



time

luxR / LuxR



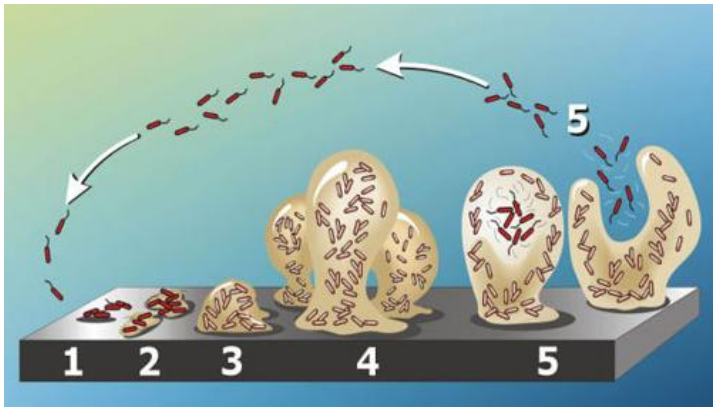
time

Network design accelerates HCD➔LCD response:

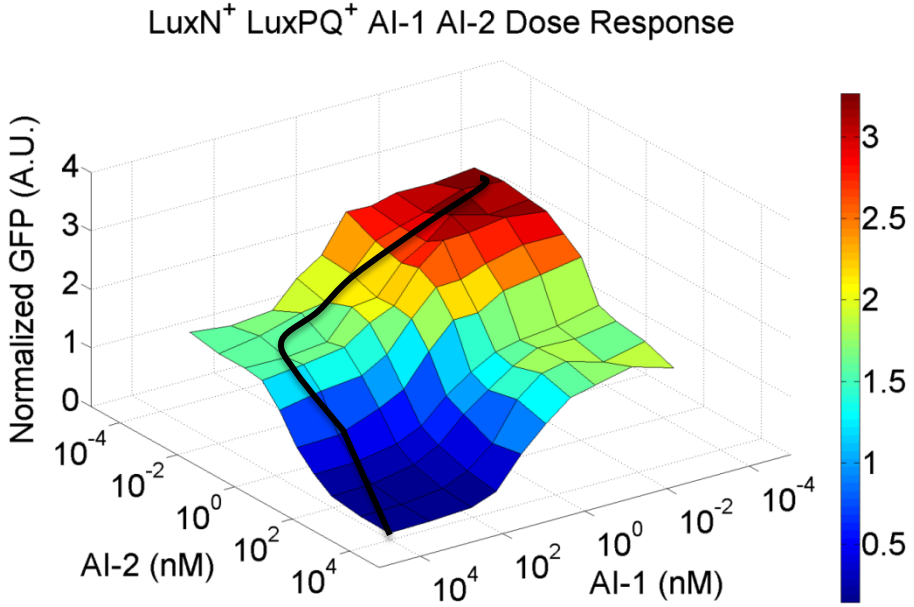
- Multiple Qrrs
- LuxR co-activation of Qrrs
- Qrr repression of LuxO
- Cap on total LuxR
- Negative feedback via AphA limits Qrr accumulation

So why is the QS network so complex?

Lifecycle of bacteria in a biofilm



Courtesy of Davis Lab at Binghamton Univ.



- **Multiple autoinducers and feedbacks may allow multi-stage developmental program.**
- **Feedbacks can help cells focus on most relevant signal and respond quickly to HCD ➔LCD transitions.**

Summary

- **AphA/LuxR are the LCD/HCD master regulators in the Vibrio quorum-sensing network.**

- **Complex network architecture allows:**
  - **Increased information on cell density**
  - **“Attention” to specific signals**
  - **Fast response to HCD ➔LCD transition**

Acknowledgments

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Julia van Kessel

Yi Shao

Jessie Schaffer

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QUANTITATIVE
BIOLOGY

The Microbiome, metagenomics, and clustering-free 16S RNA analysis

Ned Wingreen

Mikhail Tikhonov

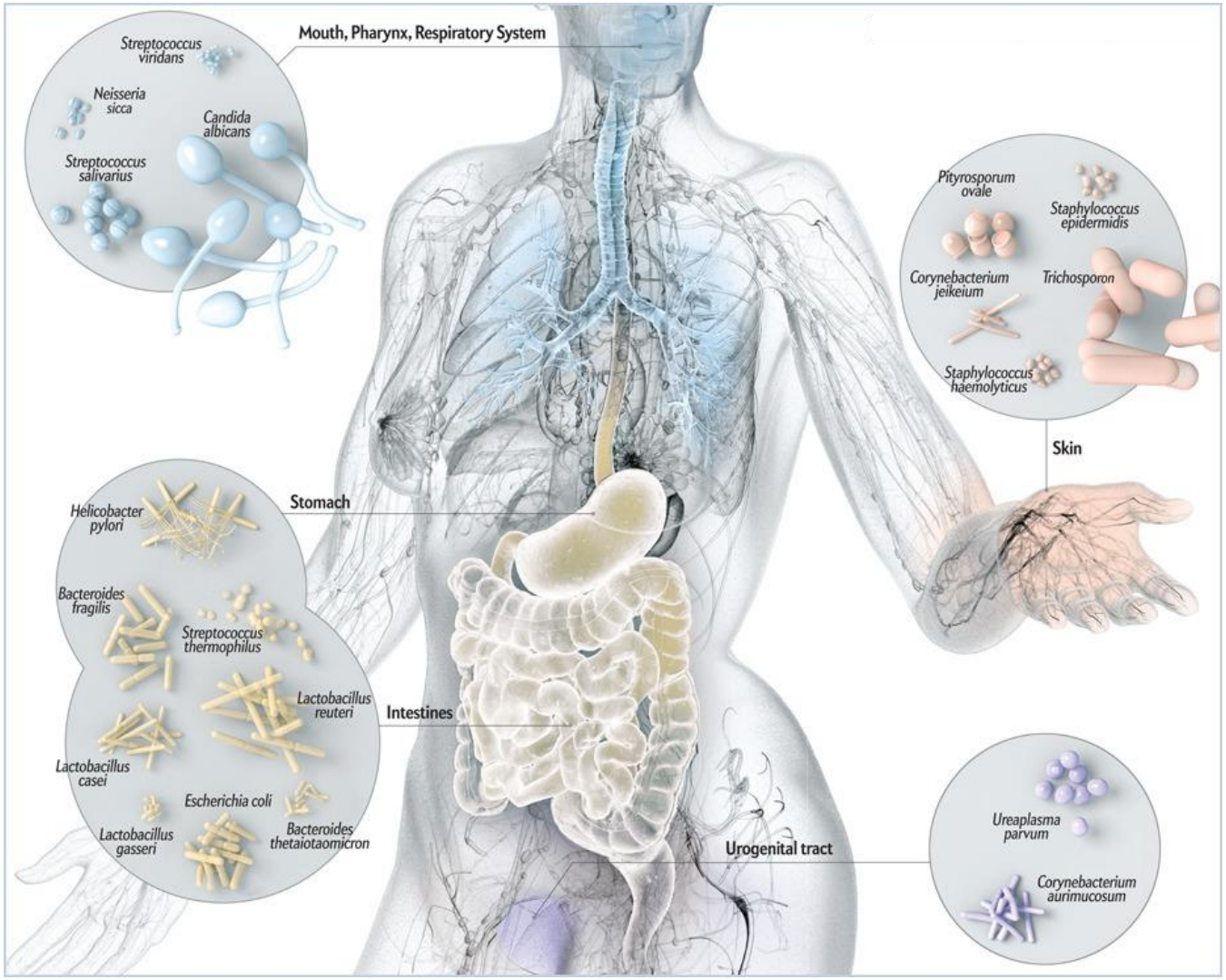
Robert Leach

Princeton University

Outline

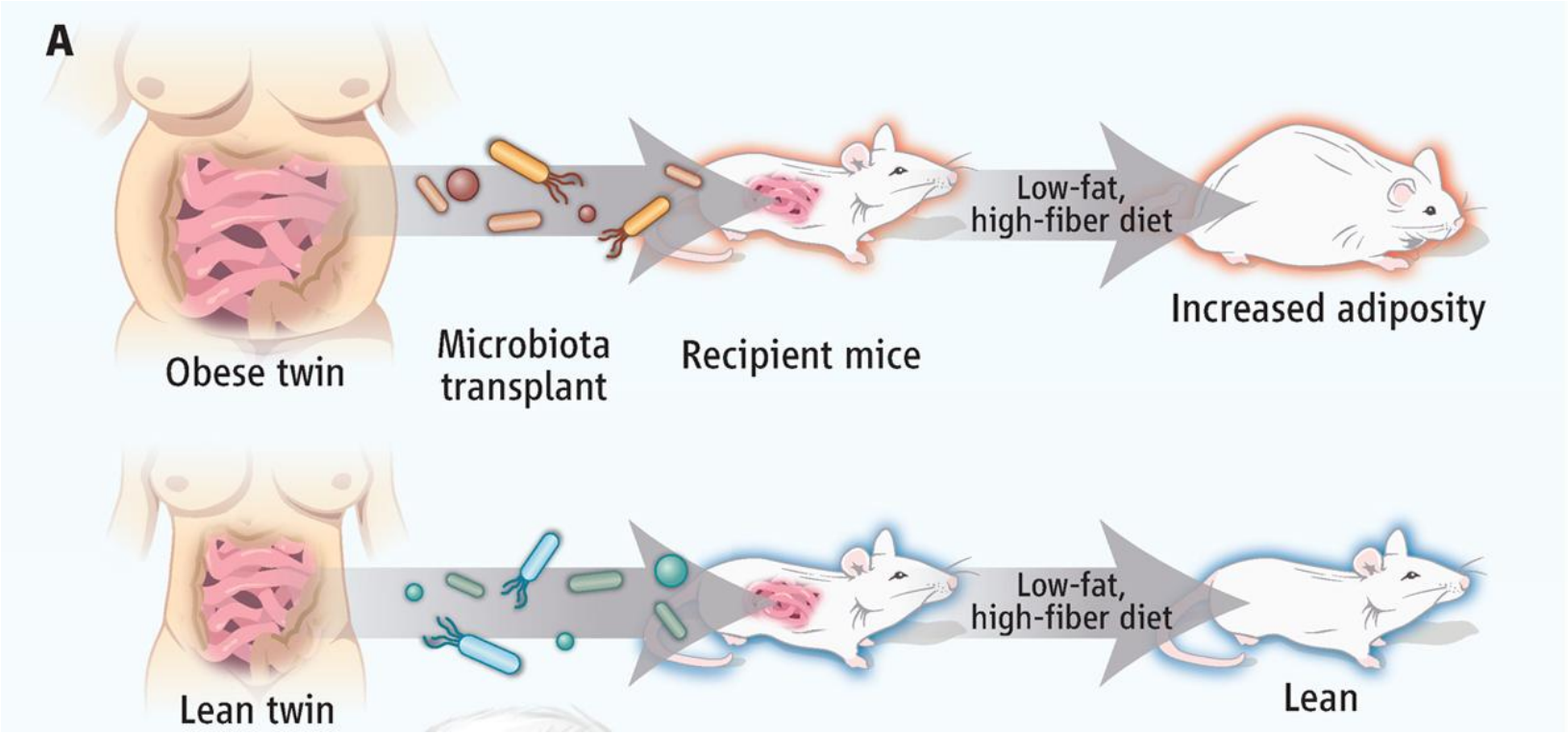
- The Microbiome
- 16S RNA metagenomics
- Clustering-free analysis of 16S data
- Sequence similarity vs. dynamical similarity
- Conclusions

The Human Microbiome



http://www.scientificamerican.com/article/microbiome-graphic-explore-human-microbiome/

Impact of the Microbiome



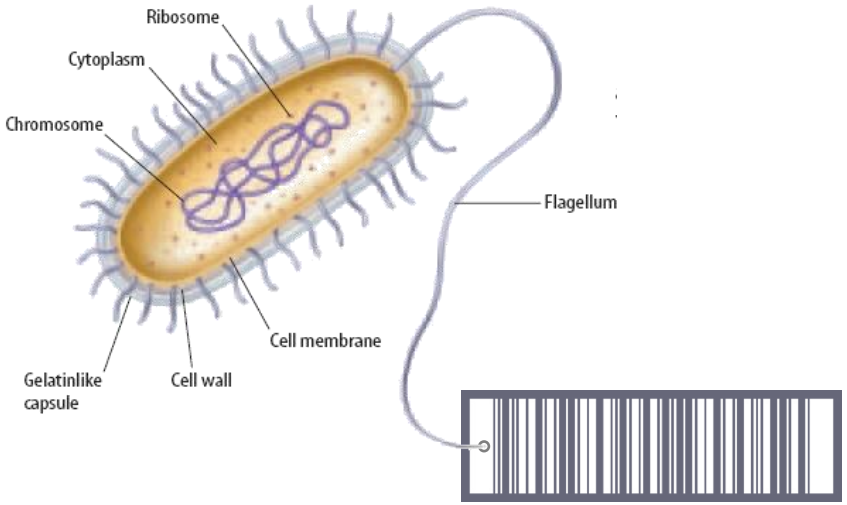
Ridaura *et al*…Jeffrey Gordon, Science 2013
Walker & Parkhill, Science 2013

16S metagenomics

Problem: most bacterial species can't be cultured

Solution: 16S ribosomal RNA

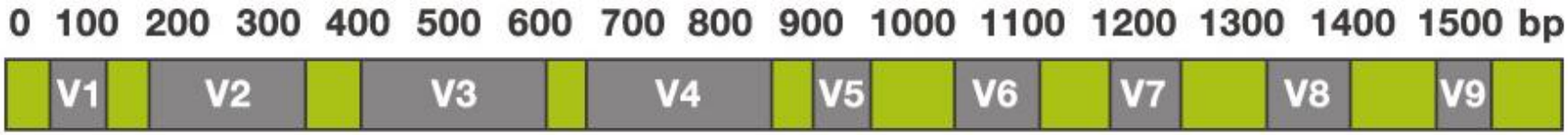
Woese & Fox, PNAS 1977

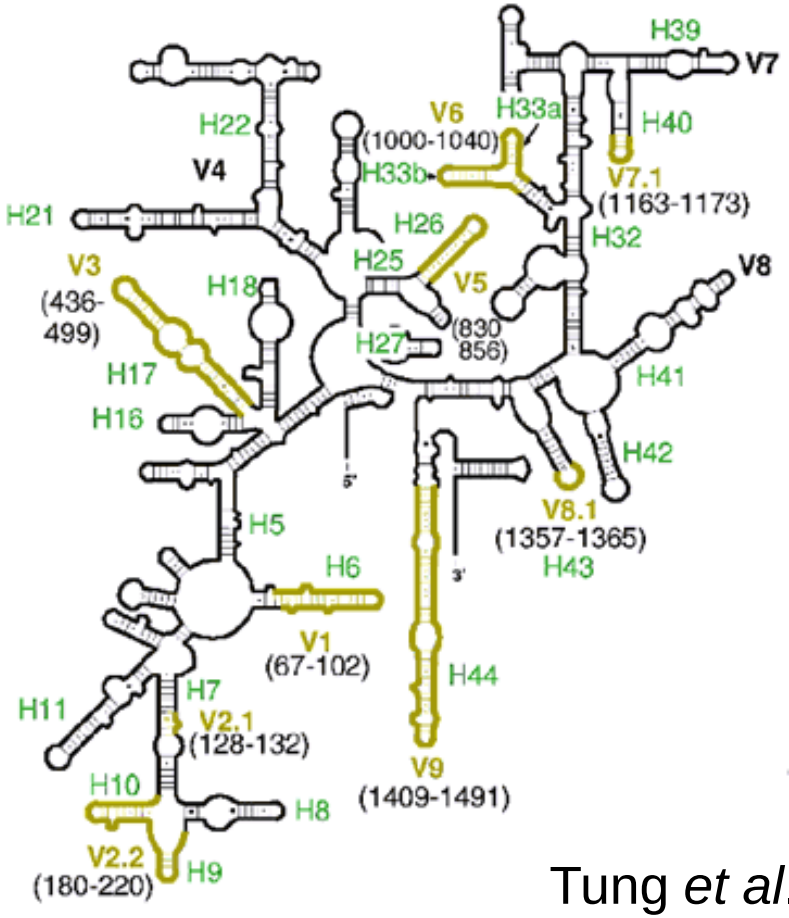


Big questions

- Origin, maintenance, and significance of diversity?
  - Role of “rare” species
- Factors shaping community?
  - Environment, interactions, host immunity, chance
- Relation to health and disease?

16S RNA gene

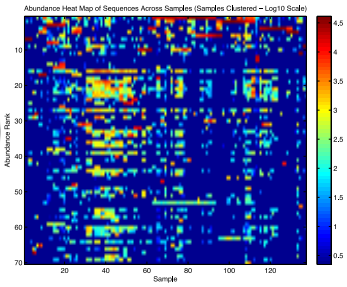




Tung *et al*., Nat Struct Biol 2002

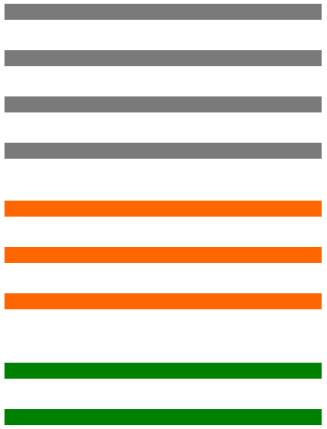
Usual 16S work flow

Extract DNA → PCR & Sequence → Process & Analyze



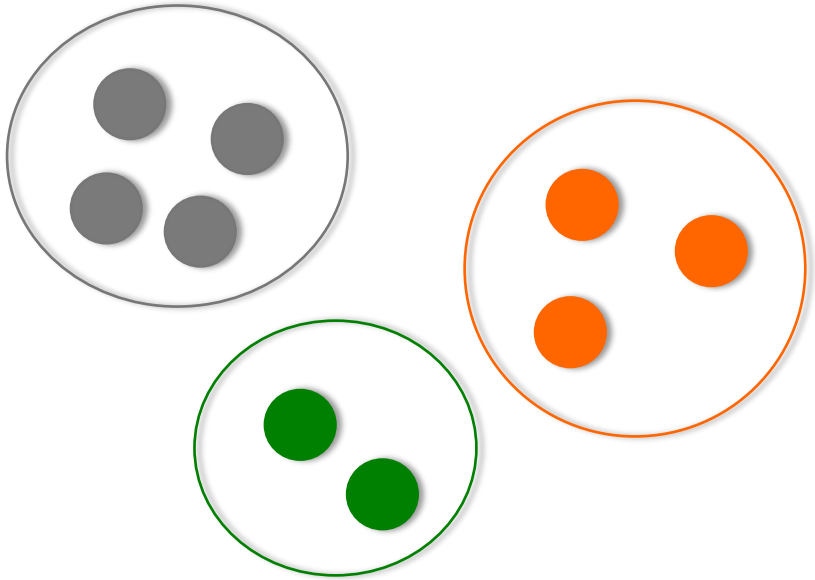
Data Visualization

Sample



Raw sequence reads

Form OTUs →



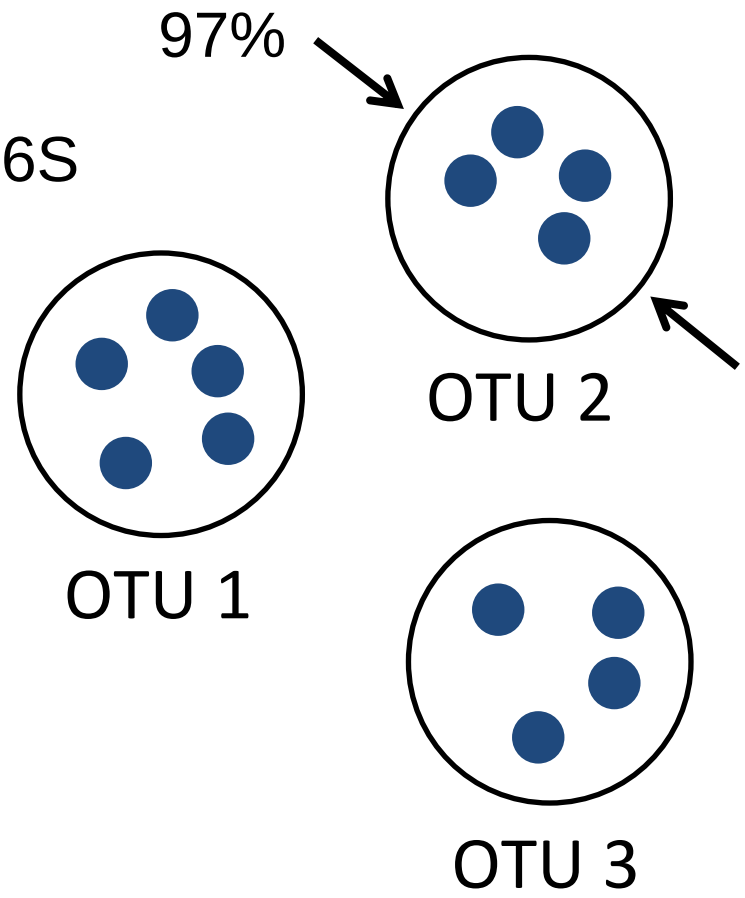
Operational Taxonomic Units (OTUs)

Justification?

1. Noise
2. Ecological similarity of close 16S

Applications?

1. Mapping to known species
2. Co-occurrence patterns



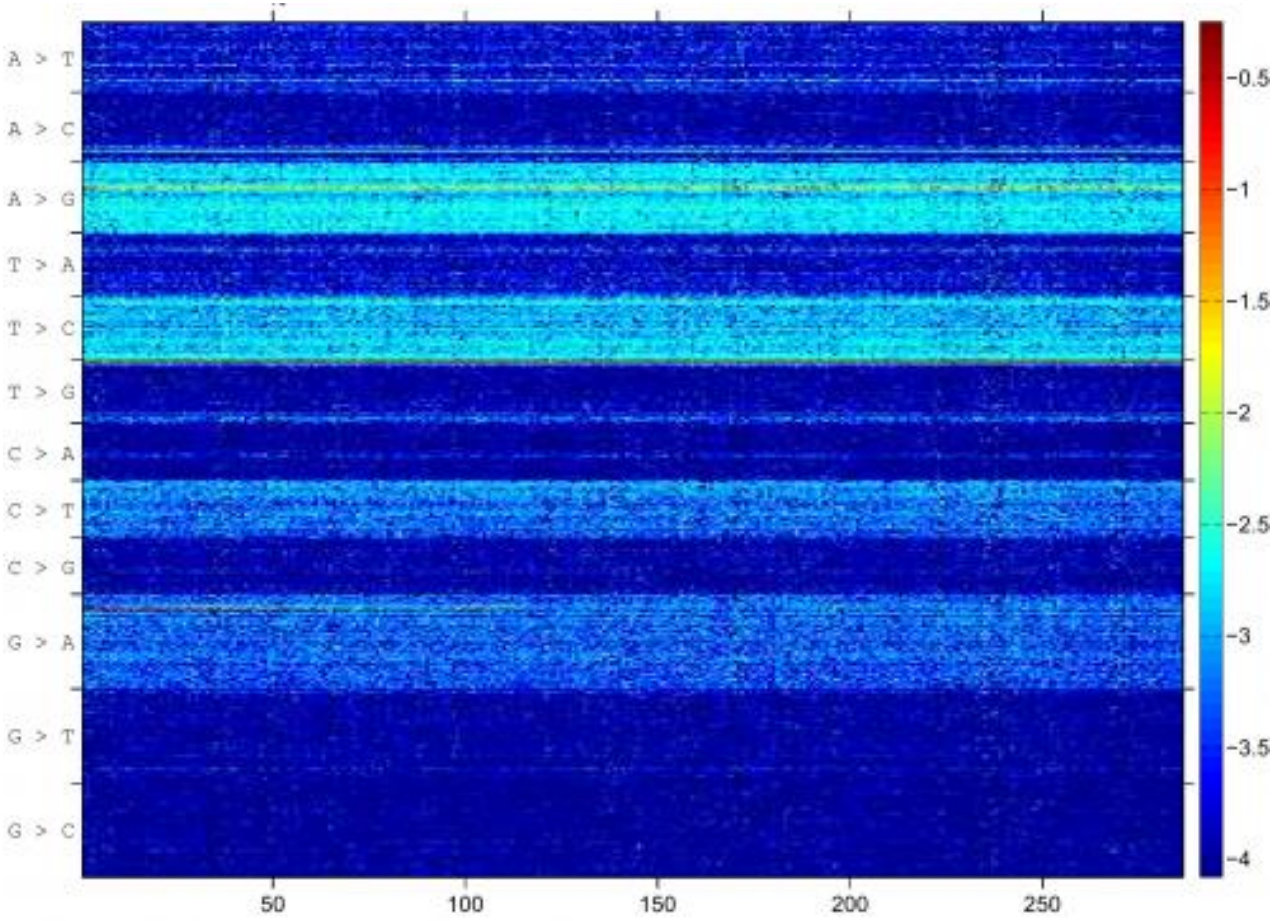
However, OTUs are ill-defined.

Do we need OTUs?

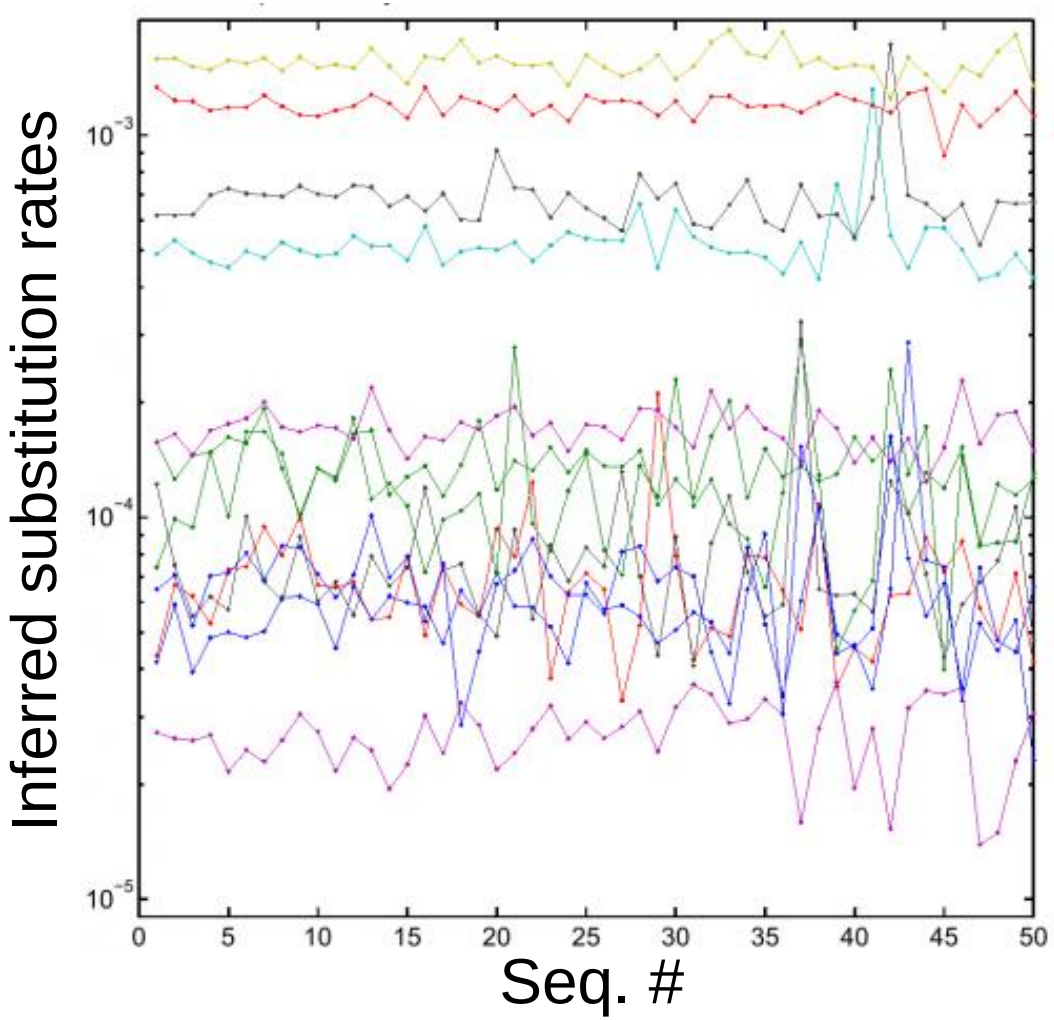
16S analysis without OTUs

Data: daily sampling of the tongue community of two cohabiting individuals for > 1 year (Caporaso *et al*., Genome Biol (2011))

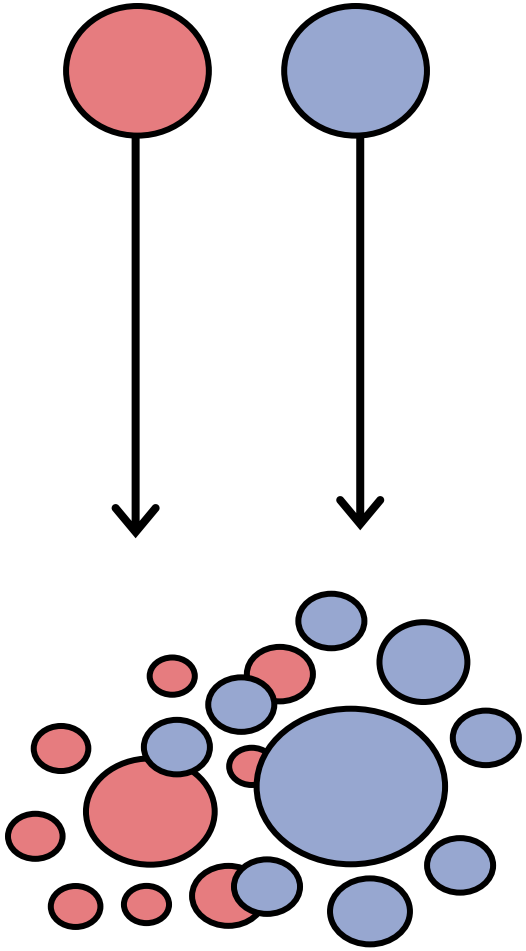
Log_{10} abundance of 360 neighbors of Seq. #1 (most abundant 130 nt sequence)



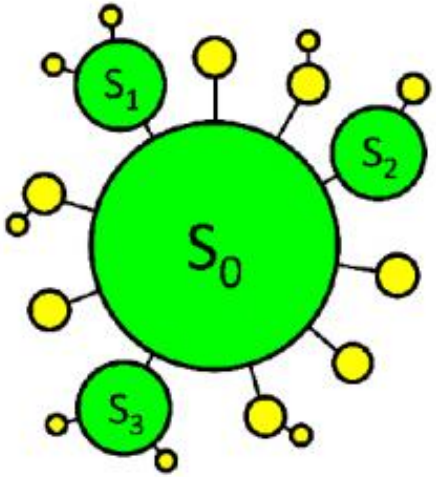
Error rates are low and reproducible

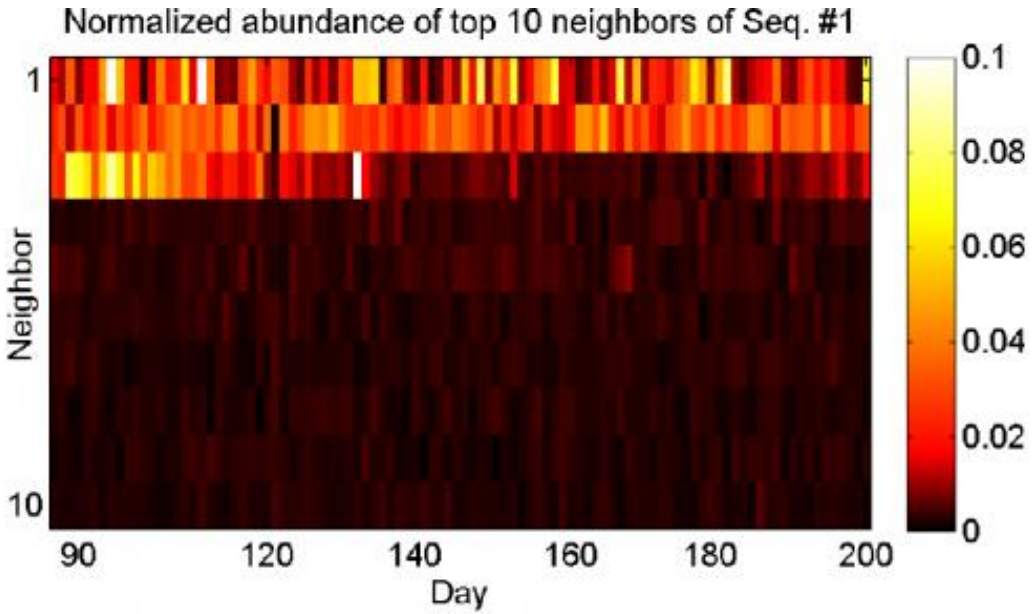


Inferred error rates can be used to identify real sequences



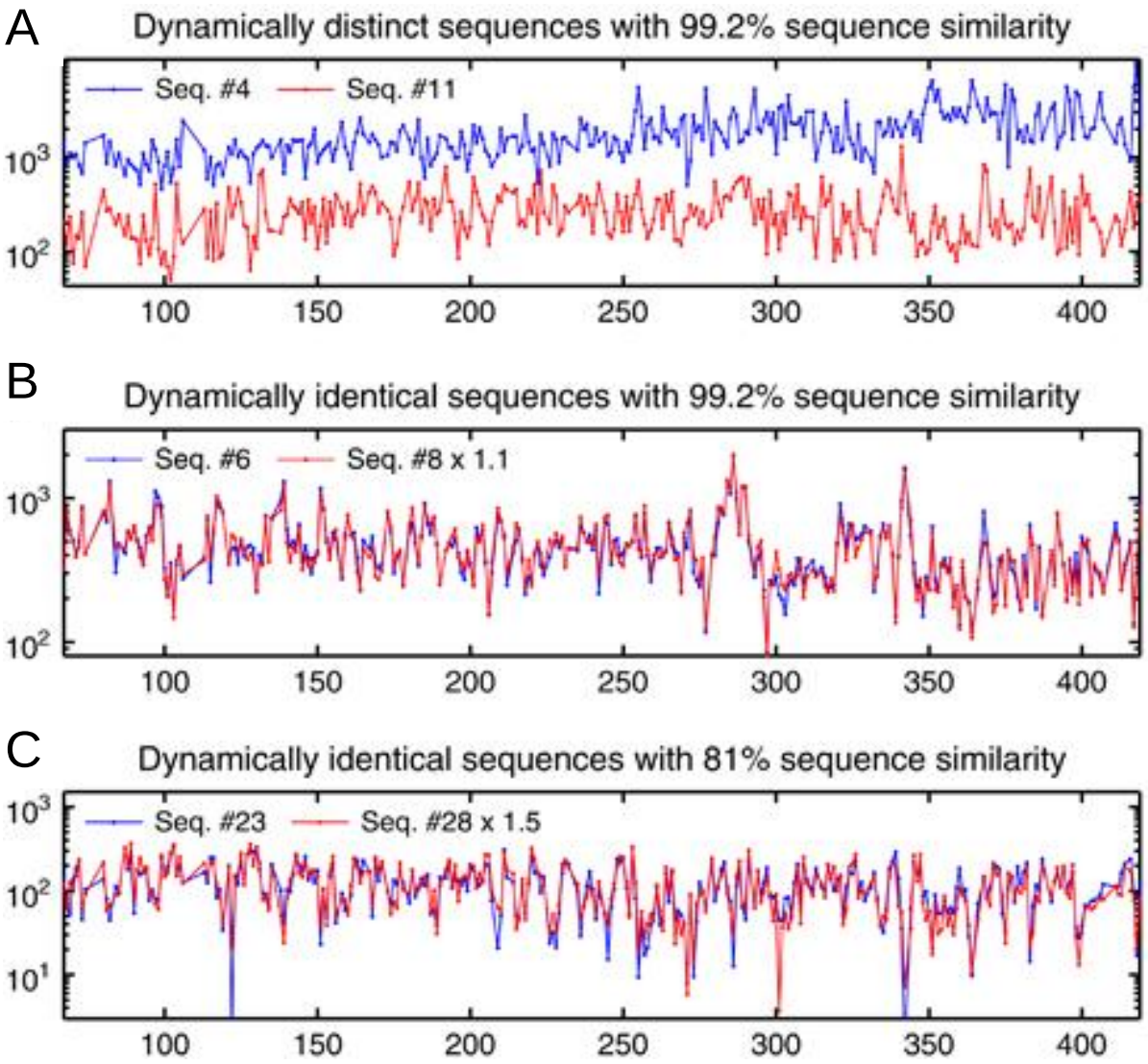
Real sequences differing by only 1 nt have different dynamics

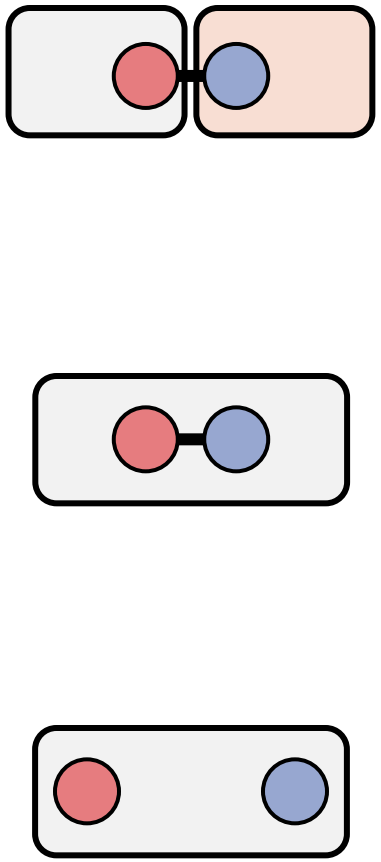




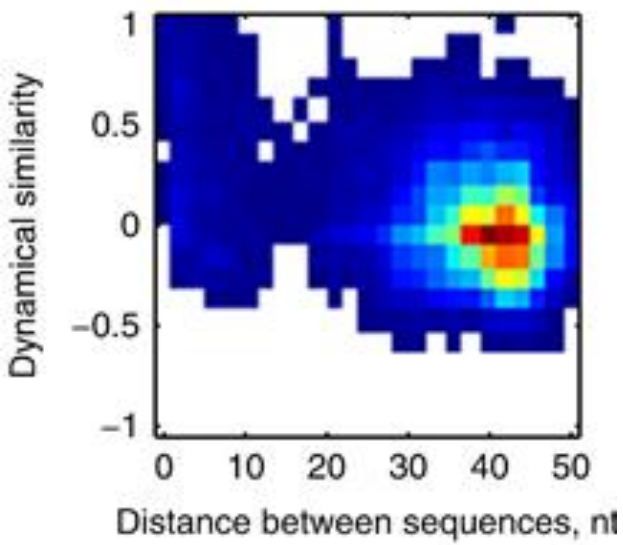
Typically resolve > 20 distinct real sequences per OTU

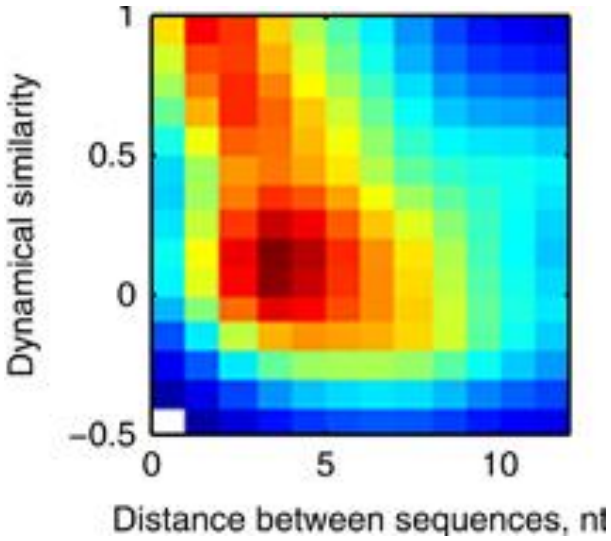
Dynamical similarity vs. sequence similarity: examples





Dynamical similarity vs. sequence similarity

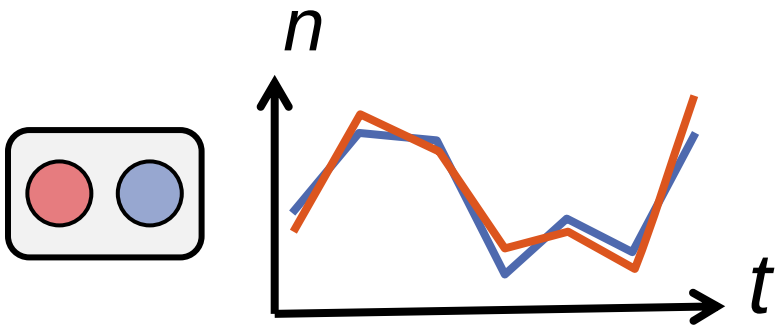




"Dynamical similarity" = Pearson correlation between time traces (normalized by maximum possible given Poisson noise)

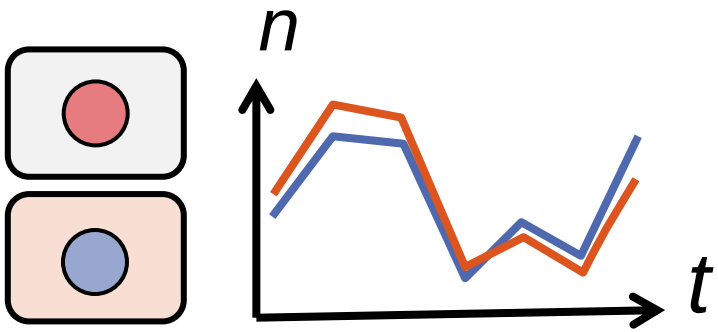
Same bacterium or a dynamically similar strain?

Δ(*t*): Zero 1-day autocorrelation



All difference due to measurement noise

Δ(*t*): Nonzero 1-day autocorrelation

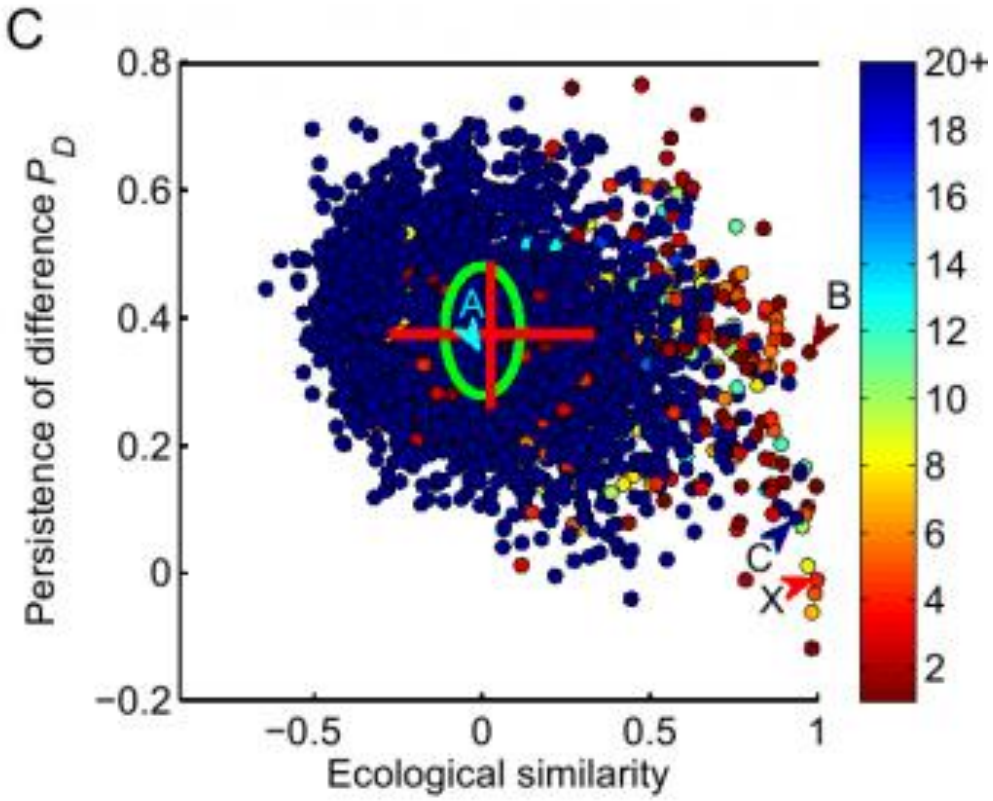


Abundance difference may persist

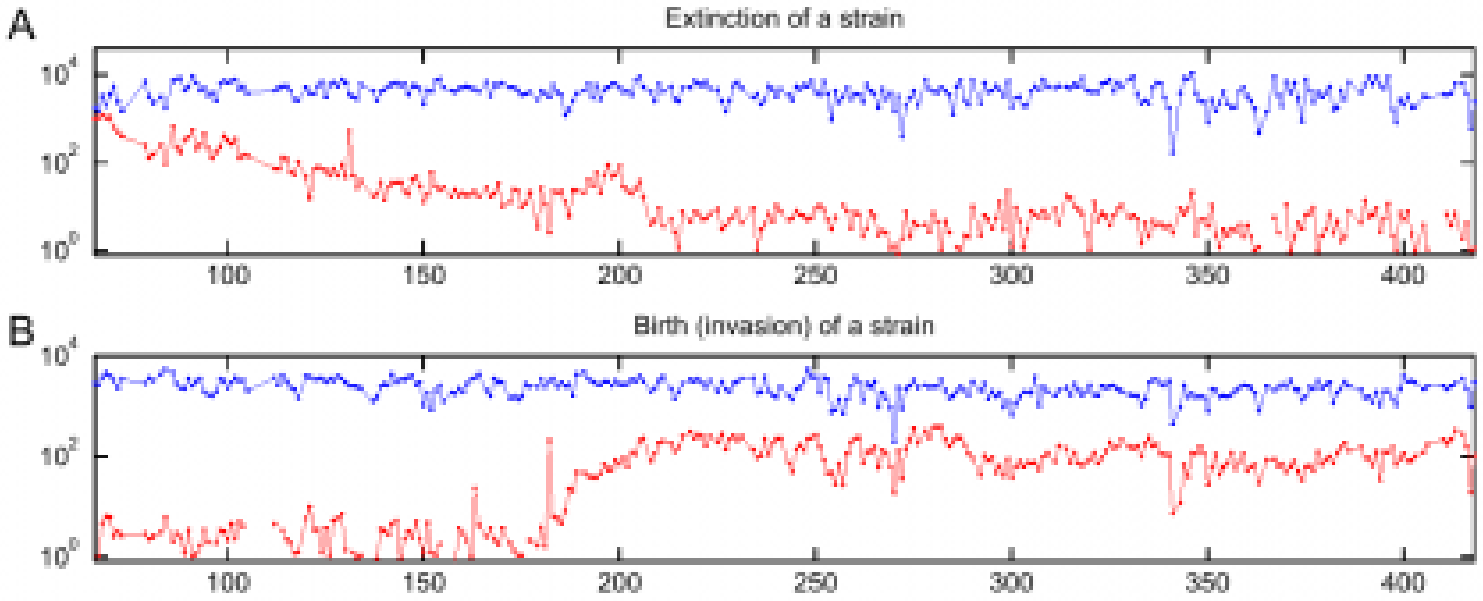
Relative abundance fluctuation $\Delta(t) = (n_2 - n_1) / [(n_1 + n_2)/2]$

"Persistence of difference" $P_D = \langle \Delta(t)\, \Delta(t+1) \rangle / \langle \Delta^2(t) \rangle$

Can identify 16S paralogs (REPLACE FIGURE!)



Slow dynamics of sequences 1 nt from abundant sequence



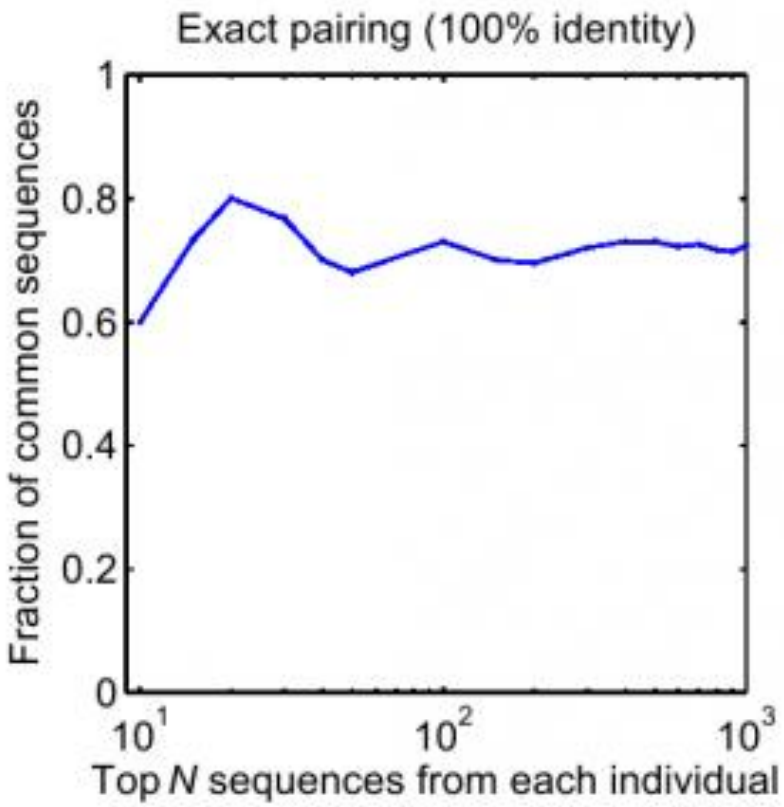
Is 130 nt enough?

In principle, distinct subpopulations could share the same 130 nt sequence.

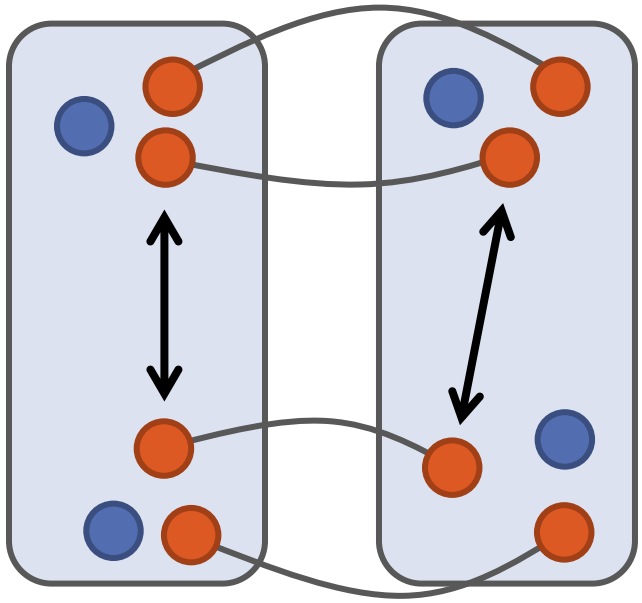
Then exact sequence identity might be no more informative than 1 nt difference, which we know allows for dynamical differences.

What can we do?

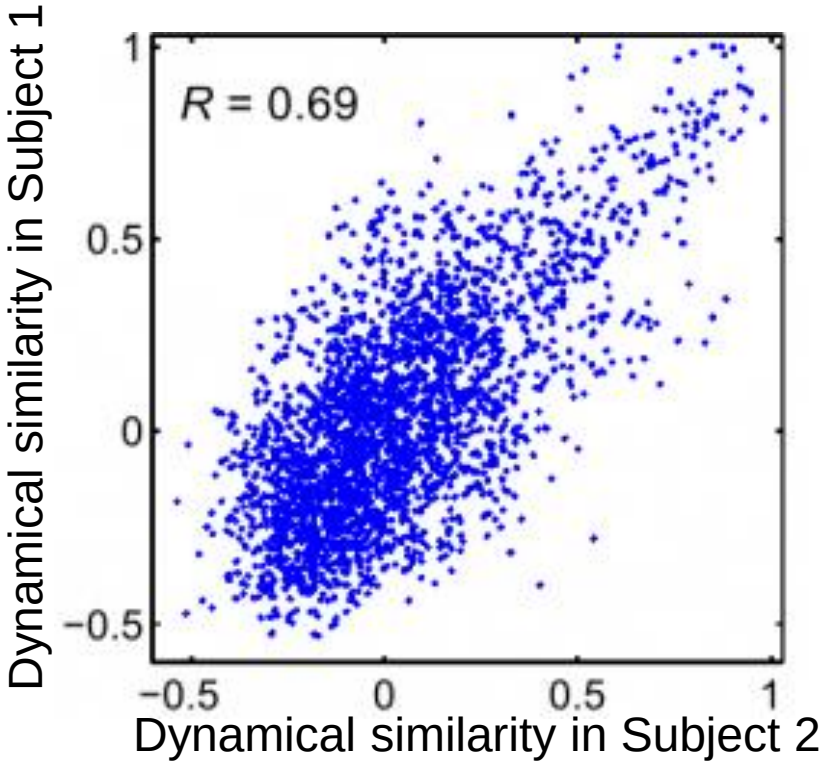
Exploit shared strains between two human subjects

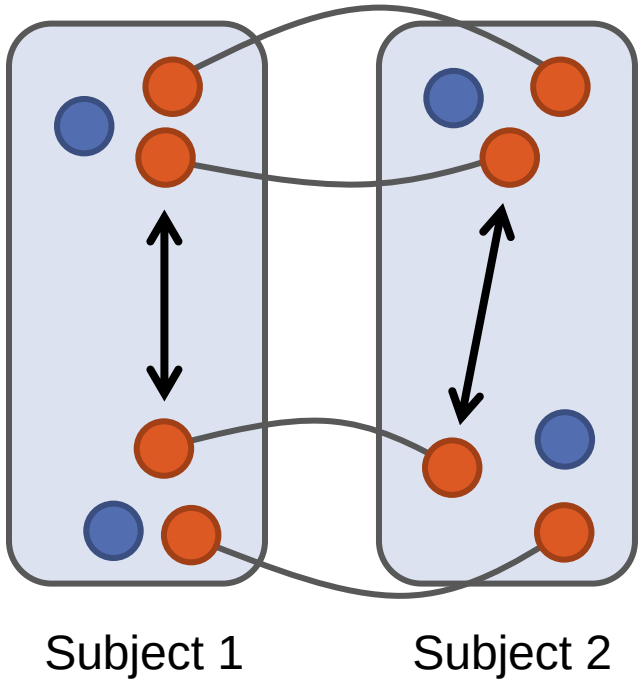


Significant strain exchange

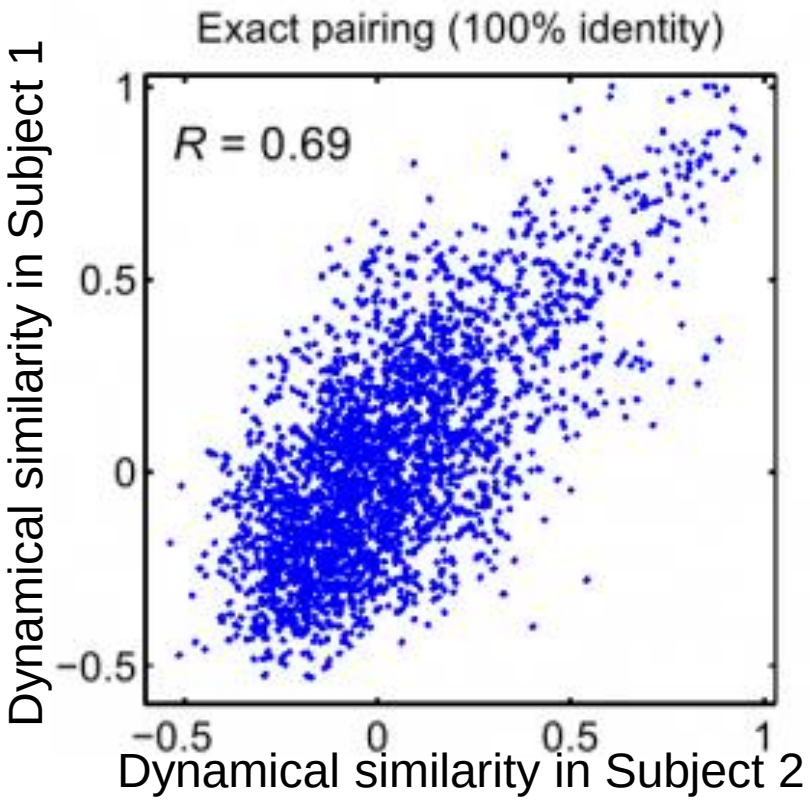


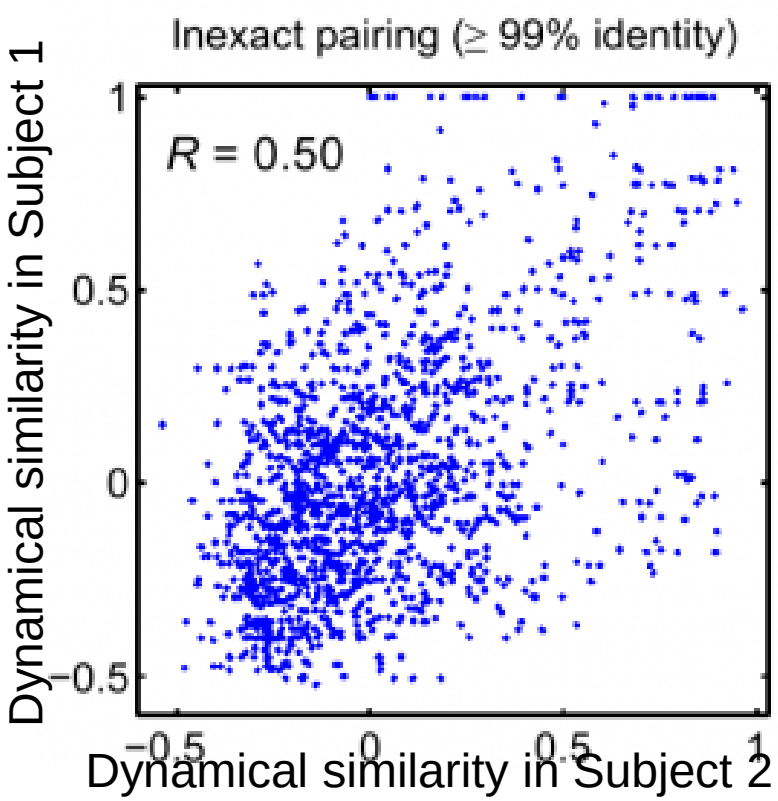
Shared strains share dynamical similarity





1 nt mismatch enough to degrade correlation of dynamical similarity





16S tag identity and near-identity are fundamentally different

Near-identity:

~10^6 generations

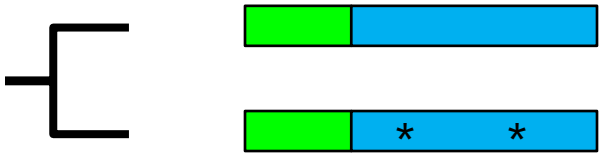
16S tags differ by 1 nt

Identity:

16S tags identical

or, more likely,

16S tag genome



Summary and conclusions

- Microbiomes are ubiquitous – bacteria live in communities
- Metagenomics: rich source of data (16S and “shotgun”)
- Cluster-free filtering for time-series & multi-sample 16S data
- Applied to tongue microbiome data (Caporaso *et al.* (2011)):
    - 20+ real sequences per OTU
    - 16S paralogs vs. dynamically similar strains
    - Slow dynamics of subpopulations
- Many big questions to address…

Thank you!

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Tikhonov *et al.*, ISME Journal (2014)