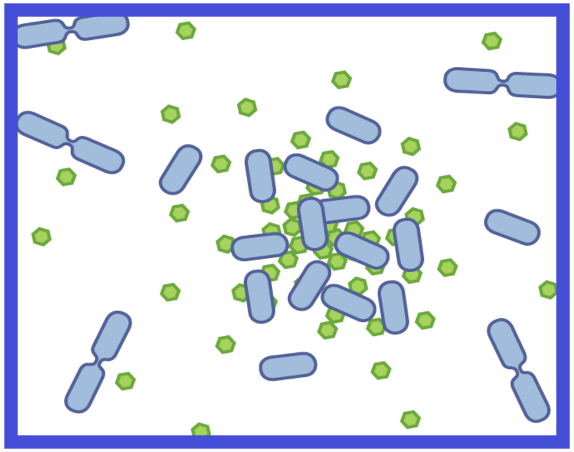
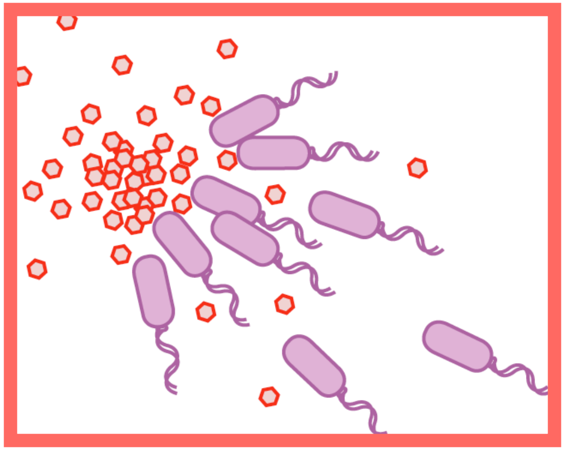
RICE UNIVERSITY
Houston, TX

Quorumtaxis:

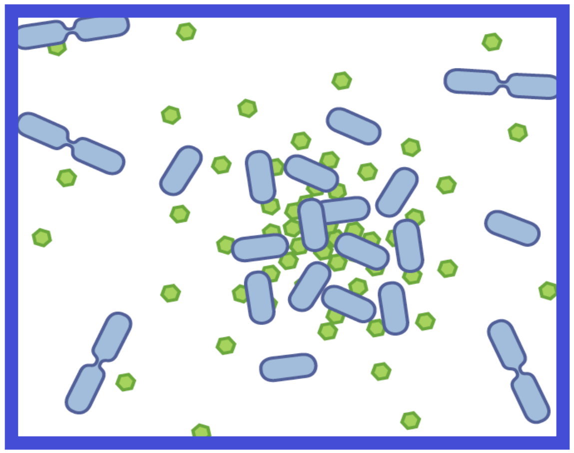
Programming *E. coli* to Eavesdrop, Stalk, and Kill *B. subtilis*

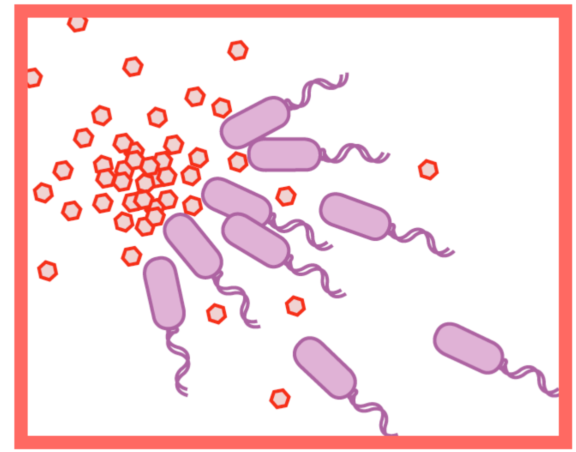
Quorum-sensing + Chemotaxis



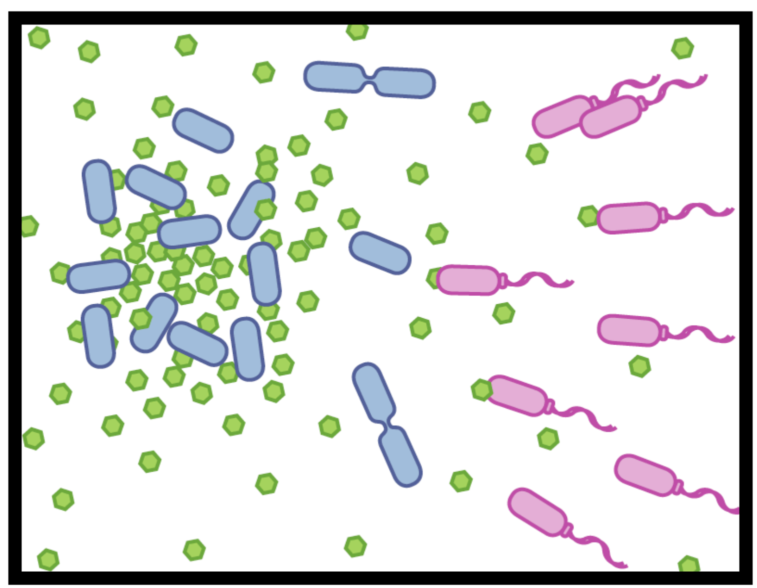


Quorum-sensing + Chemotaxis

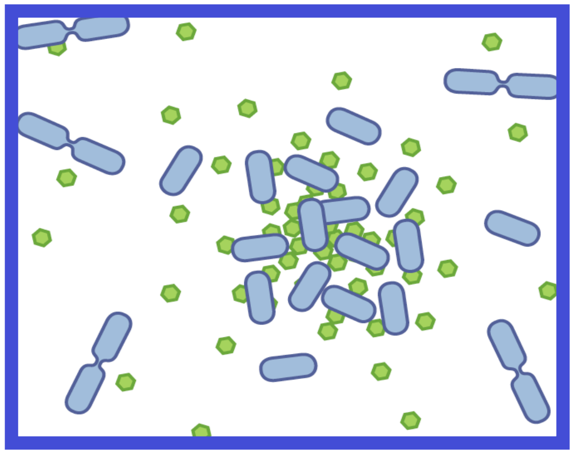


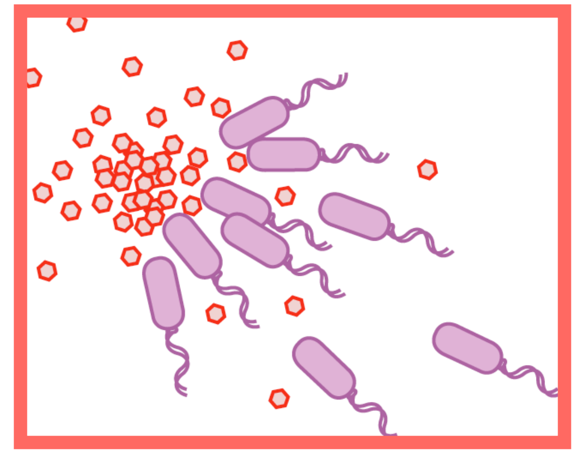


= Quorumtaxis

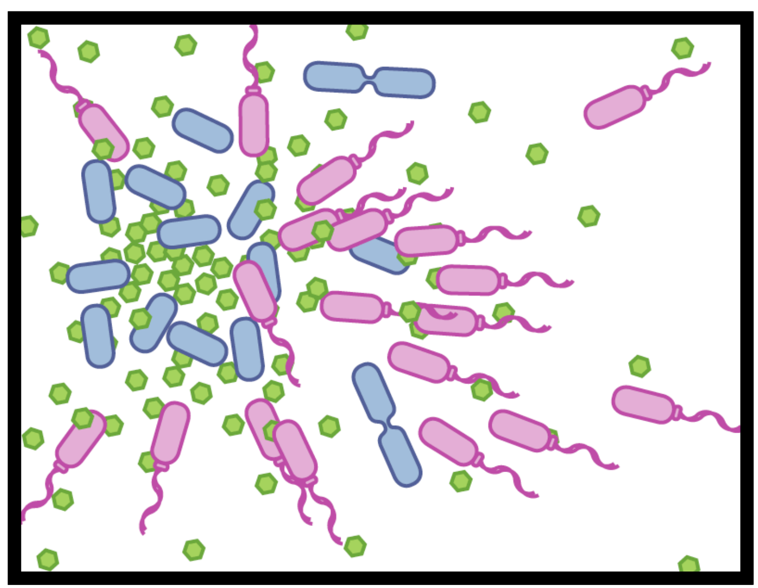


Quorum-sensing + Chemotaxis

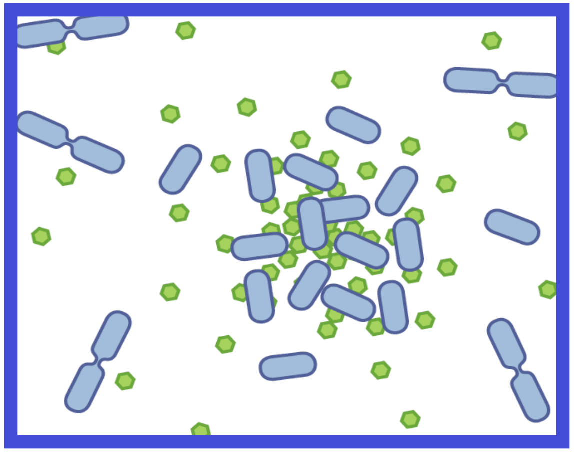


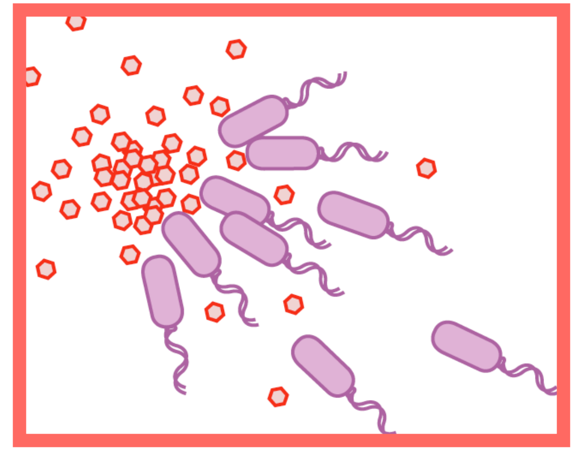


= Quorumtaxis

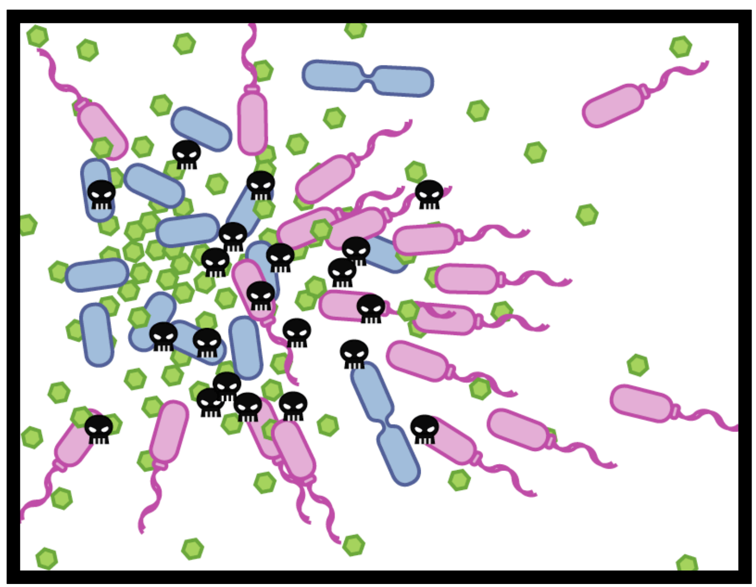


Quorum-sensing + Chemotaxis

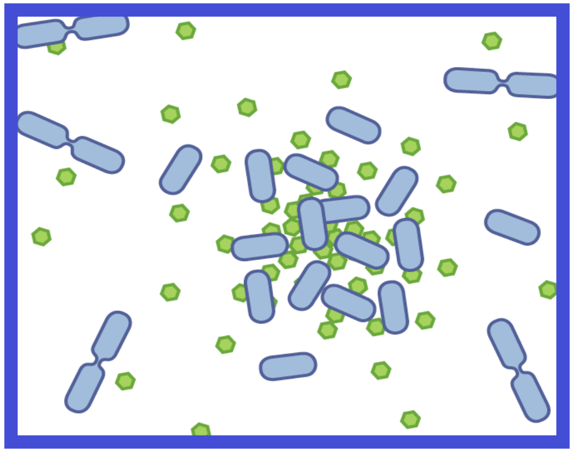


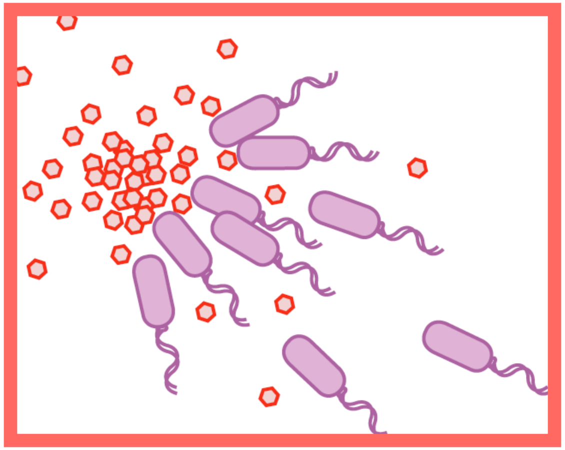


= Quorumtaxis

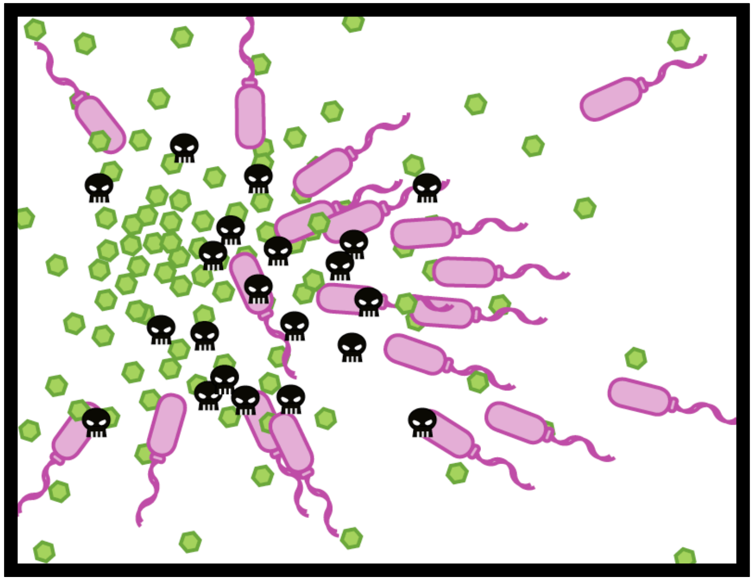


Quorum-sensing + Chemotaxis





= Quorumtaxis

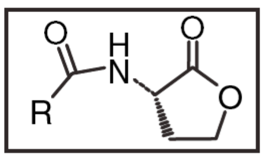


What is a Good Pheromone to Use for Quorumtaxis?

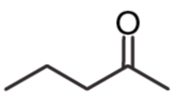
Gram-negative bacteria

Acyl homoserine lactone autoinducers

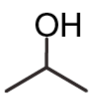
Core molecule



R groups

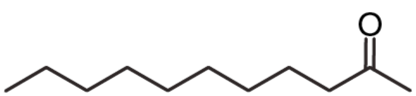


Vibrio fischeri



Vibrio harveyi

Pseudomonas aeruginosa

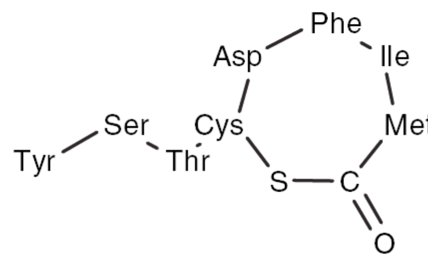


Pseudomonas aeruginosa

AHL-based pheromones are metabolic products with limited diversity.

Gram-positive bacteria

Oligopeptide autoinducers



Staphylococcus aureus

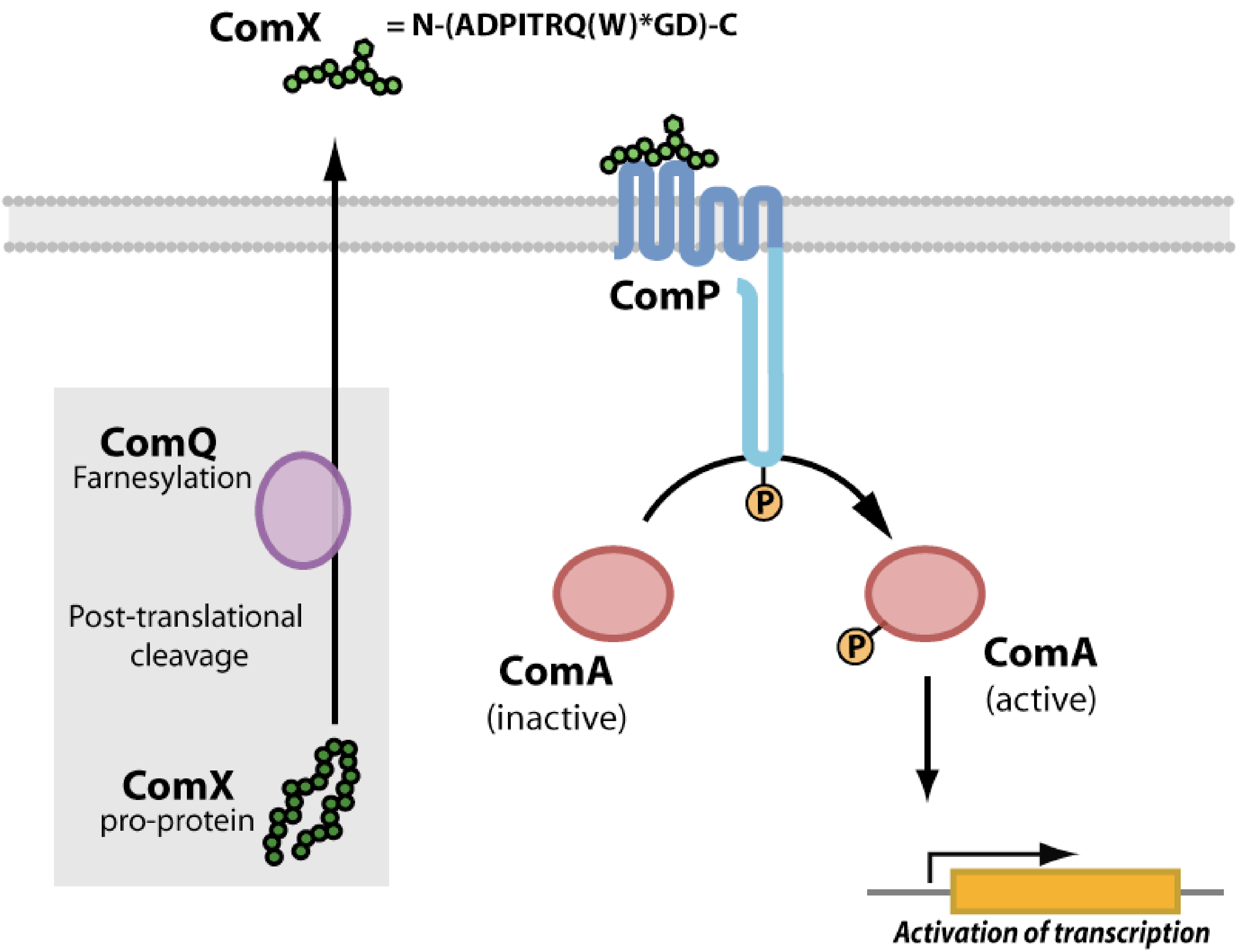
ADPITRQW*GD — *Bacillus subtilis*

EMRLSKFFRDFILQRKKO — *Streptococcus pneumoniae*

Oligopeptides are genetically encoded with a tremendous potential for diversity.

Science (2006) 311: 1113–1116

The *B. subtilis* Peptide-based Quorum Sensing System has 4 Protein Components



The ComXP Systems Could be Used to Create Diverse Quorumtaxis Systems

ComX precursors exhibit tremendous sequence diversity,

```
Bs strain     ComX precursor (C-terminus)
 RO-E-2    .......DKLIPSIVDIFNKKMTLSKKCKGIFWEQ   58
 RO-H-1    ..........QSECIINGFK---GLEIYSMLDWKY   53
 RO-B-2    ..........QLKSIVDAFG---GLQIYTNGNWVPS  53
 RO-OO-2   ..........QLKSIVDAFR---GMQIYTNGNWVPS  53
 RO-C-2    ..........EKKAILYAFEQ---GEVPRTSKWPPI  53
 RO-FF-1   ..........ETQLIIEGFEGIEEVKRGNAGKWGPE  53
```

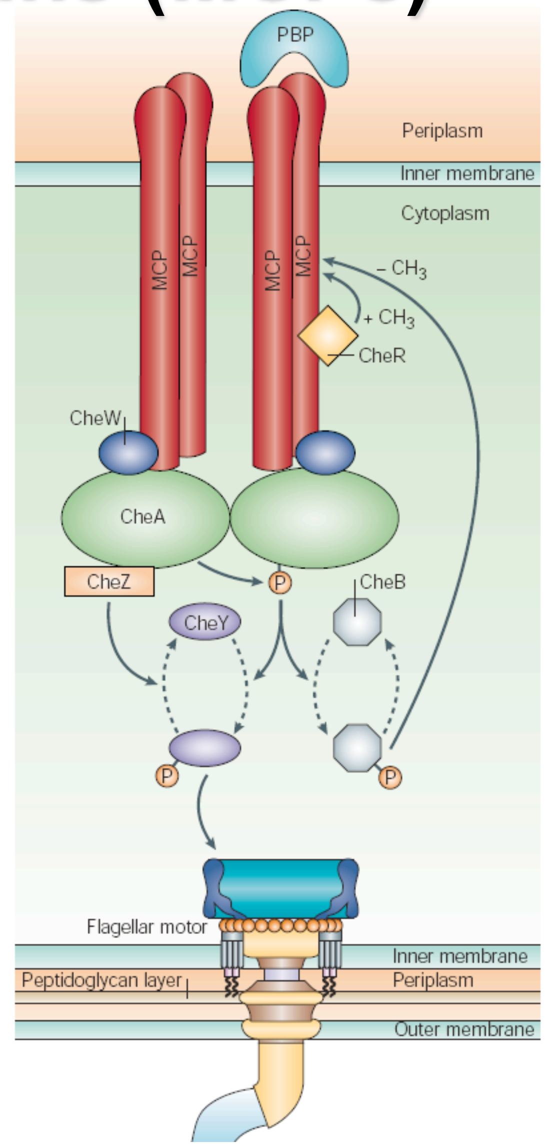
…and orthogonal ComX systems have evolved that do not communicate, eliminating cross-talk between species and reducing background.

J. Bact. (2001) 183(2):

ComP Receptor is Related to *M*ethyl-accepting *C*hemotaxis *P*roteins (MCPs)

Gene	Protein	Response
tsr	Tsr	serine
tar	Tar	aspartate
trg	Trg	ribose
tap	Tap	peptides
aer	Aer	oxygen

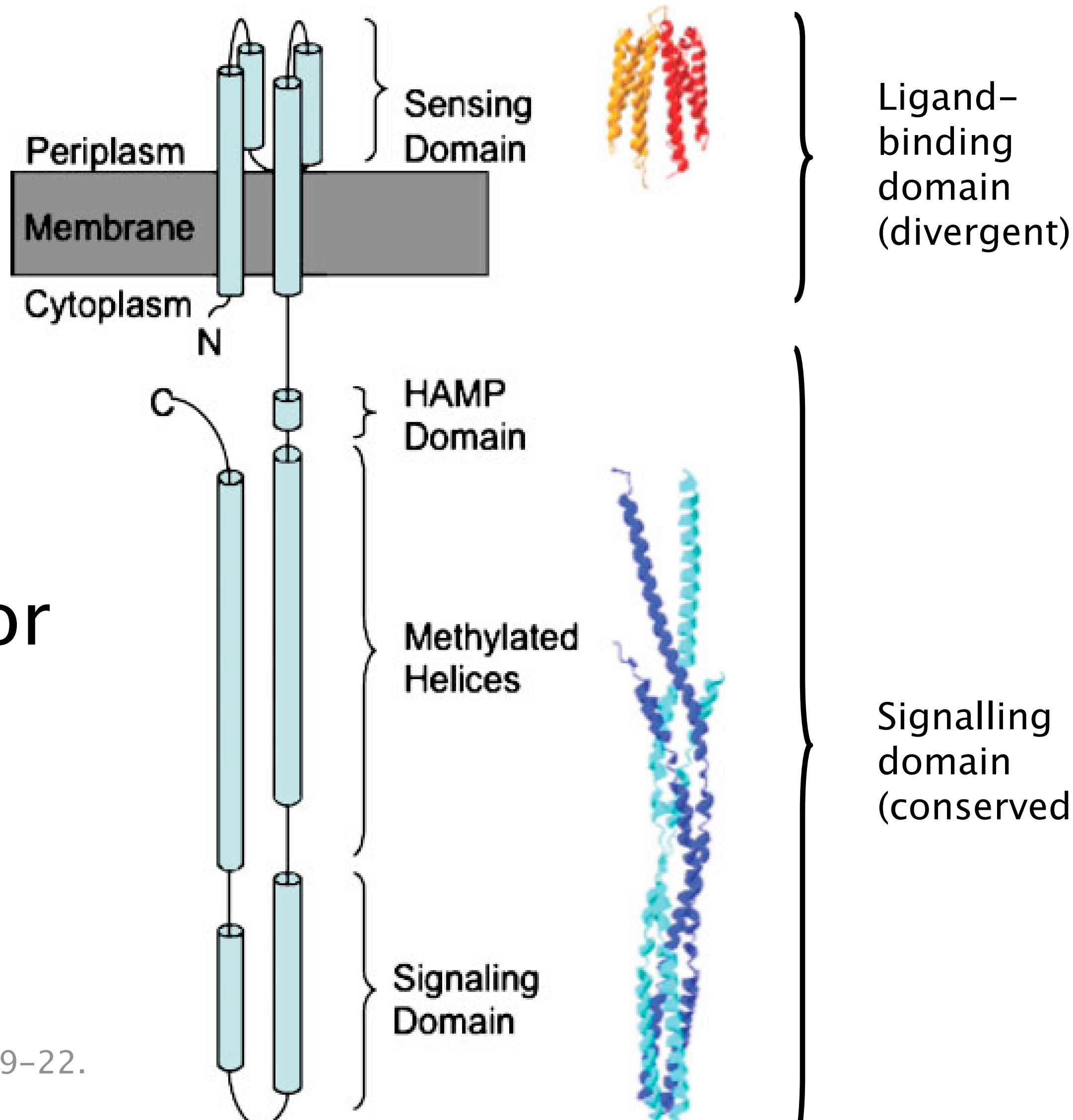
§ ComP and Tsr exhibit ~11% identity.



Nature Reviews Mol Cell Bio (2004) 5:

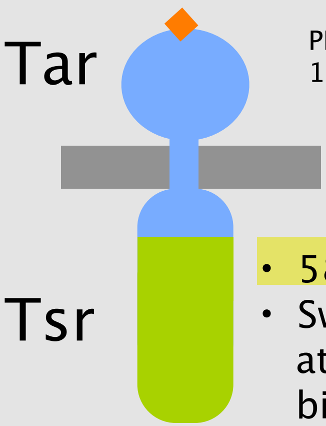
MCPs Have a Modular Architecture

Tsr Receptor



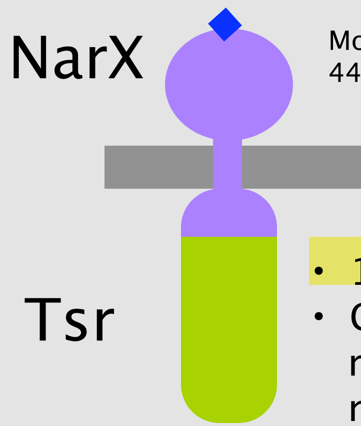
BioEssays (2005) 28: 9-22.

MCP-like Proteins Have Been Recombined to Create Novel Receptors



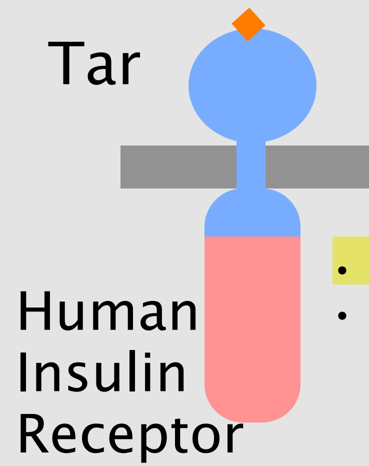
PNAS (1985) 82: 1326–1330.

- 58% Identity
- Switch of attractant binding.



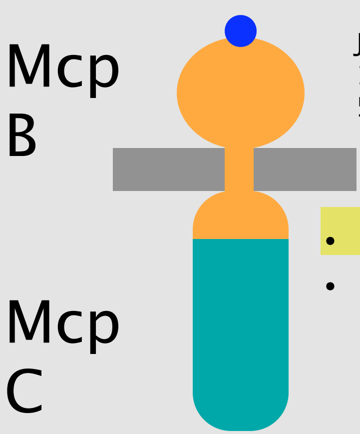
Mol. Microbiol. (2002) 44(3): 709–719.

- 11% Identity
- Chemotactic response to nitrates.



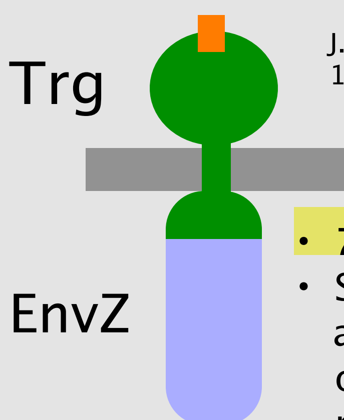
PNAS (1989) 86: 5683–5687.

- 3% Identity
- Aspartate activation of a tyrosine kinase.



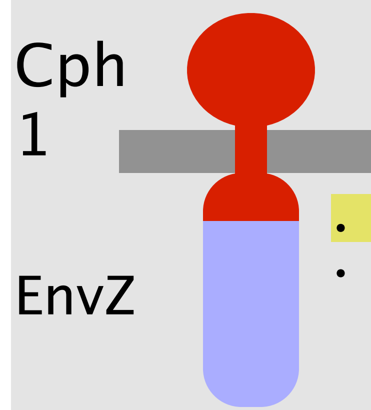
J.Bact. (2004) 186: 5950–5955.

- 25% Identity
- Swapping of Asp, Pro domains of B. subtilis receptors.



J. Bact. (1993) 176: 1157–1163.

- 7% Identity
- Sugar activation of ompC promoter.



Nature (2005) 438: 441–442.

- 12% identity
- Light activation of ompC promoter.

Strategy

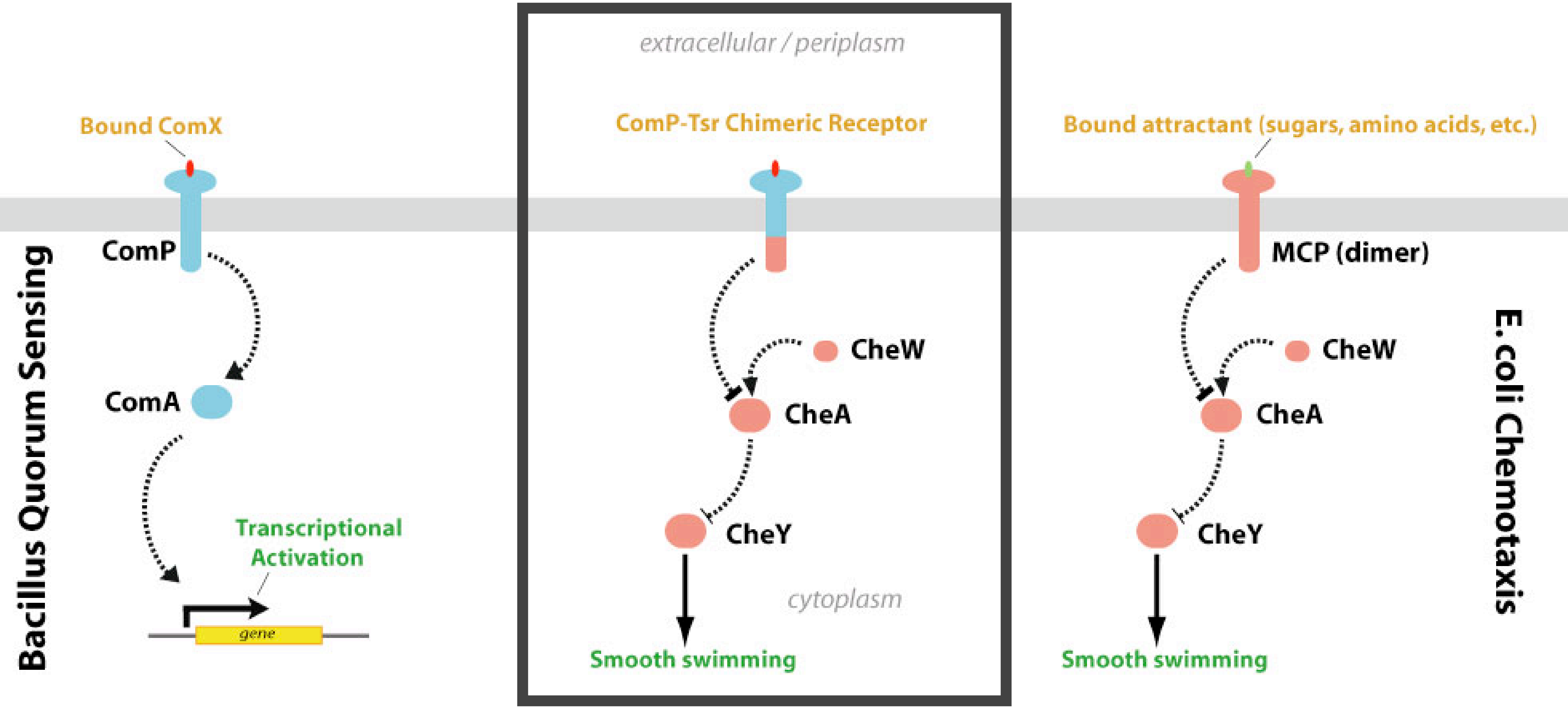
Phase I

- § Use BioBricks and ComP–Tsr chimeras to construct quorumtaxis circuit.
- § Use chemotaxis models to design and optimize quorumtaxis circuit.

Phase II

- § Extend circuit to include destroy output, e.g., SdpC toxin production.

Module Integration by a Chimeric Receptor

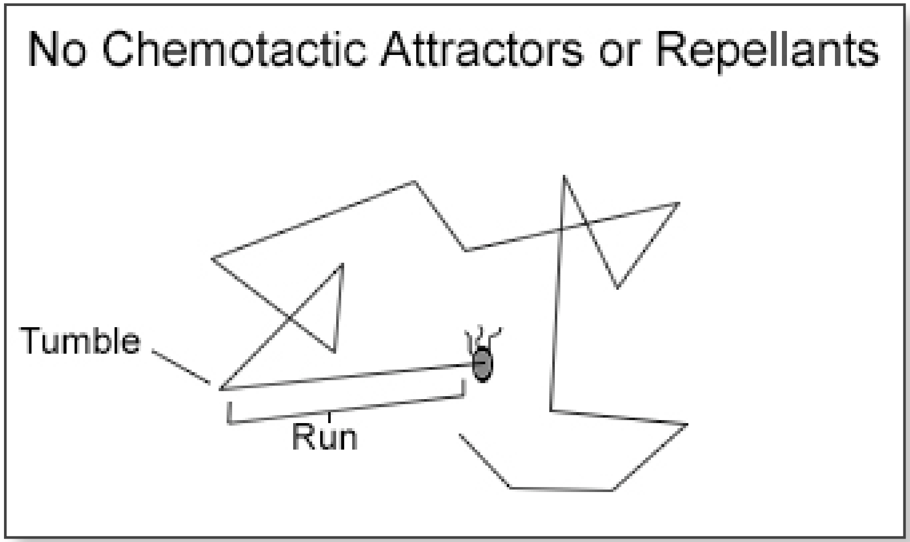


Issues That May Arise With Chimeric Receptors

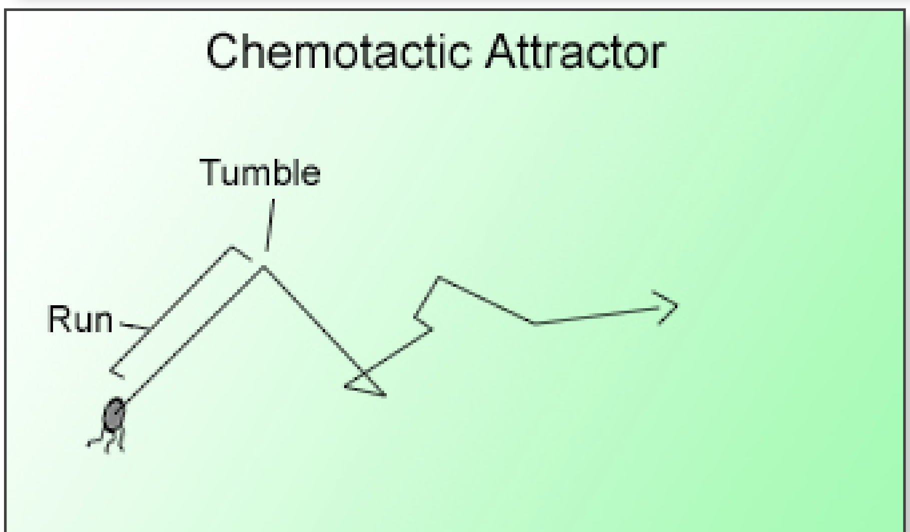
- ComP-Tsr chimeras may be weakly functional because they:

    1. Weakly bind ComX.
    2. Bind ComX, but transmit the signal poorly to the cytoplasmic domain.
    3. Bind ComX, transmit the initial signal to the flagella, but are unable to adapt to gradients of ComX.

Bacterial Chemotaxis Works by Modulating Run Lengths



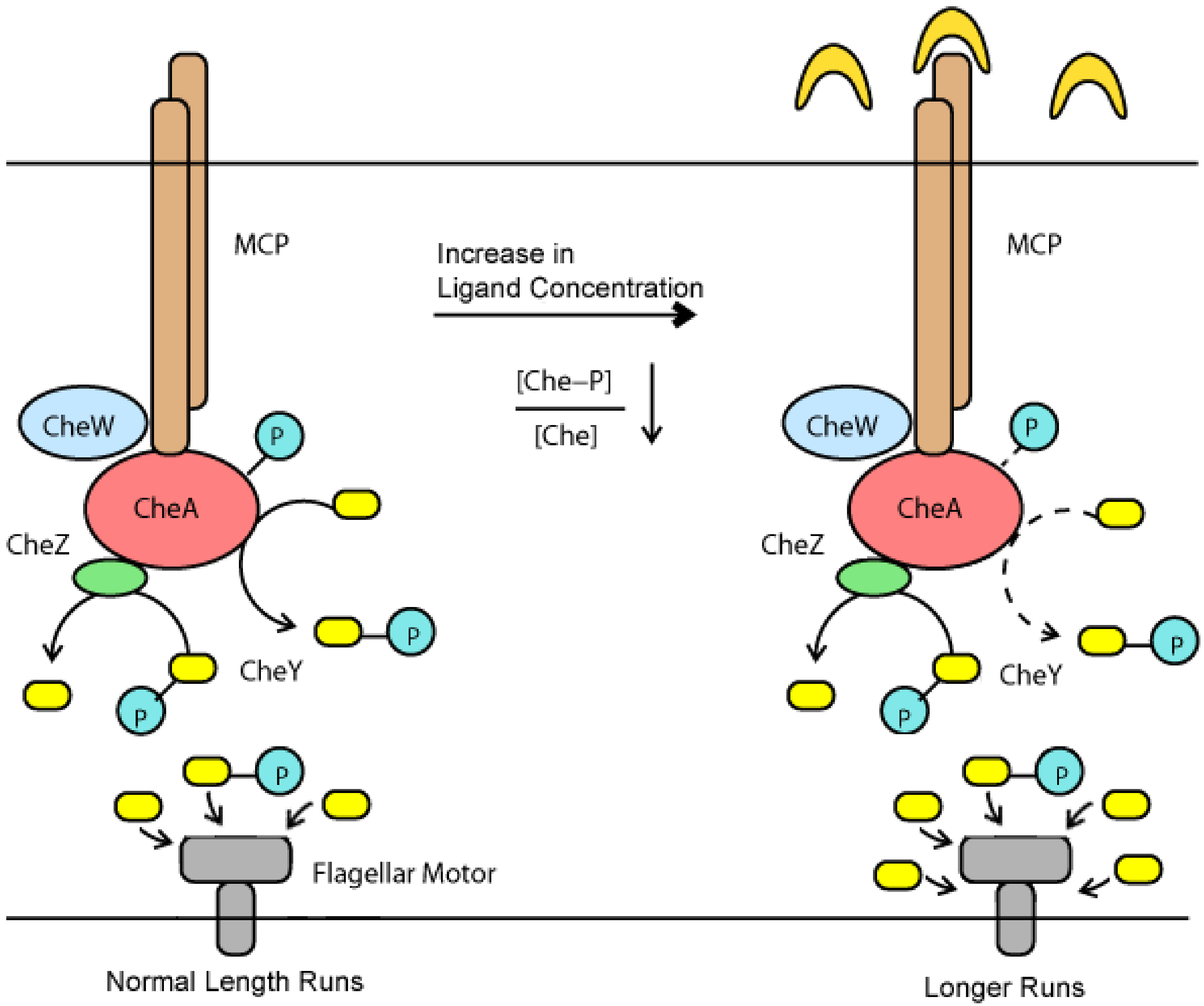
- Runs with an average length of 1 second between tumbles.



- Run lengths are increased when moving up the gradient, and decreased when moving down.

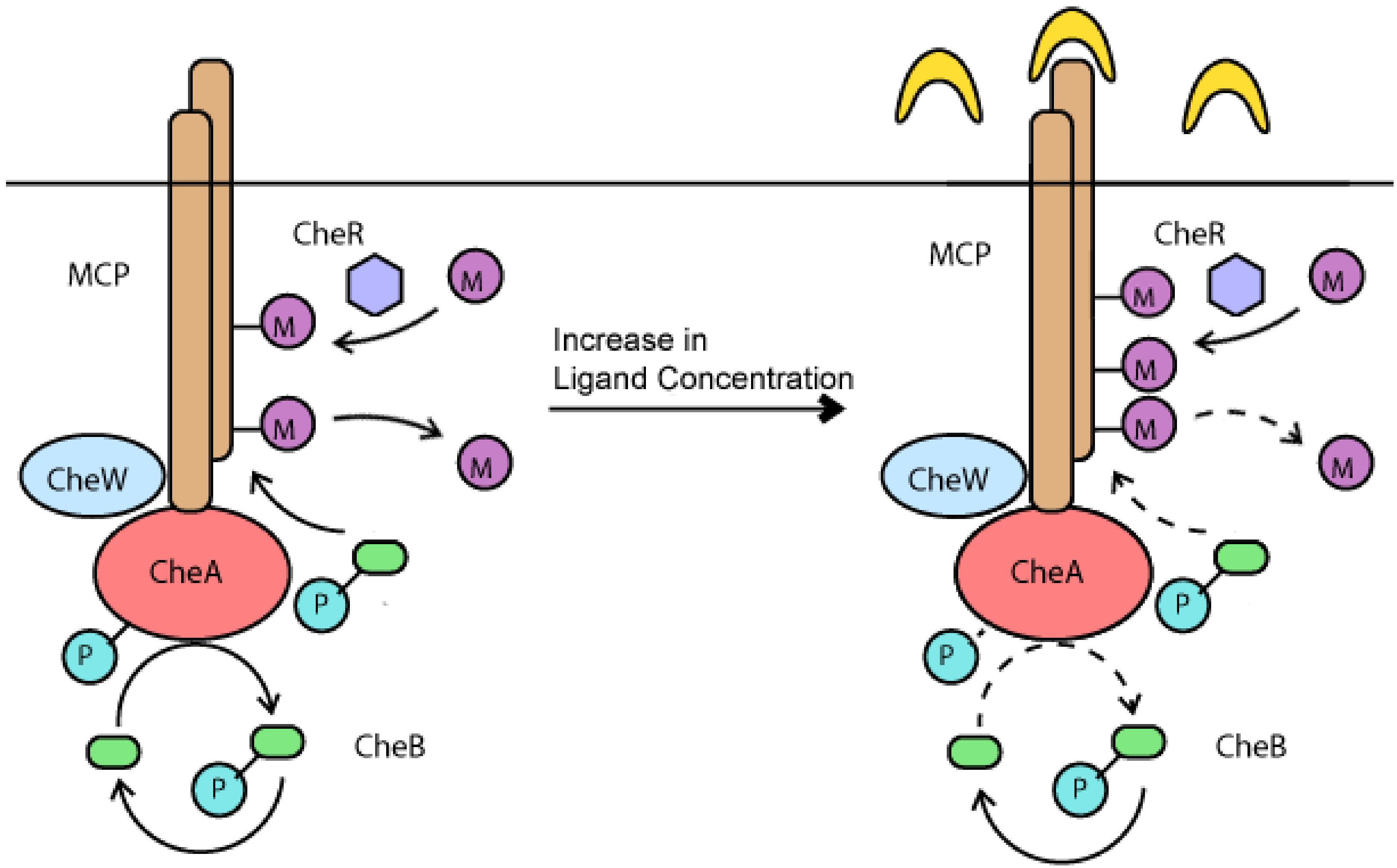
Adapted from MIT OCW: Course 7.81/8.591/9.531 – A. van Oudenaarden. (Oct 2004)

Flagellar Motion is Controlled by Fast Reactions Involving CheY



Adapted from Nature. 5, 1024-1037 (2004).

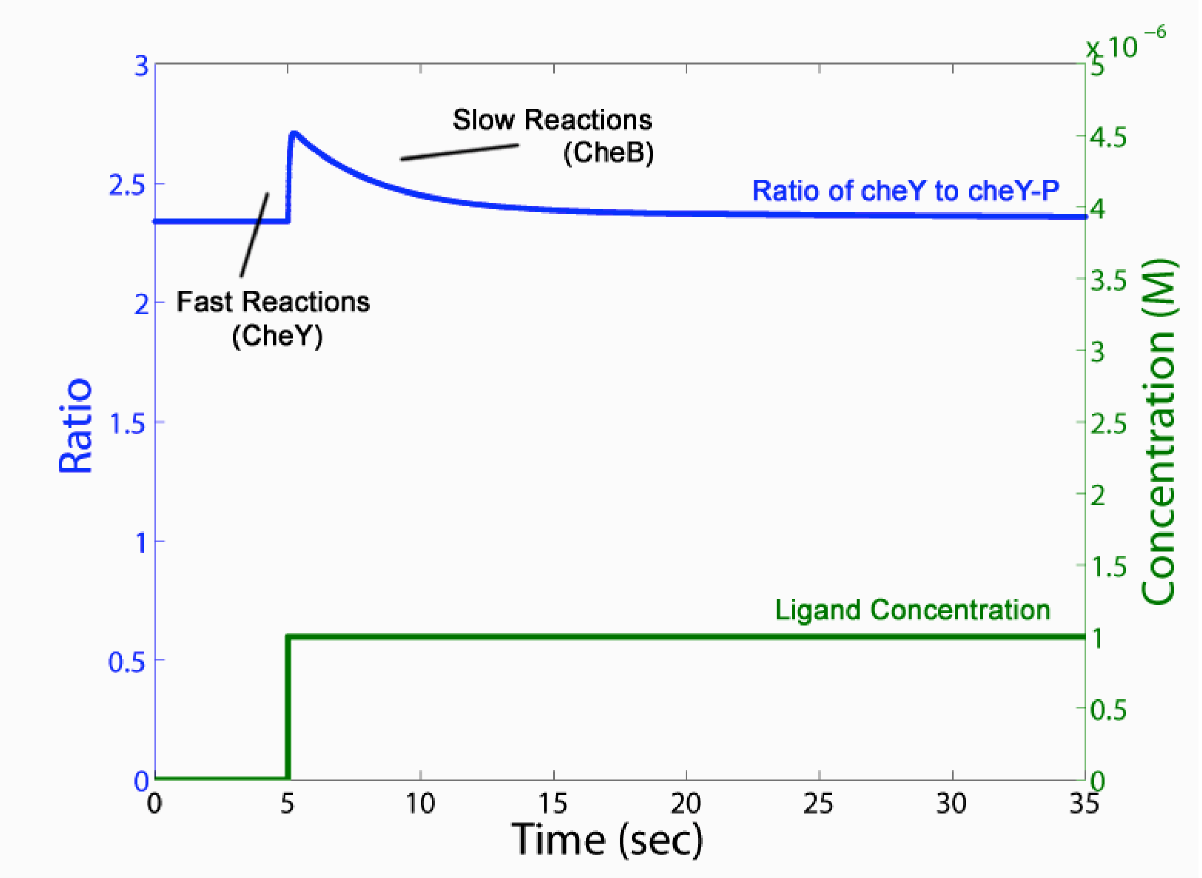
Adaptation is Facilitated by a Relatively Slow Chain of Reactions Involving CheB



Adapted from Nature. 5, 1024-1037 (2004).

Chemotaxis Can be Modeled by Combining the CheY and CheB Reaction Sequences

- Our model uses 17 differential equations and takes into account over 20 reactions.
- Reactions are modeled using the Law of Mass Action and Michaelis-Menten enzyme kinetics.



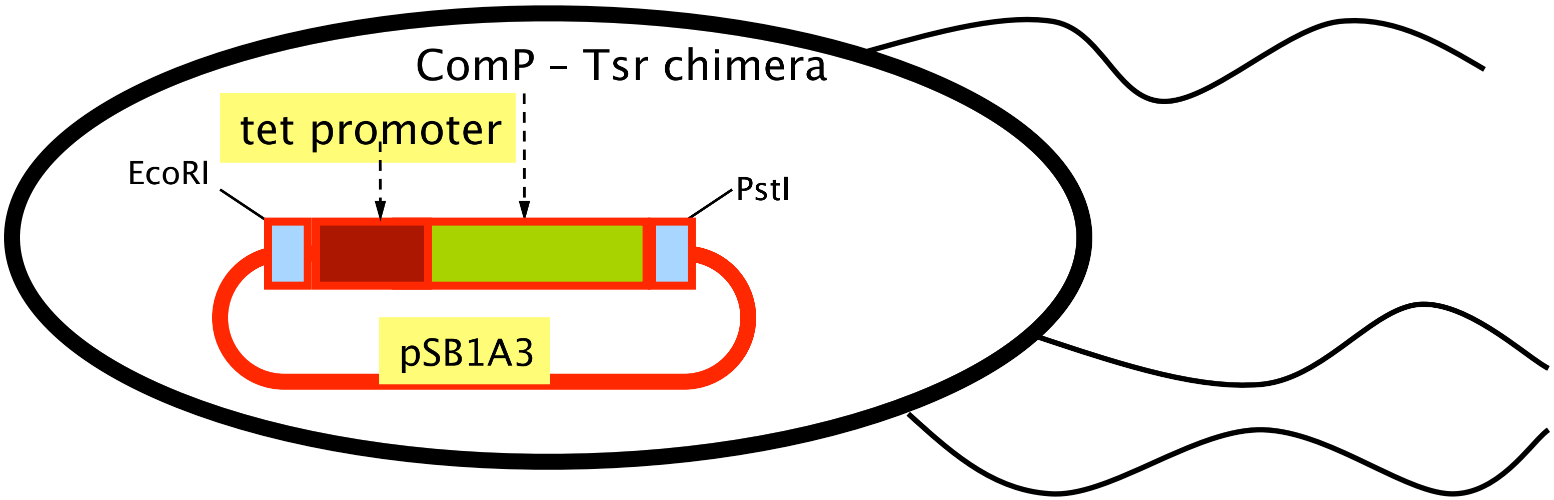
We Will Use the Model to…

- Identify ways in which our chimeric receptors differ from natural MCPs based on experimental data.
- Determine which of the working chimeras best suits our project goals.
- Characterize the specifics of our chimeras in order to make them easier to understand and use.

Outline of Experimental Strategy

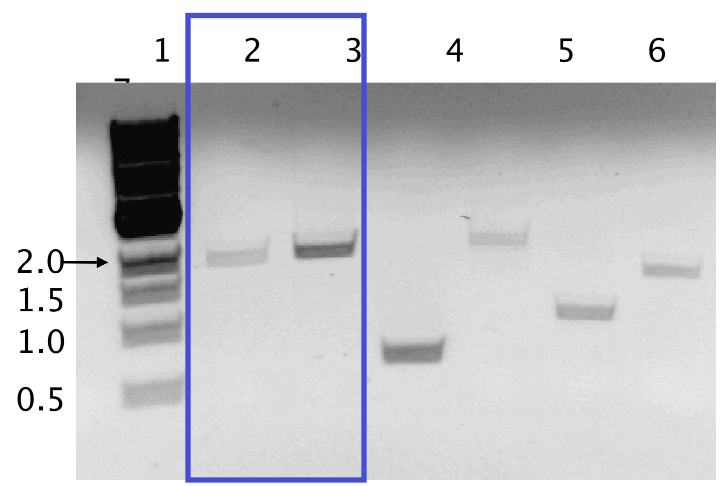
1. Amplify genetic components.
2. Create ComP/Tsr chimera library and build quorumtaxis circuit.
3. Screen for functional quorumtaxis circuit.

Step 1: Amplify BioBricks



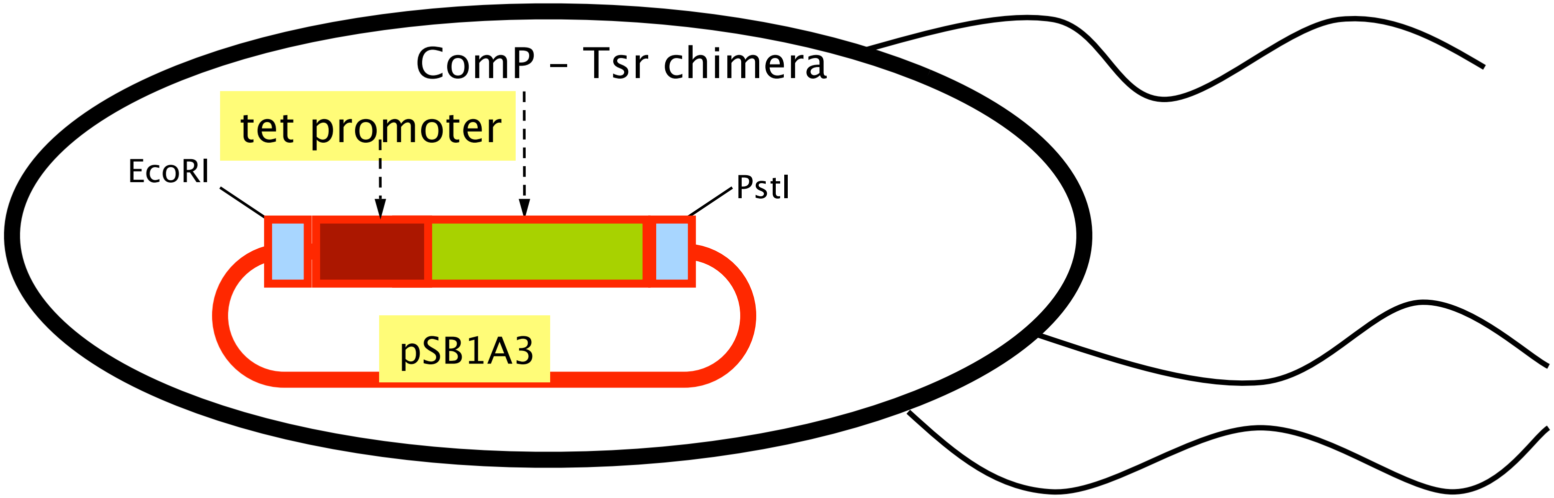
Lane	Sample	Size
1	1 kb std	--
2	pSB1A3 plasmid	2157 bp
3	pTetRBS + pSB1A3	2231 bp
4	ComA	650 bp
5	ComP	2400 bp
6	ComQX	1100 bp
7	Tsr	1600 bp

1% agarose gel

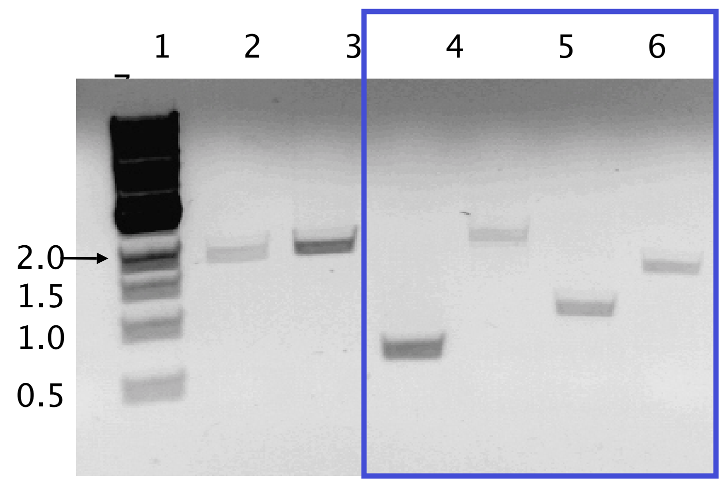


Step 2: Create Com and Tsr BioBricks

(from *B. subtilis* 168 and *E. coli*)



1% agarose gel



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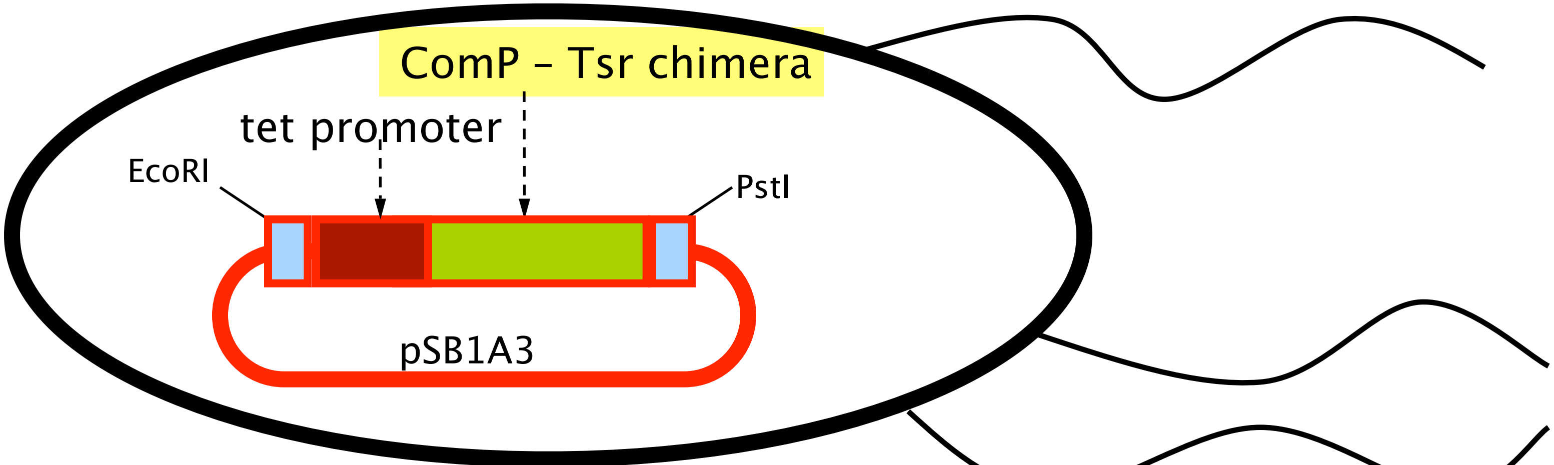
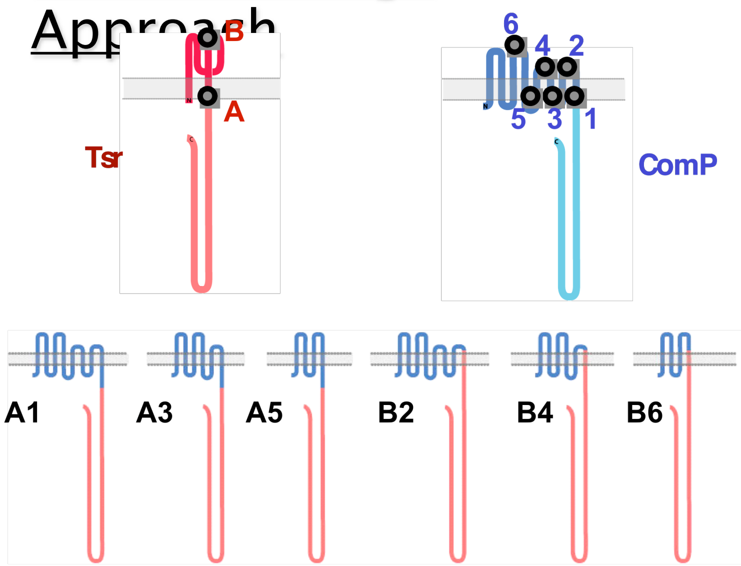
4	ComA	650 bp
5	ComP	2400 bp
6	ComQX	1100 bp
7	Tsr	1600 bp

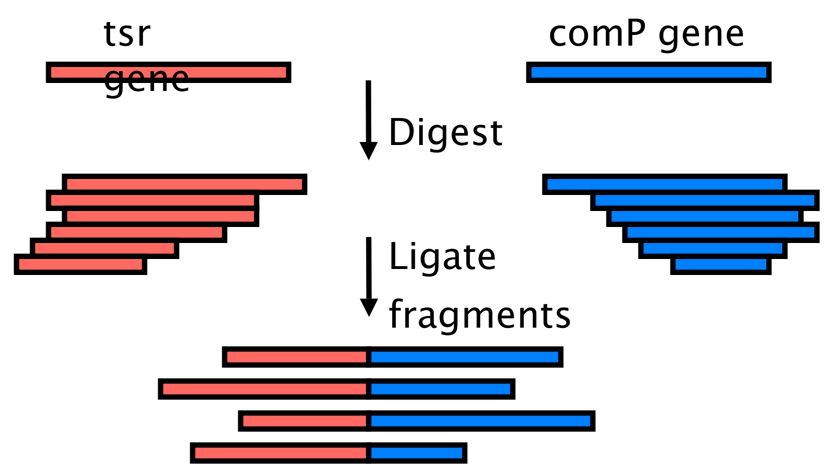
Step 3: Create ComP-Tsr Chimeras



Rational Design Approach



Combinatorial Library

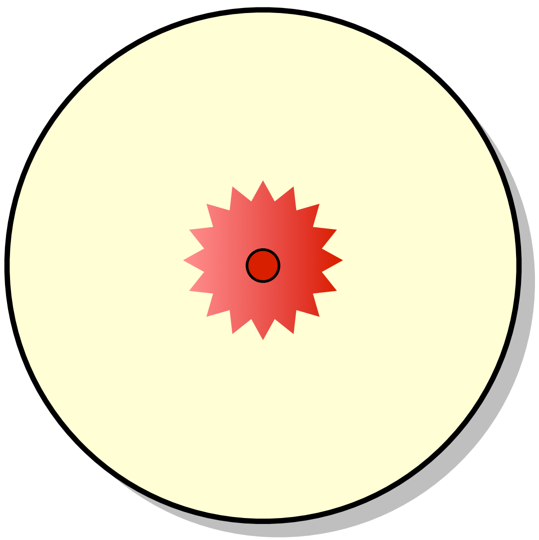


Adapted from Nature Biotechnology (1999) 17: 1159

Step 4: Test Δ*mcp E. coli* Swarming

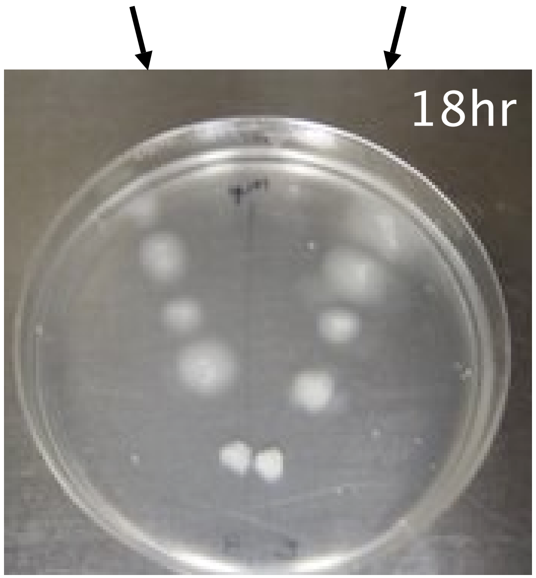
(strain that will be used to screen chimera function)

Swarming assay

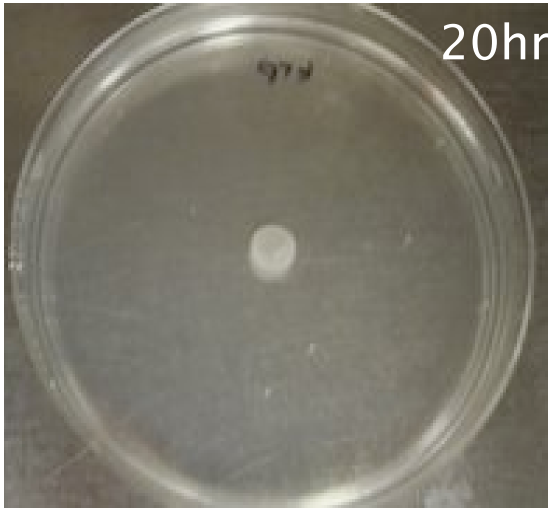


Spot cells on soft agar plate and assay outward growth.

B. subtilis & E. coli



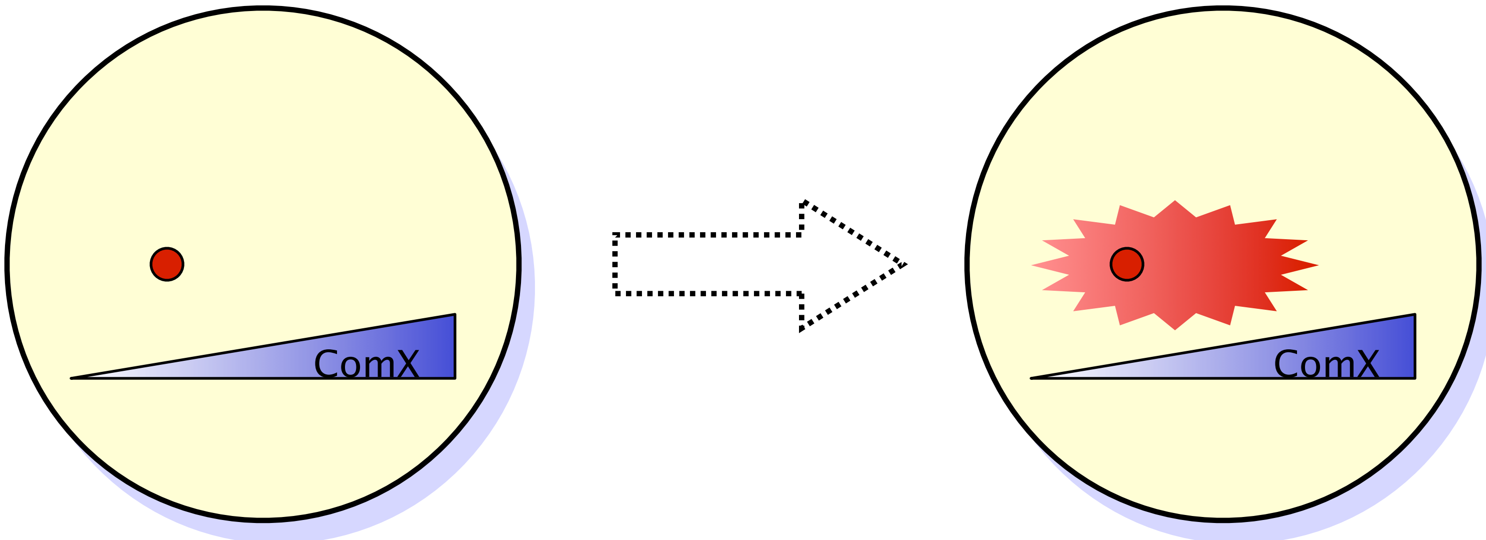
E. coli strain RP8611 (Δmcp)*



* acquired from Prof. John S. Parkinson, University of Utah

Step 5: Screen for Functional Chimeras

Quorumtaxis



Spot Δmcp E. coli on soft agar plate containing B. subtilis culture extract (i.e., ComX)

Expected swarming pattern

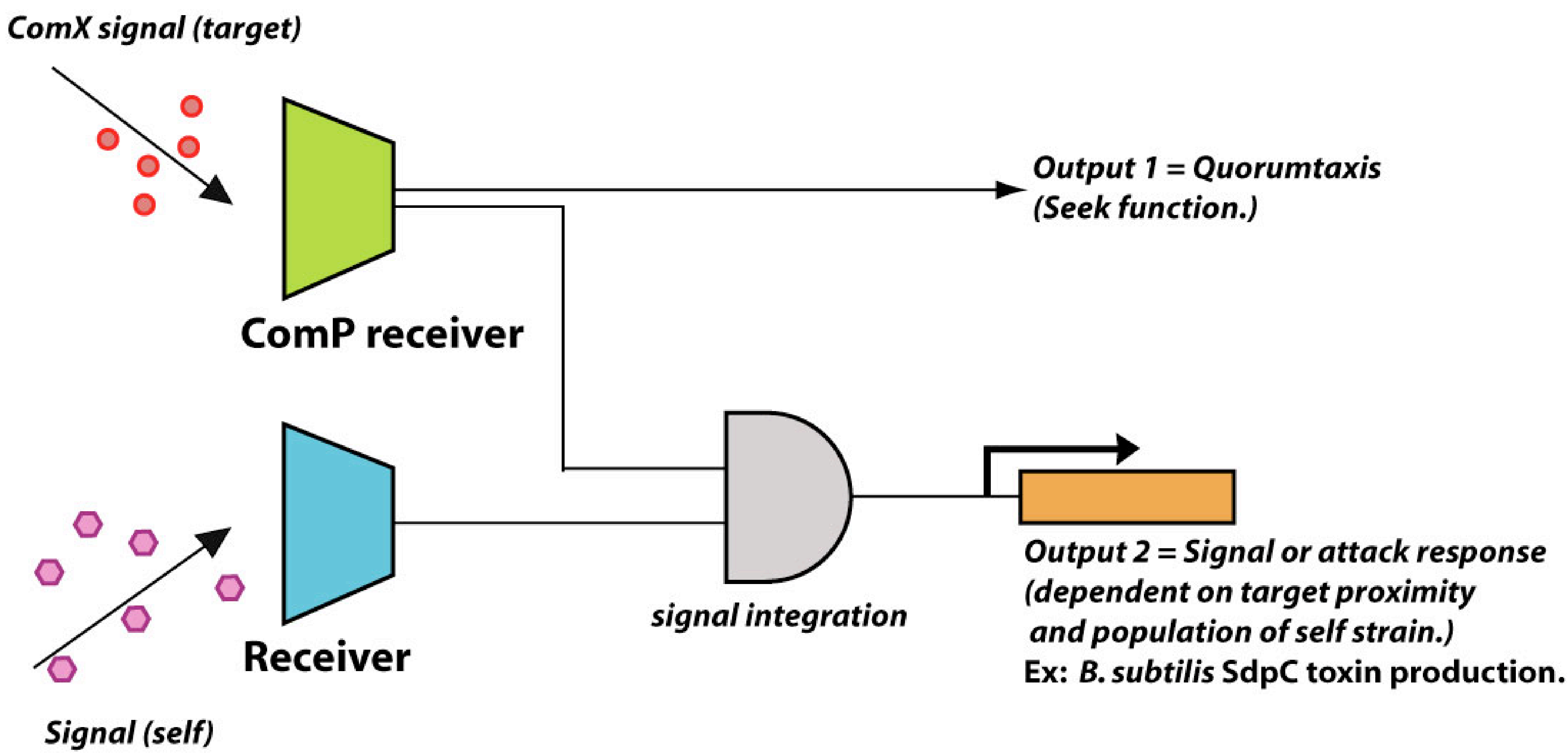
Progress and Future Work

1. Amplify genetic components.

2. Create ComP/Tsr chimera library and build quorumtaxis circuit.

3. Screen for functional quorumtaxis circuit.

Phase II: Develop 'Destroy' Output



BIOCHEMISTRY AND CELL BIOLOGY

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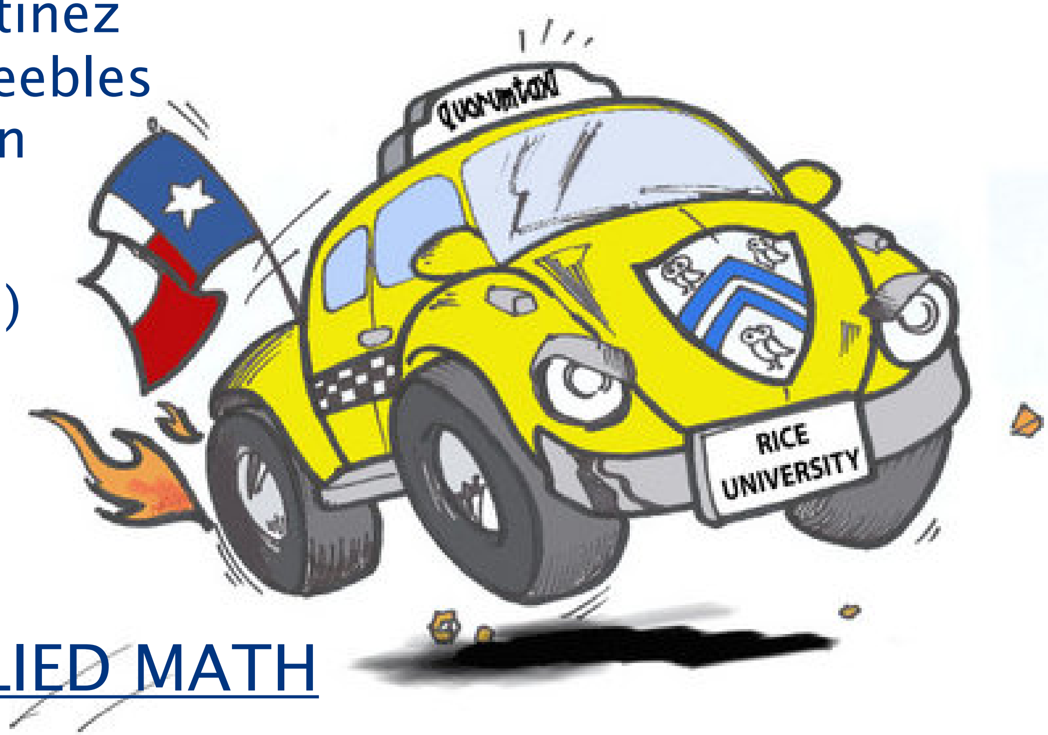
Ka-Yiu San

The Rice Taxis Drivers



COMPUTATION AND APPLIED MATH

Steve Cox
Jay Raol
Thomas Segall-Shapiro

CAIN PROJECT

Elizabeth McCormack

CHEMICAL AND BIOMOLECULAR ENGINEERING

Ken Cox
Dario Prieto
Miinkay Yu

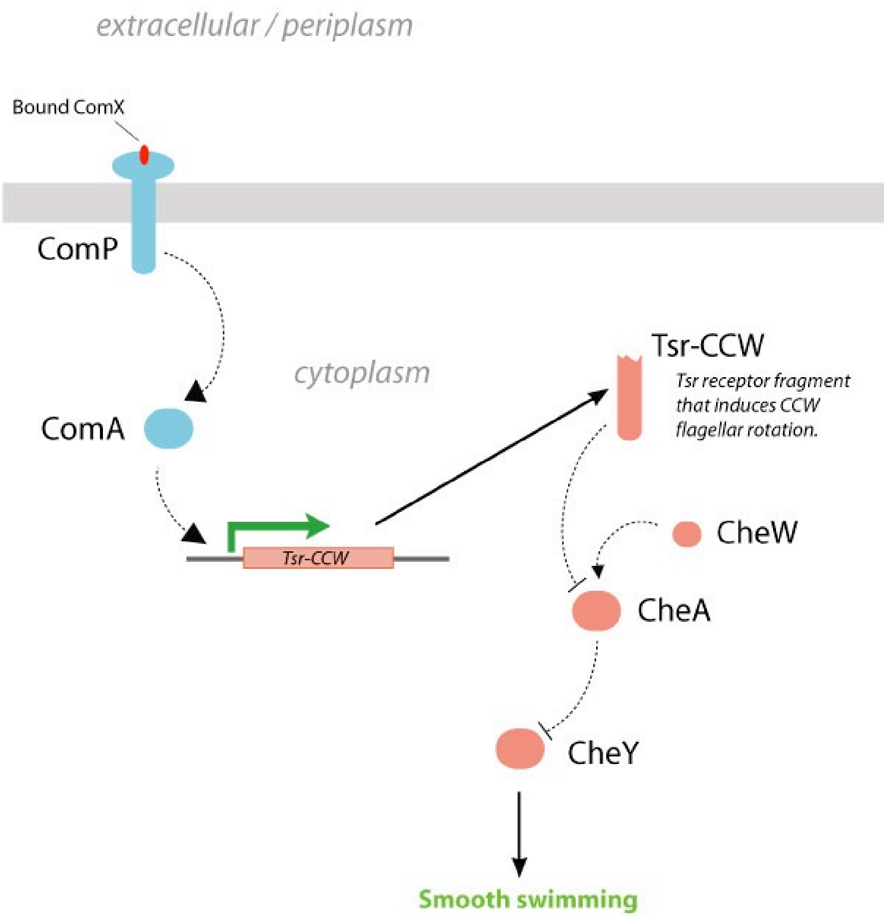
iGEM

Andrew Hessel

Extra Slides

Alternative Strategy

Transcriptional Control of Chemotaxis



- Engineer E. coli cell to express ComP and ComA components of the B. subtilis quorum sensing pathway.
- Reception of ComX by ComP
- Activation of ComA, which induces transcription of a Tsr-CCW fragment leading to a smooth swimming phenotype