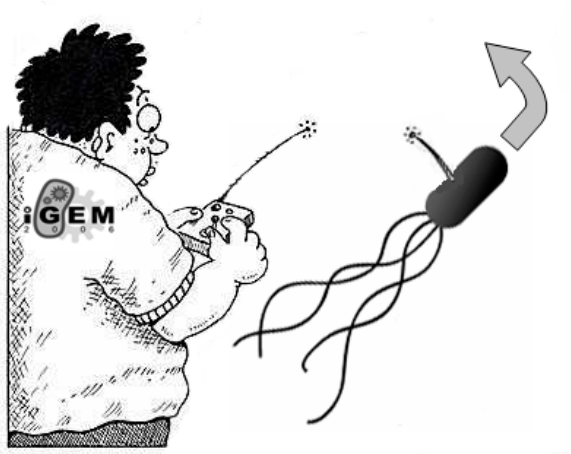
Remote Control of Bacterial Chemotaxis

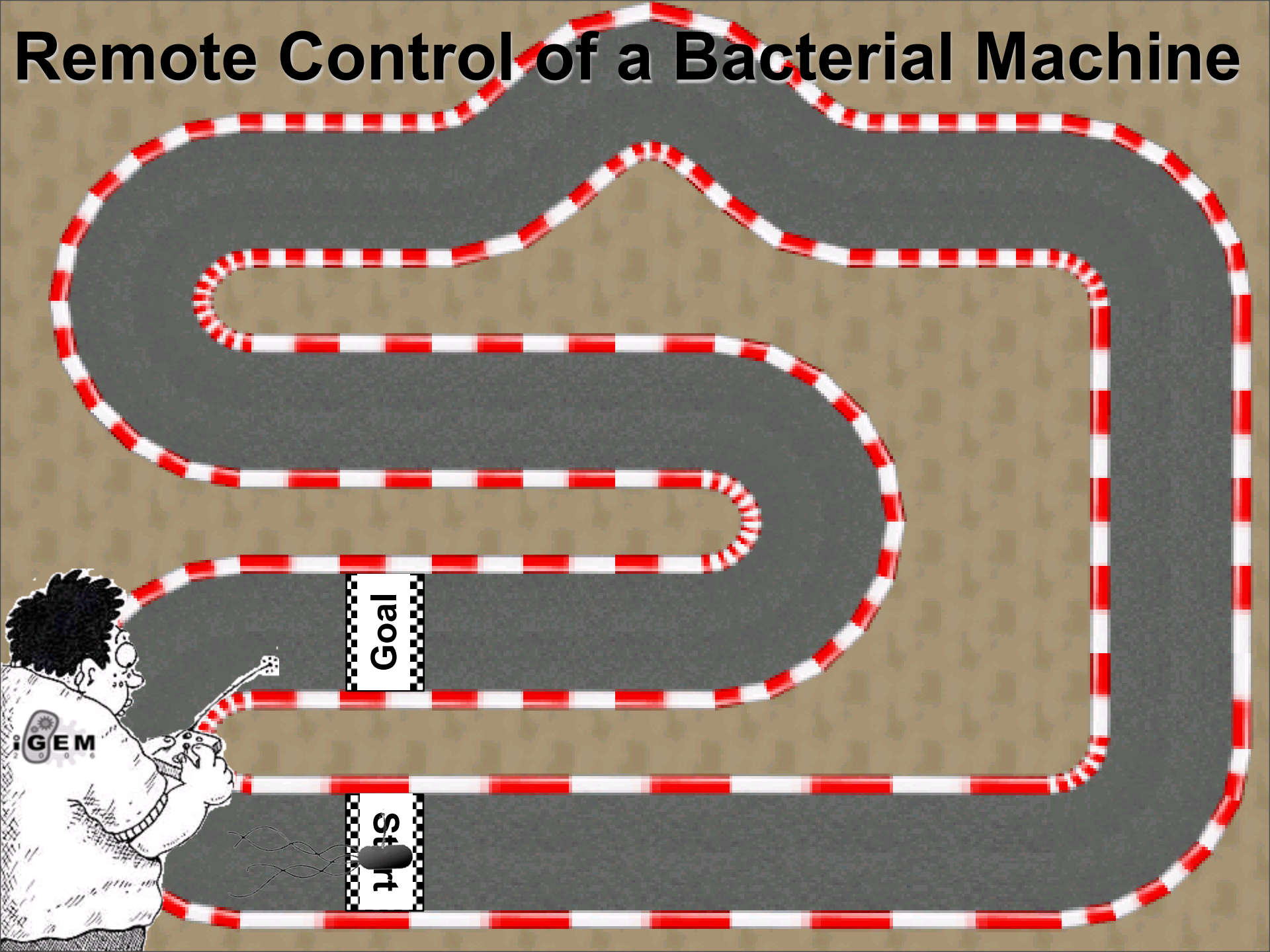
UCSF iGEM Team 2006



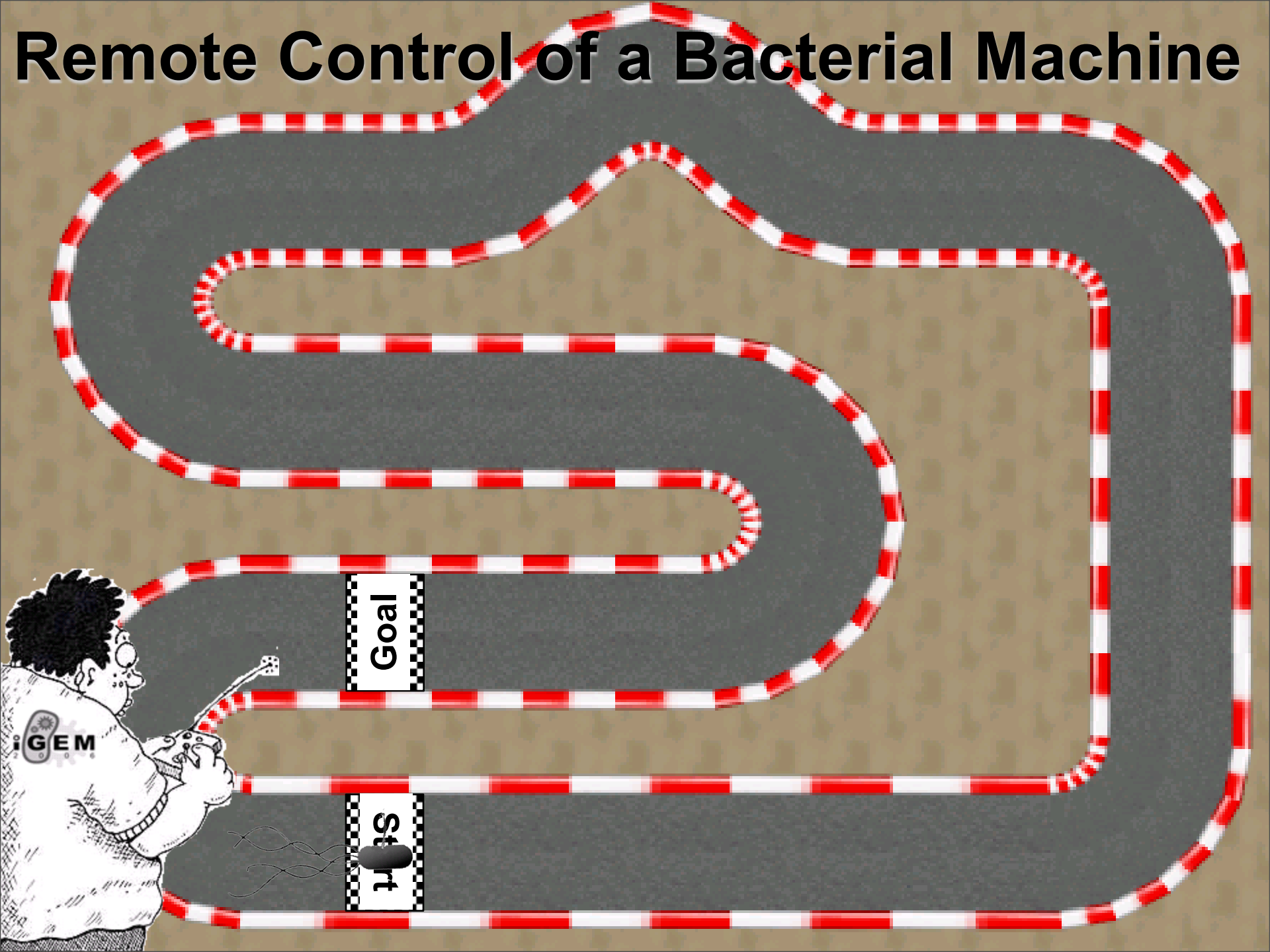
Patrick Visperas
Matthew Eames
Eli Groban
Ala Trusina

Christopher Voigt, Tanja Kortemme, Chao Tang, Chris Rao

Remote Control of a Bacterial Machine

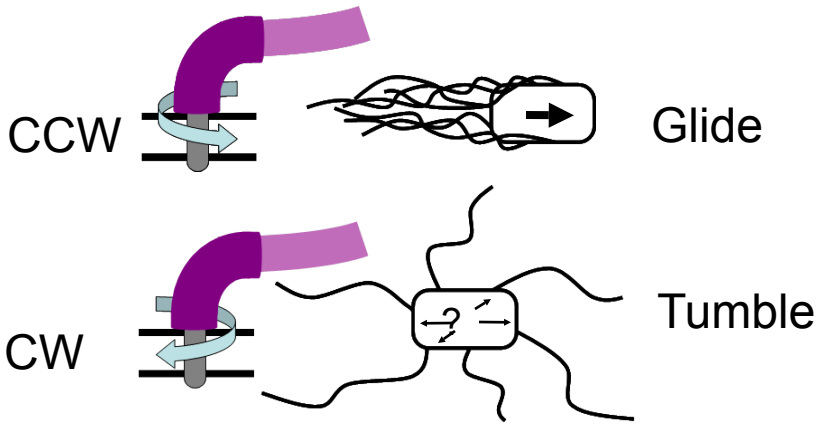


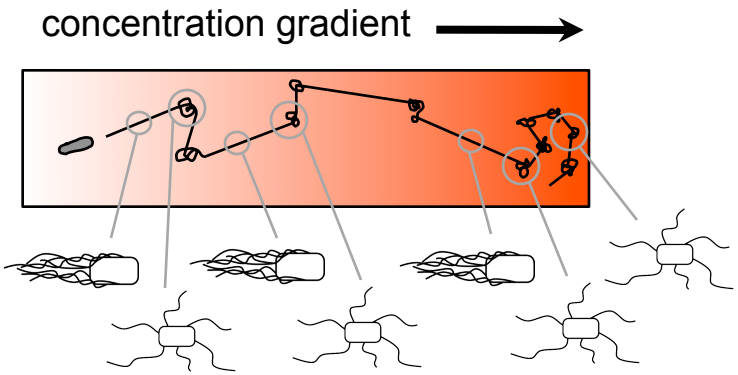
Remote Control of a Bacterial Machine

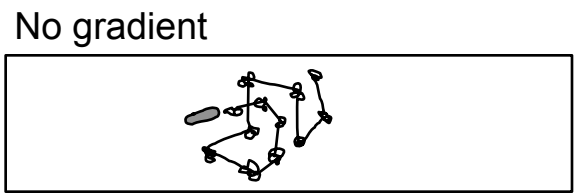


How Bacterial Chemotaxis Works

Bacteria Swim Up Gradients







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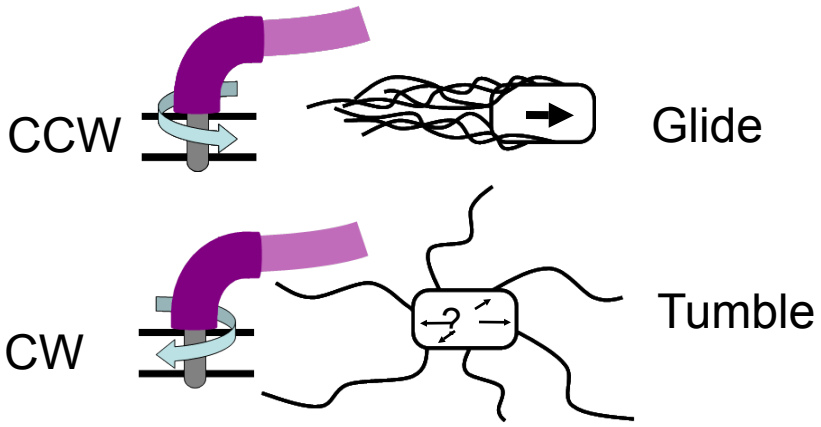
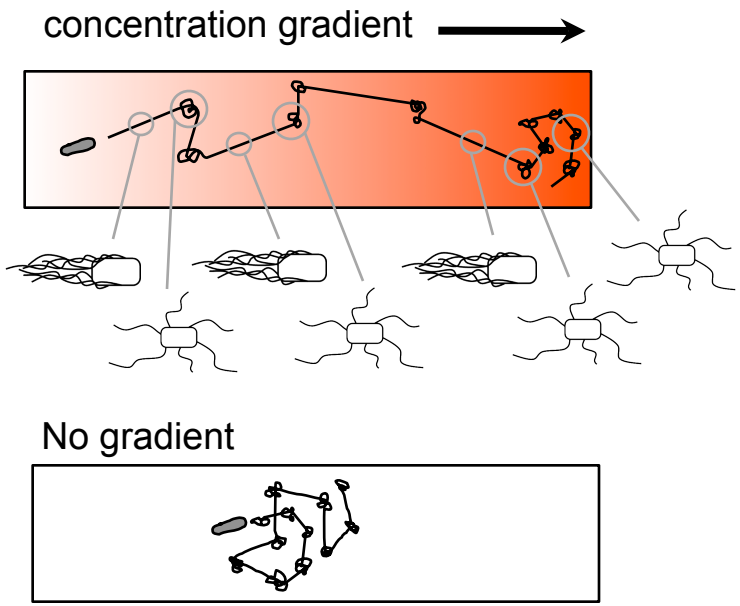
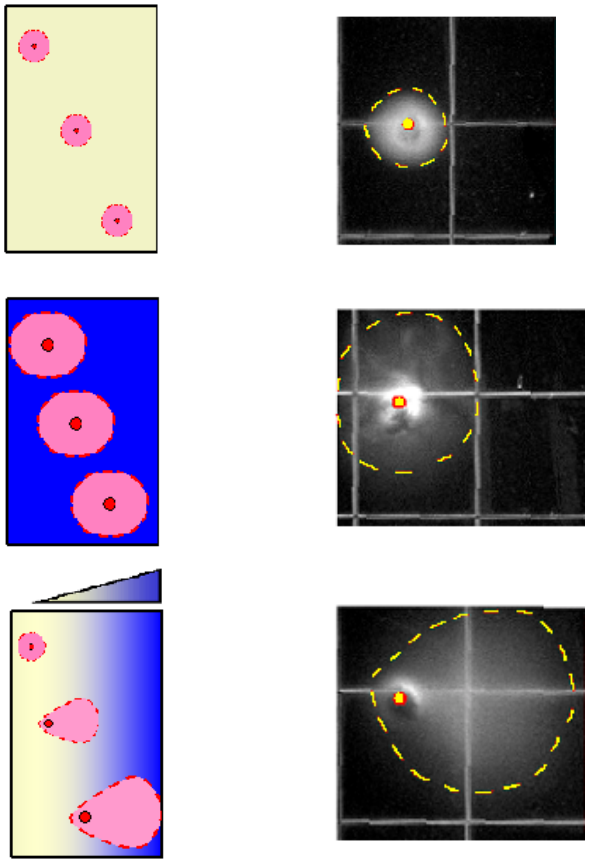


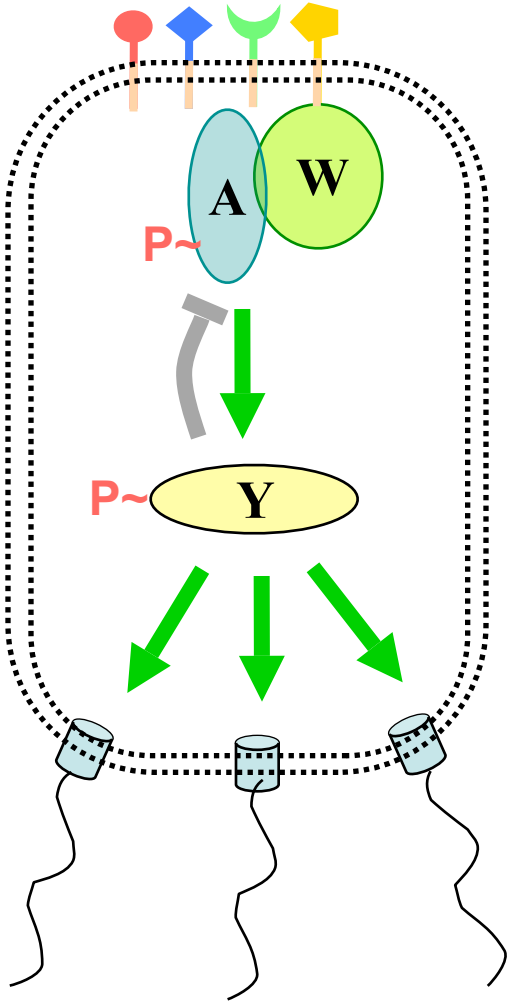


How Chemotaxis is Observed

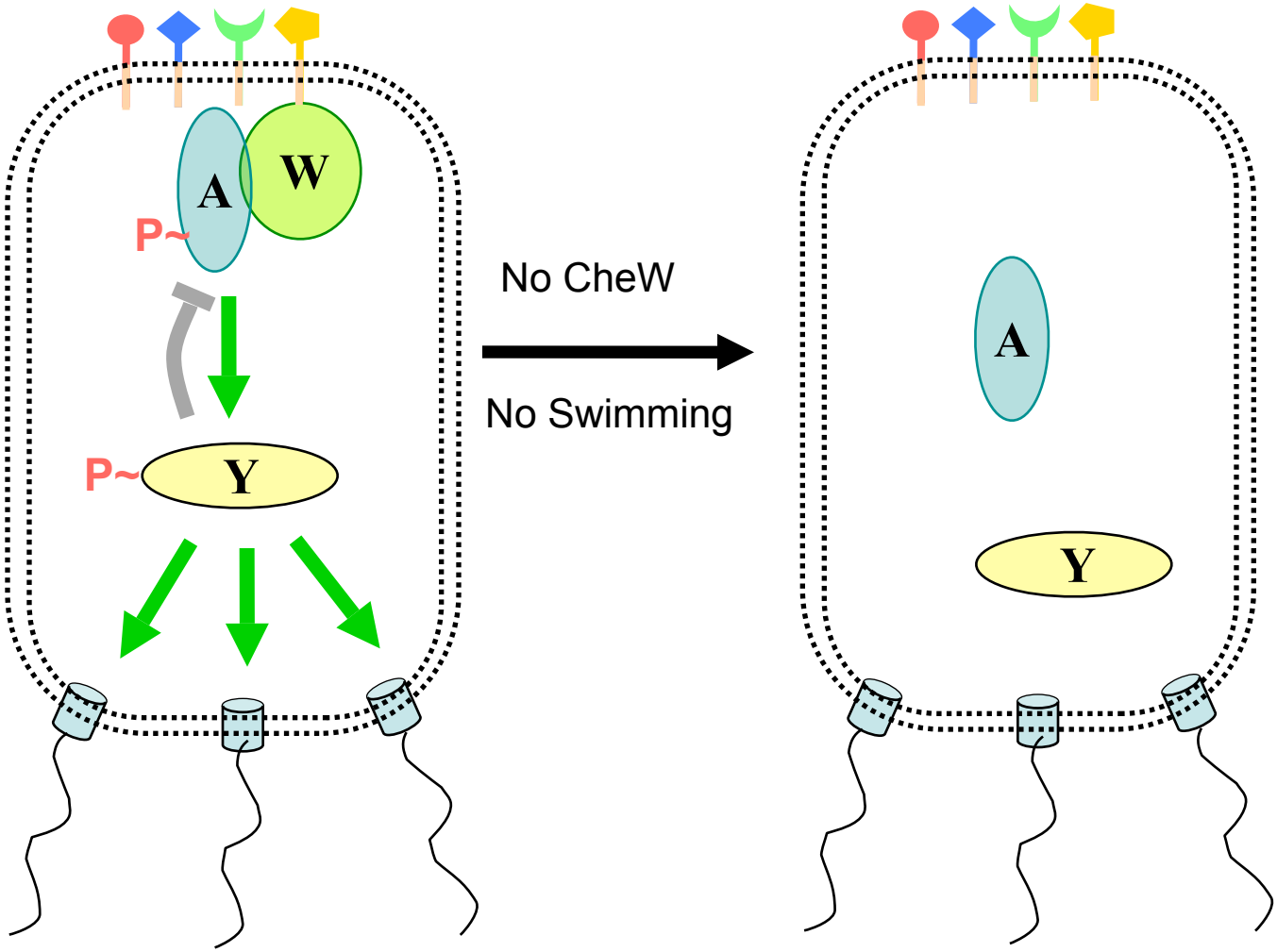
Goulian Motility Assay (U Penn)



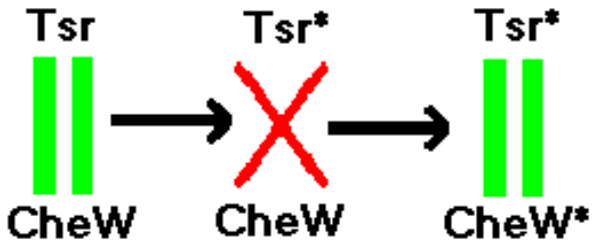
The Regulatory Network

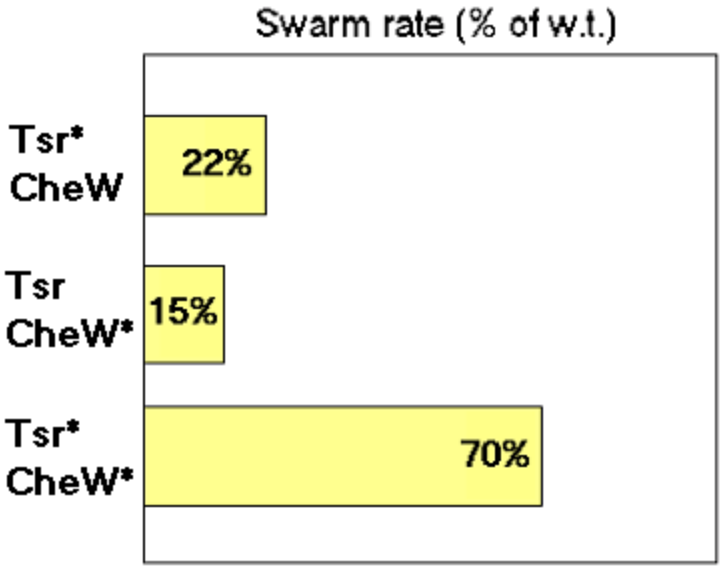


The Regulatory Network

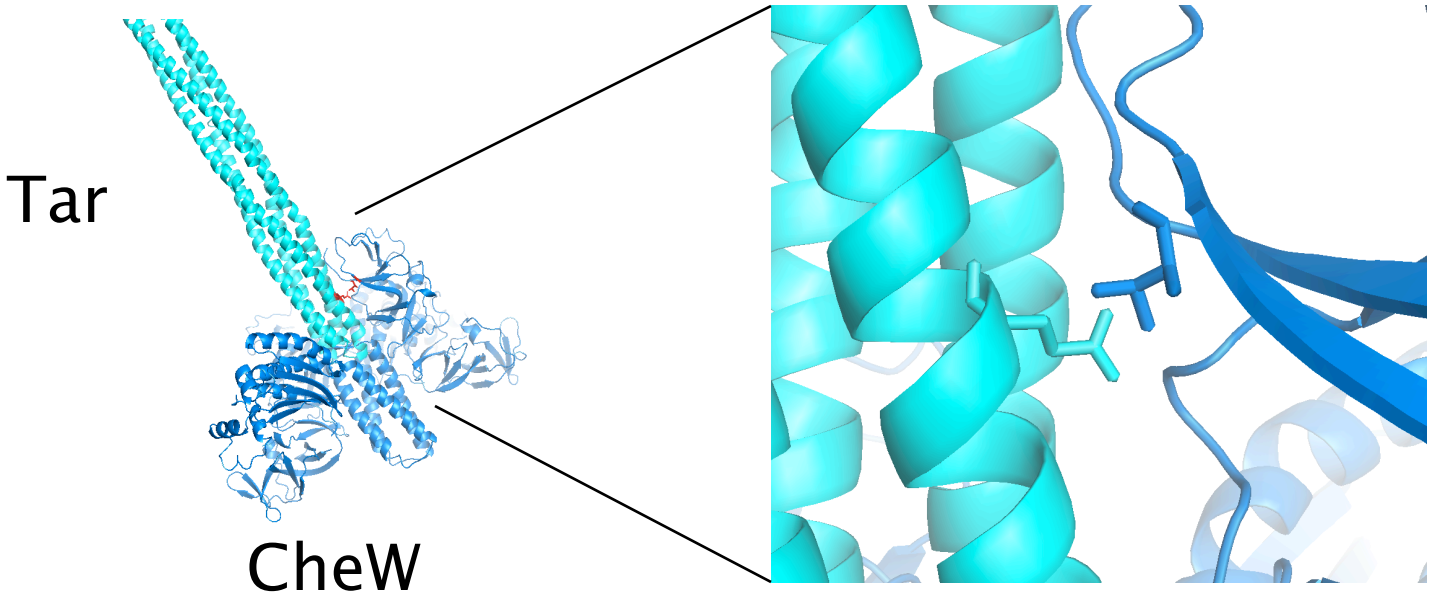


Binding Partners





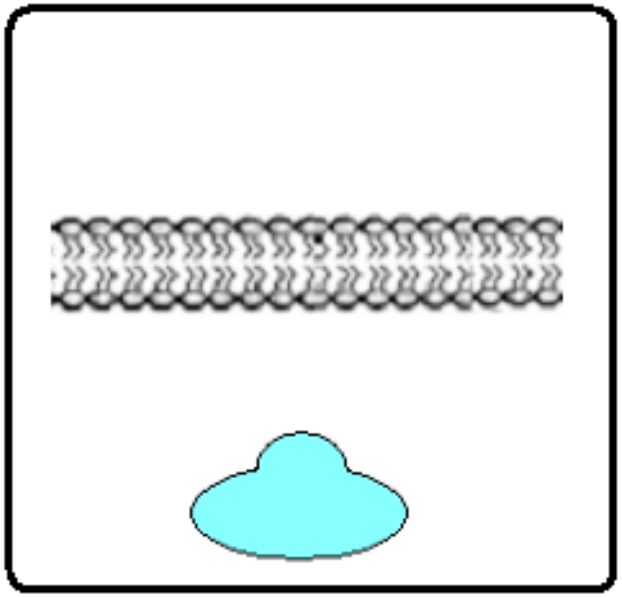
- Orthogonal binding pair Tsr–CheW (Liu et al, 1991)
- We mapped these mutations onto the Tar–Chew complex



Tsr mutation can be mapped to Tar

Gradient

Aspartate



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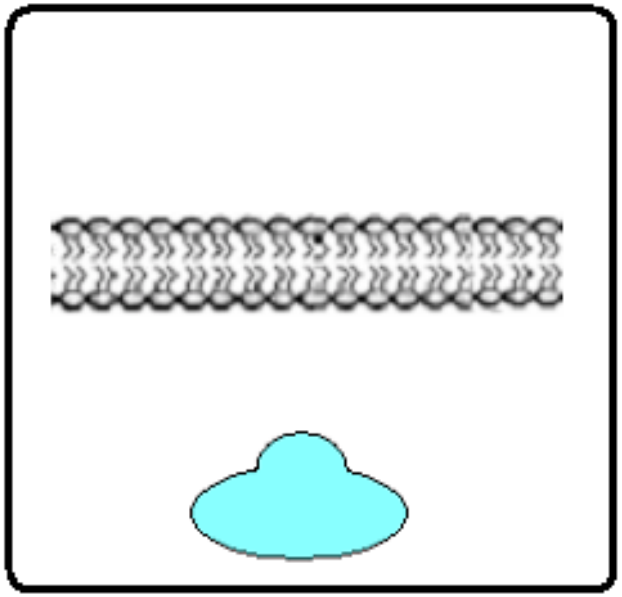
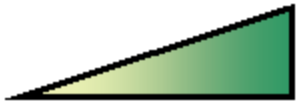
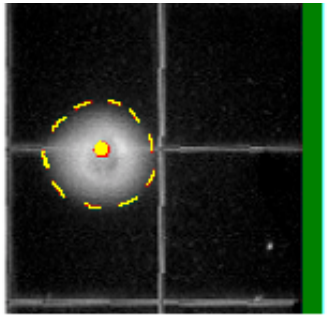




No Tar



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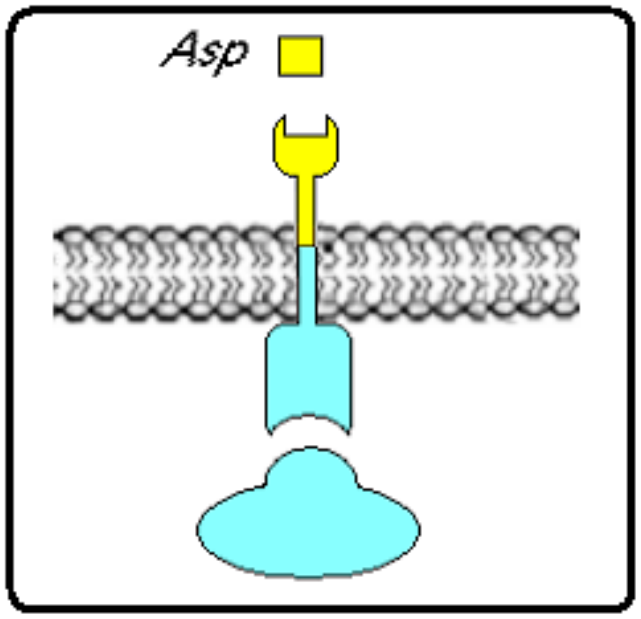

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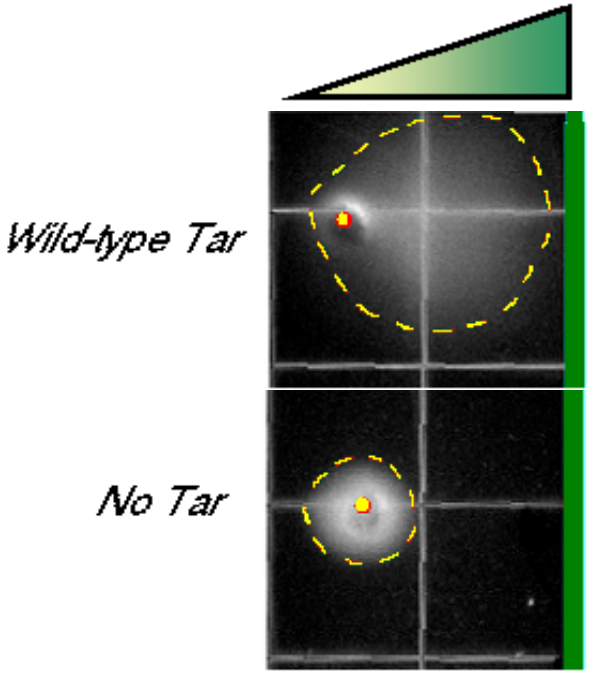

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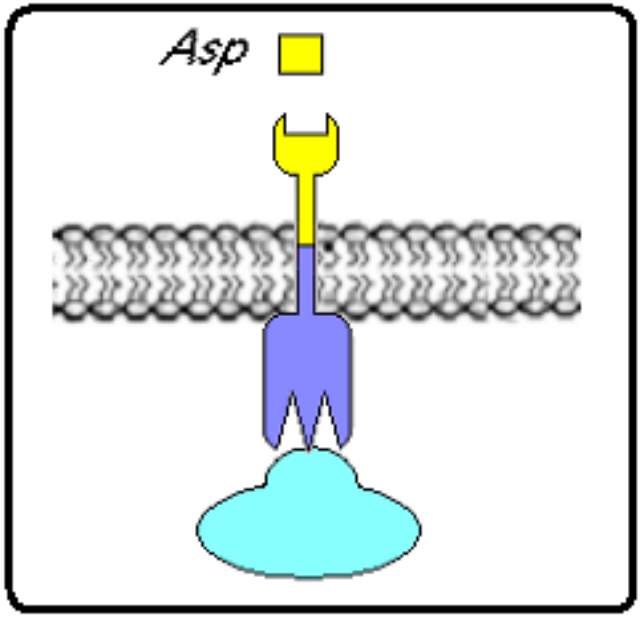

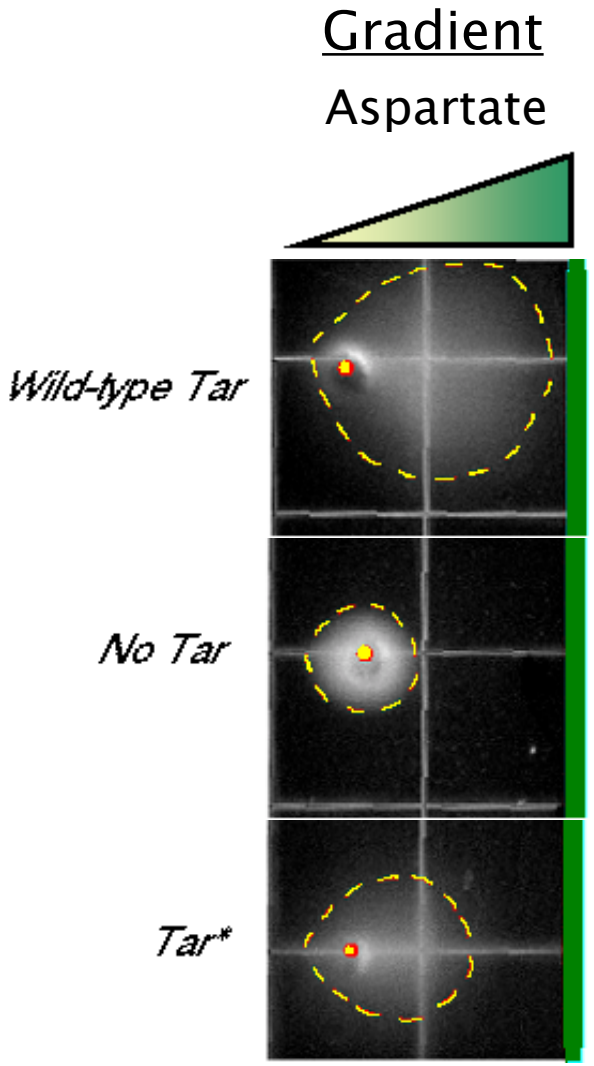

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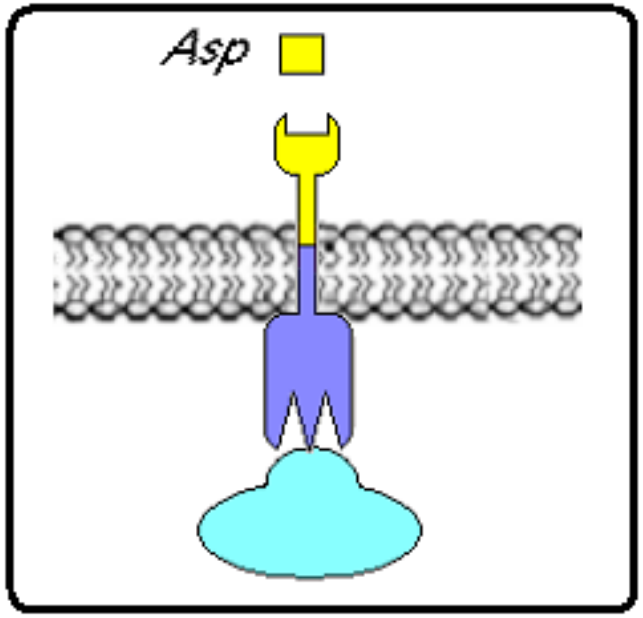
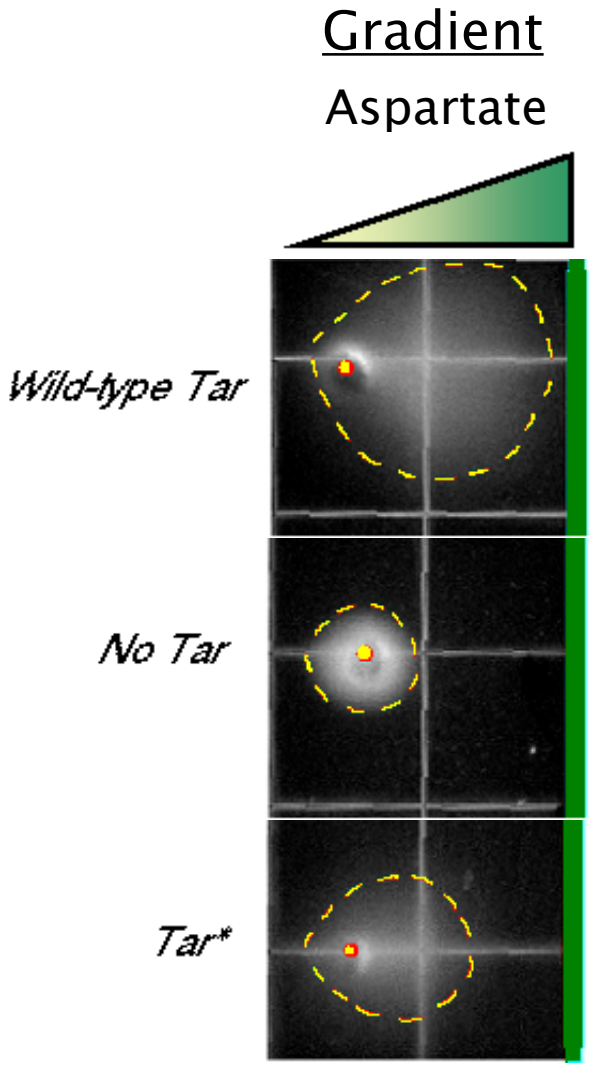
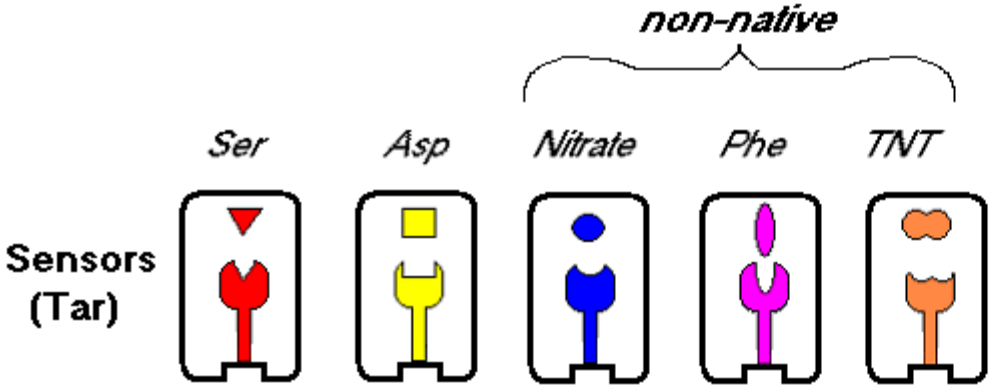


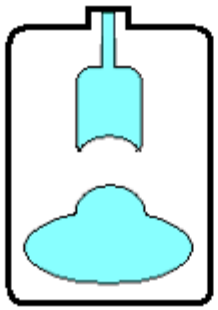


• point mutation in Tar* reduces motility to approximately 40% that of wild-type

Part Design

- New orthogonal signaling pairs

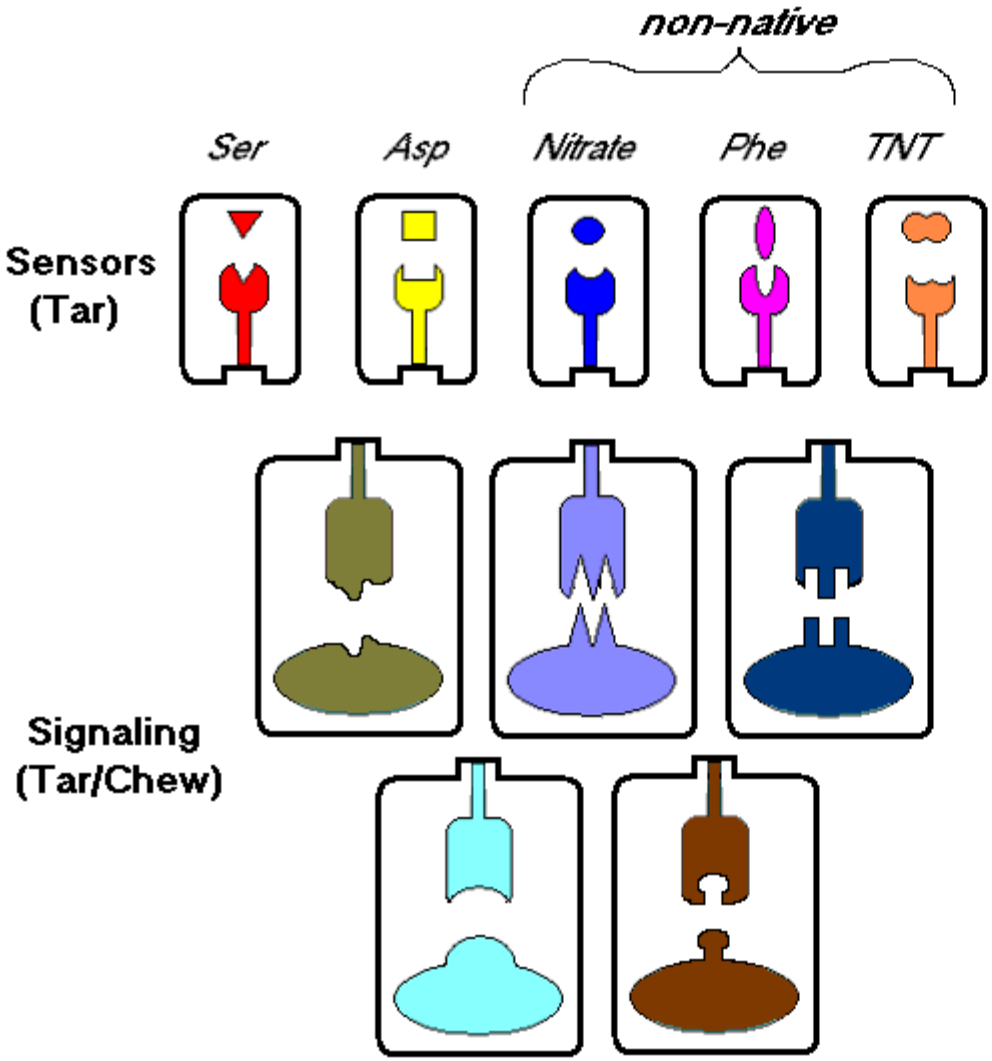


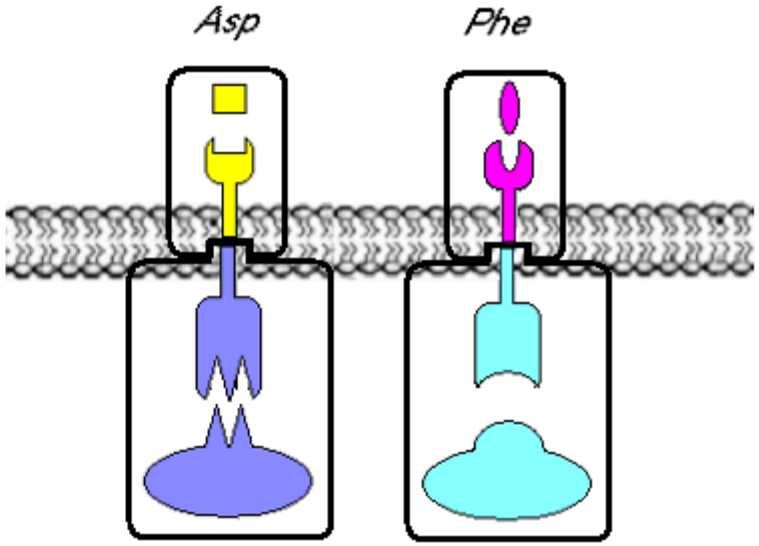


"Codon randomization" is used to reuse scaffolds without fear of recombination

Part Design

- New orthogonal signaling pairs

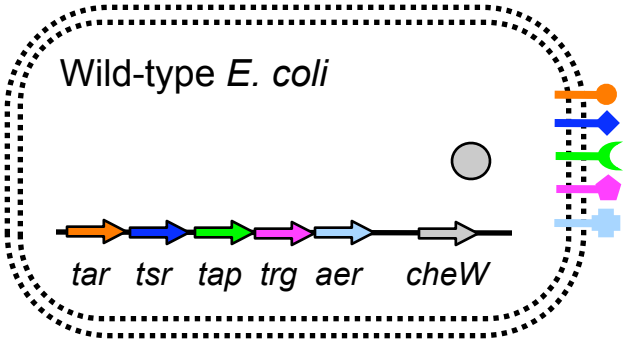




"Codon randomization" is used to reuse scaffolds without fear of recombination

Chassis Engineering

• Build *E. coli* strain lacking all sensors and CheW, but retaining the other chemotaxis proteins



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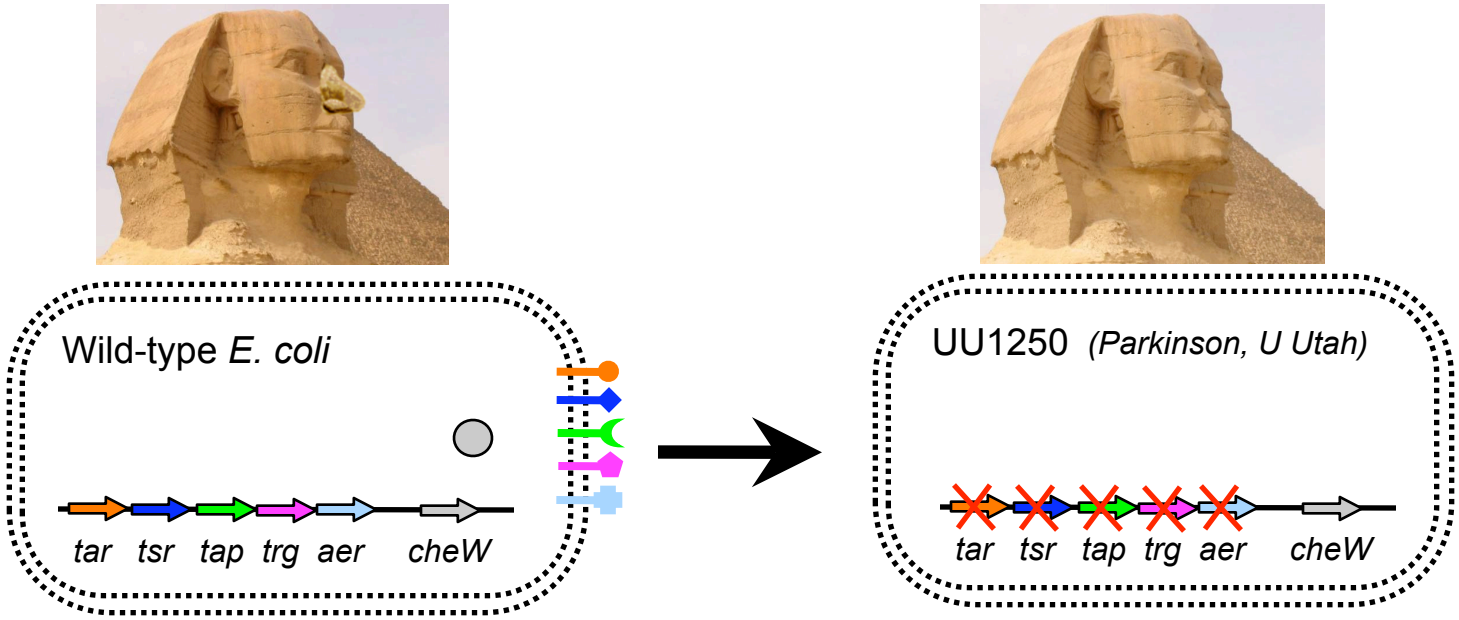

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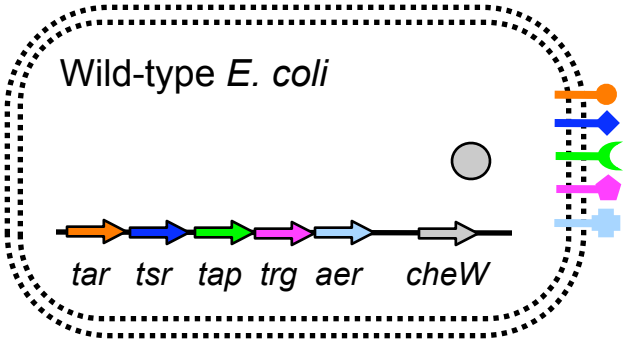
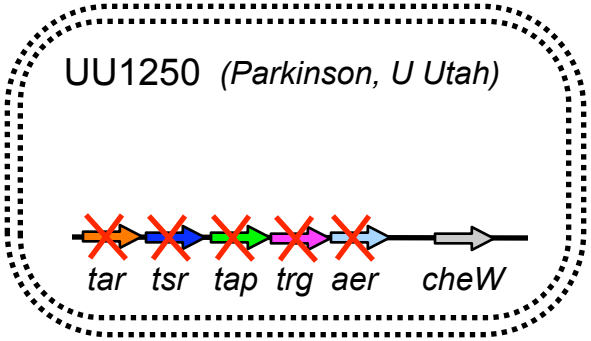
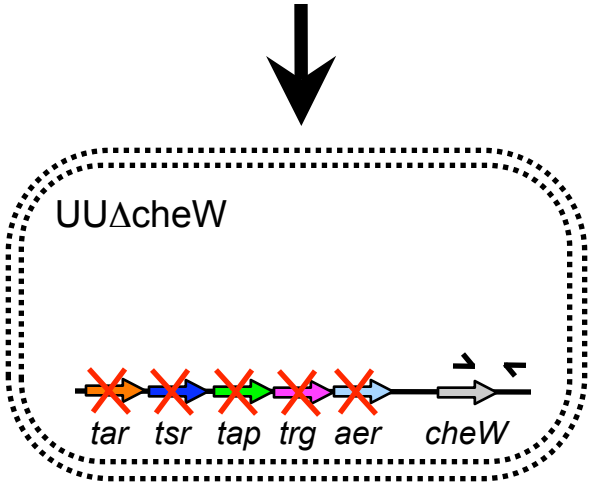
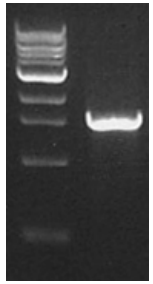








Chassis Engineering

• Build *E. coli* strain lacking all sensors and CheW, but retaining the other chemotaxis proteins

Wild-type *E. coli*

tar tsr tap trg aer cheW

UU1250 *(Parkinson, U Utah)*

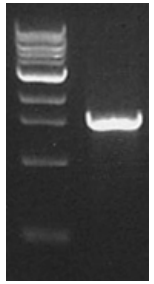
tar tsr tap trg aer cheW

UUΔcheW

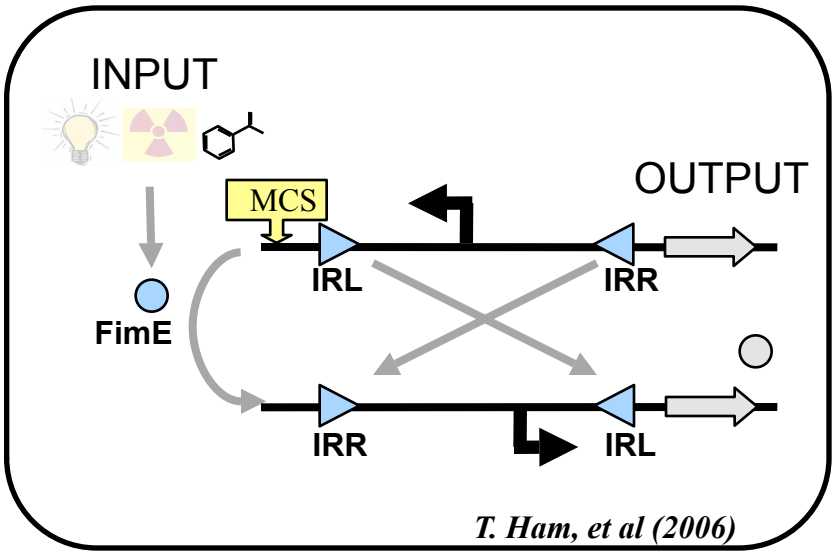
tar tsr tap trg aer cheW

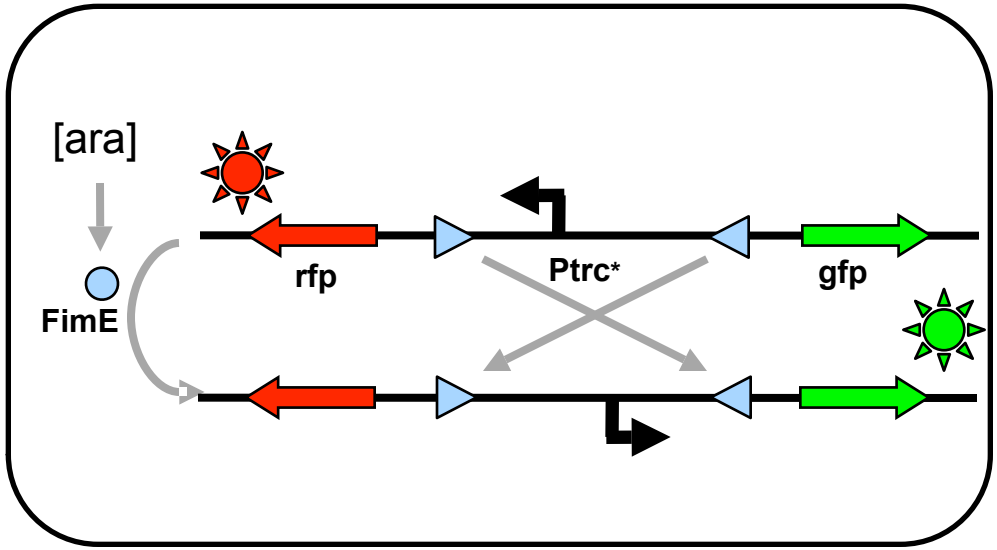
cheW*

tar*

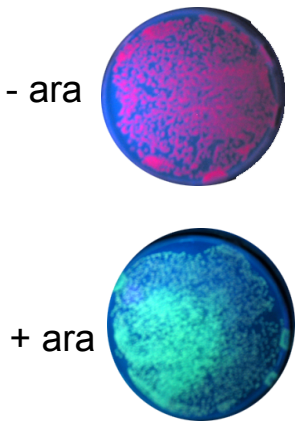


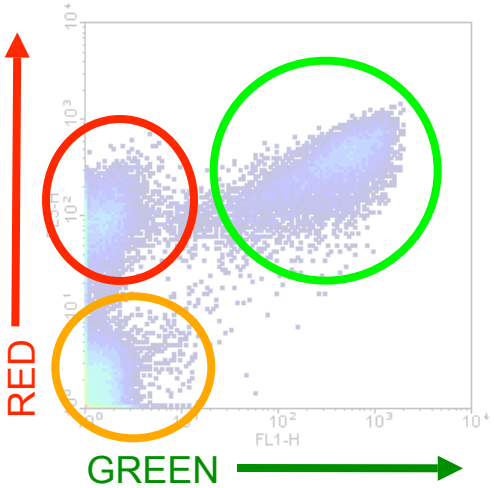
Device Characterization: The Fim-Switch

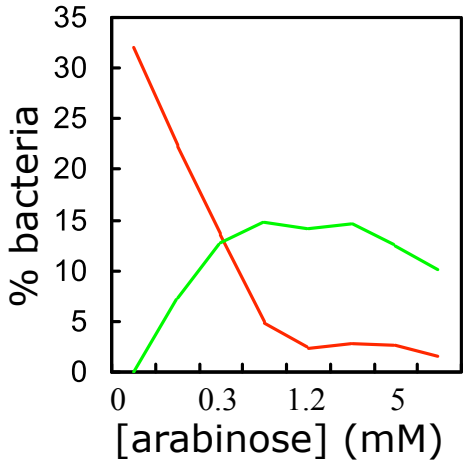




Tested in UU1250



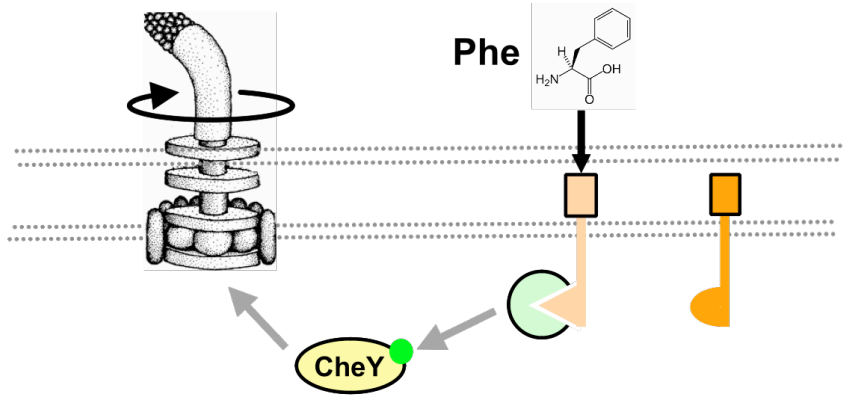


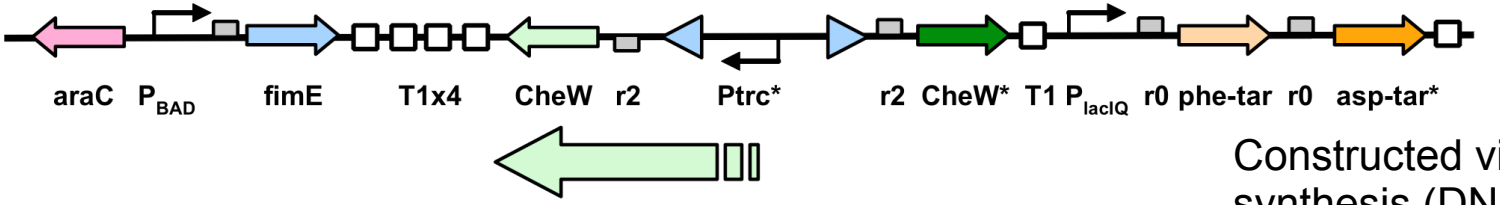


2 Possible Problems

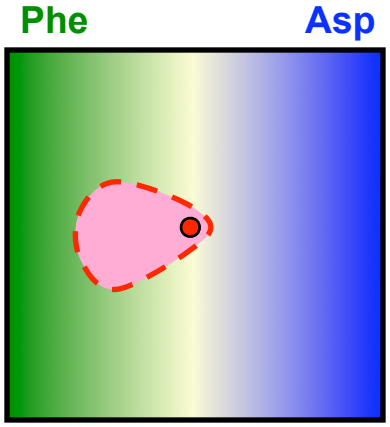
1. The plasmid is unstable
2. The strain contains FimE/ FimB

System Design (11 kB)



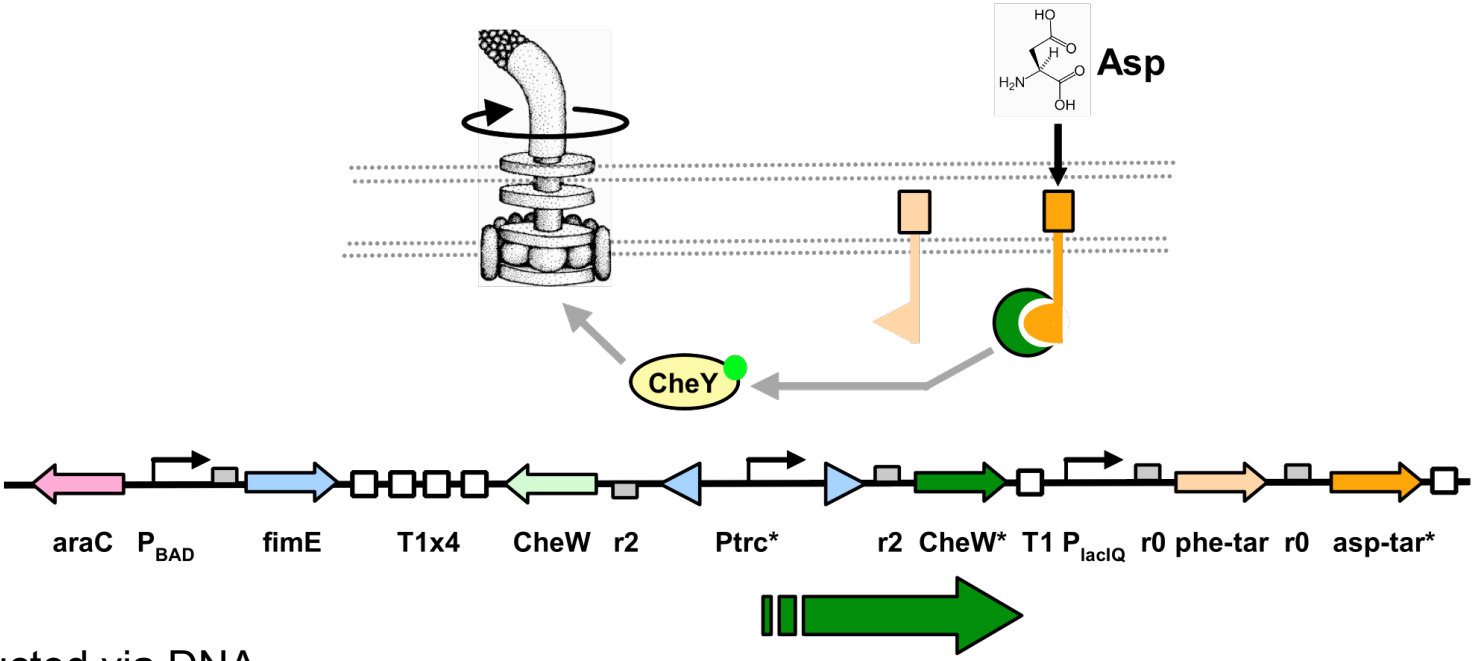


Constructed via DNA synthesis (DNA 2.0)



- arabinose

System Design (11 kB)



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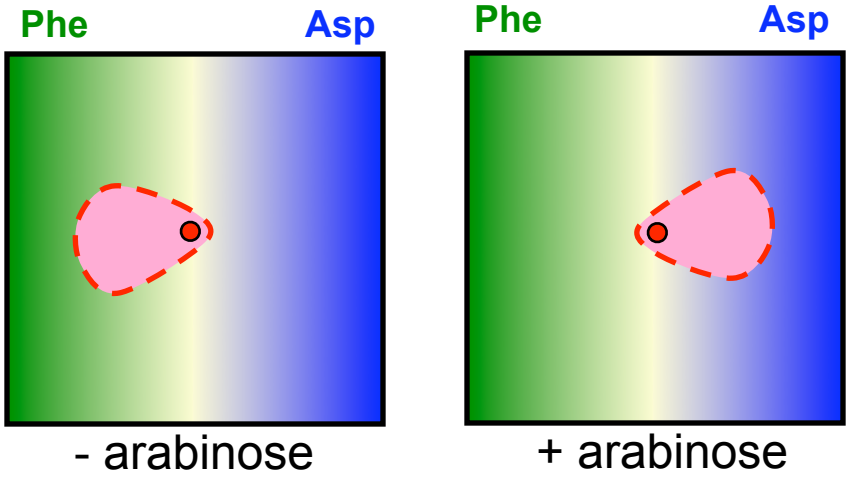
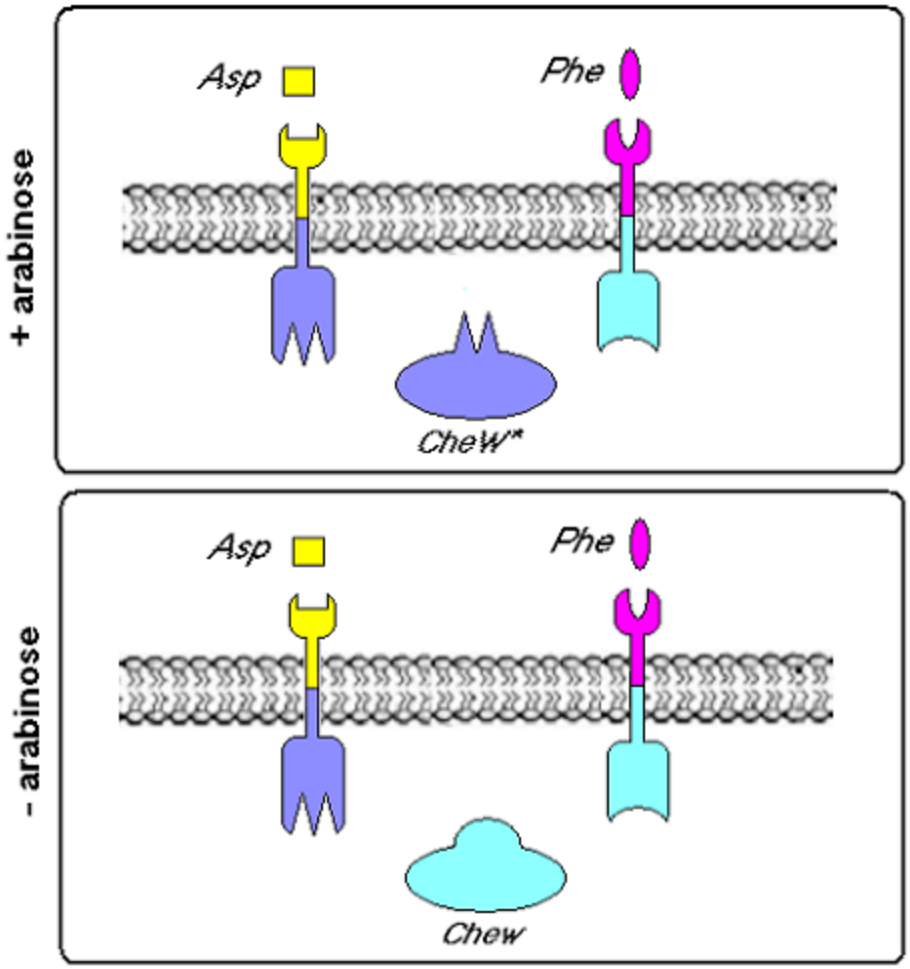
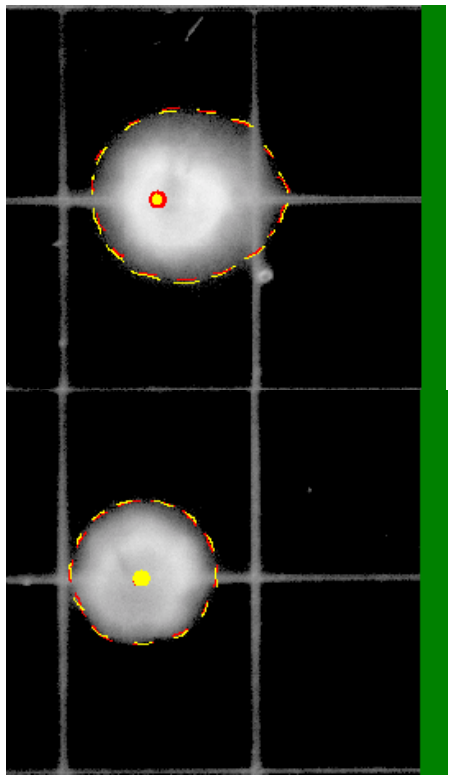


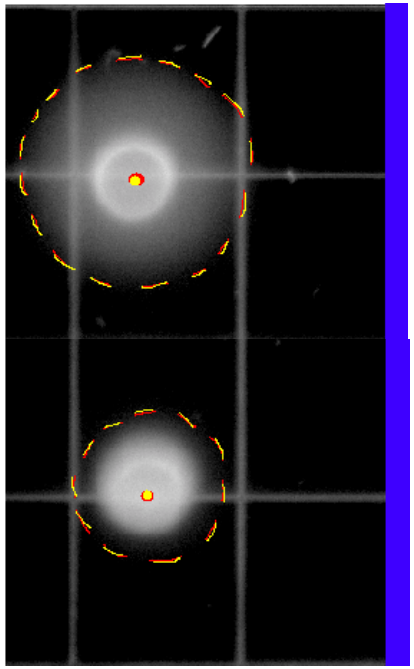
Results



Gradient
Aspartate

Gradient
Phenylalanine





Conclusions

- Orthogonal pairs are a rapid method to built signaling pathways that can operate simultaneously
- Chassis developed to rapidly screen for new protein-protein interactions
- Limited by the switch performance in this chassis
- Directional control could also be achieved by non-chemical inputs (light, radiation, etc)