

Engineering cell-cell communication via DNA messaging

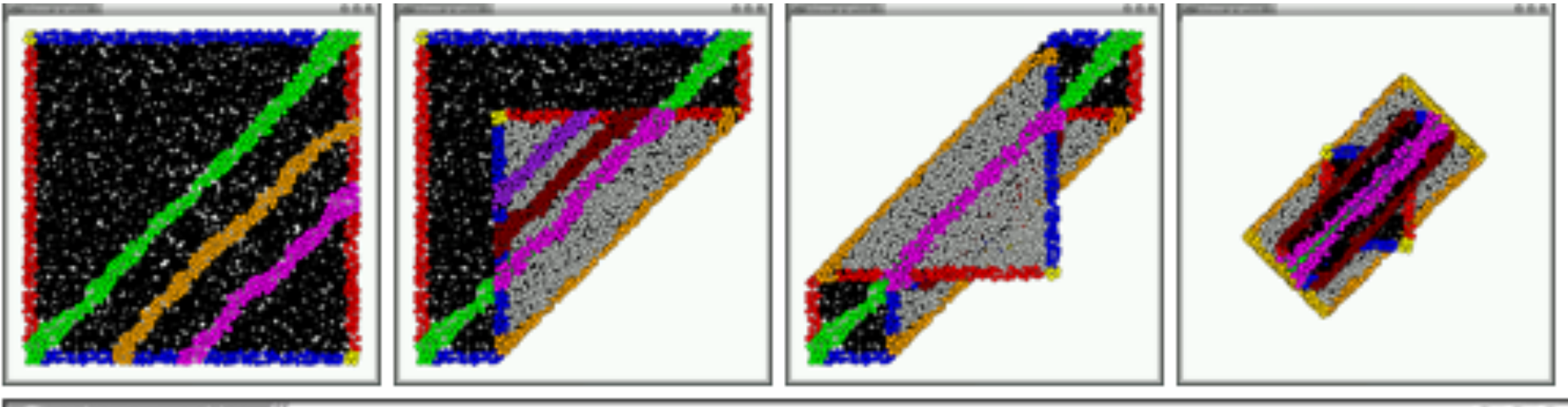
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Presented by: Paula Trepman

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Background: Amorphous Computing

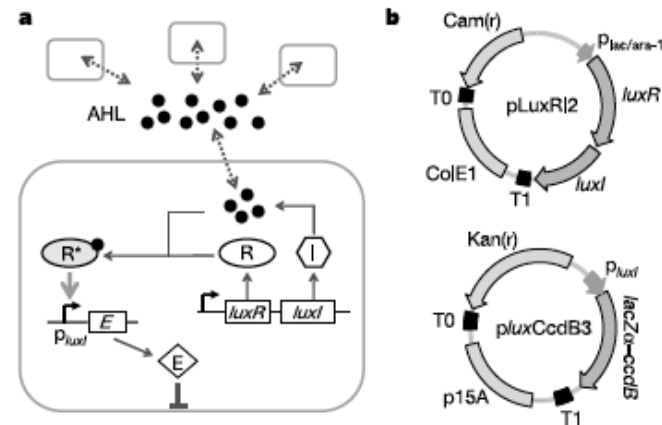
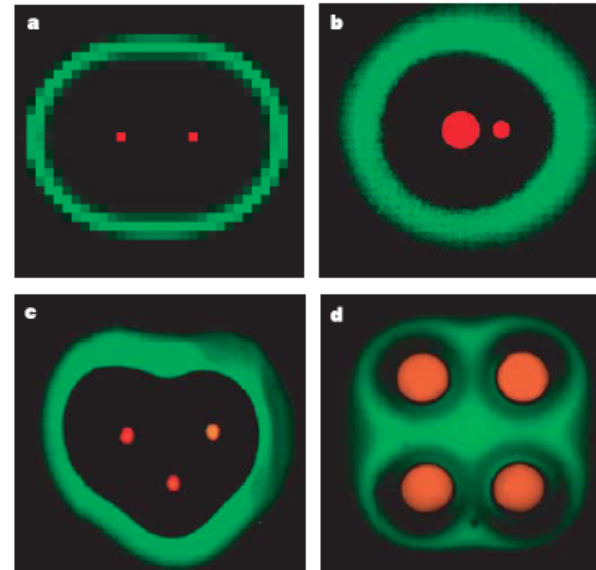
- Comprised of identical but autonomous agents
- the use local information to differentiate and act across space and time



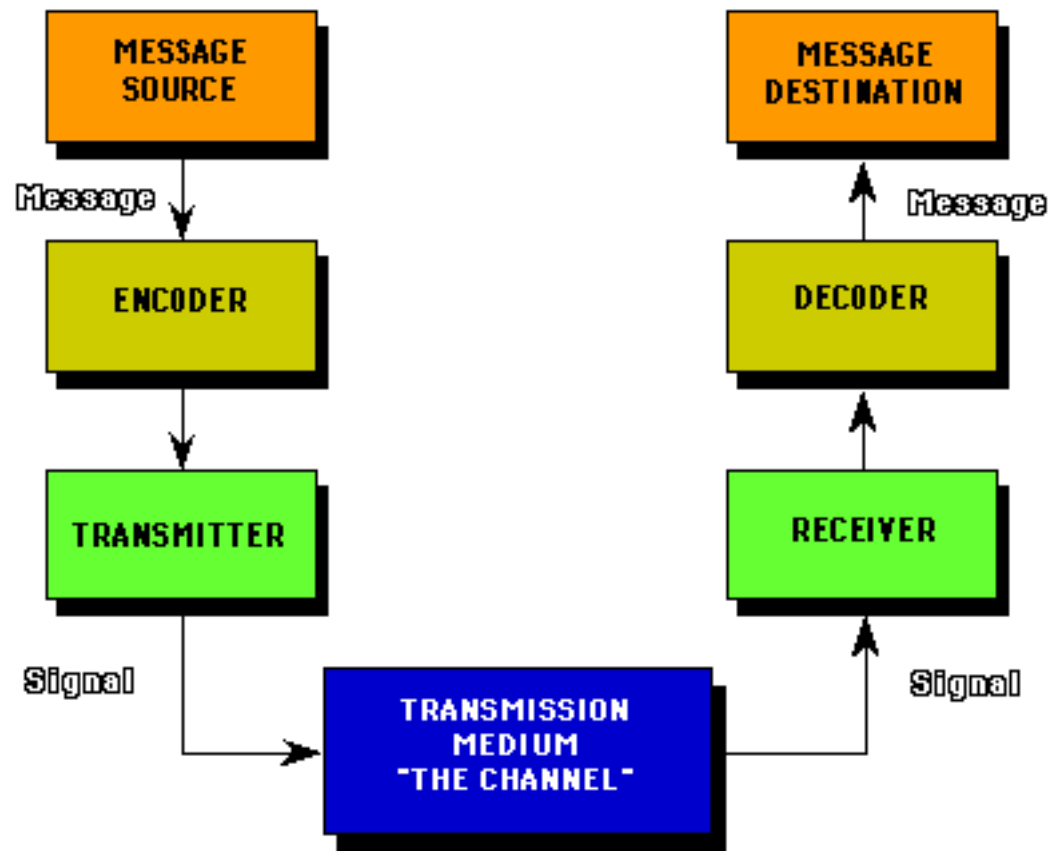
Nagpal R, MIT Artificial Intelligence Lab “Programmable Self-Assembly: Constructing Global Shape Using Biologically-Inspired Local Interactions and Origami Mathematics”

Acyl-homoserine-lactone-based systems

- Freely-diffusing molecules found in natural quorum sensing bacterial systems
- Used to establish two-dimensional patterns (Weiss et. al.)
- Edge detector (Tabor et. Al.)
- Synthetic predator-prey ecologies (You et. Al.)
- Synchronization of genetic oscillators within microbial populations (Danino et. Al.)
- Boolean logic (Tasmir et. Al.)



Shannon framework



Limitations of AHL-based systems

- Only single message transmission for a given information channel (i.e the message is the molecule)
- AHL-encoded messages are confined to transcriptional regulation as output

Limitations of existing cell-cell communication systems

a



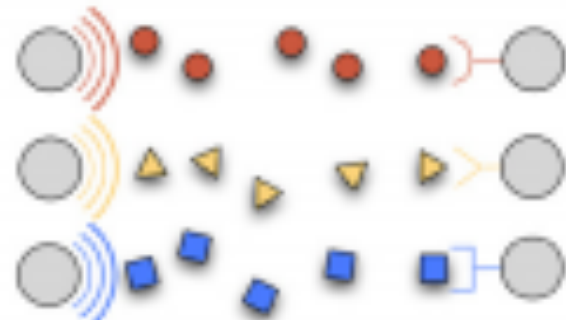
Single channel coupled to specific message

b



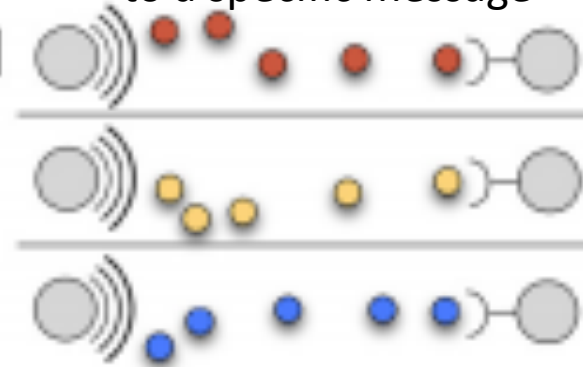
Single channel coupled to specific message
with multiple receivers

c



Multiple insulated channels each coupled
to a specific message

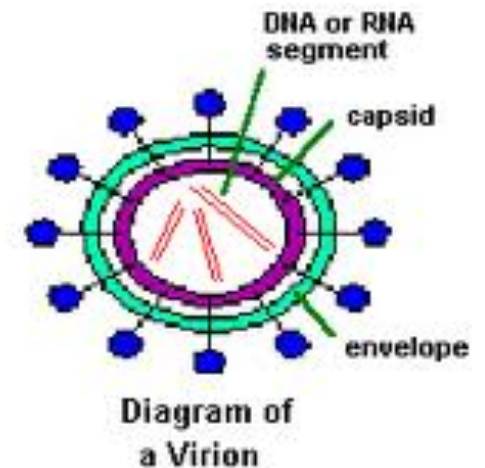
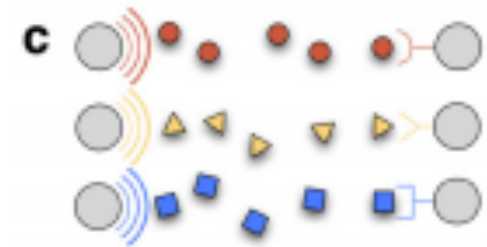
d



Single channel decoupled from any one
message

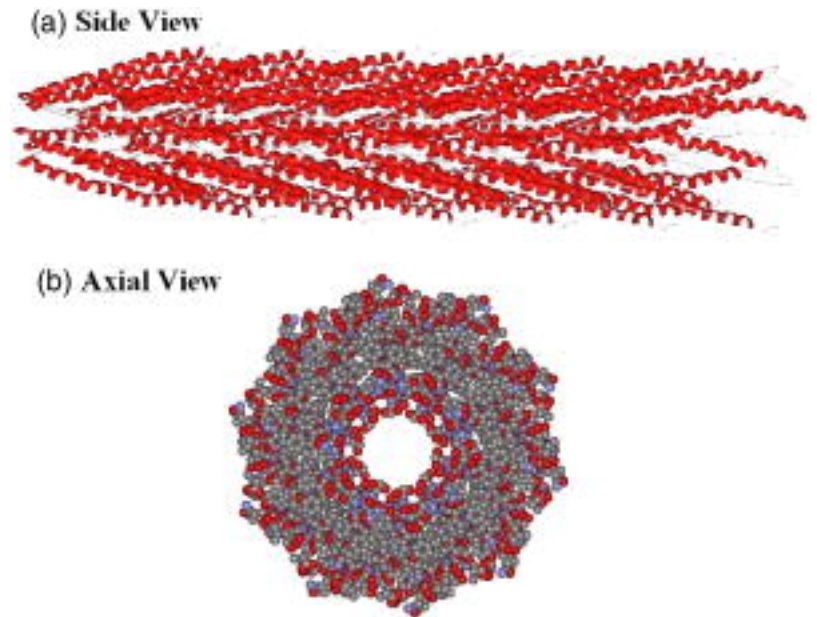
Overcoming message-channel coupling

- Increasing number of available channels
 - May cause non-specific interaction and interference
- Many natural organisms can directly or indirectly exchange or uptake nucleic acids
 - Virus-mediated exchange of genetic material
 - released from infected cells
 - Do not require direct sender-receiver interaction

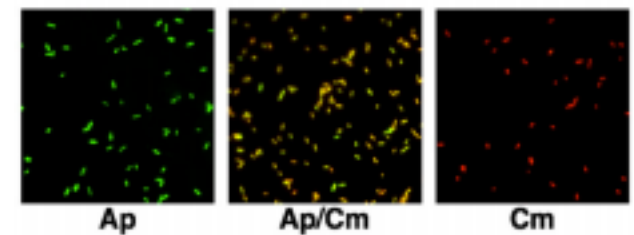
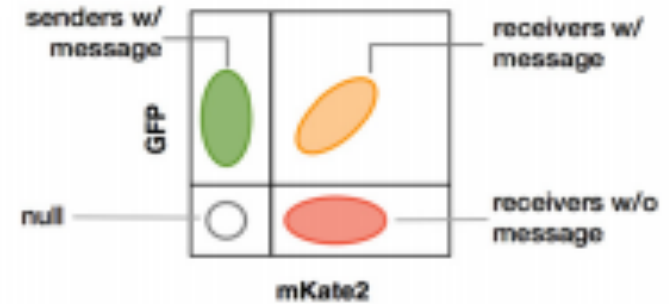
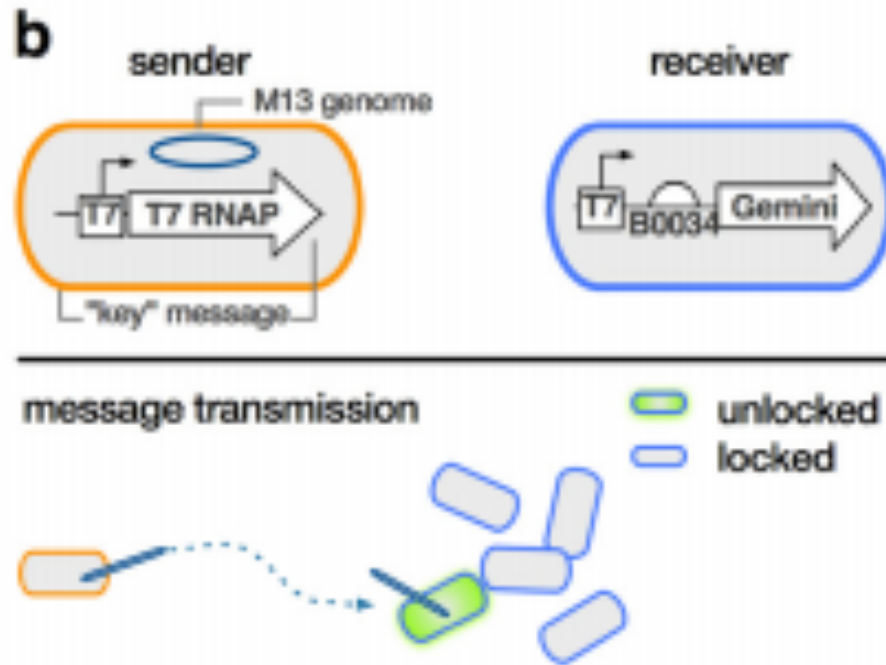


Bacteriophage M13 Ideal for Message Transmission

- Progeny particles secreted from infected cells without destroying host cell
- Can package:
 - ssDNA of various lengths
 - **heterologous DNA** containing M13 packaging sequences
- Binding of and Infection by M13 requires pilus from F-plasmid



M13-based communication system employs fluorescence and antibiotic resistance coupling for assaying message transmission



sender cell:

- Green Fluorescent Protein
- Ampicillin resistance

receiver cell:

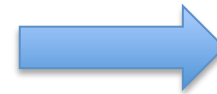
- Red Fluorescent Protein (mKate2)
- Chloroamphenicol resistance marker

Cells that have received the message:

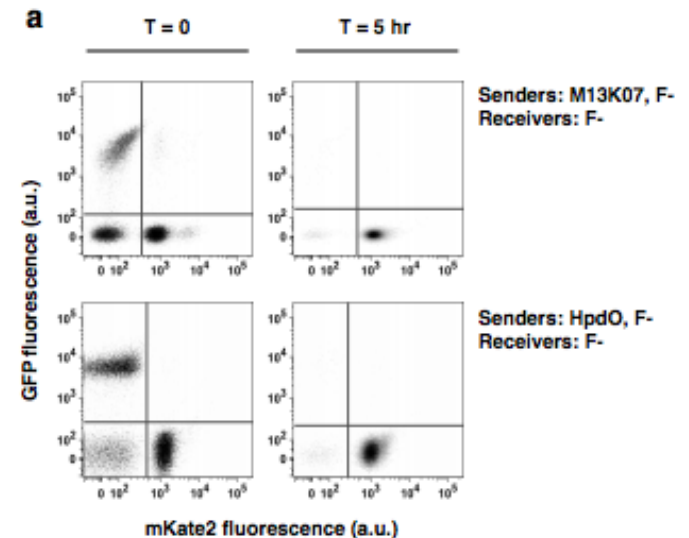
- Appear yellow
- Exhibit both Ampicillin and Chloroamphenicol resistance

Testing in Liquid Culture to Confirm Requirement of M13 packing, particles, and F+ receiver cells

- Co-culture of F+ sender cells and F+ receiver cells **in absence of M13**
 - NO cells expressed both GFP and RFP
 - NO cells grew in combined presence of ampicillin and chloroamphenicol
- Co-culture of F- sender cells and F- receiver cells
 - NO cells expressed both GFP and RFP
 - NO cells grew in combined presence of ampicillin and chloroamphenicol
- Kanamycin resistant, modified helper phage (HPdO) lacking packaging sequence
 - No transmission of messaging phagemid

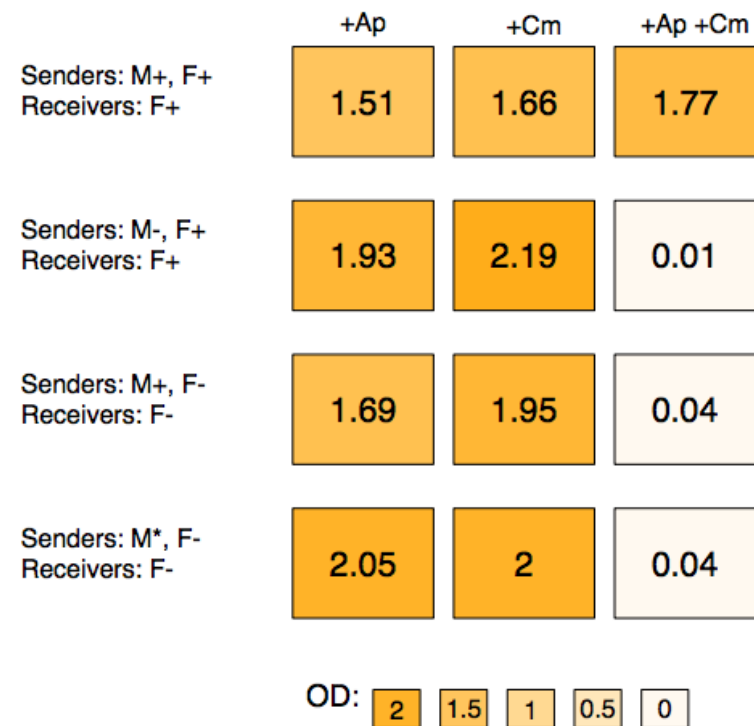


M13 is required for message transmission

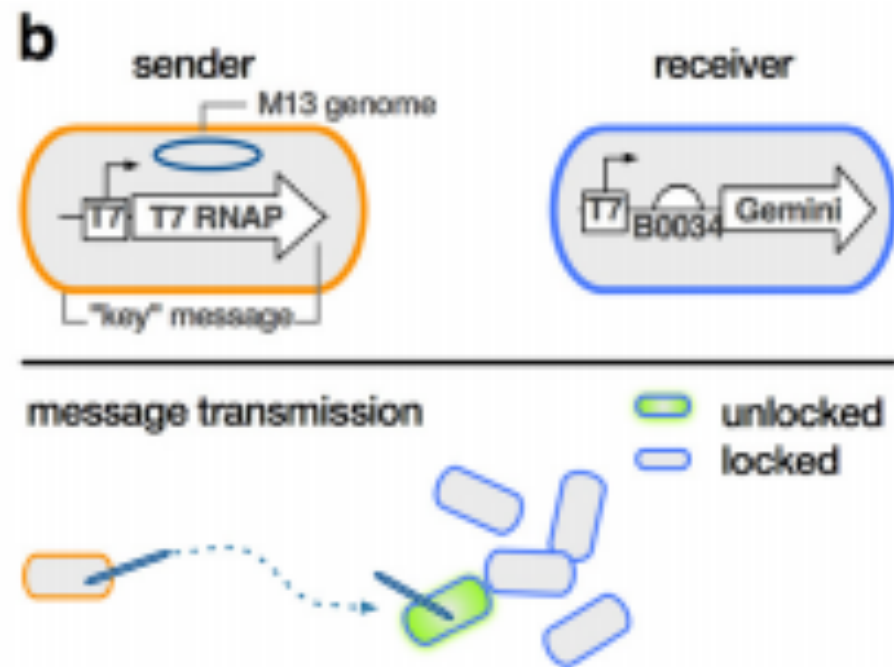


Testing in Liquid Culture to Confirm Requirement of M13 packaging, particles, and F+ receiver cells (cont'd)

- F+ Sender cells transformed with M13K07 (“helper phage” with M13 gene products) and weak M13 packaging sequence
 - **92.4%** of mKate2 + receiver cells also expressed GFP
 - Successful growth in ampicillin + chloramphenicol



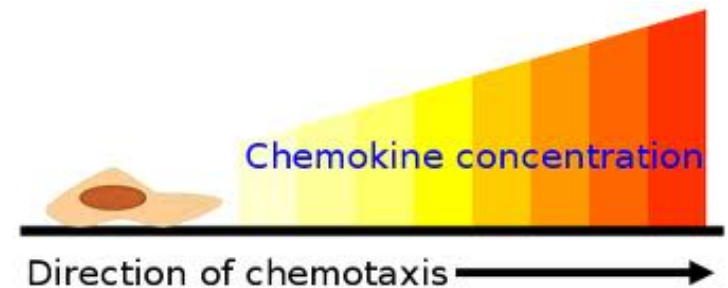
M13-based messaging can send arbitrary genetic sequences



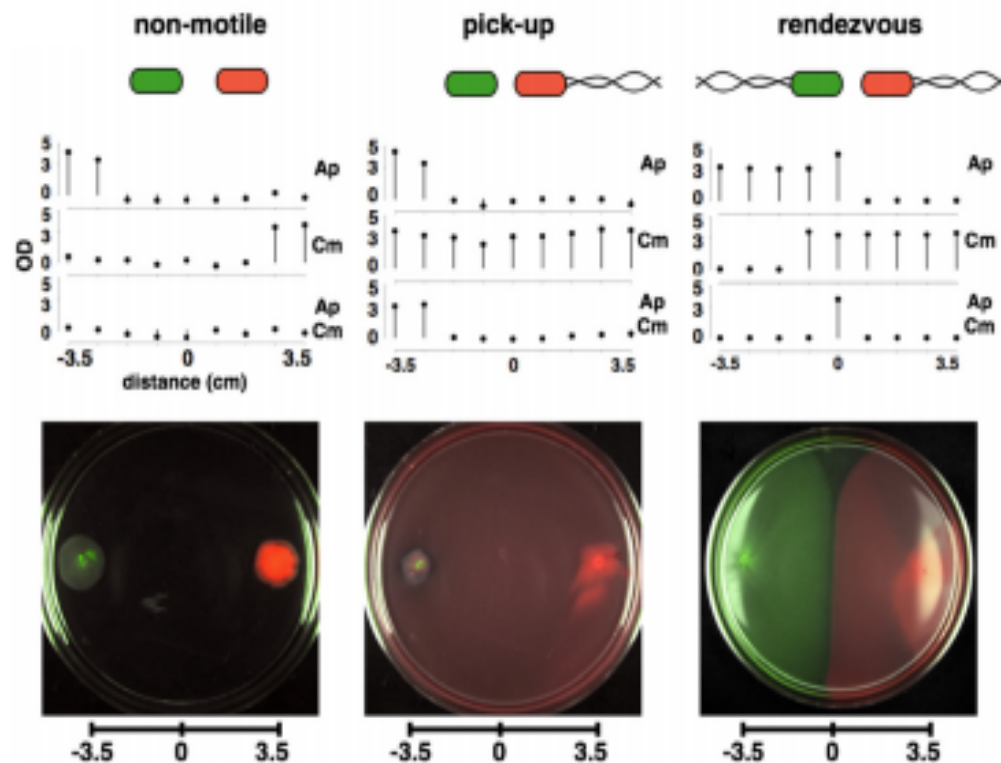
- “lock and key” mechanism
- Sender cell message (T7 RNA polymerase expression) required to activate receiver cell reporter
- M13-based messaging can successfully transmit genetic sequences

Differing message transmission in M13 system

- Lower message receipt in semisolid media rates due to:
 - Required passive diffusion of M13 messaging particles within agar microenvironment
 - Chemotaxis driven by nutrient depletion due to cell growth
- By size comparison, diffusion faster in AHL than M13-based system



Use of Bacterial Chemotaxis Allows for Longer-Range Messaging

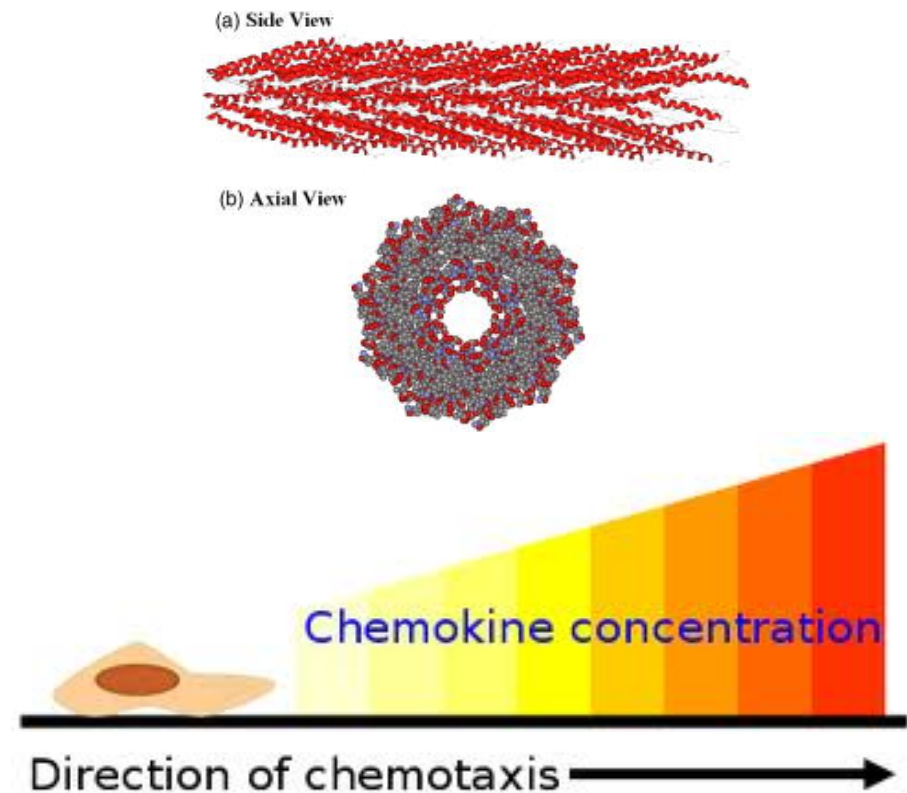


- Passive diffusion in M13 system only ~2mm run length at 24 hours
- Chemotactic coupling increased length across which DNA messages can be transmitted

- Transmission across a 7 cm gap on semisolid media: **33%**
Receiver Cells Transduced

Novel method for genetic messaging

- M13 phage-based system enables variety of genetic messages
- Chemotactic coupling enhances speed of cell-cell communication



Significance

- Ability to send genetic messages between cells more quickly
- Improved versatility over AHL messaging
- M13K07 helper phage also causes production of some secondary “M13K07” messages that convert receiver cells to potential rebroadcasting cells

Potential Improvements and Future Research

- Conditional control of M13 gene product synthesis from external factor
- Messaging vectors with varying DNA copy numbers
- Variable strength M13 packaging sequences
- Autonomous trans-kingdom communication of DNA messages

Discussion Questions

- Are transcriptionally-coupled, AHL-based communication systems limiting?
- Is there a demand for this M13-based system that can transmit genetic sequences?
- What types of genetic sequences might be useful to transmit between cells?
- Is this speed of cell-cell communication fast enough to be useful?