Bacteriophages for intercellular communication in bacterial cultures

Manish Kushwaha

Bacteriophages for intercellular communication in bacterial cultures
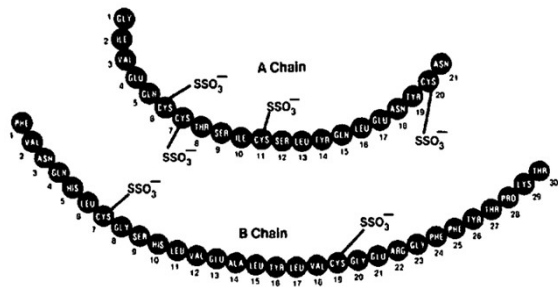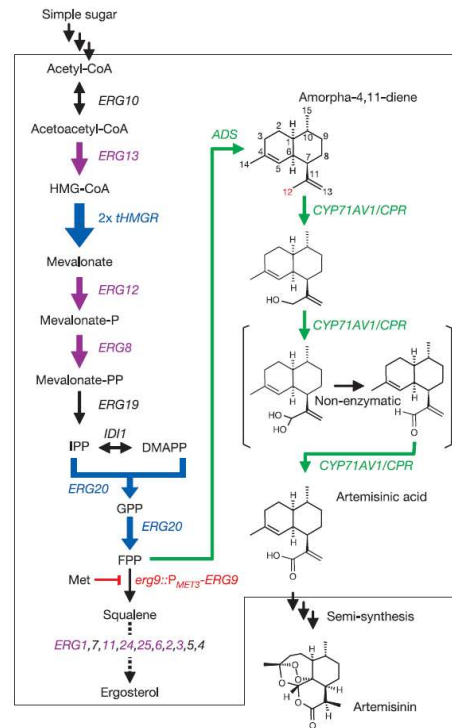
Manish Kushwaha
Micalis Institute, INRAe Jouy-en-Josas
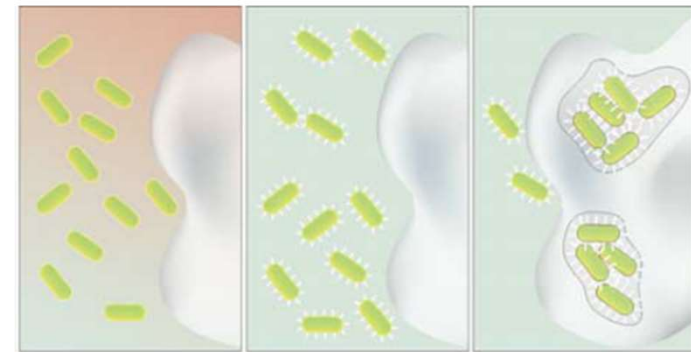
03 December 2021

Engineering the cellular machinery has many advantages

Insulin

(Chance *et al*., 1981.
Proceedings of the 7th
American Peptide
Symposium.)
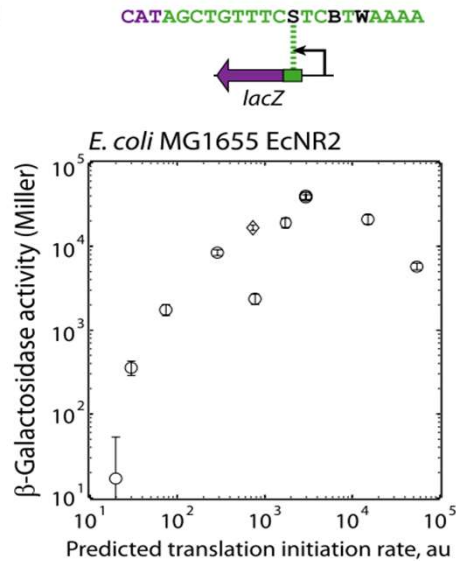
Artemisinin

(Ro *et al*., 2006. Nature.)

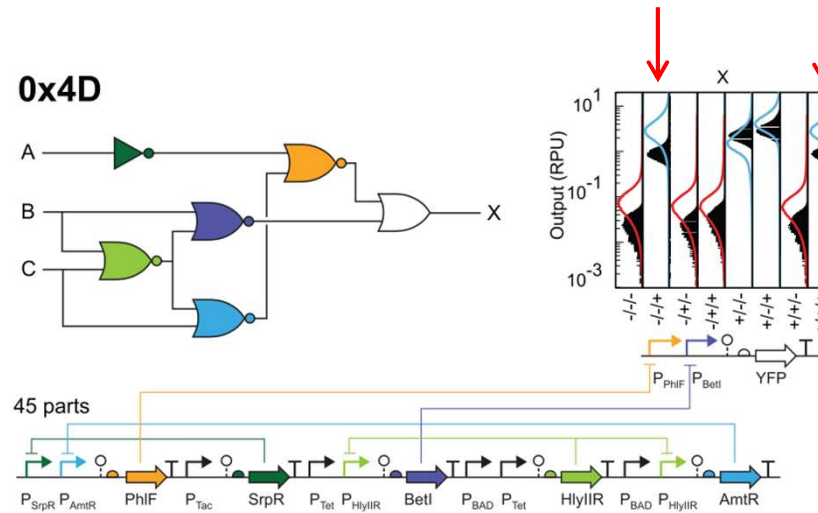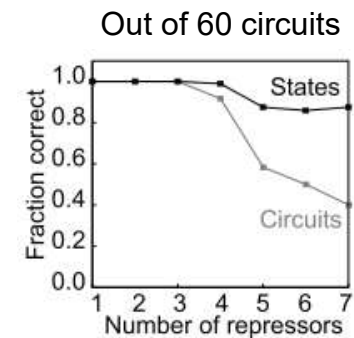## Smart systems

(Anderson *et al*., 2006. JMB.)

Engineered machinery: Expenses add up

Single Protein Expression
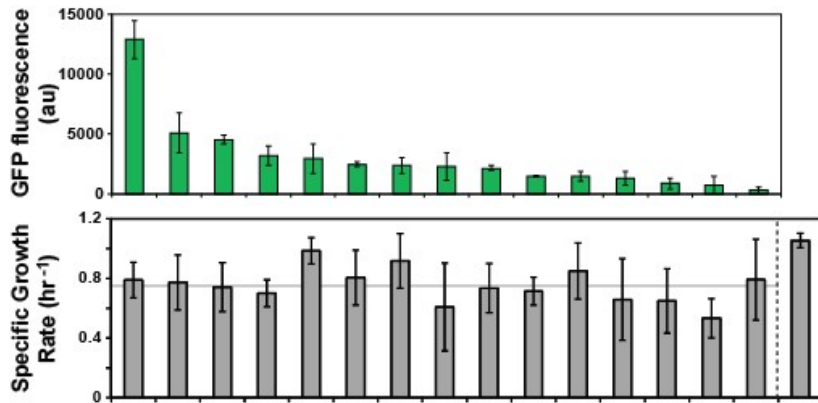(Farasat *et al.*, 2014. Mol. Syst. Biol.)

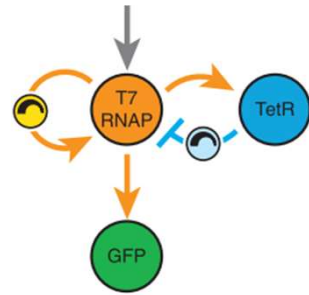**Multiprotein Circuits**
(Nielsen *et al.*, 2016. Science)

- Engineered parts increase the "load" on the system

Intra-cellular Resource Allocation

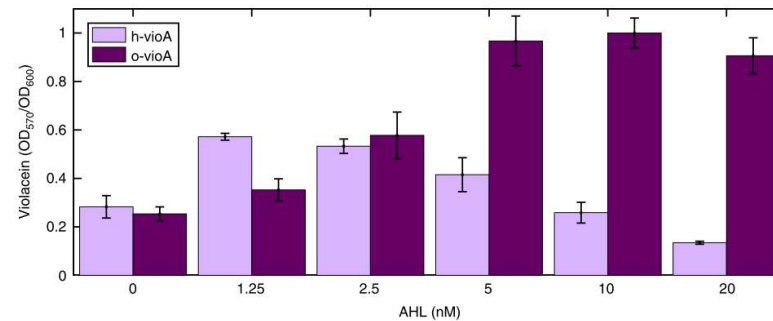

VioA translated by orthogonal ribosomes

Transcriptional Power Supply

(Kushwaha & Salis, 2015. Nat. Comm.)
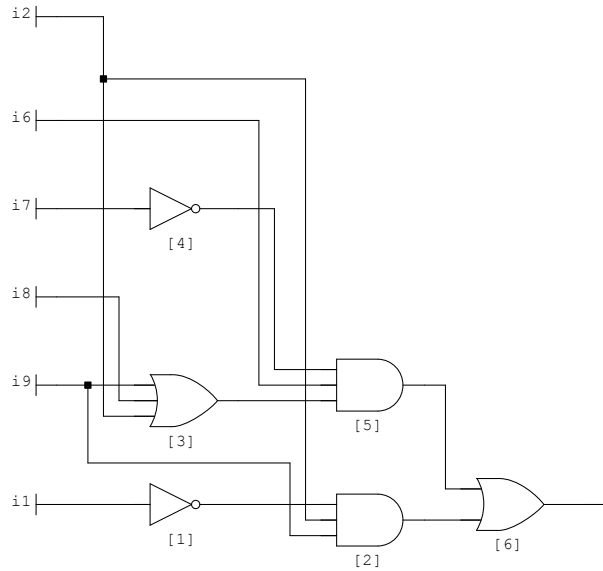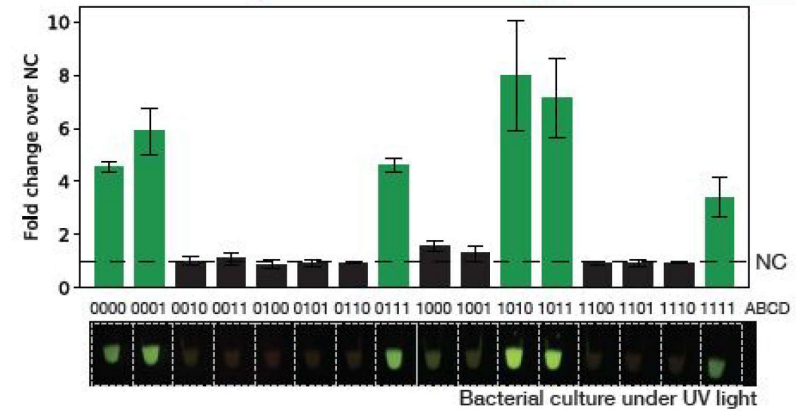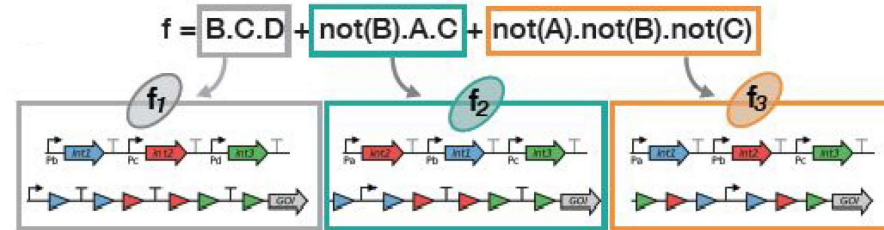
Translational Partitioning

(Darlington *et al.*, 2018. Nat. Comm.)

- Managing resource partitioning within the cell

Inter-cellular Resource Allocation

~i7 . i6 . (i9 + i8 + i2) + (~i1 . i2 . i9)

4x

$$f = B.C.D + not(B).A.C + not(A).not(B).not(C)$$
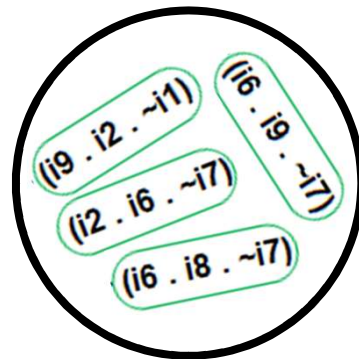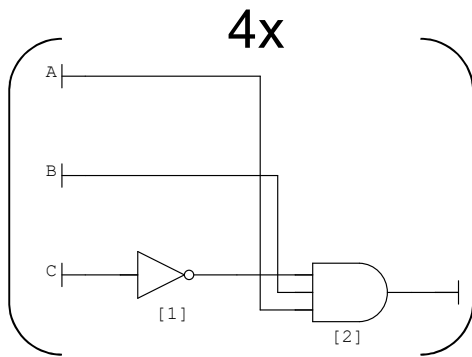
(Guiziou *et al.*, Nat. Comm. 2019)

- "Distributed computing" allows decomposition of a large problem into many smaller problems

Inter-cellular Resource Allocation
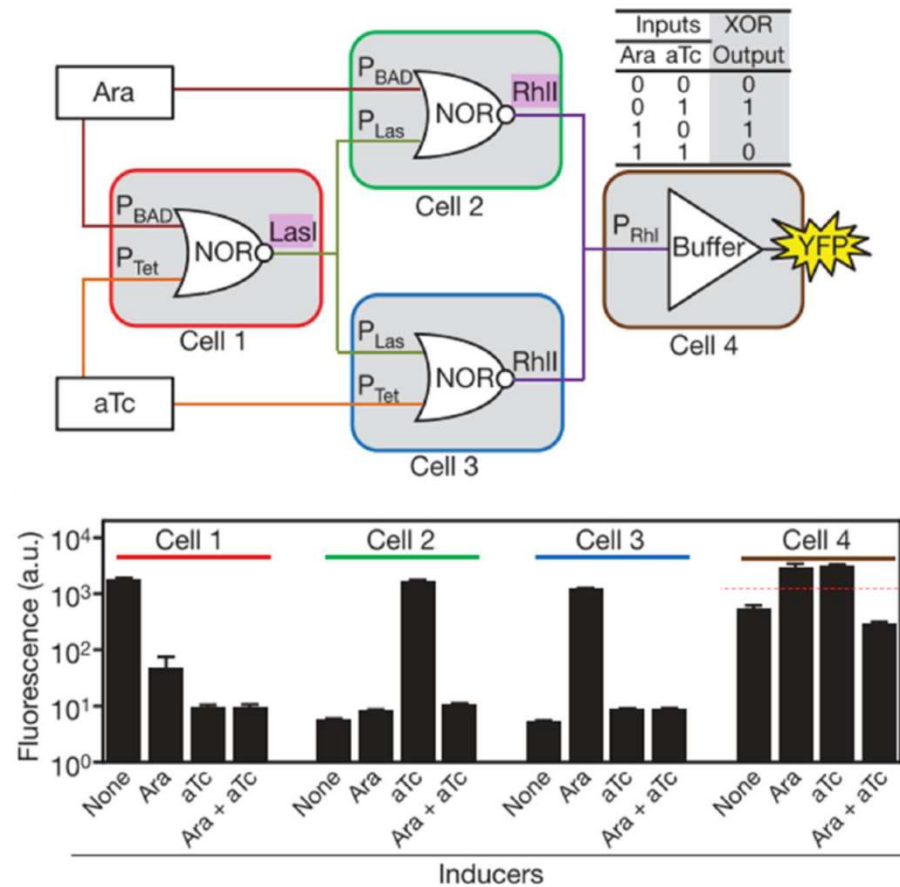
(Tamsir *et al.*, Nature. 2011)

(Regot *et al.*, Nature. 2011)

- "Distributed computing" allows decomposition of a large problem into many smaller problems

Limited external wires
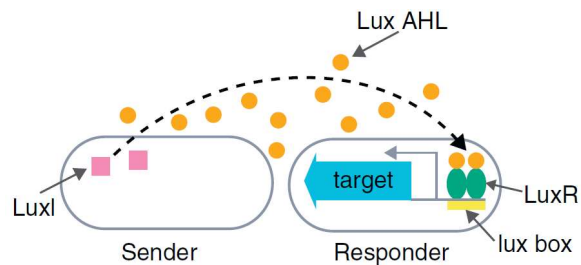
Quorum sensing molecules

(Macia *et al.*, Trends in Biotech. 2012)

Hormone molecules

(Regot *et al.*, Nature 2011)
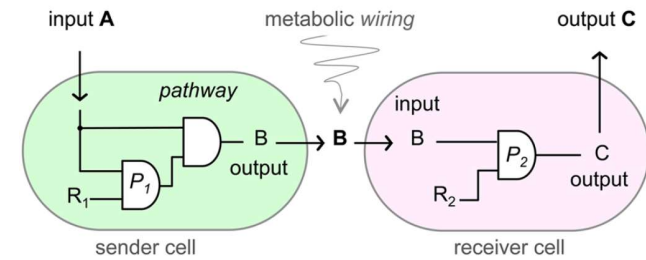
Metabolites

(Silva-Rocha *et al.*, ACS SynBio. 2013)
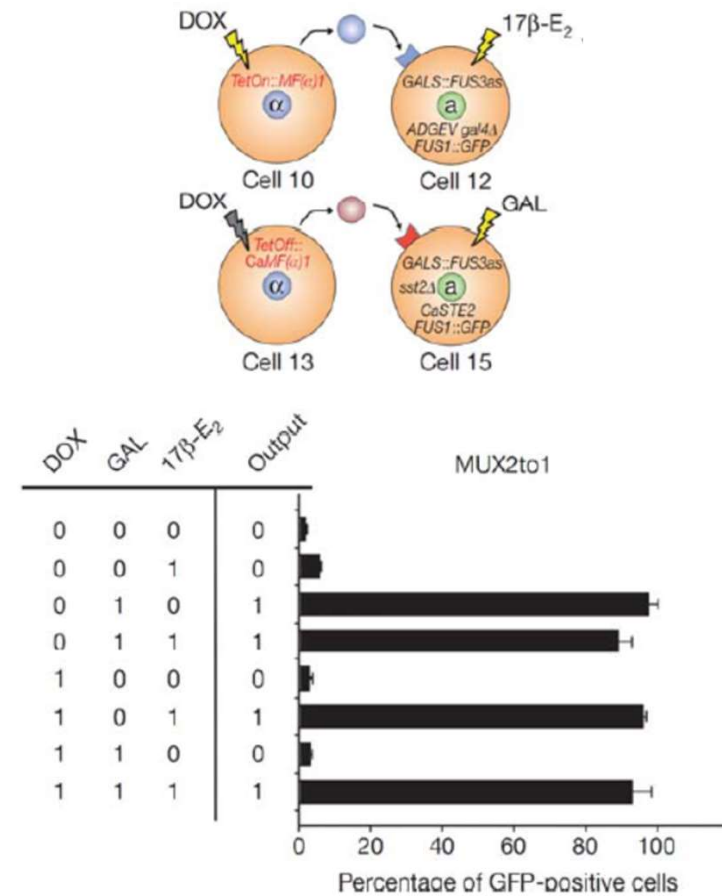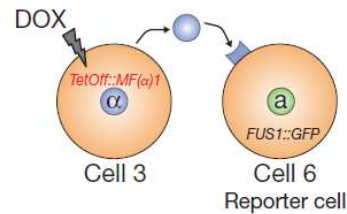
- A limited set of orthogonal external wires exist for cell-to-cell communication

Viruses as messaging vehicles?

The New York Times

Bad News Wrapped in Protein: Inside the Coronavirus Genome

By Jonathan Corum and Carl Zimmer   April 3, 2020

A virus is "simply a piece of bad news wrapped up in protein," the biologists Jean and Peter Medawar wrote in 1977.

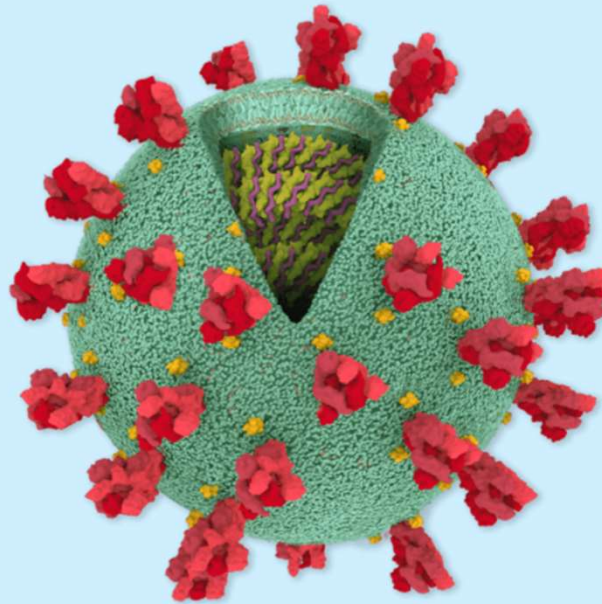# Viruses as messaging vehicles?
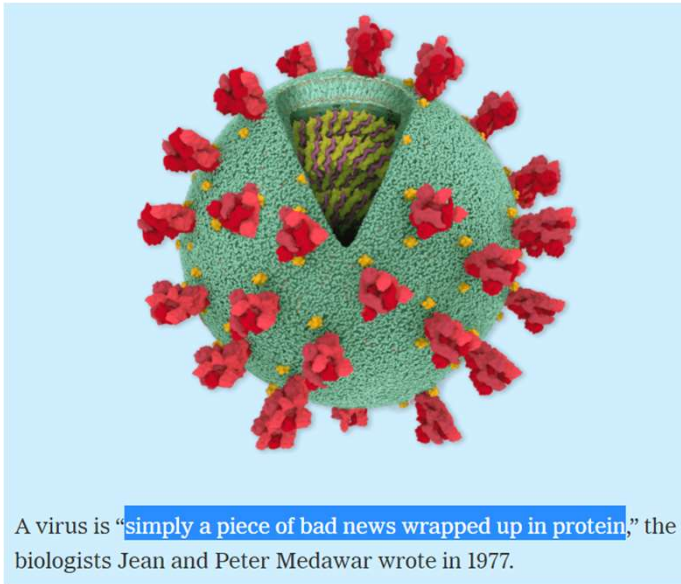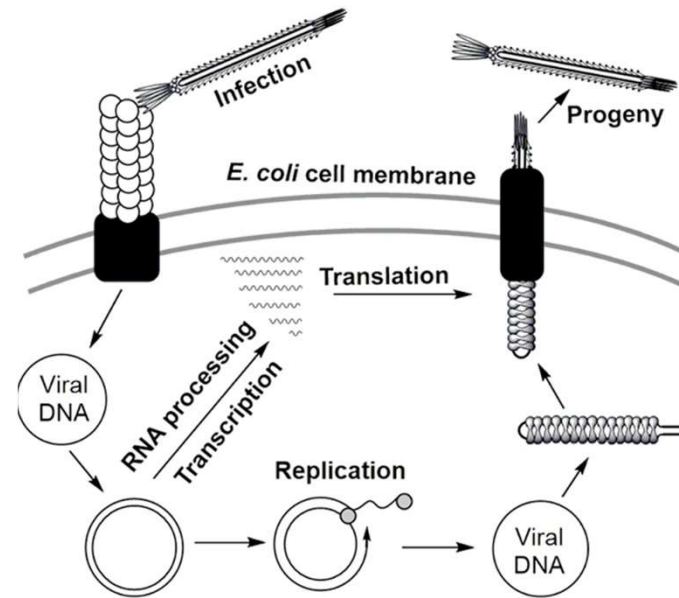

M13 bacteriophage

A virus is "simply a piece of bad news wrapped up in protein," the biologists Jean and Peter Medawar wrote in 1977.

NYT. April 3, 2020

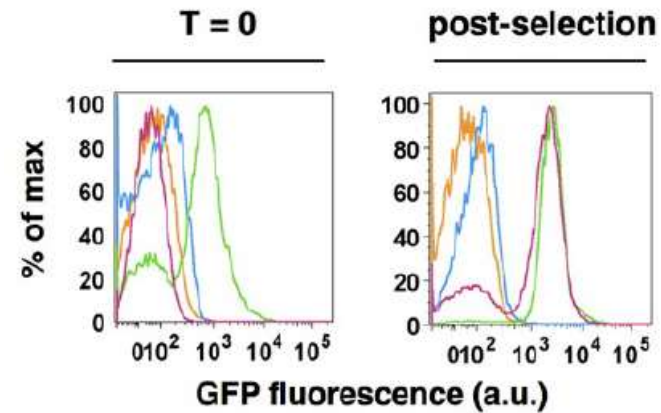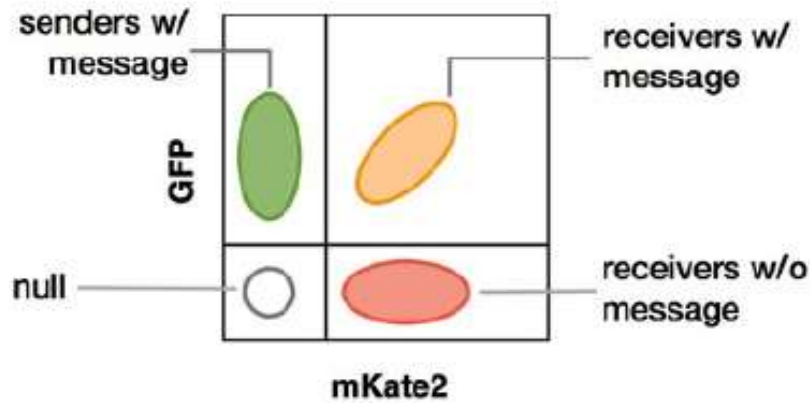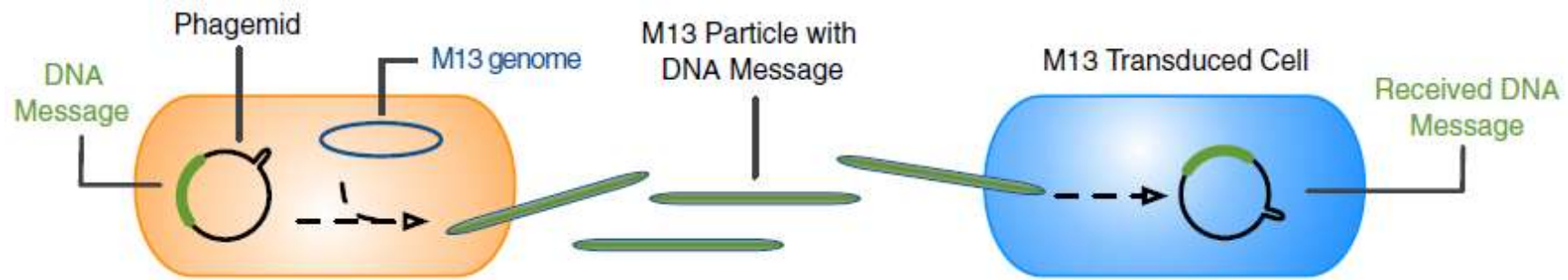(Smeal *et al.*, 2017)

Viruses as messaging vehicles?

(Ortiz & Endy. J. Biol. Engg. 2012)

- Using DNA as signaling molecule

Engineering the M13 phage for communication

(Pathania *et al.,* In preparation.)

Modelling Growth Kinetics

early fit

late fit

$$C \to C + C$$

$$C + R \to C + C$$
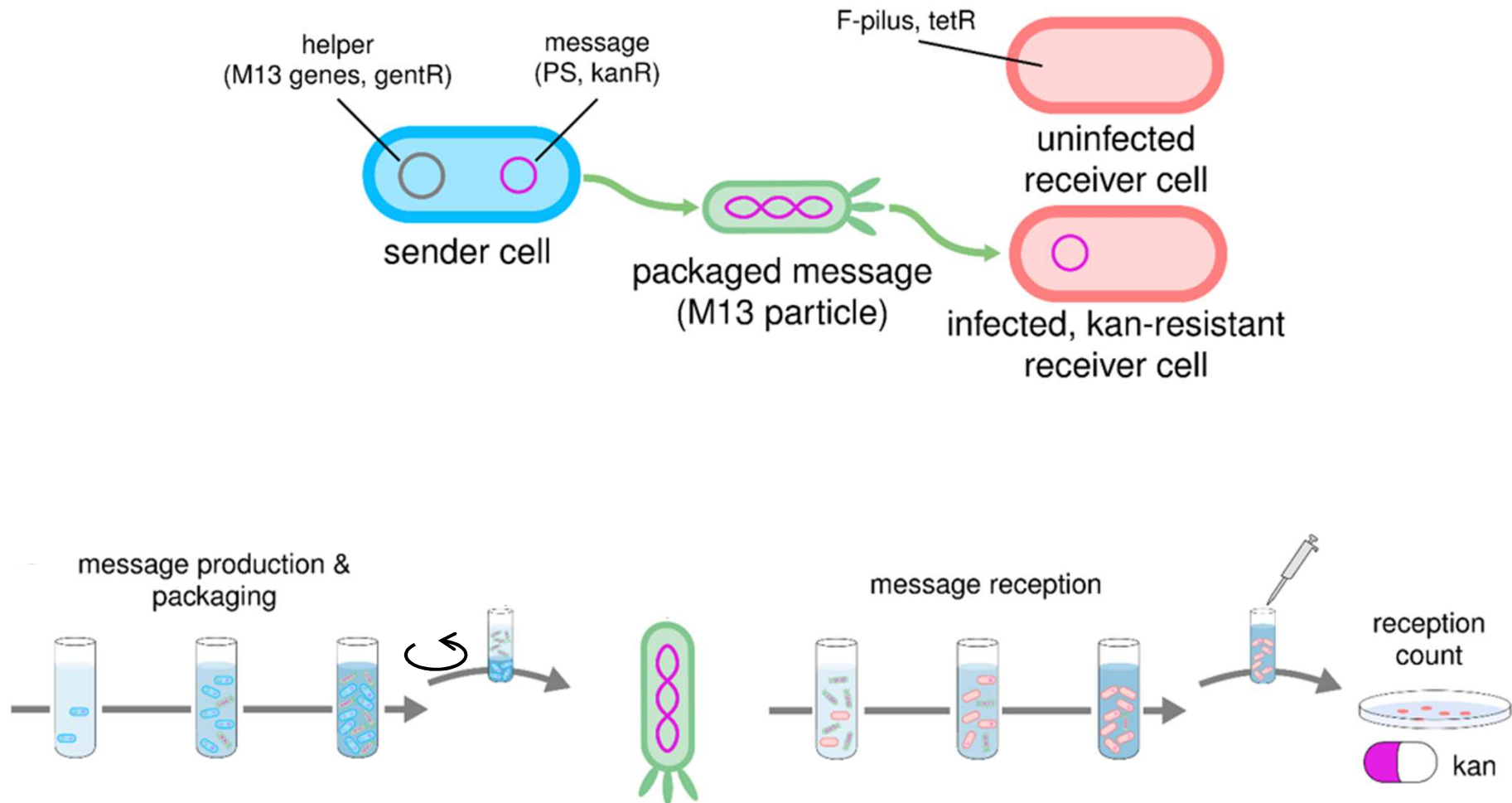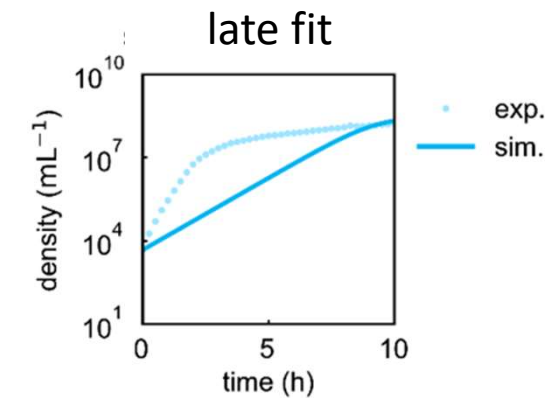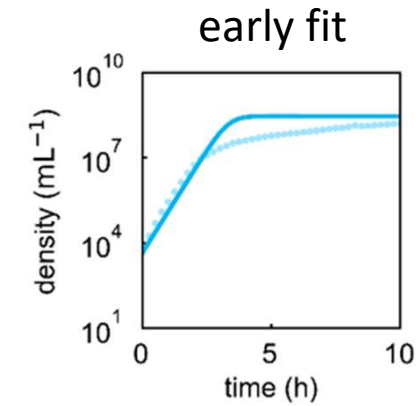
- 1-phase model
- At T=0, $[C](0) = 10^6 \text{mL}^{-1}$
  $$[R](0) = 10^8 \text{mL}^{-1}$$

exp.

sim.

(Pathania *et al.*, In preparation.)

Modelling Growth Kinetics

2-phase growth rates

early phase
late phase

2-phase model

early: $C + R_1 \rightarrow C + C$
late: $C + R_2 \rightarrow C + C$

early
late

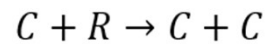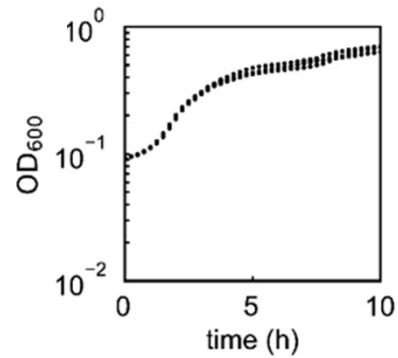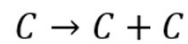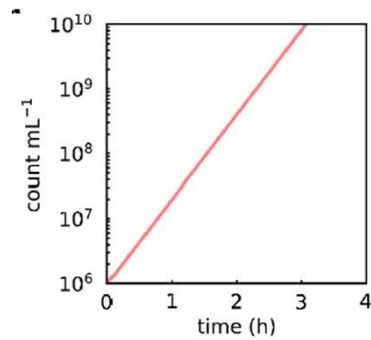top10 | top10 + phagemid | top10 + helper | top10 + phagemid + helper | uninfected | infected

(ER2738)

Sender   Receiver
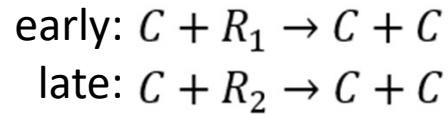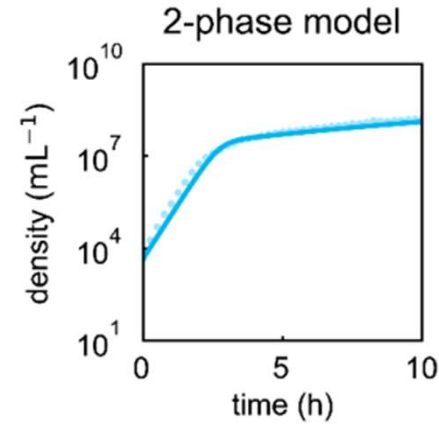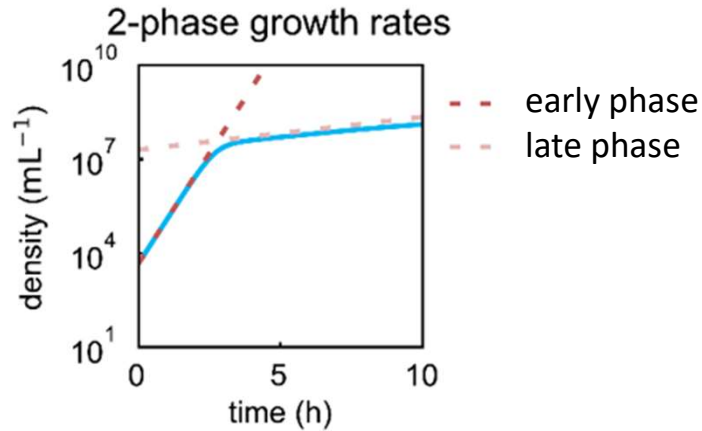
- 2-phase model
- At T=0, $[C](0) = 10^6 \text{mL}^{-1}$
  $[R_1](0) = 10^8\ ml^{-1}$
  $[R_2](0) = 10^9\ ml^{-1}$

(Pathania *et al.*, In preparation.)

Modelling Secretion Rates
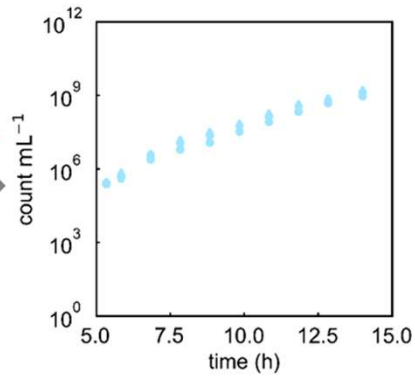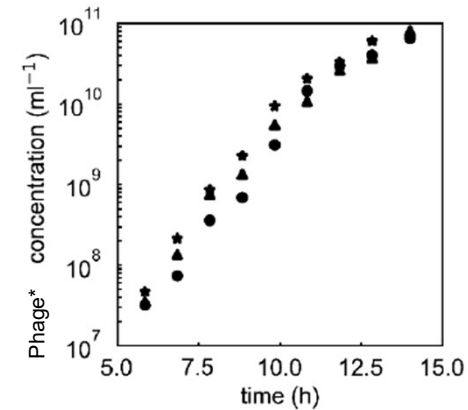
Sender Growth

Phage Growth
(*as determined by the CFU method)

$$C + R_1 \rightarrow C + R_1 + P$$
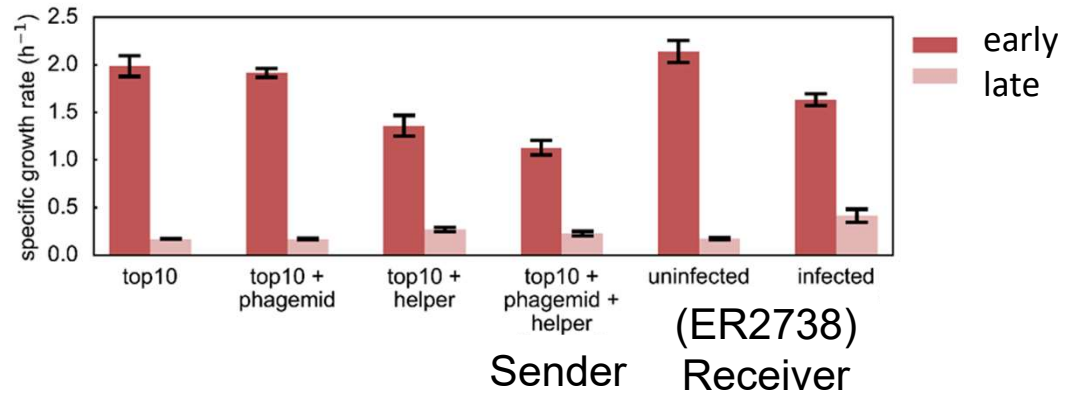$$C + R_2 \rightarrow C + R_2 + P$$
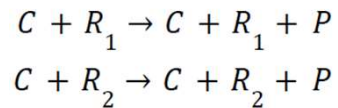
- Secretion depends on Sender Growth Phase

(Pathania *et al.*, In preparation.)

Modelling Infection Rates
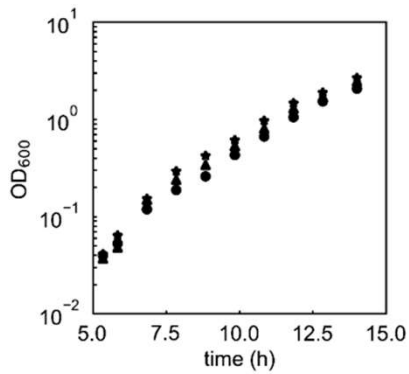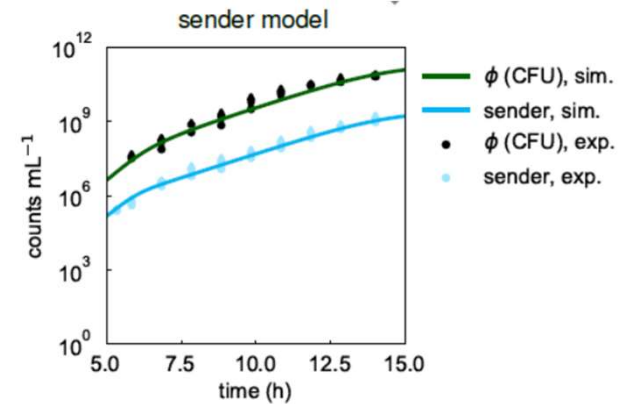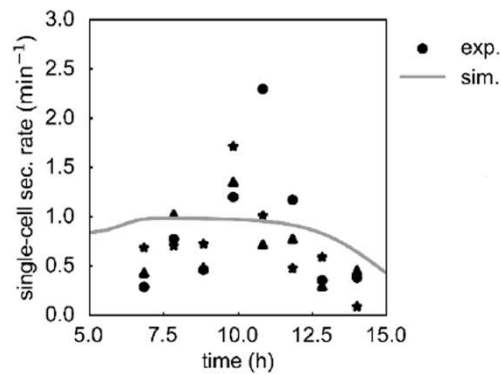

receivers harvested from different OD, resuspended to same OD

kan
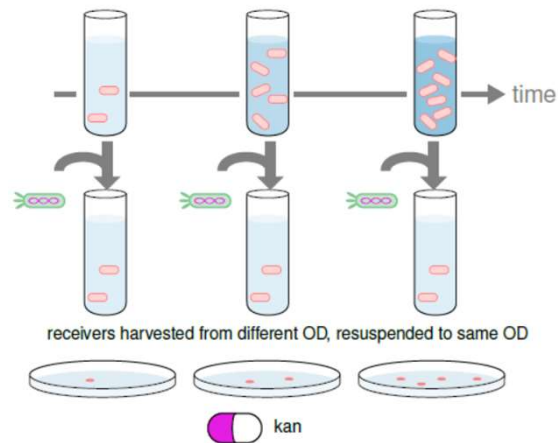
- Batch culture Receiver cells harvested at different ODs
- Then re-adjusted to OD of 1
- Infected with the same concentration of phages

- Model: Two infectability states (low 40% and high 100%)
- Model: Death due to kanamycin proportional to growth rate

$$P + C \rightarrow C'$$
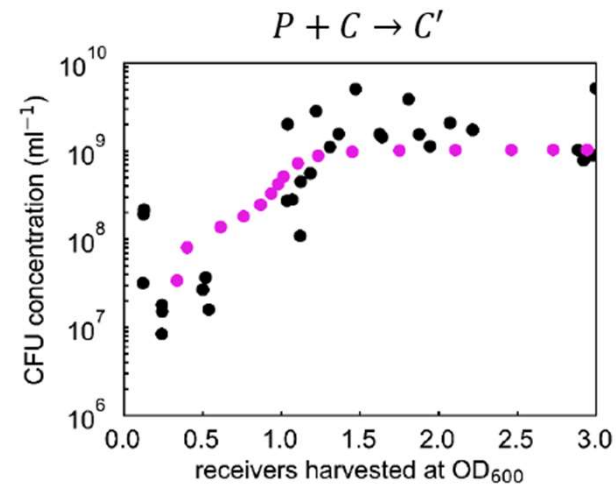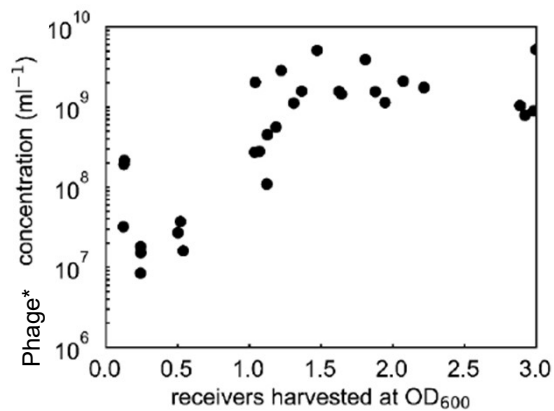
- Infection depends on Receiver Growth Phase

(Pathania *et al.*, In preparation.)

Modelling Infection Rates

receivers harvested from same OD, resuspended to different OD
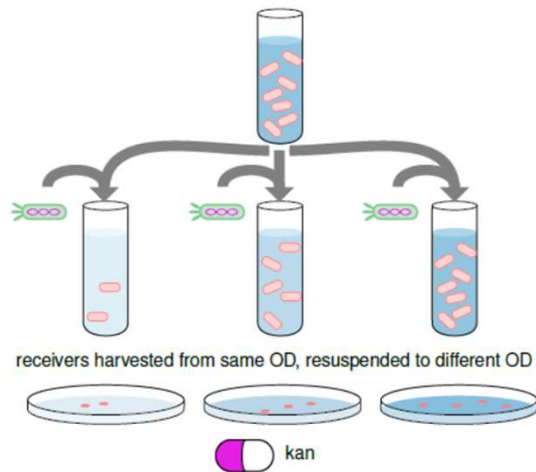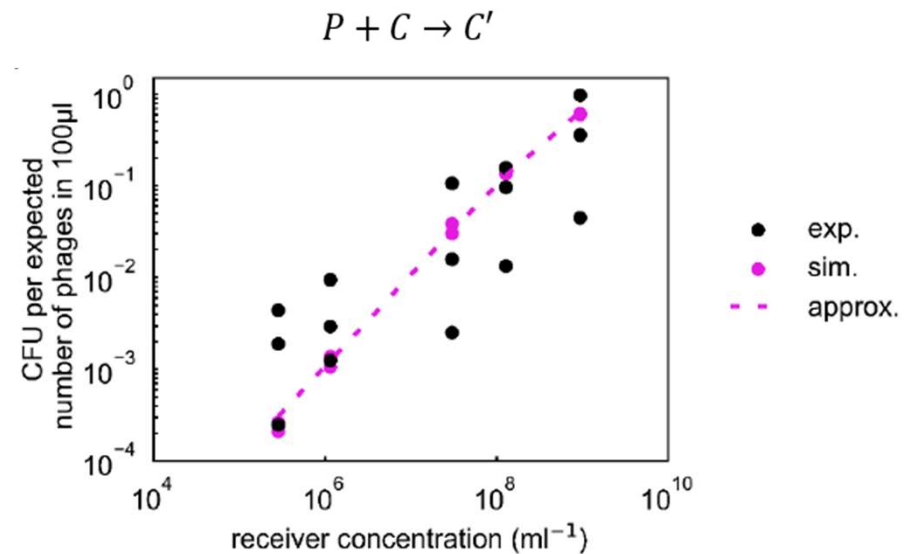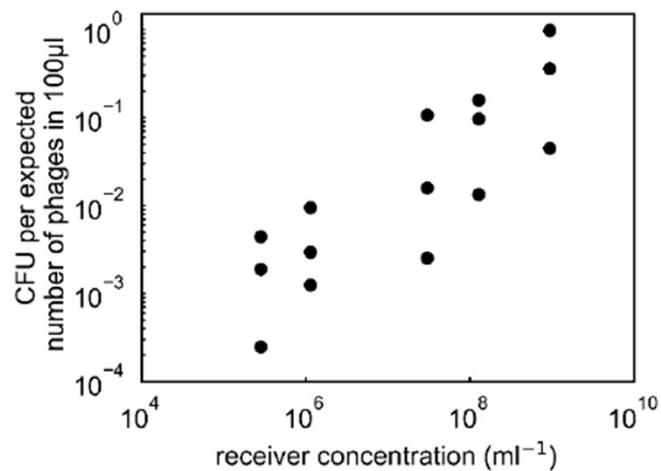
- Receiver cells harvested at OD of 1
- Then re-adjusted to different ODs
- Infected with the same concentration of phages

- Model: Two infectability states (low 40% and high 100%)
- Model: Low -> High state transition rate (¼ h^{-1})
- Model: Death due to kanamycin proportional to growth rate

$$P + C \rightarrow C'$$

- exp.
- sim.
- approx.

- Infection depends on Receiver Cell Density

(Pathania *et al.*, In preparation.)

Modelling infection rates

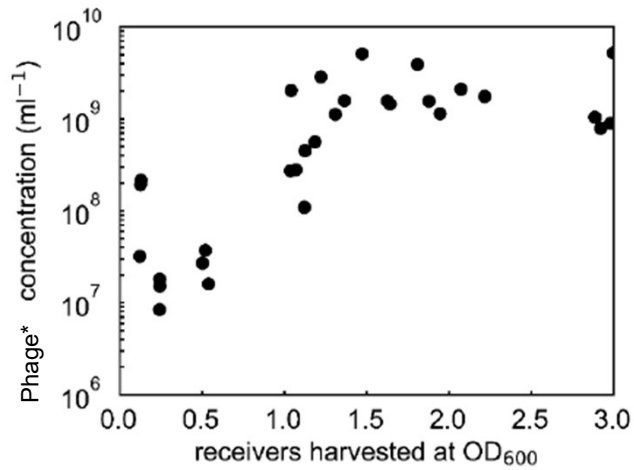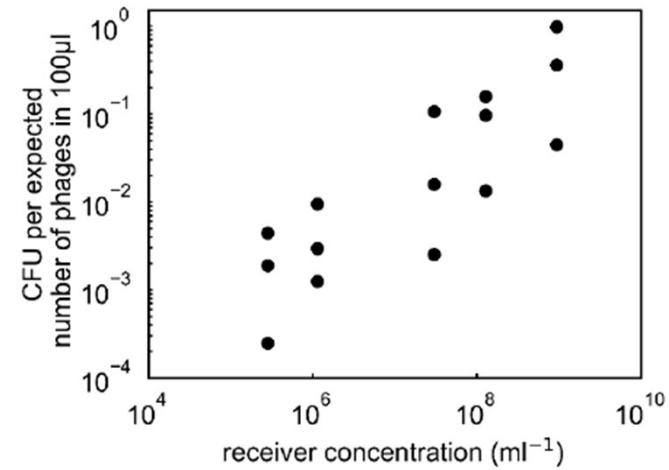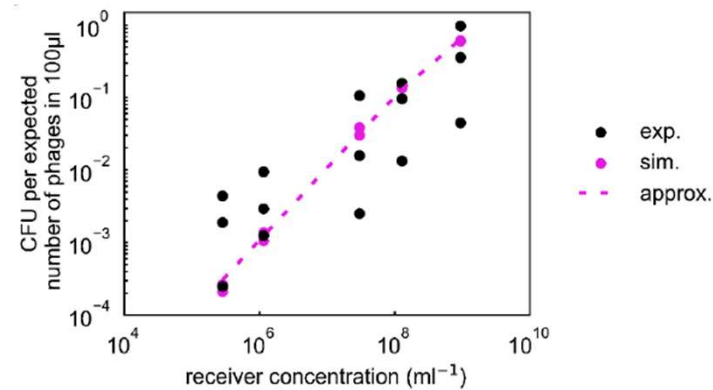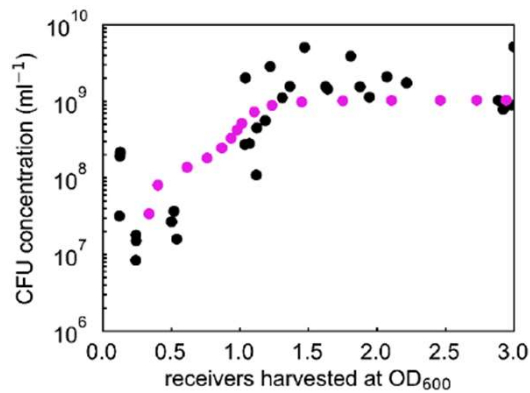- Re-adjusted to OD of 1
- Infected with same concentration of phages
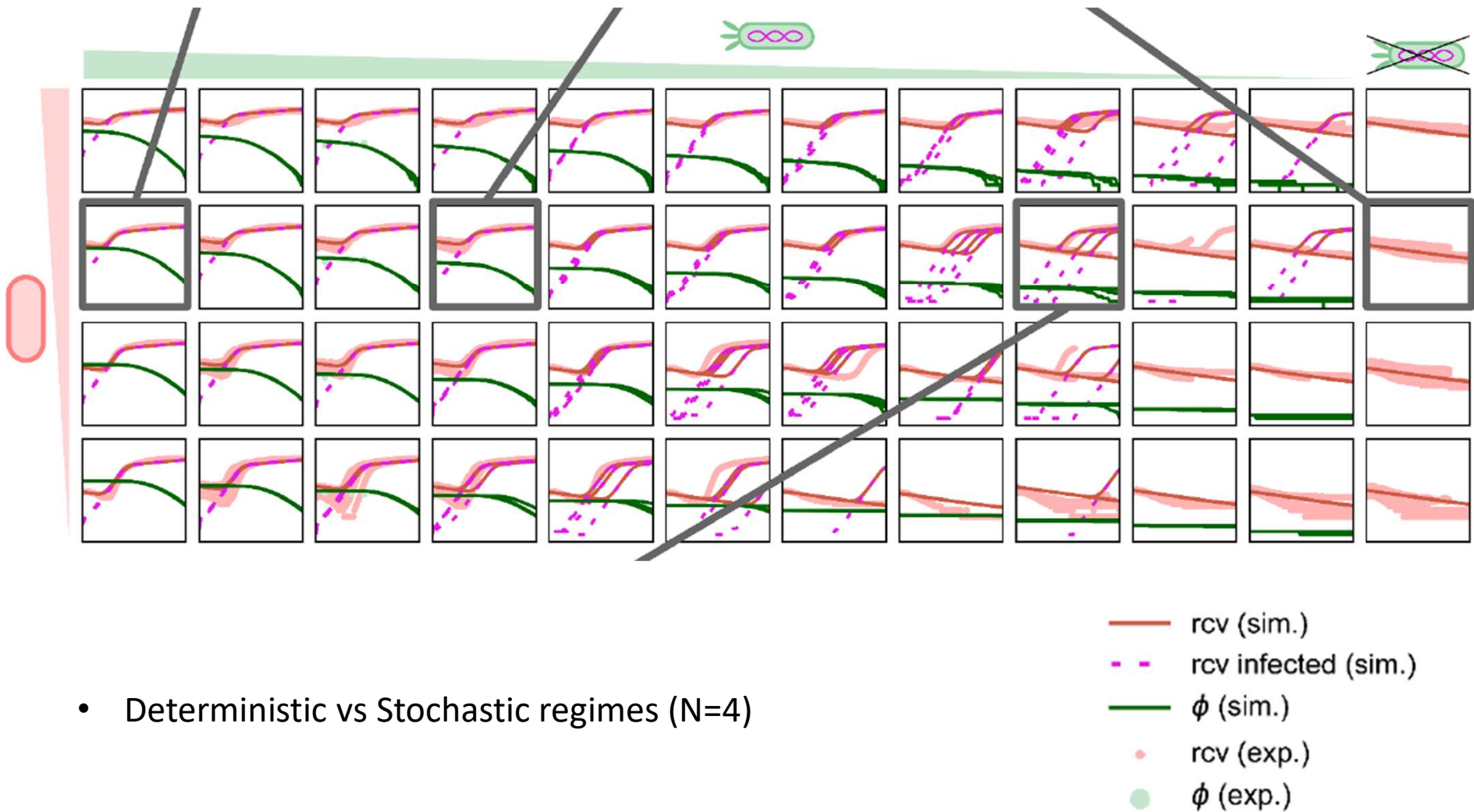
- Model: Two infectable states

- Cells harvested at OD of 1
- Re-adjusted to different ODs
- Infected with same concentration of phages

- Infection is Growth Phase dependent
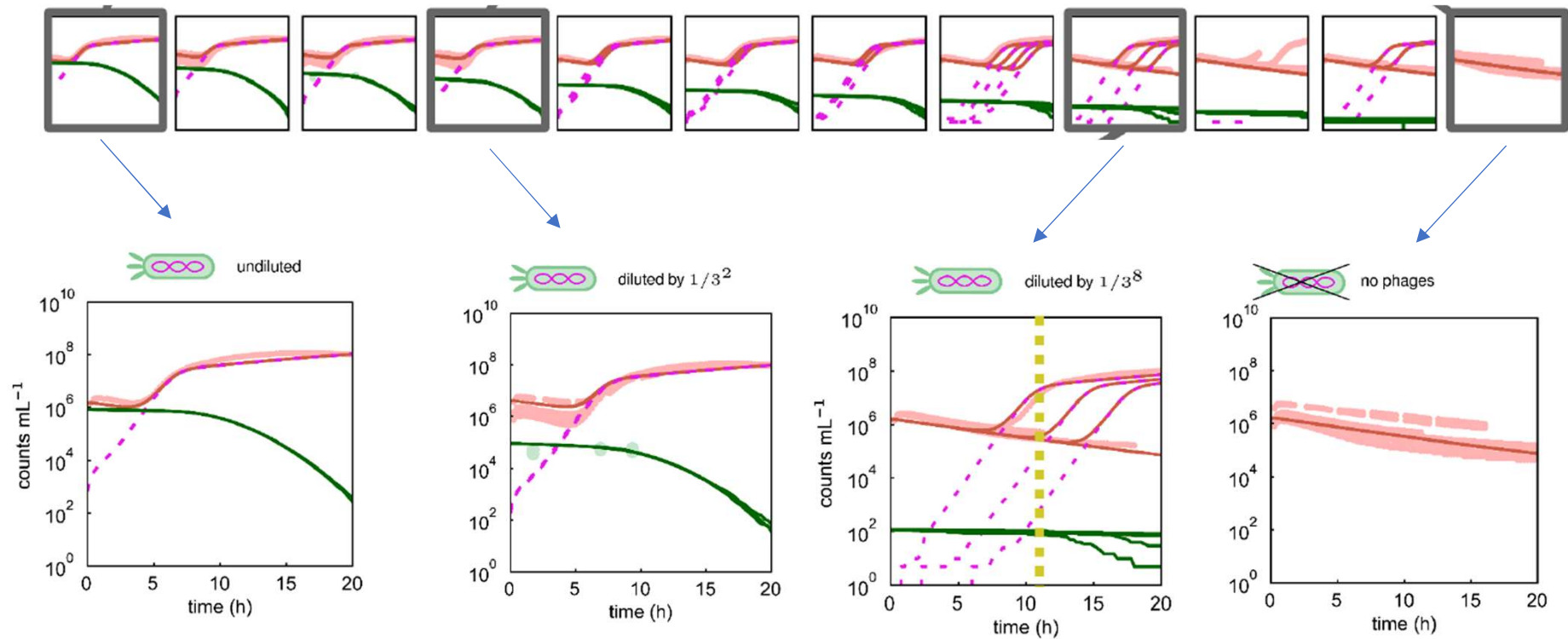
(Pathania *et al.*, In preparation.)

Modelling infection rates

- Deterministic vs Stochastic regimes (N=4)

Legend:
- rcv (sim.)
- rcv infected (sim.)
- ϕ (sim.)
- rcv (exp.)
- ϕ (exp.)

(Pathania *et al.*, In preparation.)

Modelling infection rates

- Deterministic vs Stochastic regimes (N=4)

rcv (sim.)

rcv infected (sim.)

ϕ (sim.)

rcv (exp.)

ϕ (exp.)
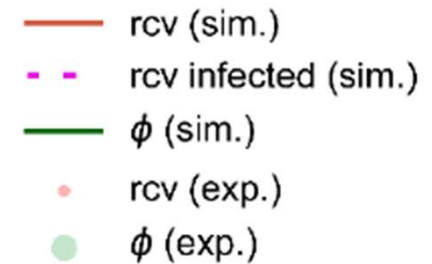
(Pathania *et al.*, In preparation.)

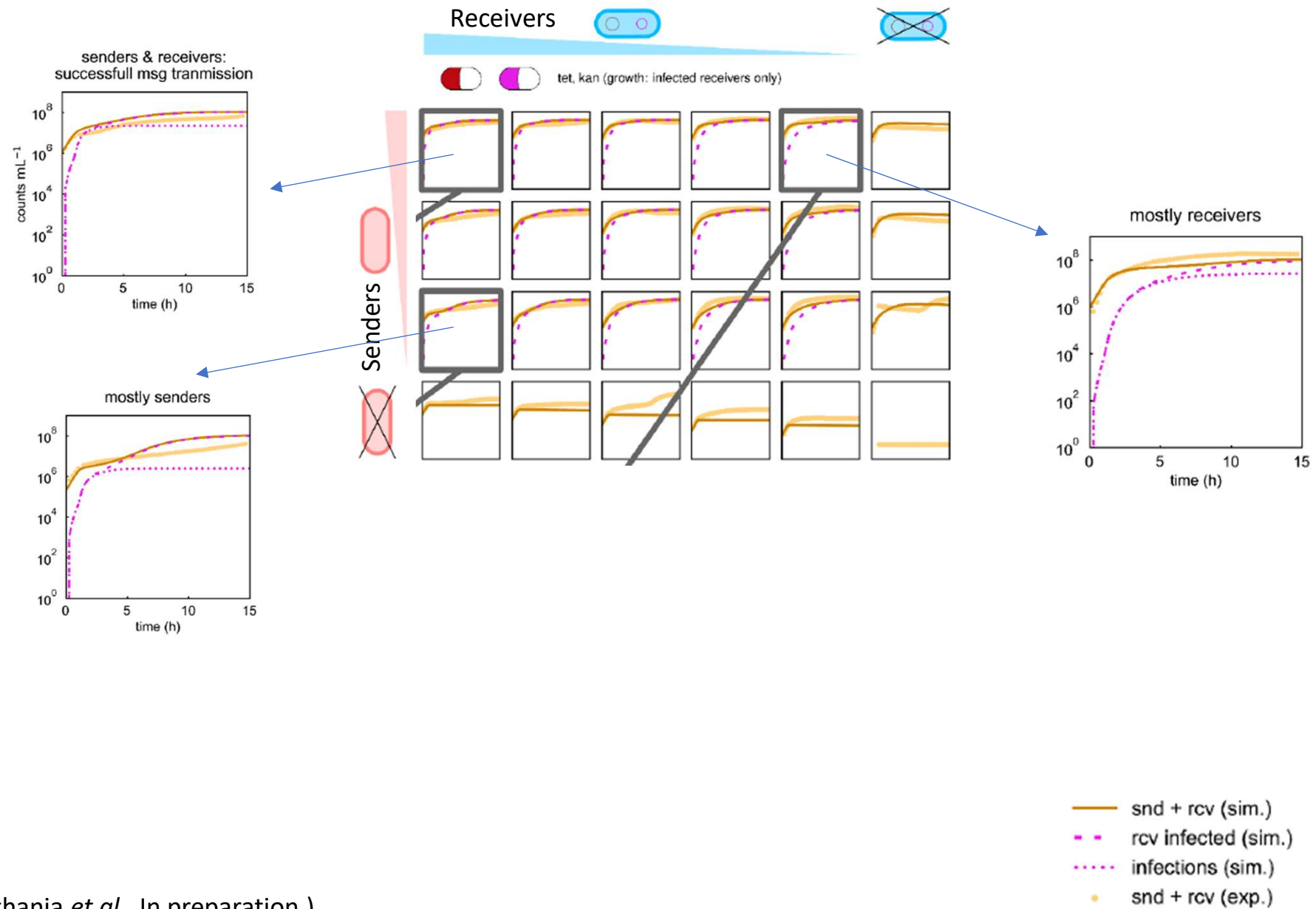# Modelling communication rates
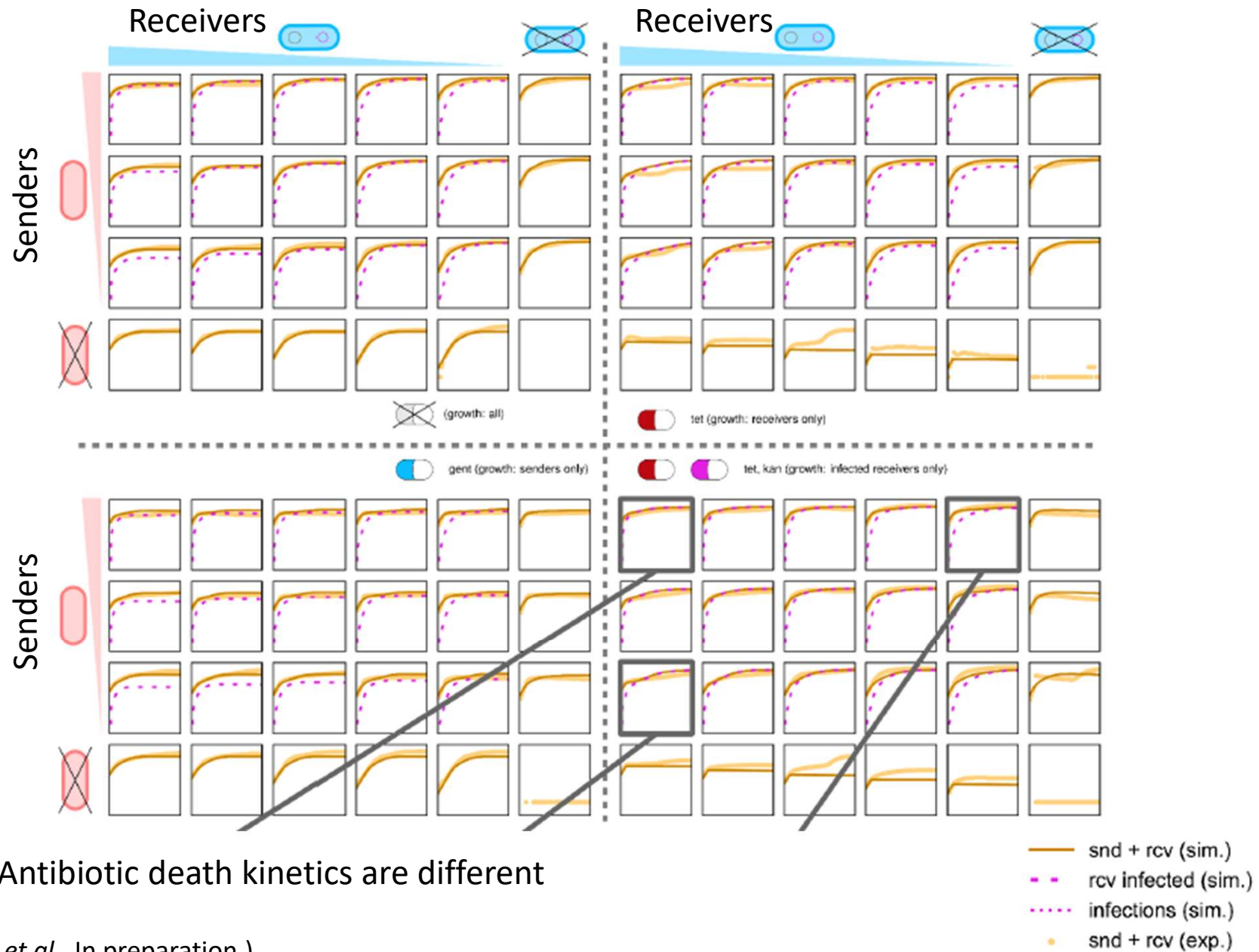
- Antibiotic death kinetics are different

(Pathania *et al.*, In preparation.)

Modelling communication rates

(Pathania *et al.*, In preparation.)

Acknowldegements

Team BioRetrosynth

Amir Pandi
Amit Pathania
Corbin Hopper
Roman Luchko
Tom Zaplana
Hadi Jbara

Matthias Fuegger
Thomas Nowak
Da-Jung Cho
Quentin Soubeyran