

# Image Segmentation and Modelling of Host-Pathogen Dynamics of *Salmonella*

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Thesis Defense

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# Outline

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## **1. Introduction**

- Host-pathogen interactions
- Host-defense and antibiotics
- Pathogen lifestyles
- Bacterial decision making
- Salmonella enterica*

## **2. Image Segmentation and Hyper-Replication**

- Timelapse fluorescence microscopy
- Image segmentation and growth analysis
- Identifying hyper-replication
- HR fraction analysis
- Implications

## **3. Mean Field Model of Invasion**

- Stages of invasion
- Model variables and parameters
- Dynamical equations
- Parameterization
- Applications

## **4. Conclusions and Outlook**

# Host-pathogen interactions

**Pathogens** are infectious agents that cause disease in **hosts**.

Population-level (spread of Zika virus)

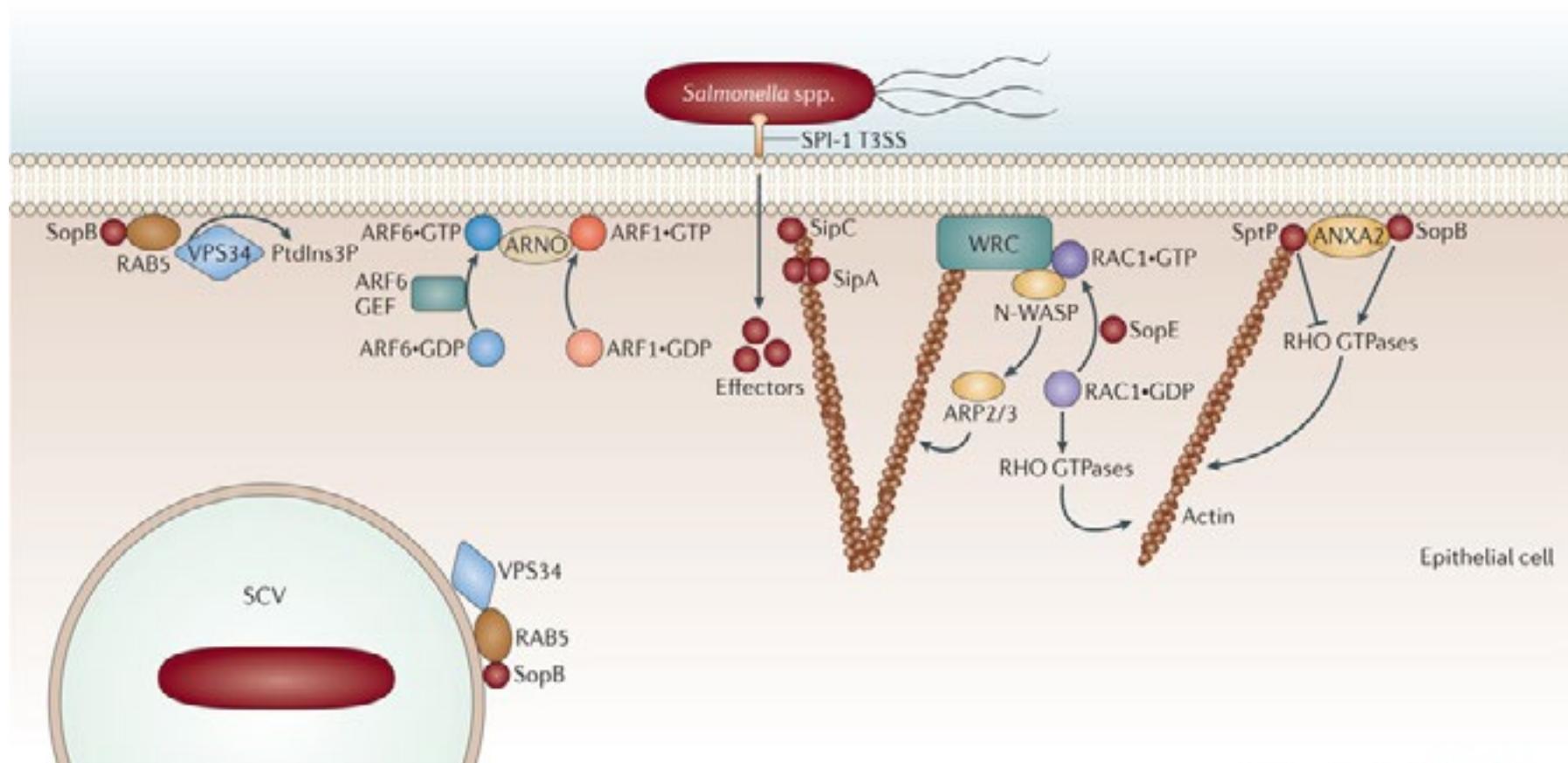


Peterson et al. *New England Journal of Medicine* (2016).

# Host-pathogen interactions

**Pathogens** are infectious agents that cause disease in **hosts**.

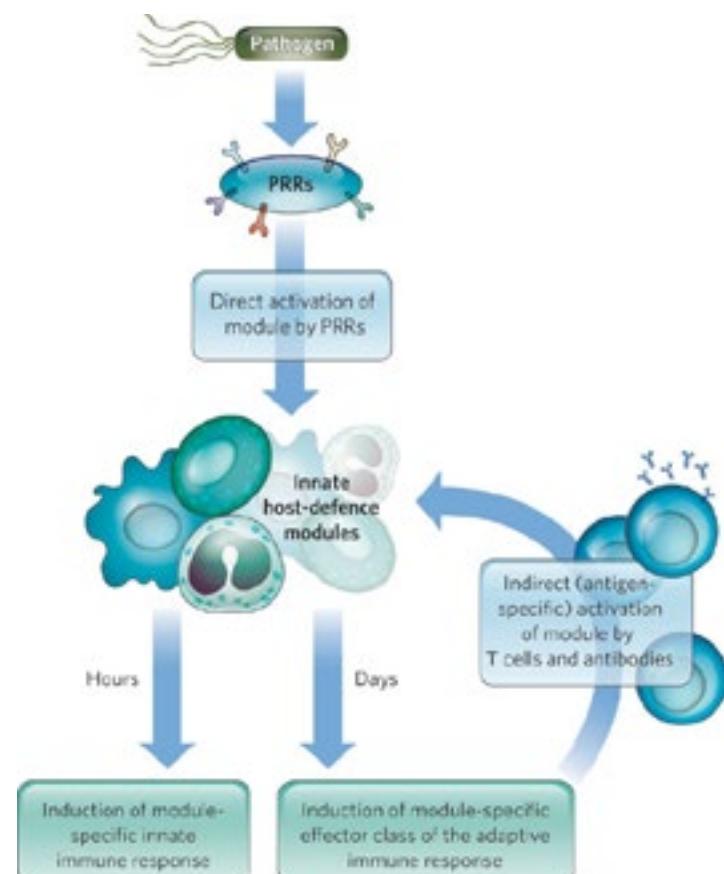
Single-cell and molecular-level (*Salmonella* invading epithelial cell)



LaRock et al. *Nature Reviews Microbiology* (2015).

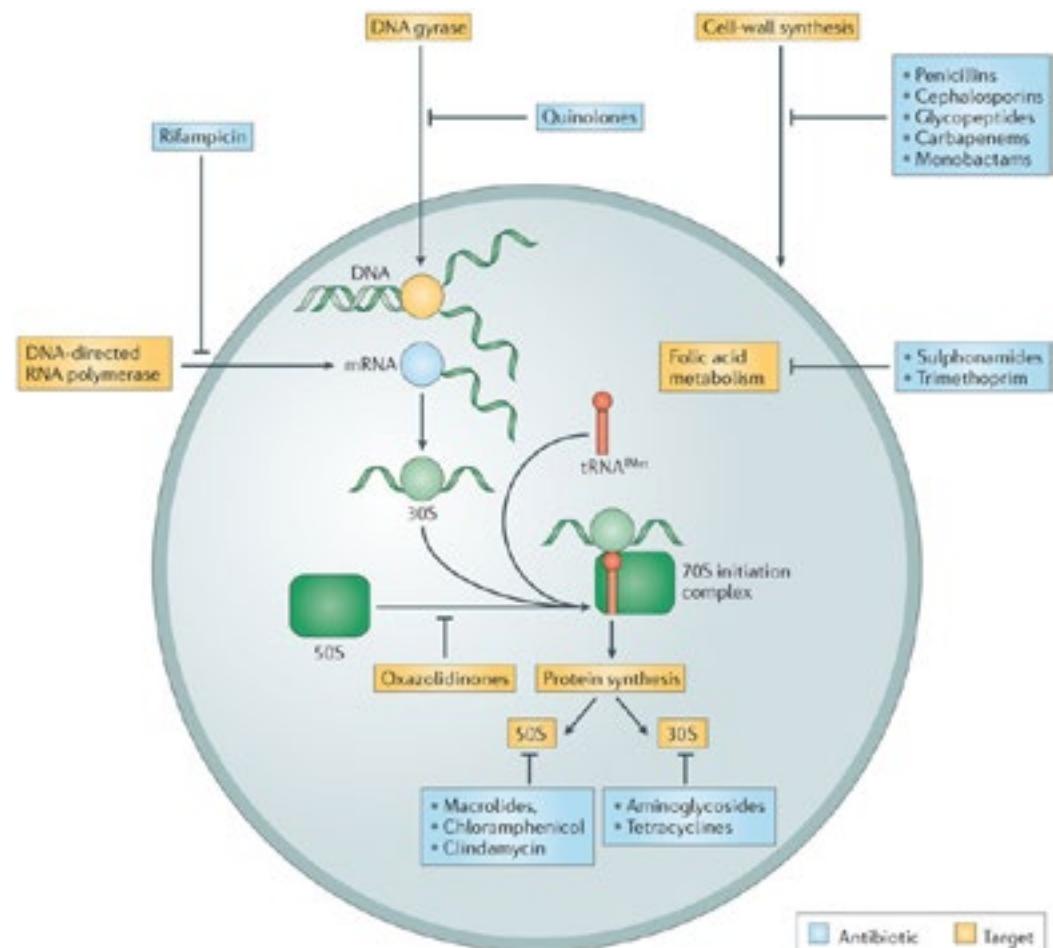
# Host defense and antibiotics

## Innate and adaptive immune system



Medzhitov. *Nature* (2007).

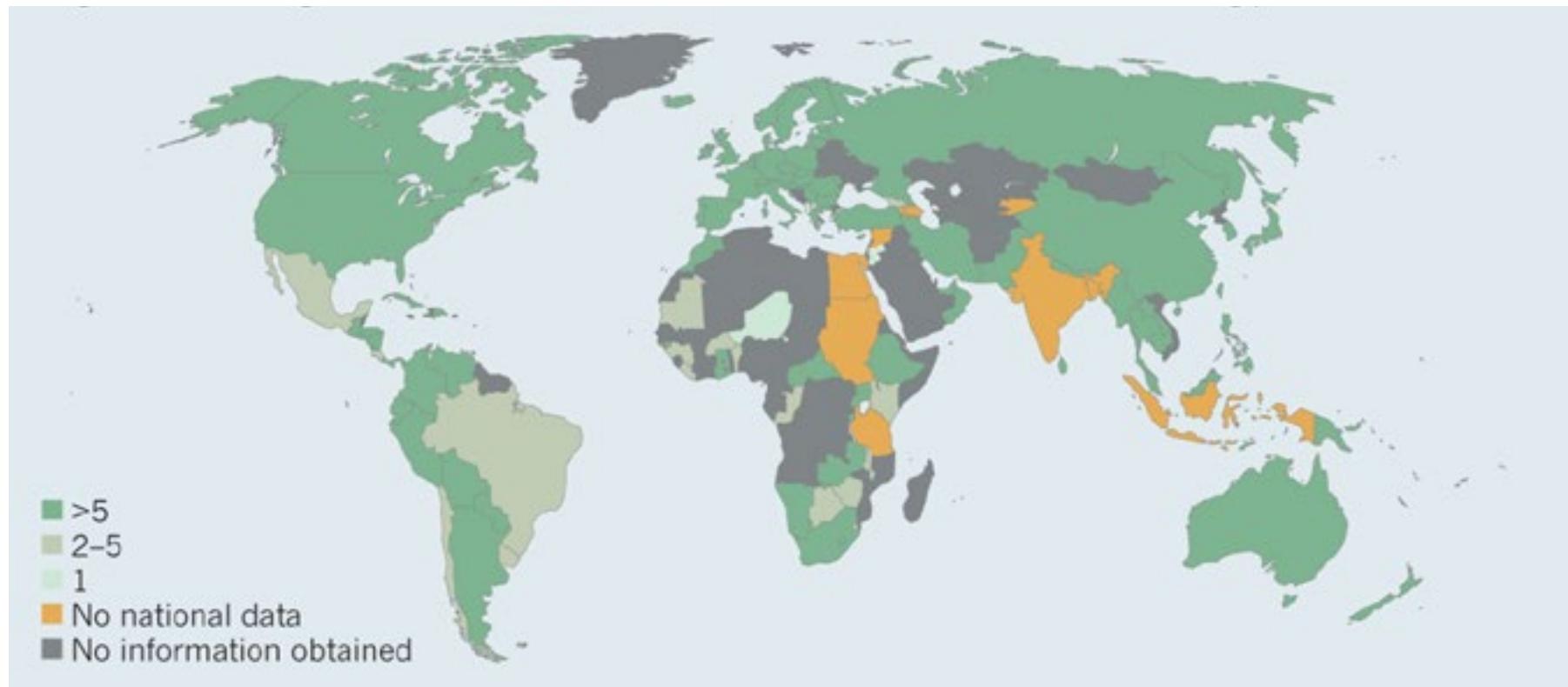
## Targets of antibiotics



Kim. *Nature Reviews Drugs Discovery* (2013).

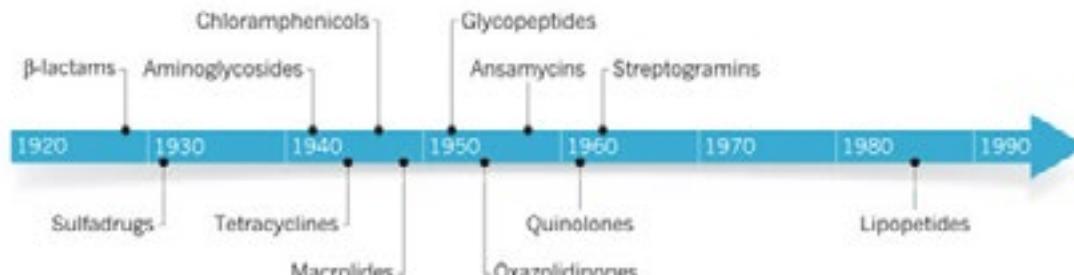
# Antibiotic resistance

Resistant strains of deadly bacteria are a growing threat worldwide.



Reardon. *Nature* (2014).

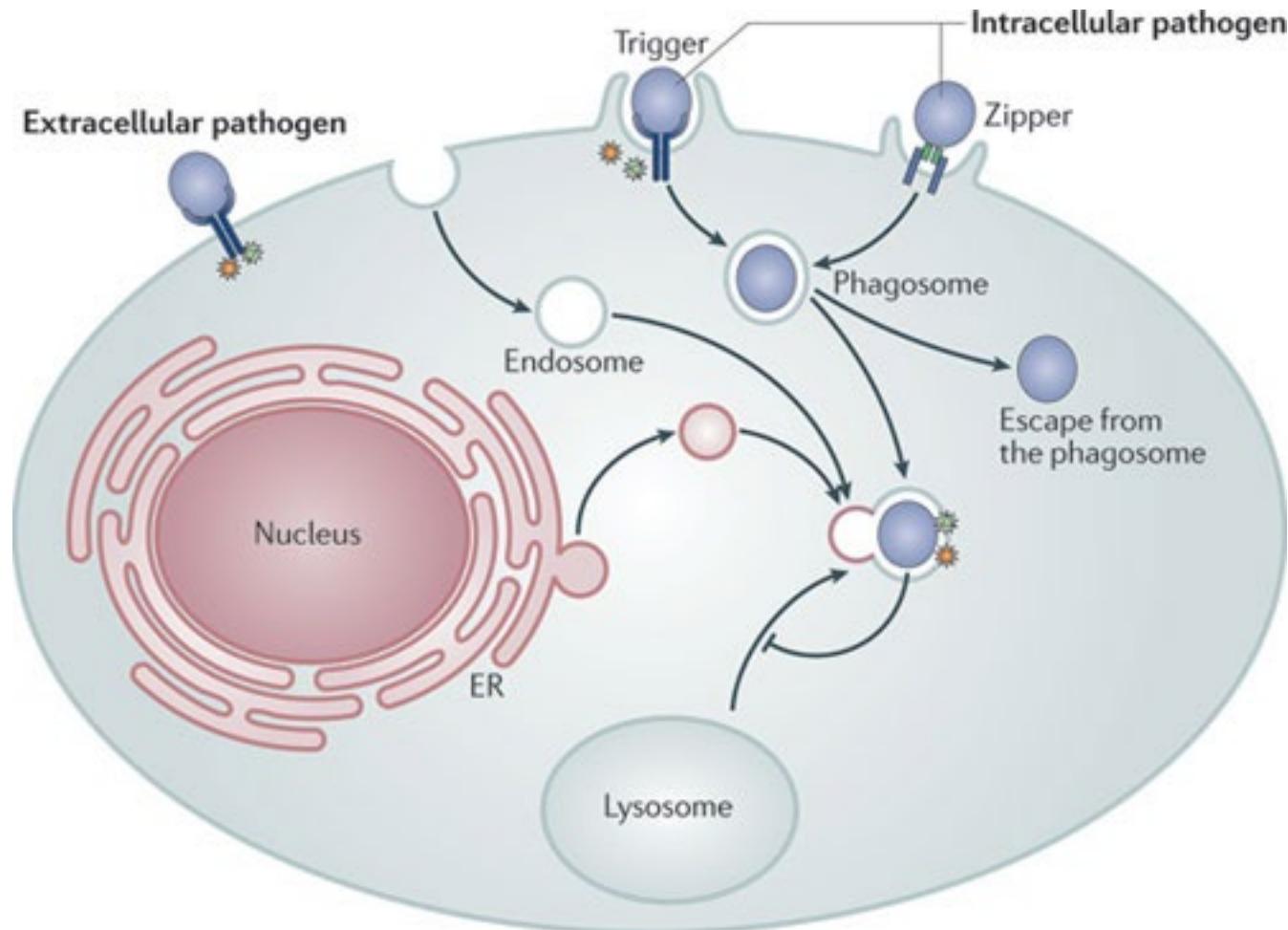
## Antibiotic discovery timeline.



Lewis. *Nature* (2012).

# Lifestyles of pathogens

Pathogens are categorized as **extracellular** or **intracellular**.



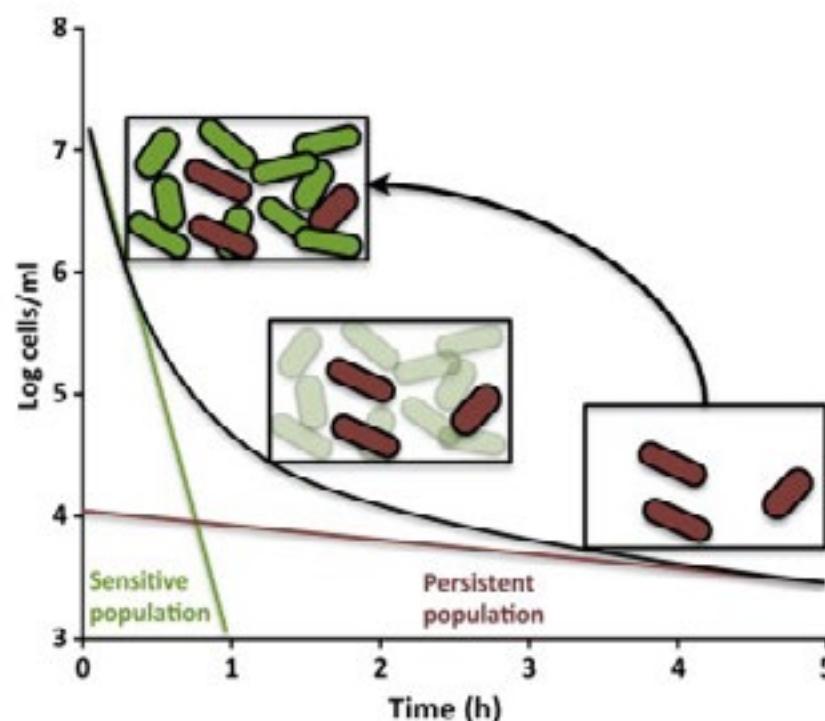
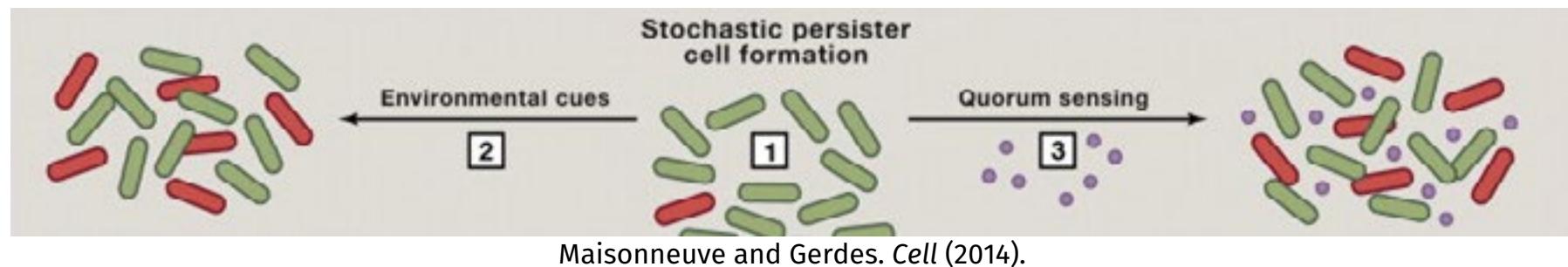
Ham et al. *Nature Reviews Microbiology* (2011).

Intracellular pathogens are further divided into **vacuolar** or **cytosolic**.

# Bacterial decision making

Living cells tend to differentiate into specialized subtypes.

Some bacterial species avoid immune response by entering a persister state.

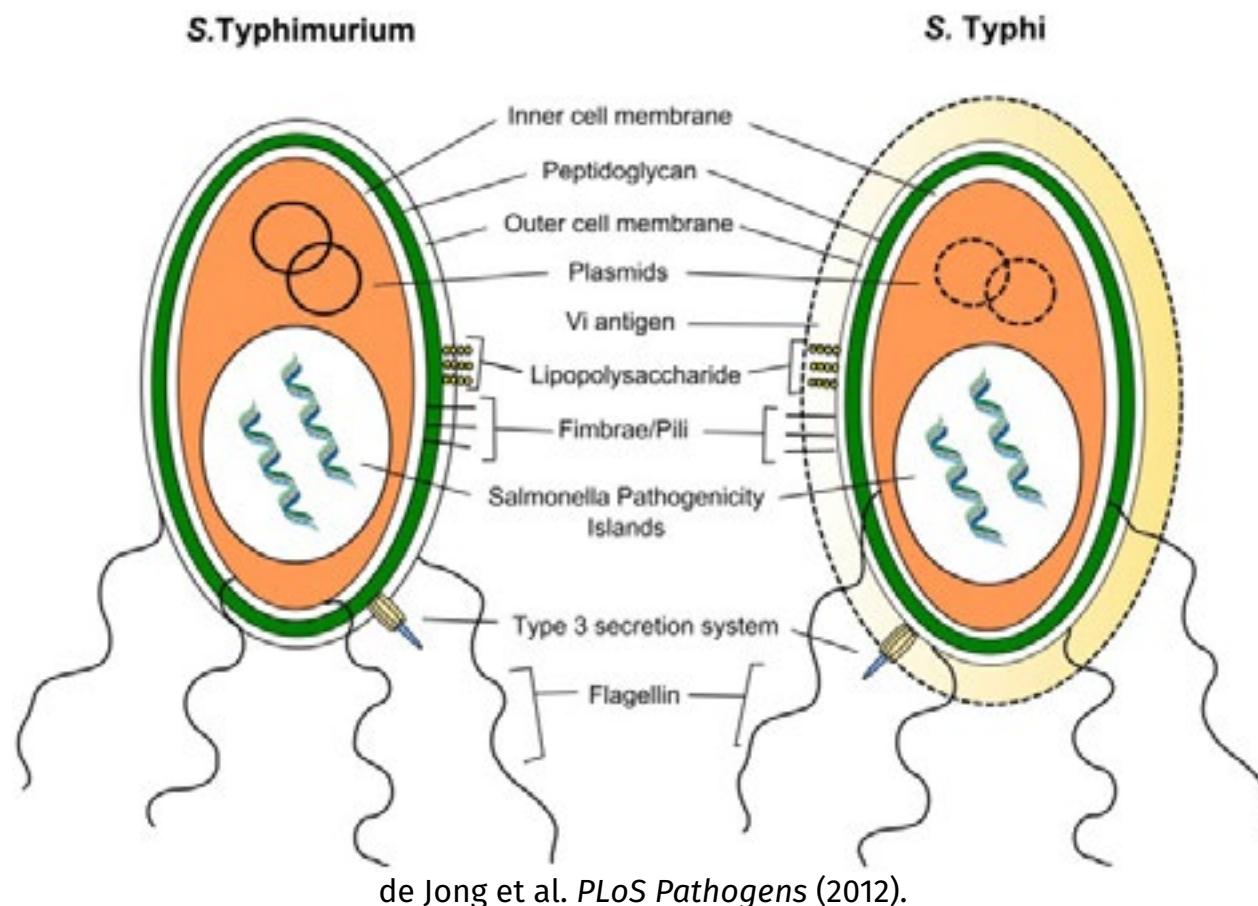


Ayrapetyan et al. *Trends in Microbiology* (2015).

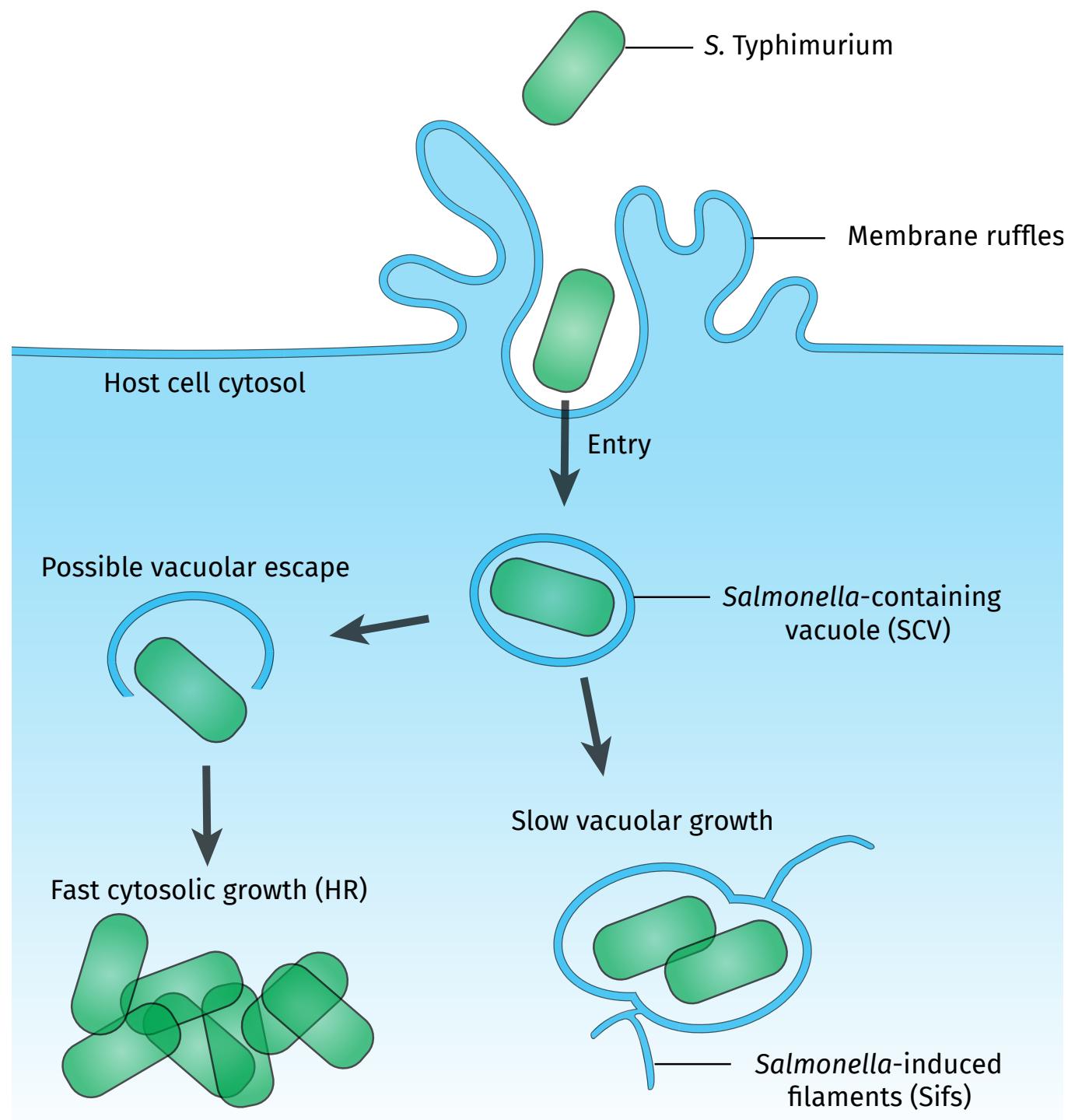
# *Salmonella enterica*

Generally classified **typhoidal** (*S. Typhi*) or **non-typhoidal** (many serovars, most common is *S. Typhimurium*).

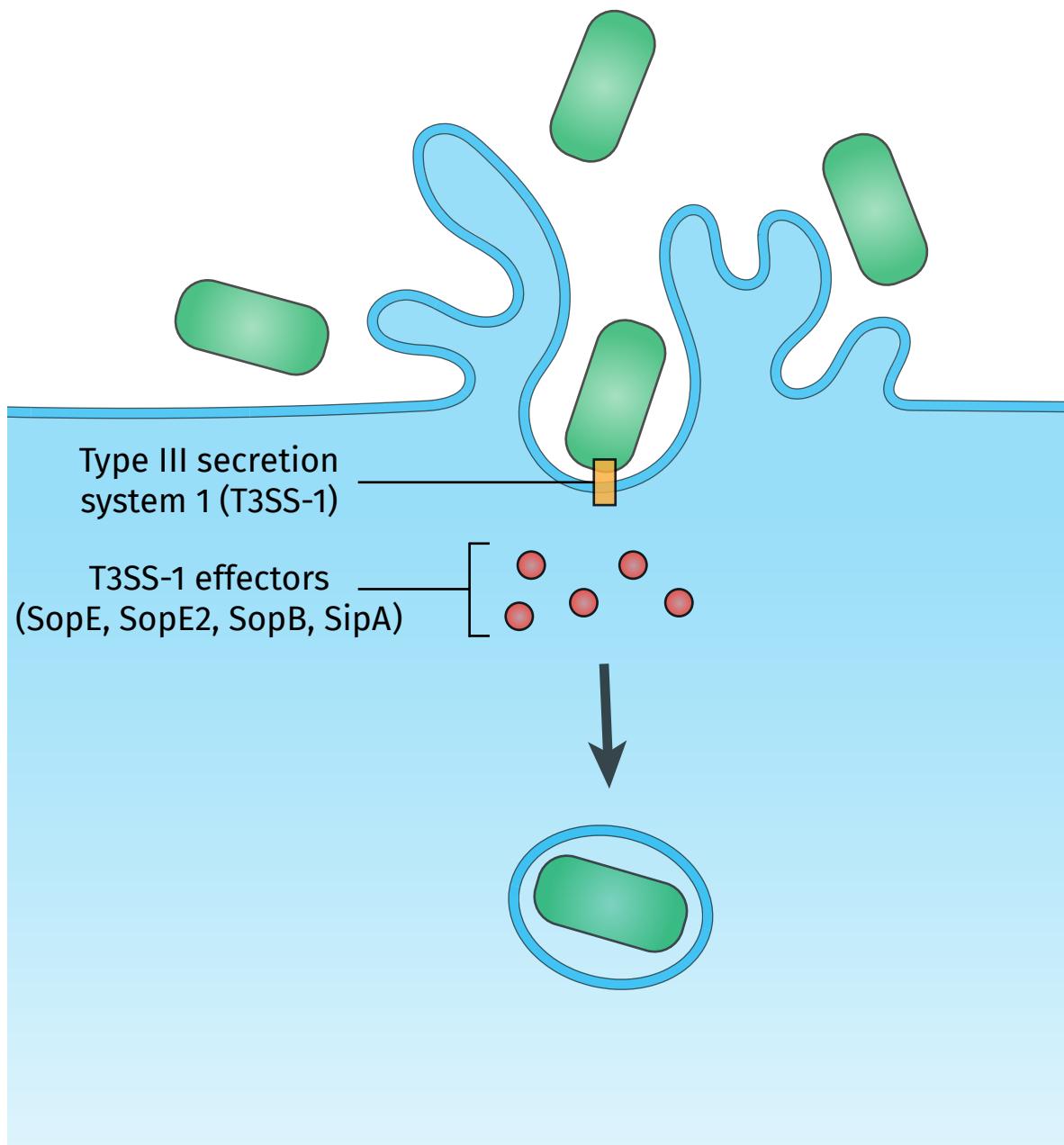
Hundreds of thousands of deaths each year attributed to **typhoid fever** and **enterocolitis**.



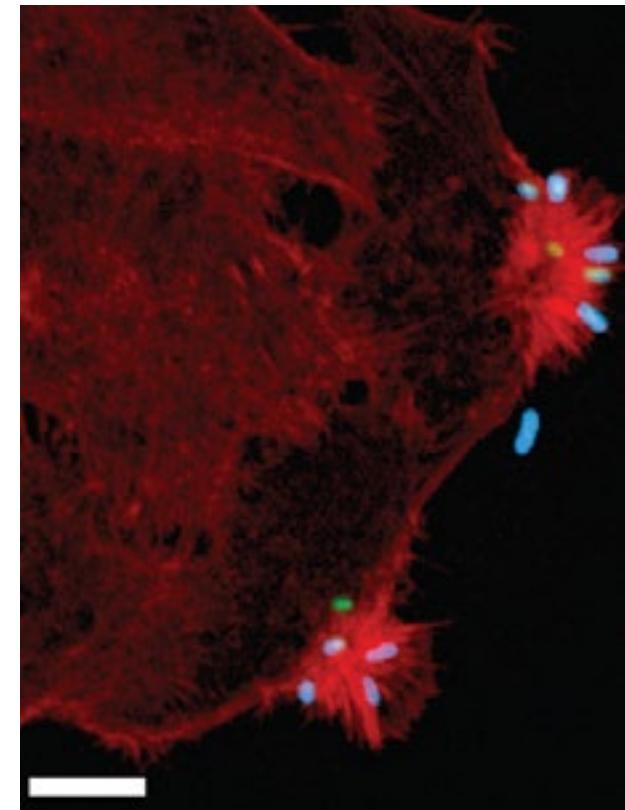
# *S. Typhimurium* invasion



# Cooperative invasion via ruffles

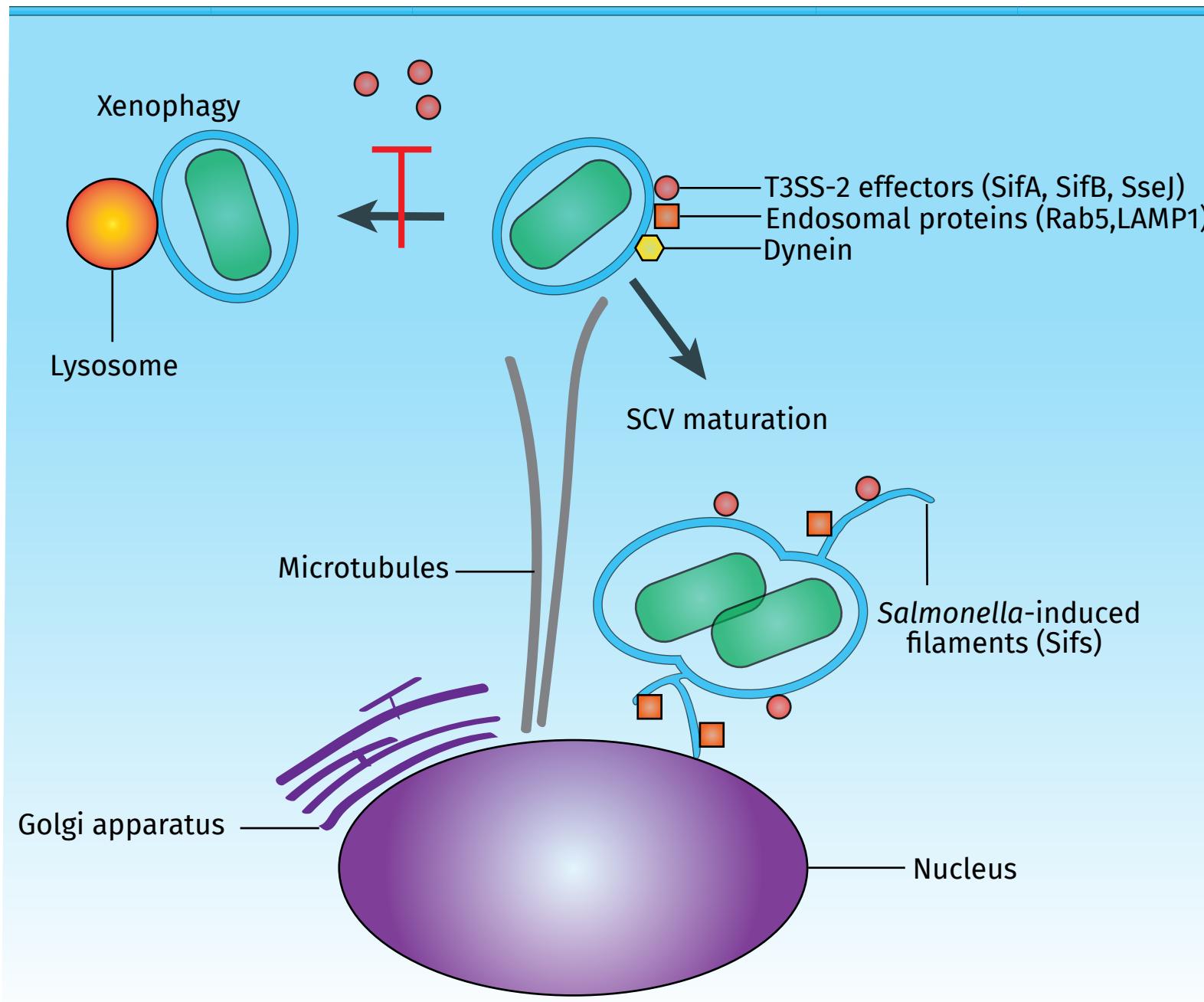


Misselwitz et al. *PLoS Pathogens* (2012).

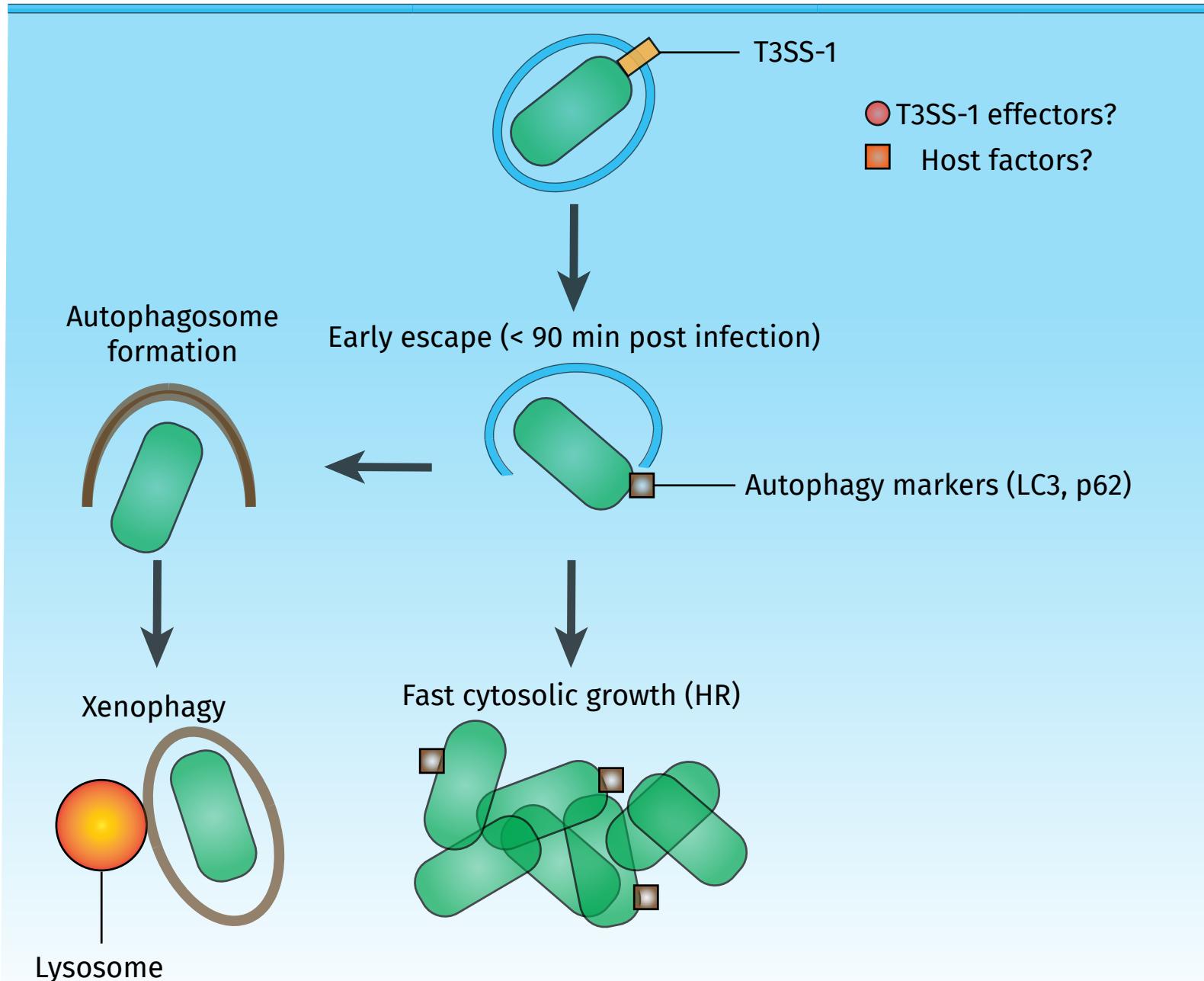


6 minutes post infection  
**Salmonella inside**  
**Salmonella outside**  
**HeLa cell actin**  
Scale bar: 10  $\mu\text{m}$

# Vacuolar maintenance

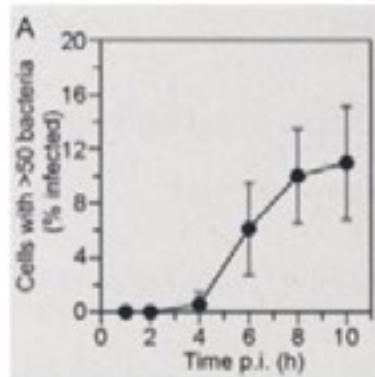
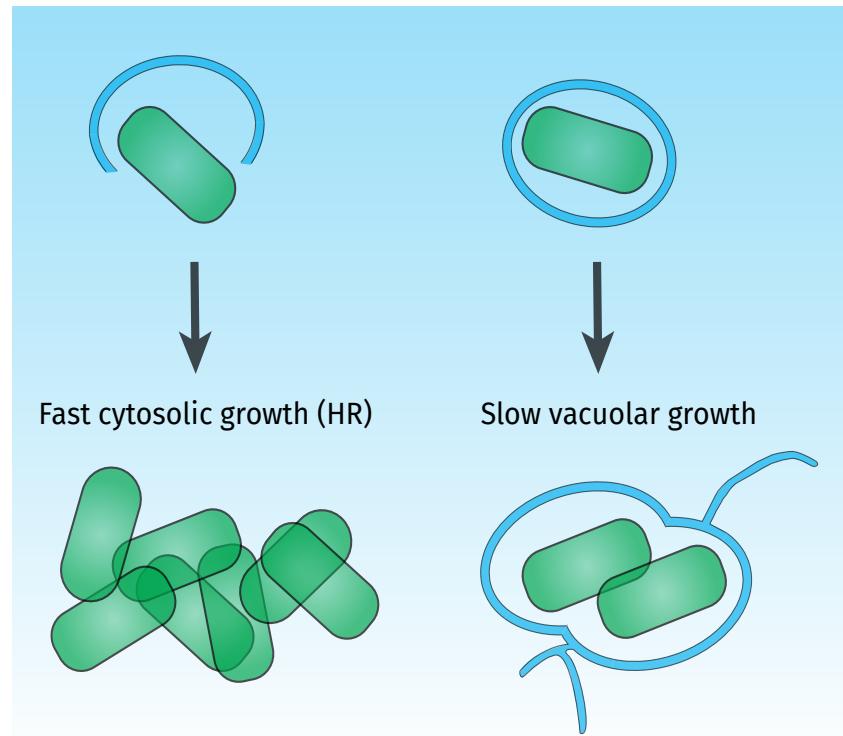


# Vacuolar escape and hyper-replication

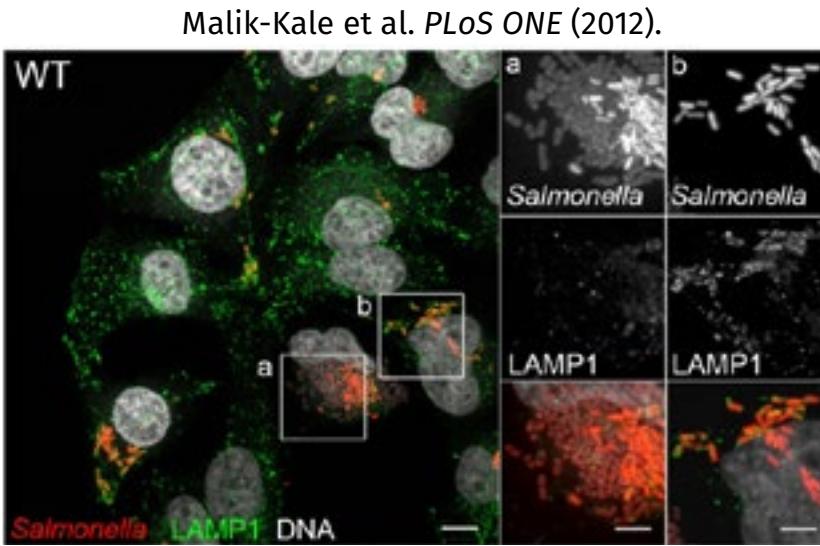


# Vacuolar escape and hyper-replication

Knodler et al. *Proc Natl Acad Sci* (2010).



HR: >50 bacteria/cell  
HR fraction  $\approx$  10%



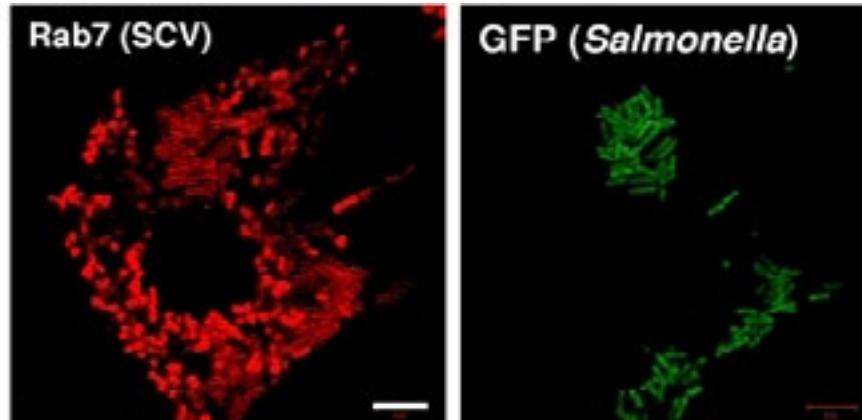
Bacteria  
Vacuole marker

Q: What are the mechanisms involved in hyper-replication?

Q: How does the **HR fraction** (% infected cells with hyper-replicative bacteria) vary with **bacterial load**?

# Independent escape hypothesis

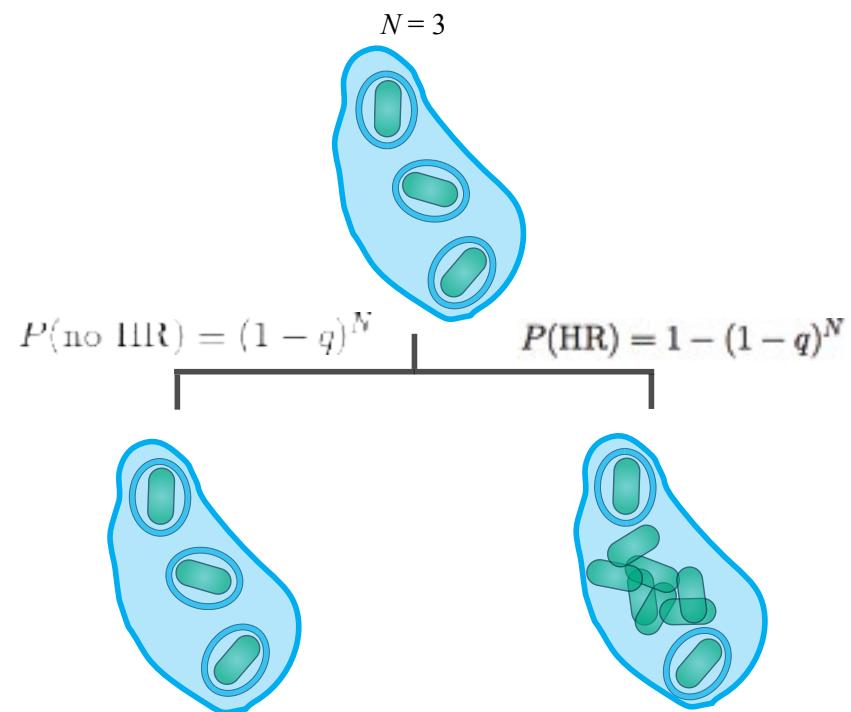
Eswarappa et al. *Infect Immun* (2010).



Vacuole marker

Bacteria

One bacterium per SCV



**Hypothesis:** Each invaded bacterium has an equal probability  $q$  of escaping into the cytosol and hyper-replicating.

e.g.  $q = 10\%$

$$\begin{aligned} P(\geq 1 \text{ HR bacterium}) &= 1 - P(\text{no HR}) = 1 - (1 - q)^N \\ &= 10\% \quad (N = 1) \\ &= 19\% \quad (N = 2) \end{aligned}$$

...

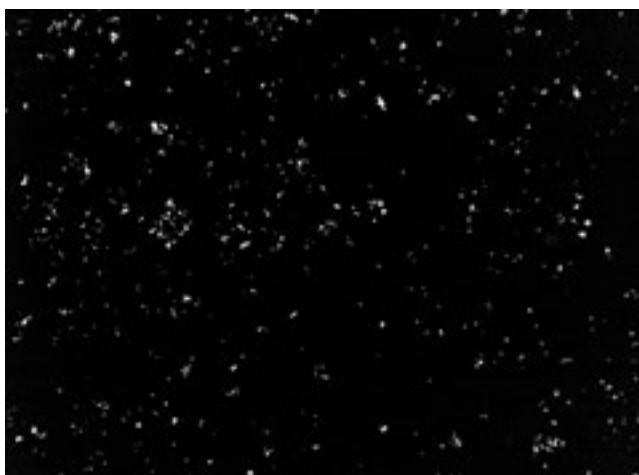
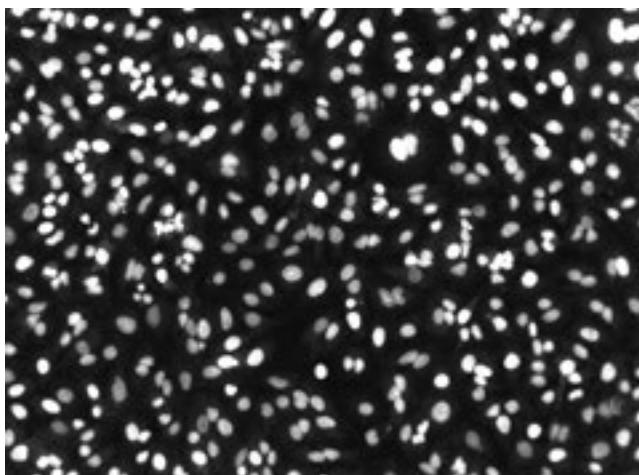
# Timelapse fluorescence microscopy

- Images taken by our experimental collaborator **Jennifer Fredlund**, in the lab of **Jost Eninga** at the **Institut Pasteur**.
- Images taken, at various time points post infection, of **DsRed wildtype *Salmonella Typhimurium*** infecting **Draq5 HeLa** in cell culture plates.

Experiment 1

High confluence, 10x

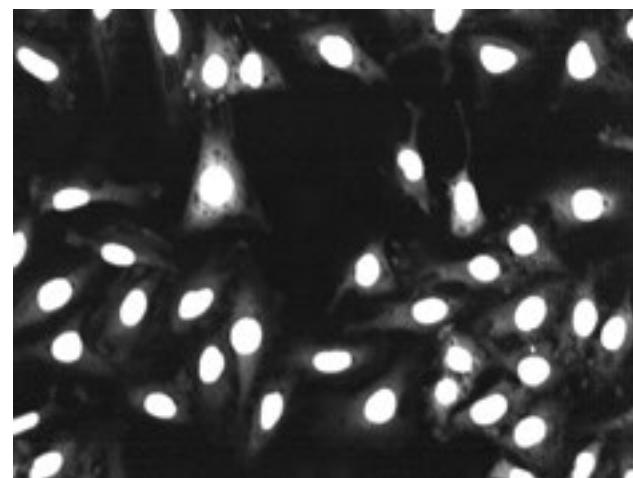
25, 50, 100 MOI



Experiment 2

Low confluence, 40x

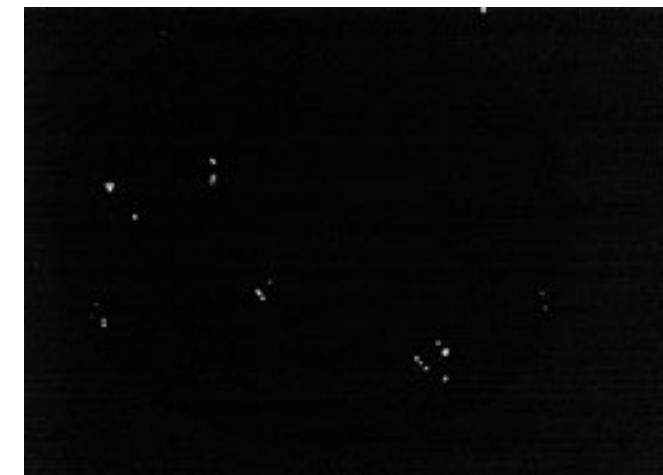
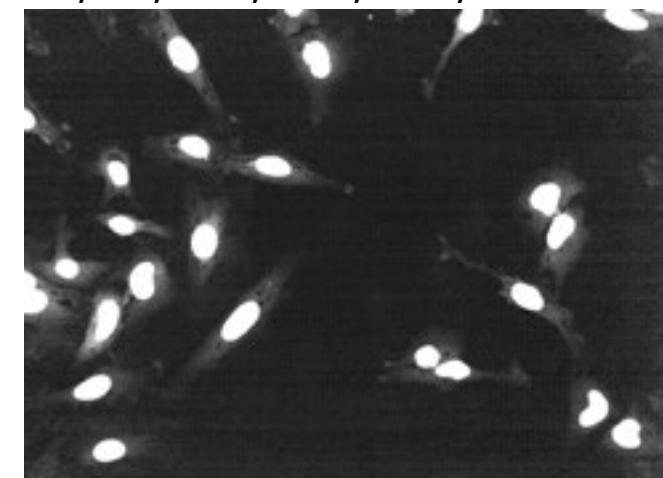
25, 50, 100, 200, 400, 800 MOI



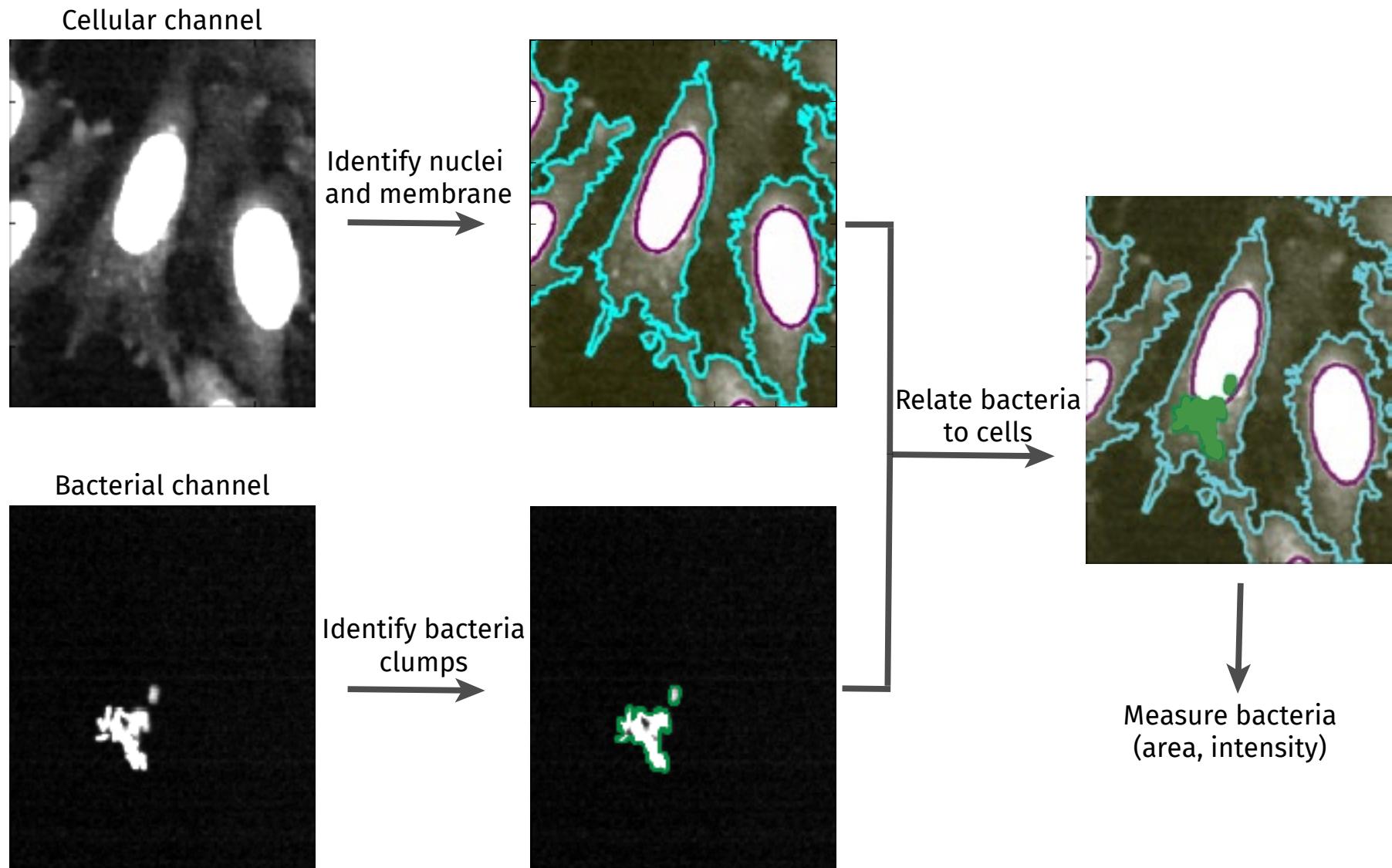
Experiment 3

Lowest confluence, 40x

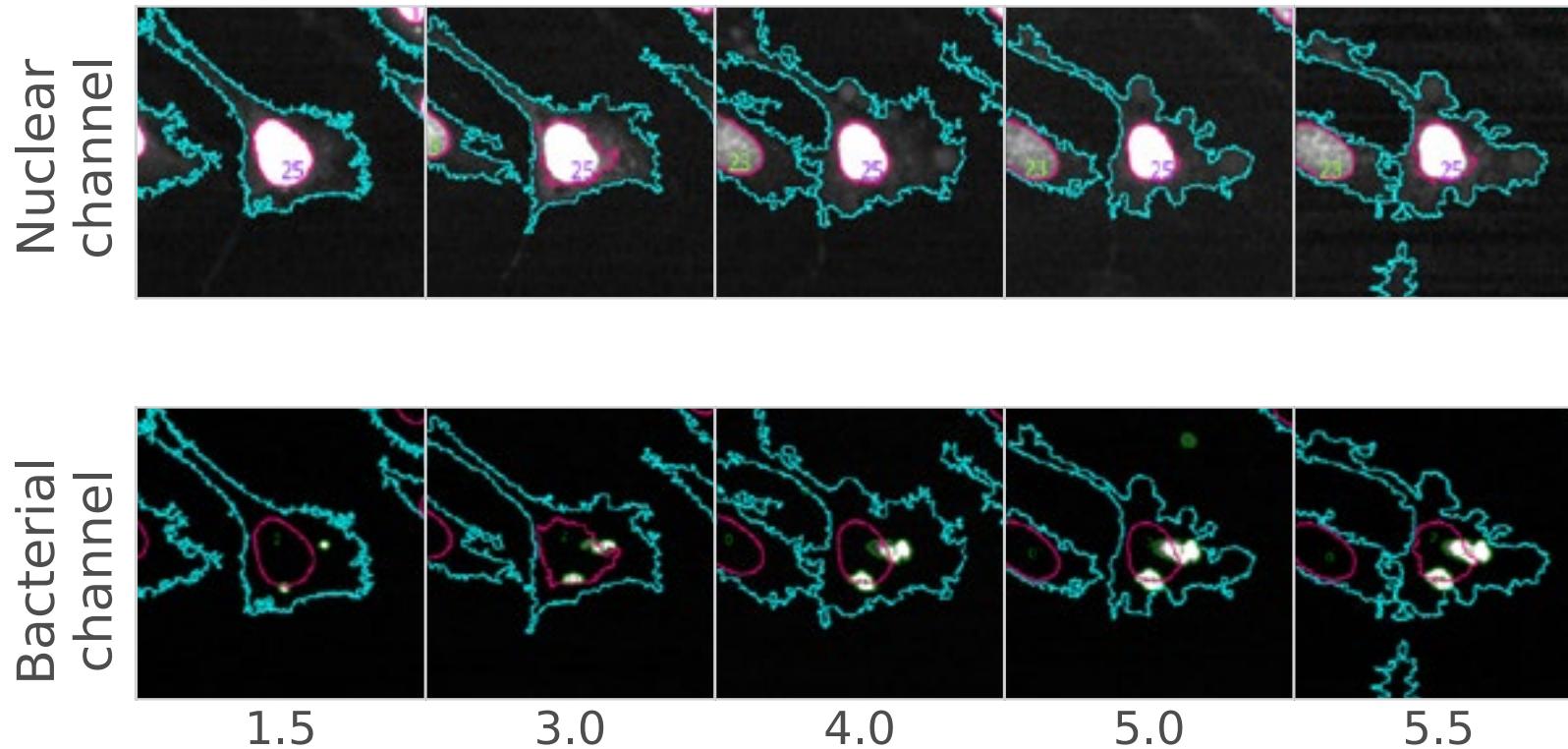
25, 50, 100, 200, 400, 800 MOI



# Image segmentation

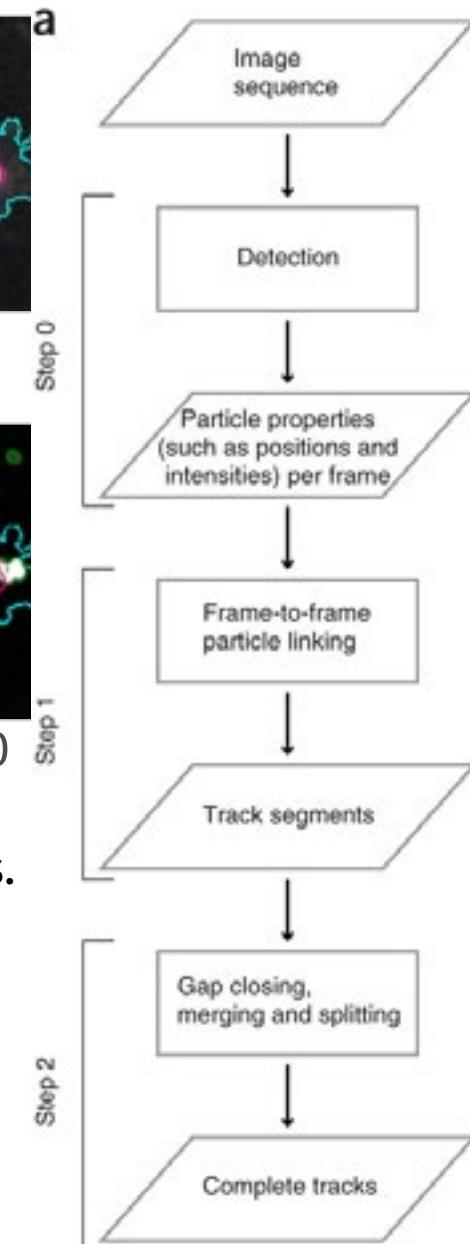
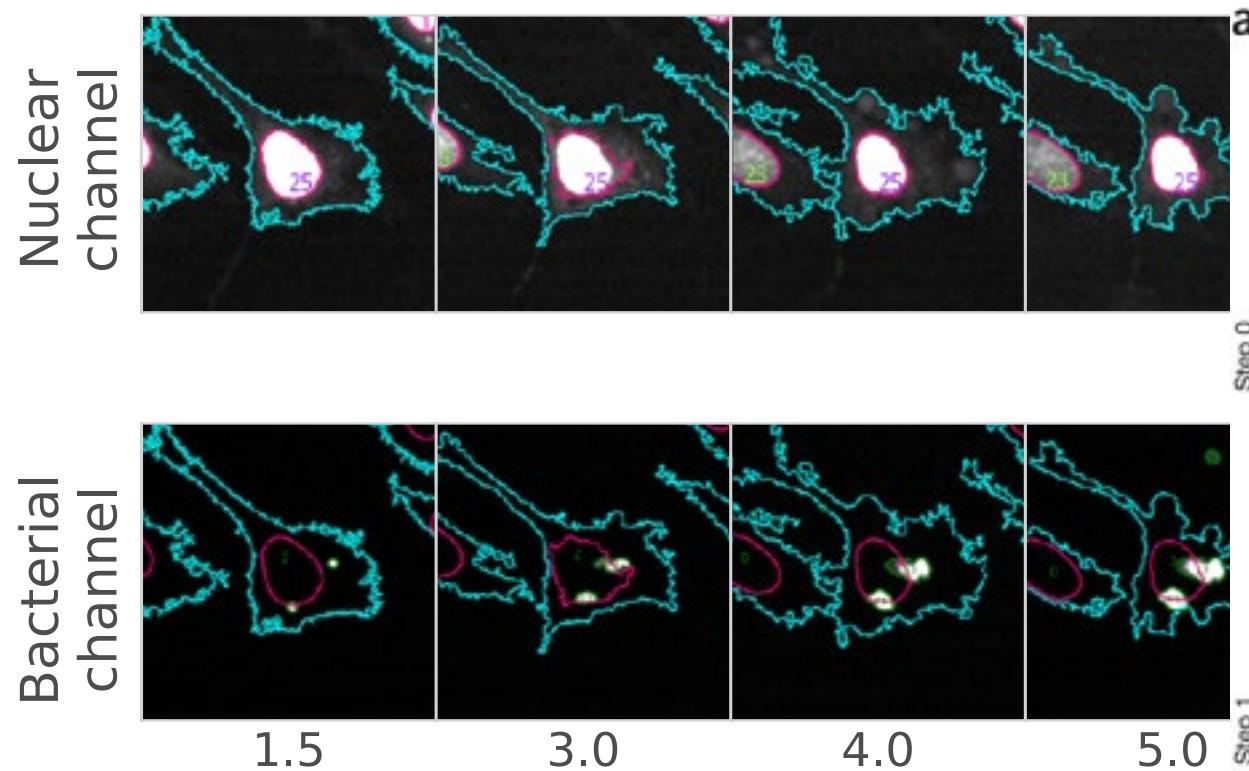


# Tracking cells



- Tracking cell trajectories is essential for growth analysis.
- High confluency, particle motion and object merging/splitting make this difficult.

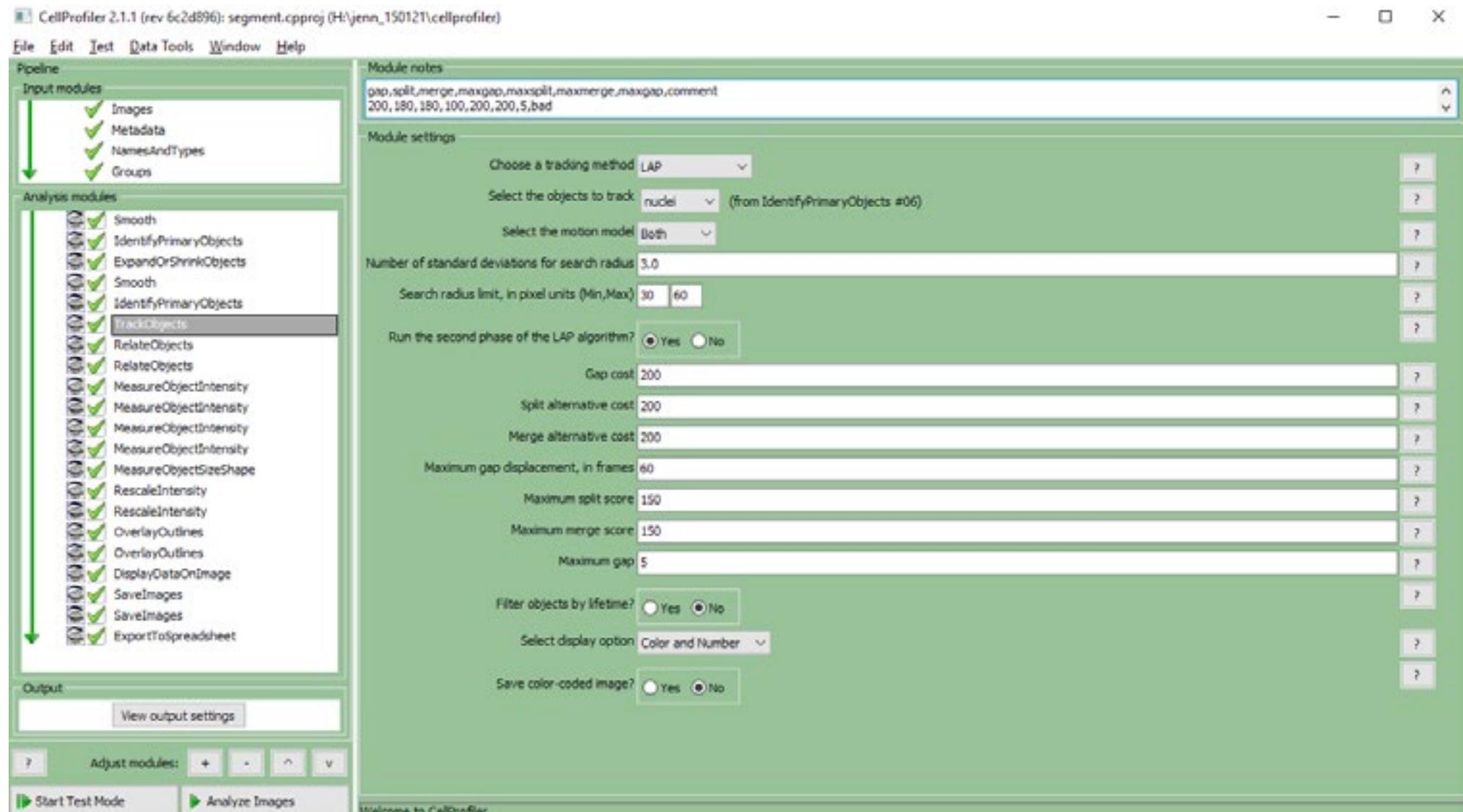
# Tracking cells



- Tracking cell trajectories is essential for growth analysis.
- High confluence, particle motion and object merging/splitting make this difficult.
- The **LAP (two-pass) method**, from the TrackObjects module of CellProfiler was used.
- Solves a global optimization problem to identify the most likely set of particle trajectories.

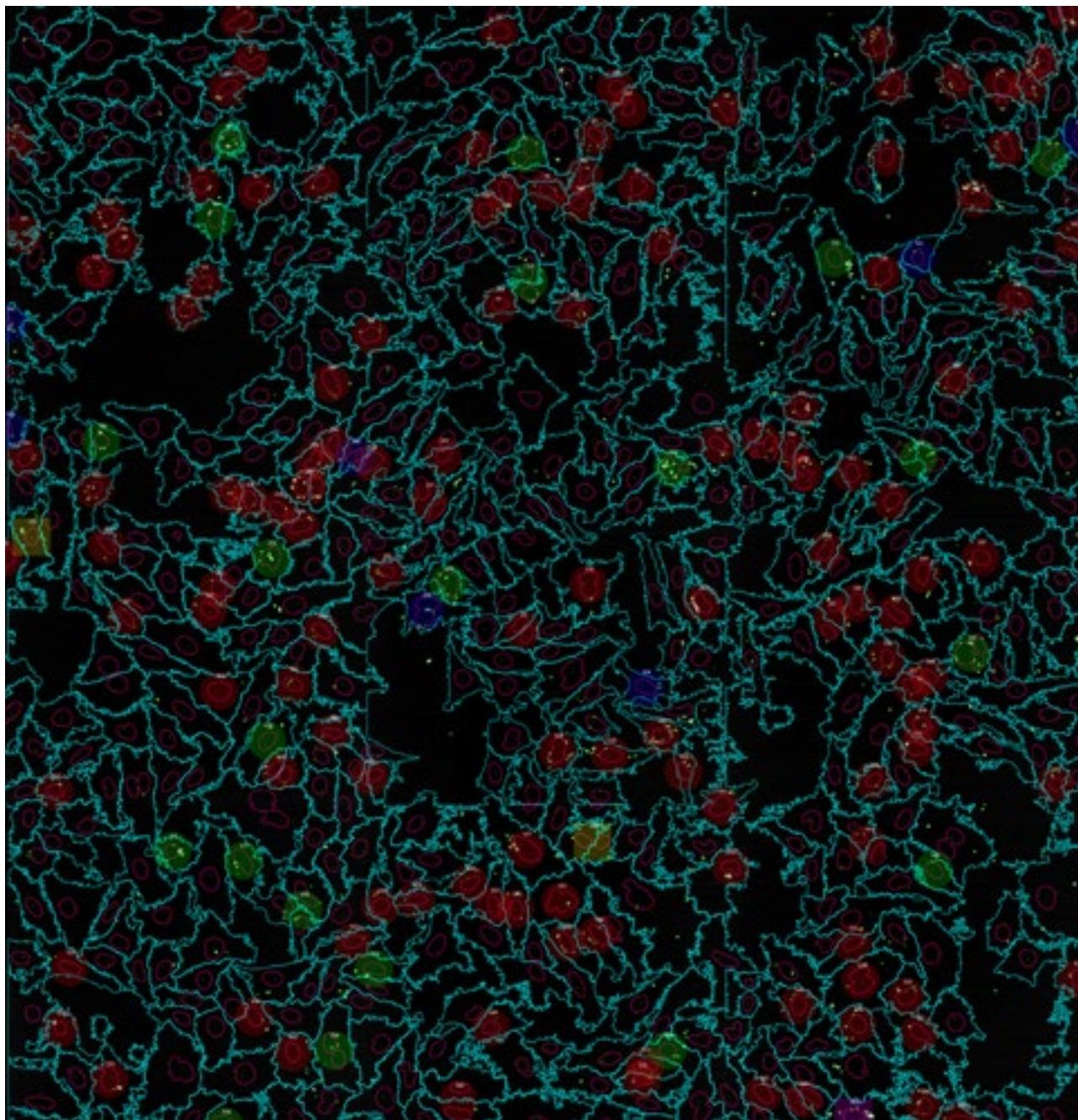
Jaqaman et al. *Nature Methods* (2008).

# Tracking cells

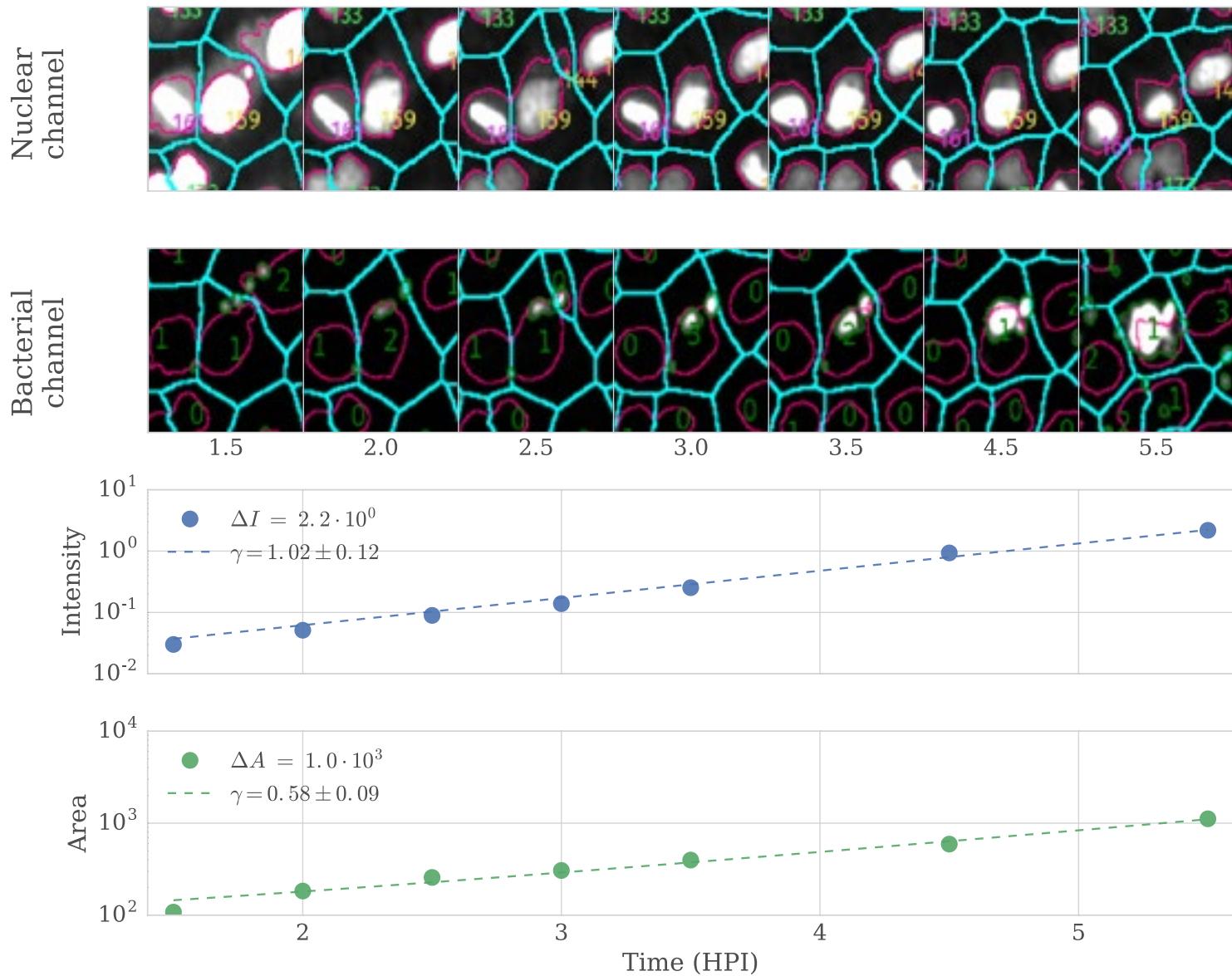


# Tracking cells

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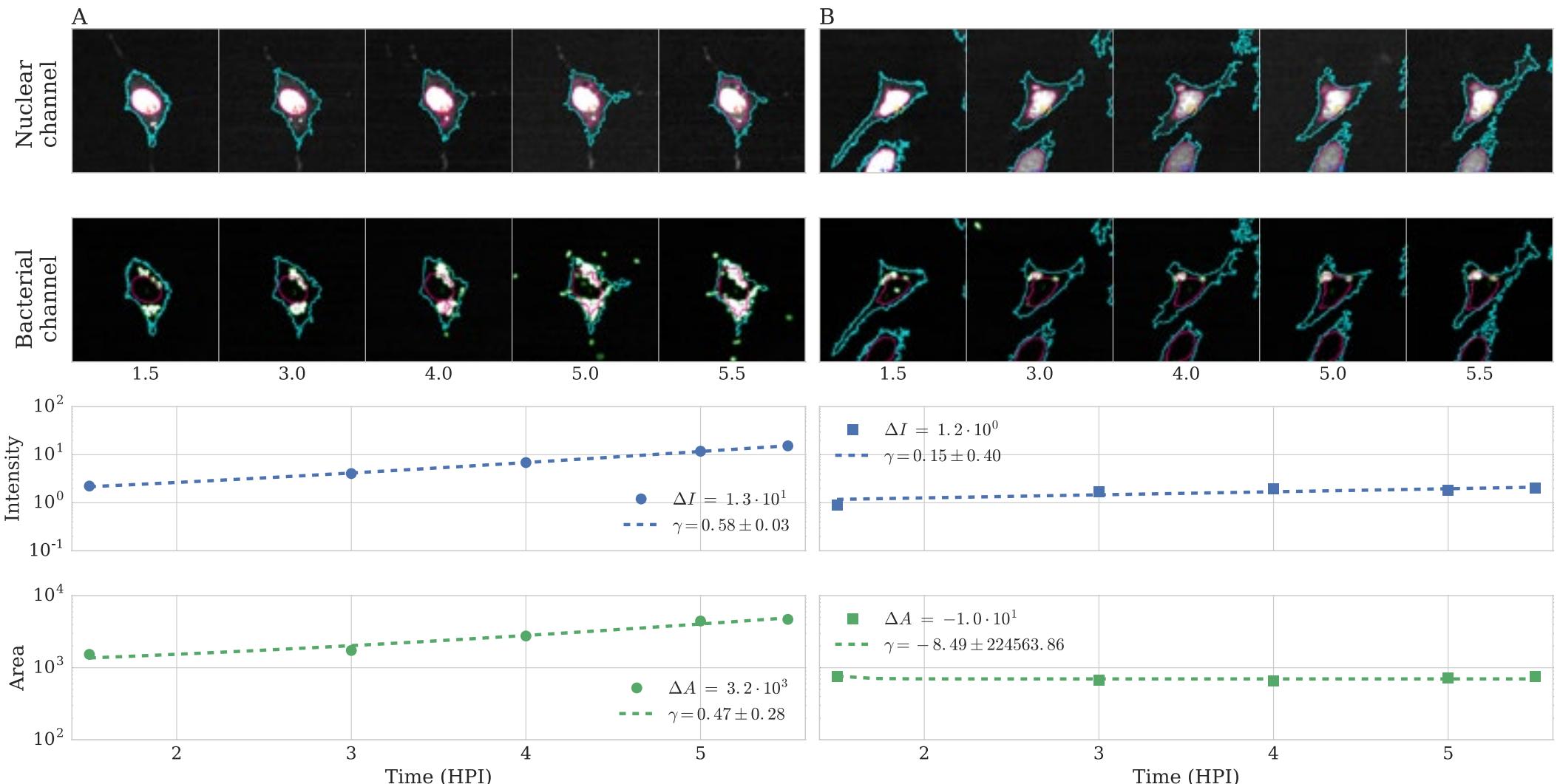


# Single-cell analysis



- Measure **net intensity growth**  $\Delta I$  and **net area growth**  $\Delta A$ .
- Fit to exponential growth (plus background) to get **growth exponent**  $\gamma$ .
- Filter out poor fits with  $\gamma < \Delta \gamma$

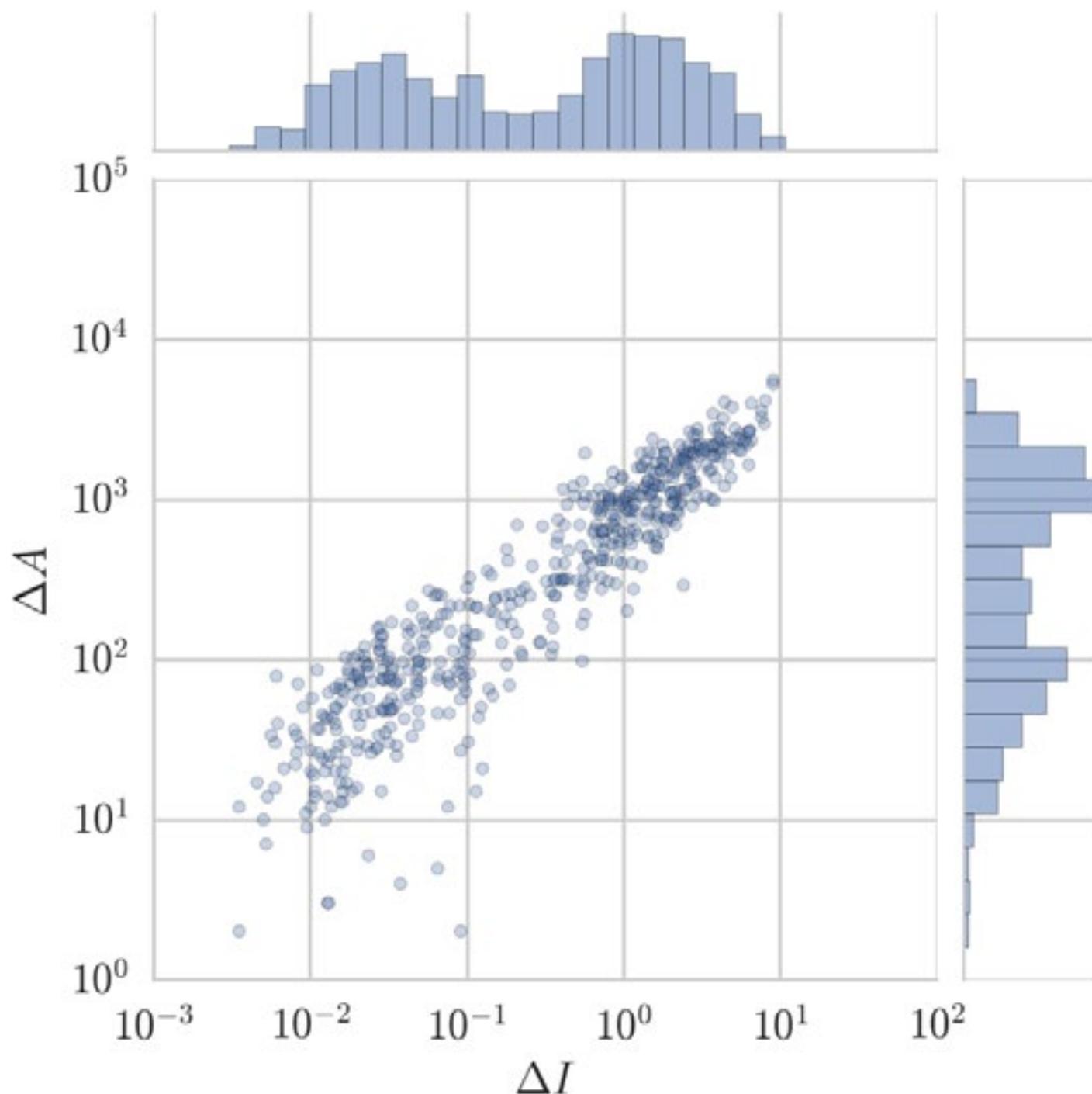
# Single-cell analysis



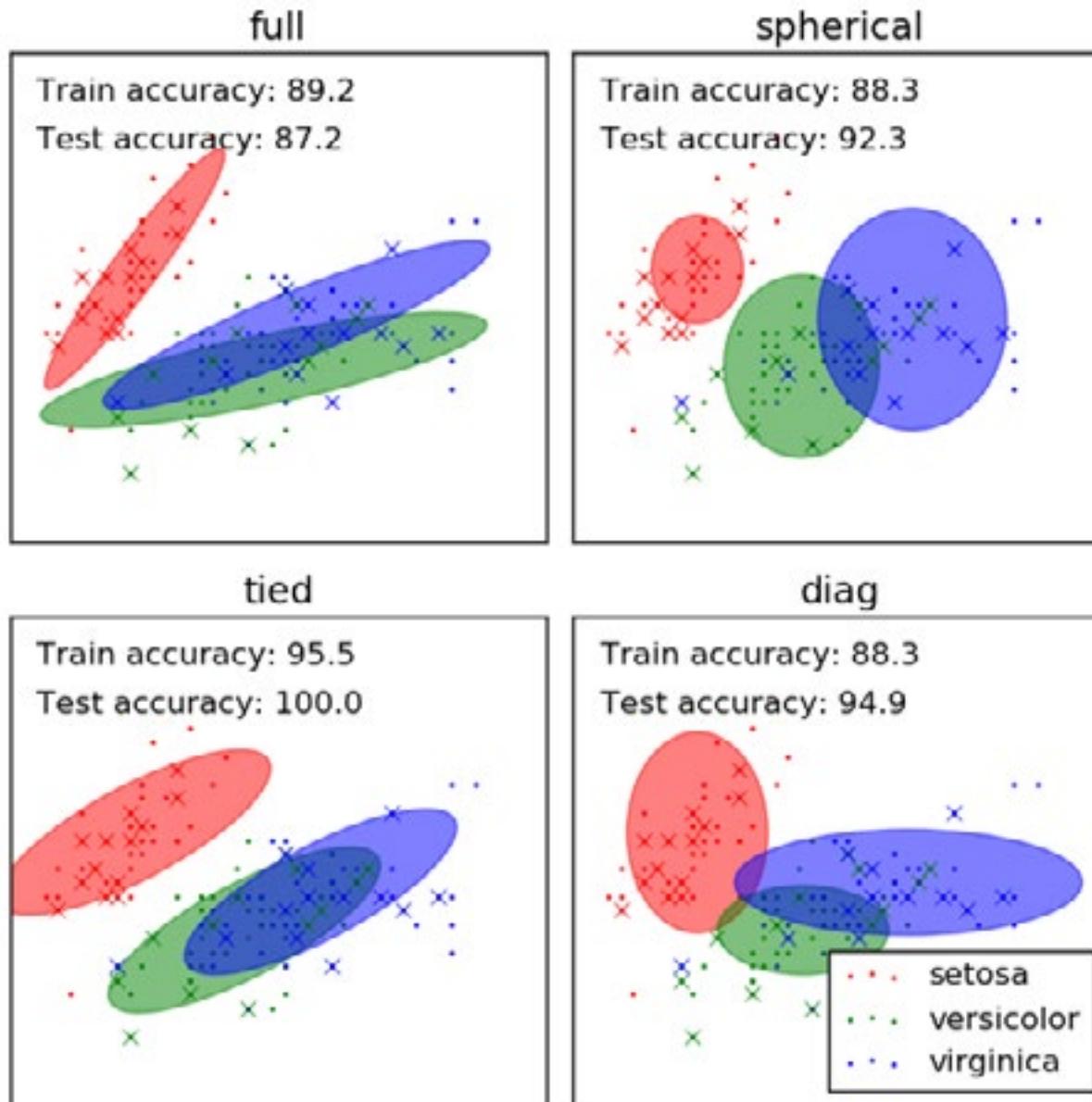
- Fast vs slow growth can be identified by eye in many cases.
- Identification should ideally be unbiased and automatic.

# Bacterial growth distributions

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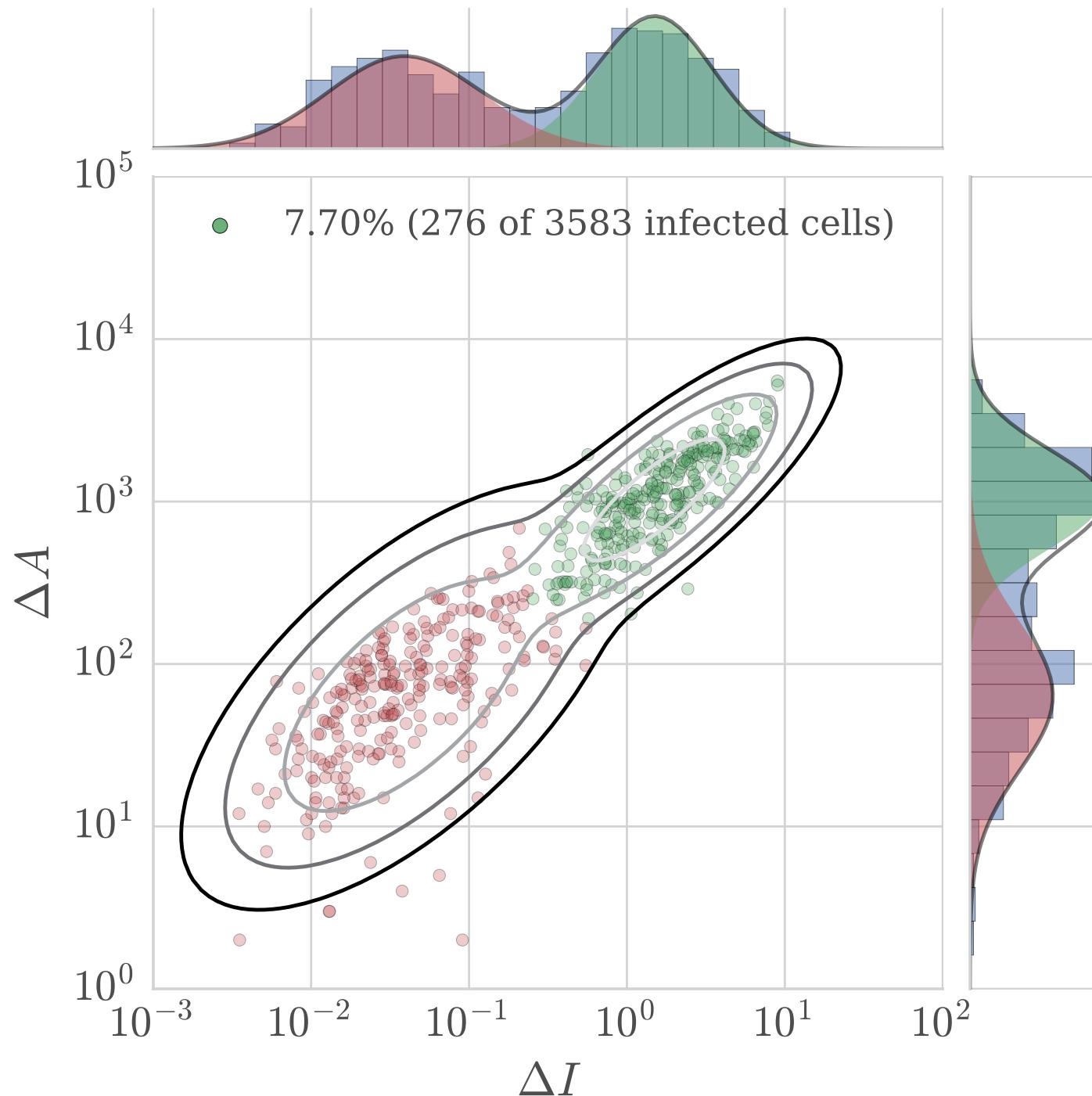


# Bacterial growth distributions

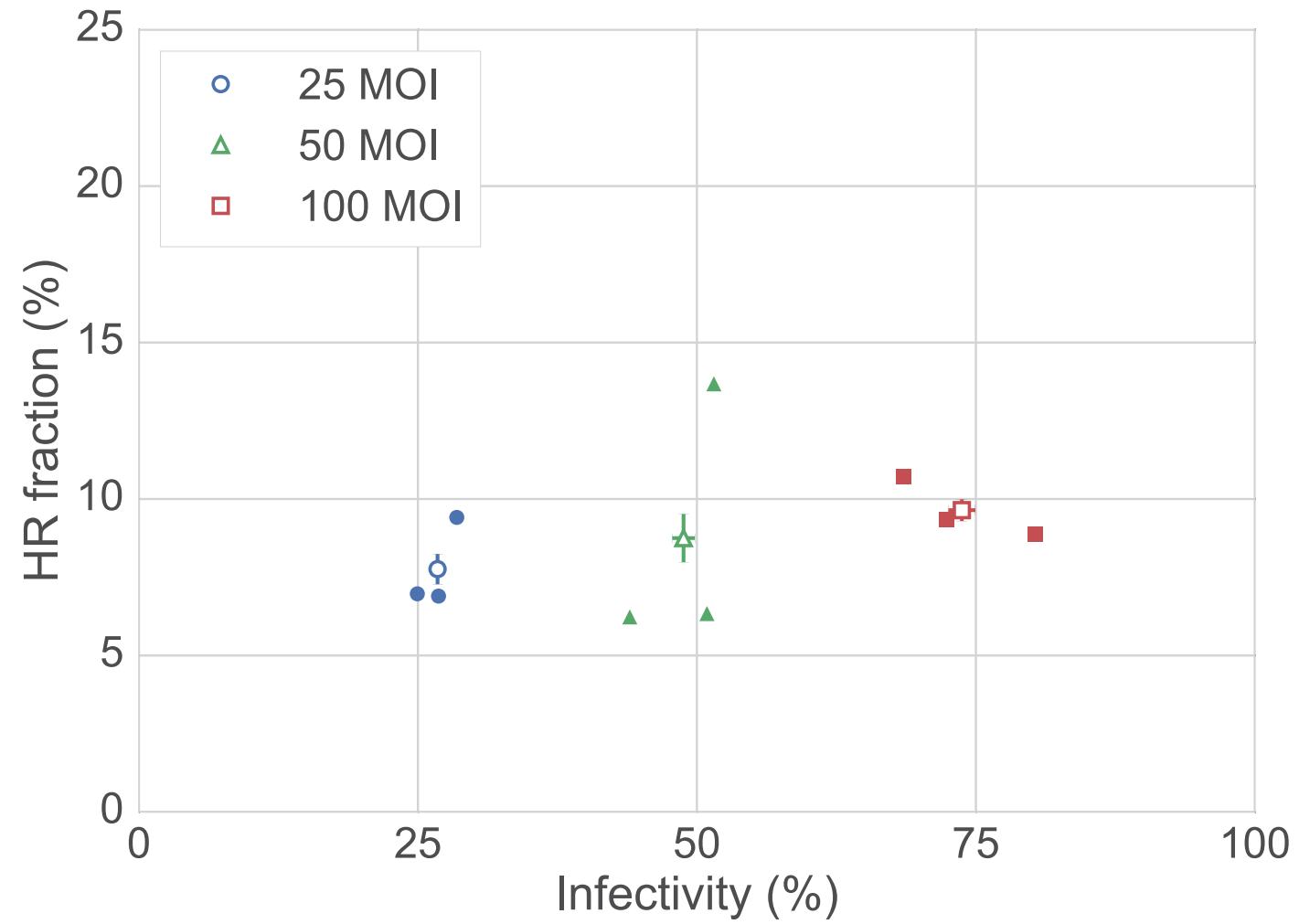
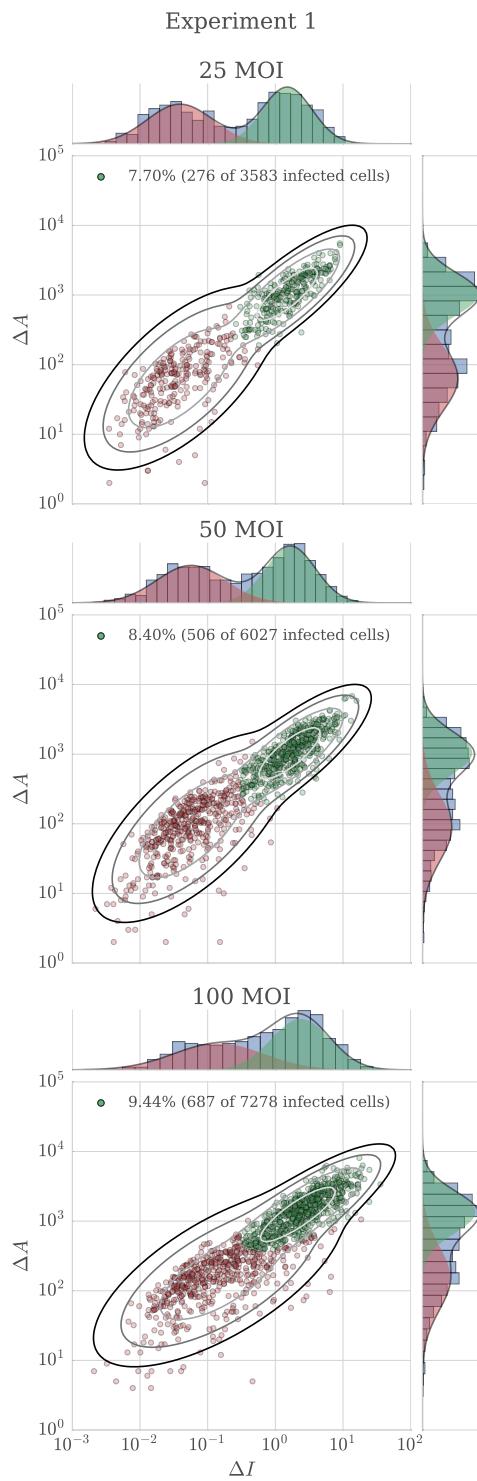


Gaussian mixture model classifications. [Scikit learn 2014](#).

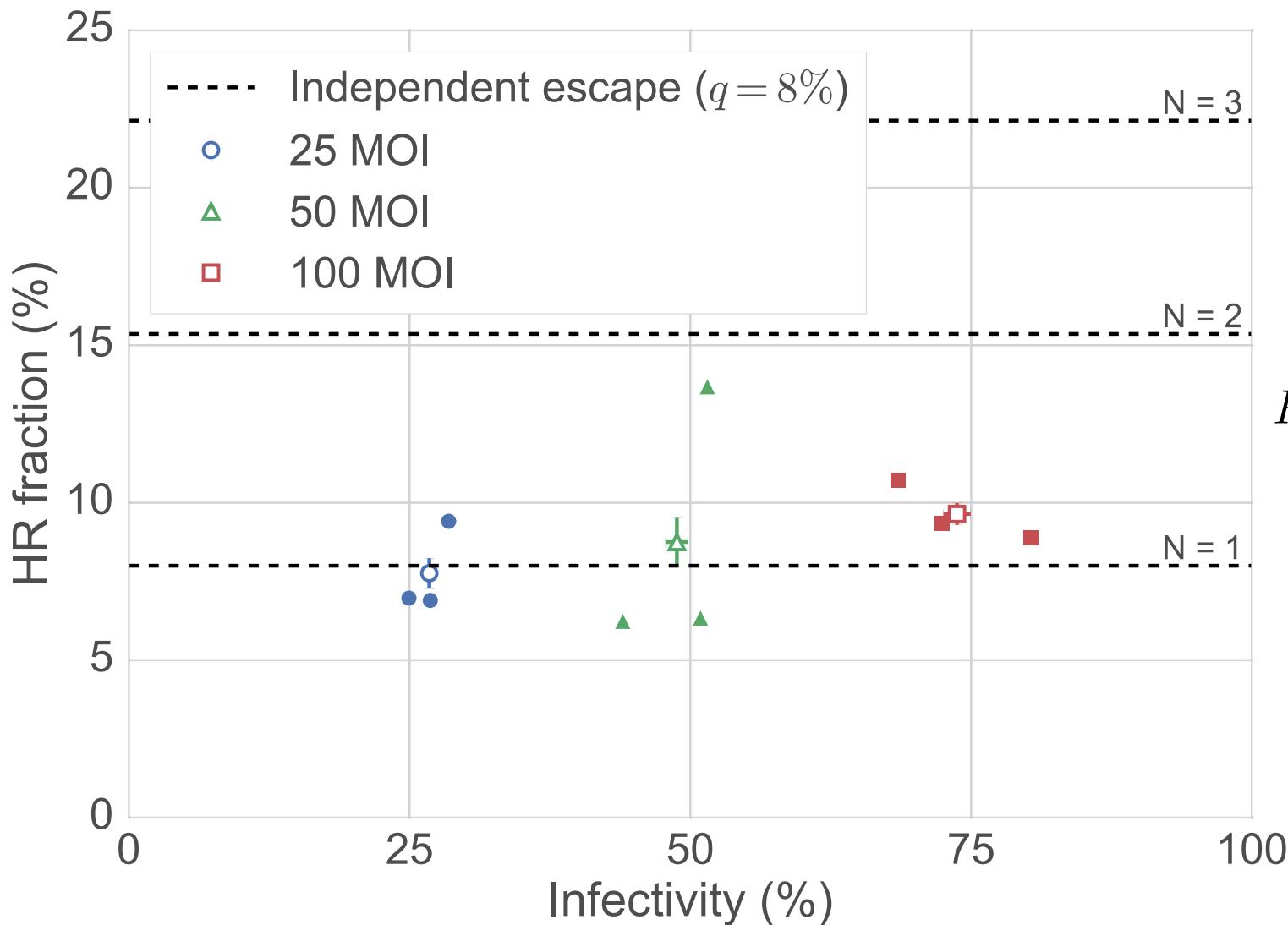
# Bacterial growth distributions



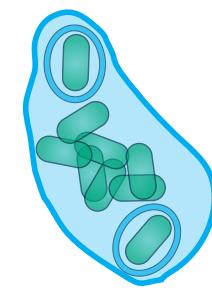
# HR fraction vs infectivity



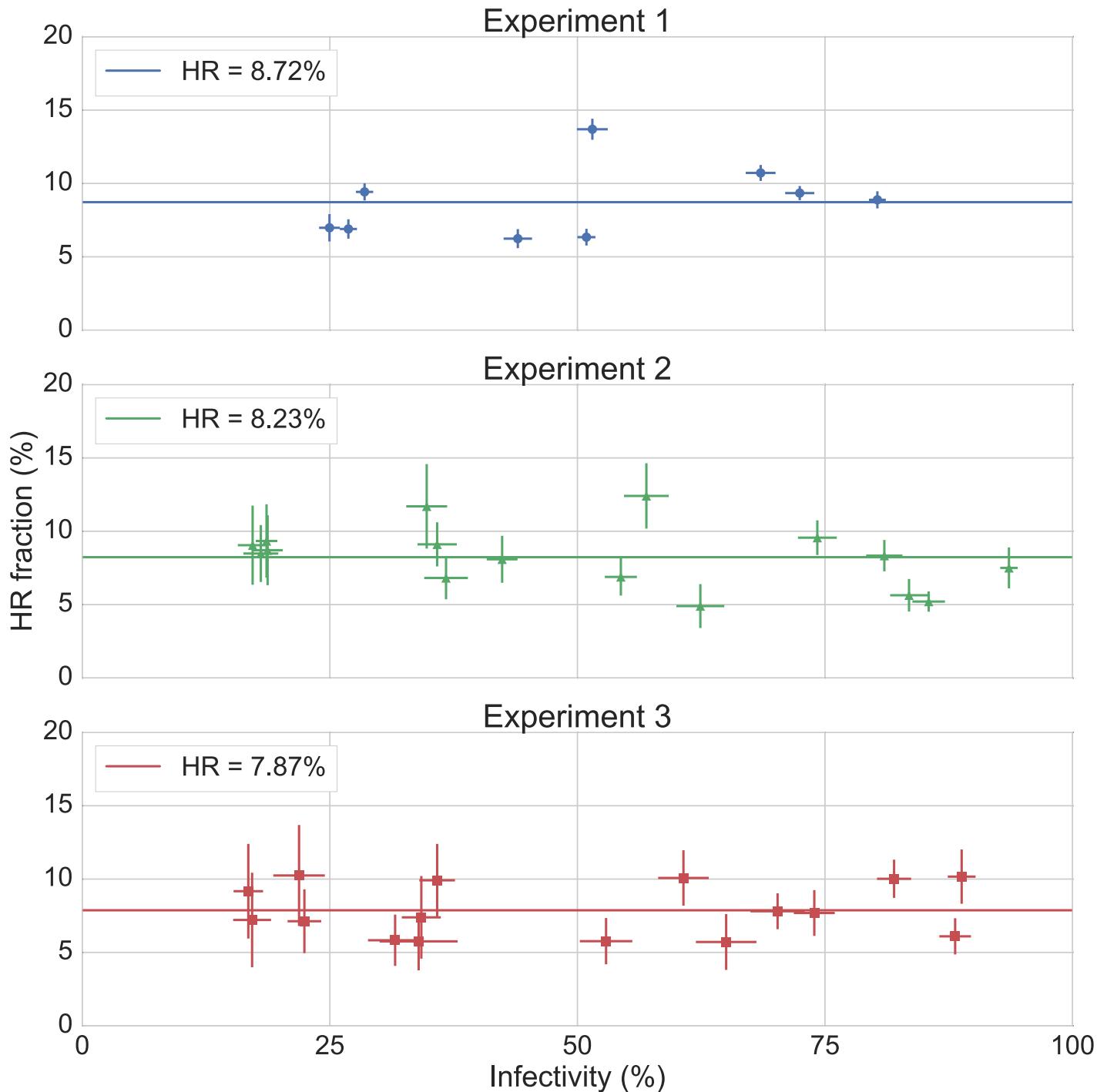
# HR fraction vs infectivity



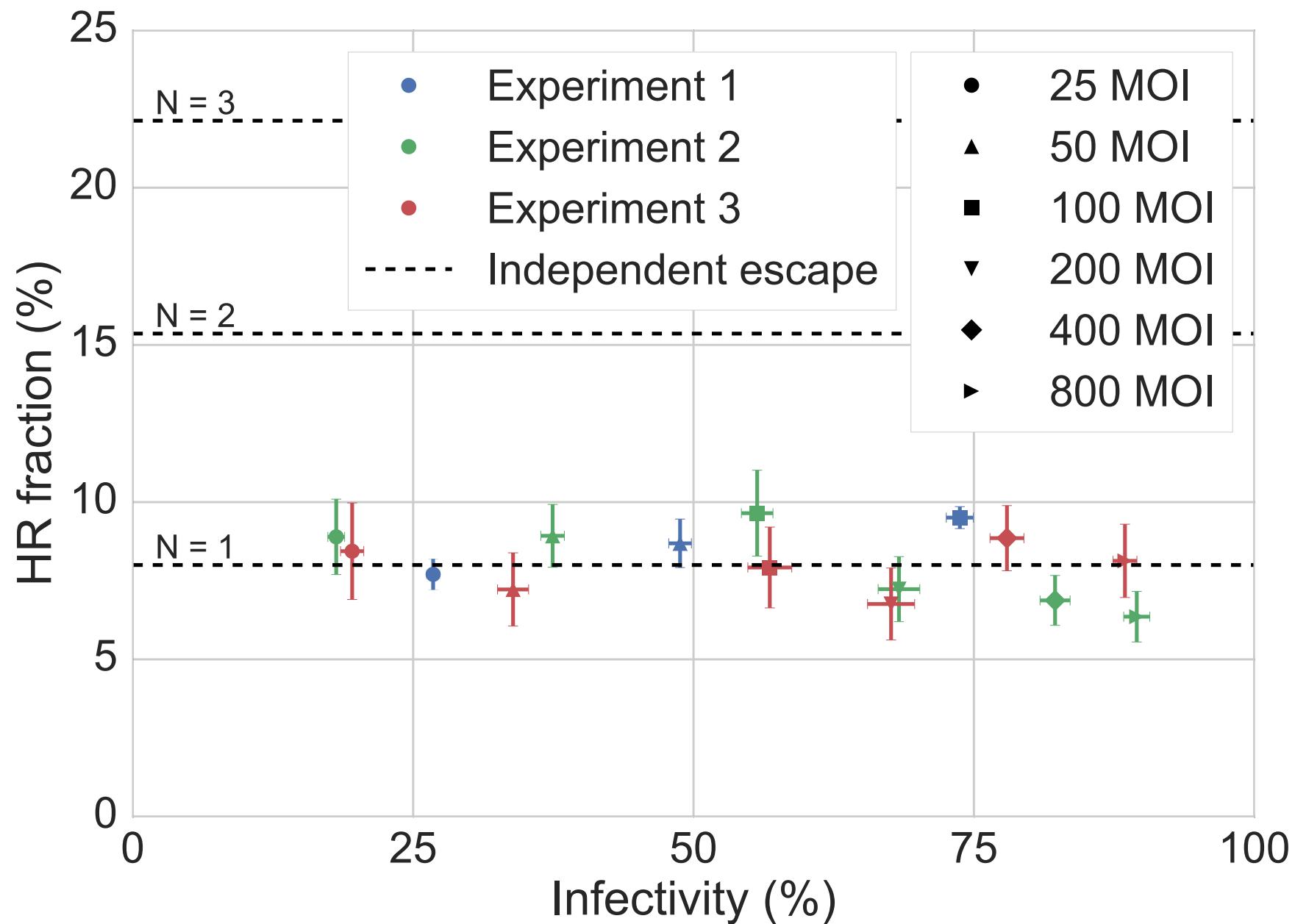
$$P(\text{HR}) = 1 - (1 - q)^N$$



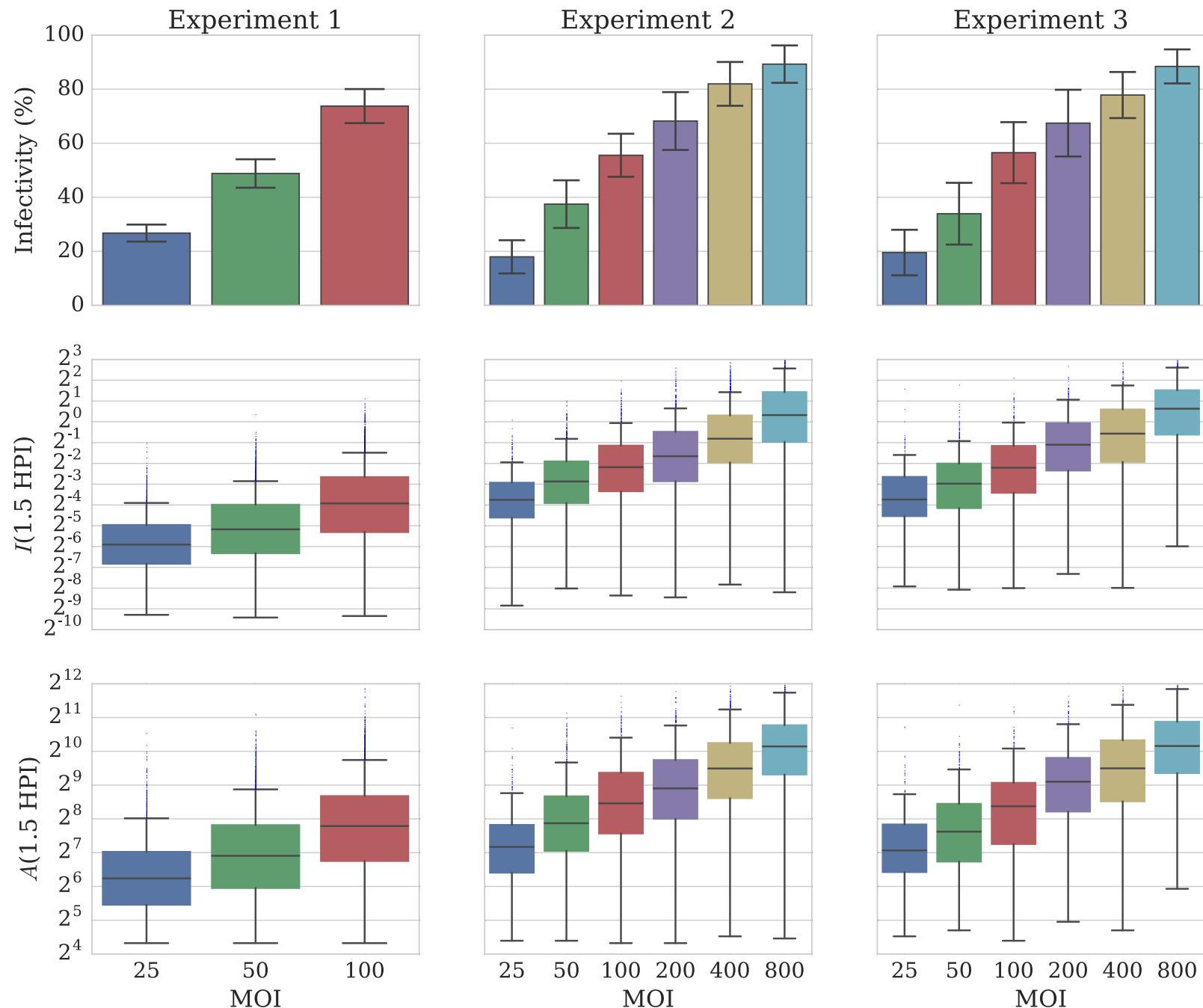
# HR fraction vs infectivity



# HR fraction vs infectivity

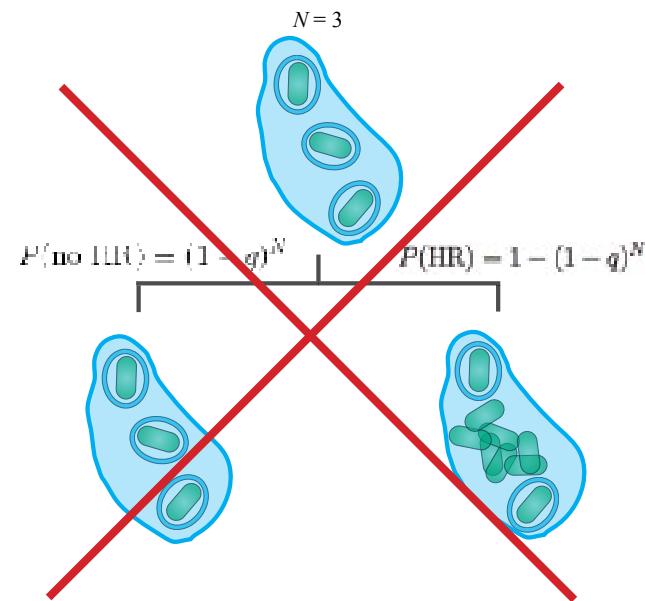


# Bacterial load vs MOI

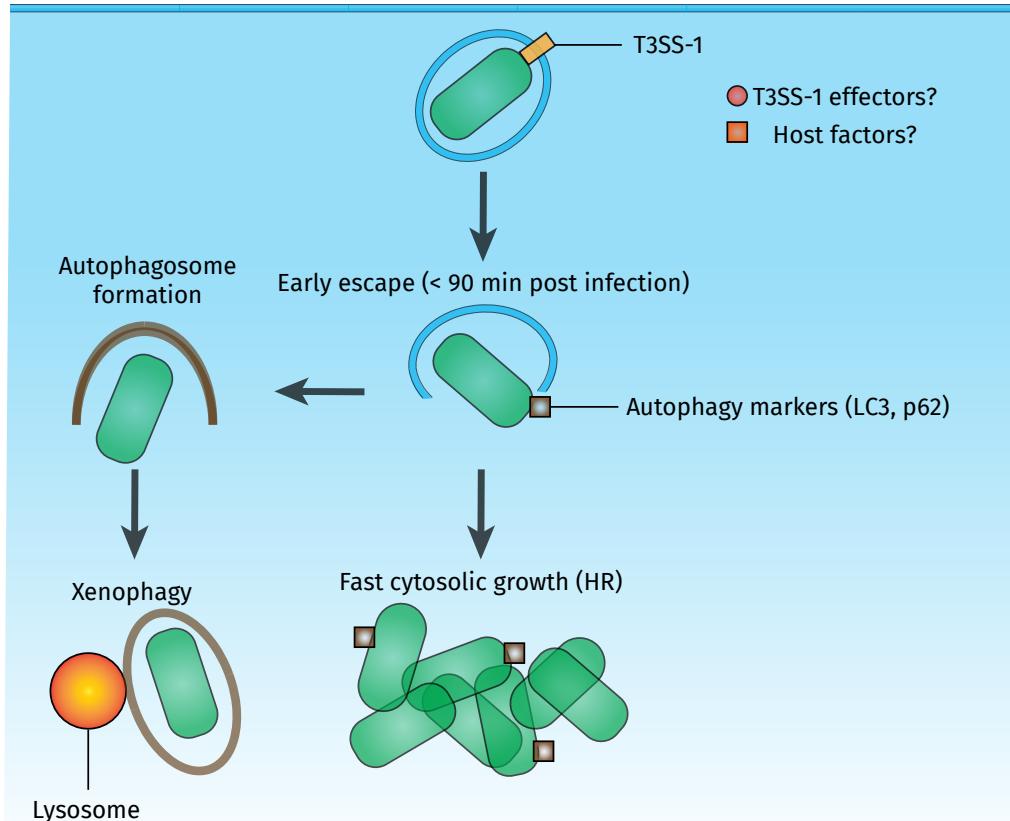


# Implications

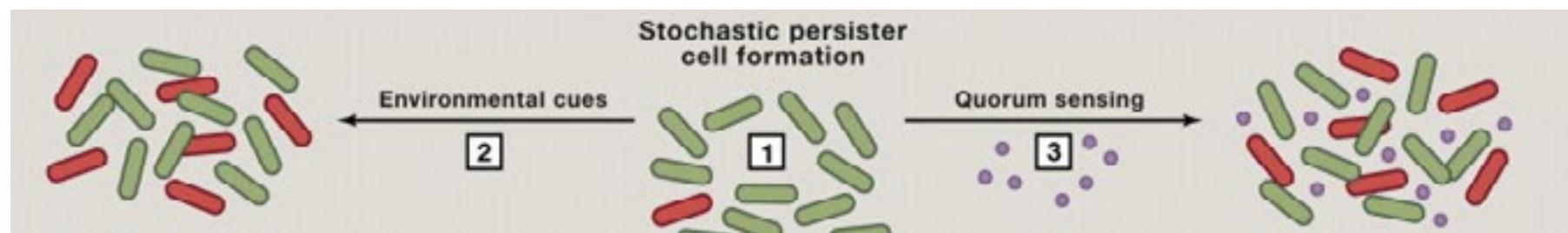
A constant HR fraction rules out the independent escape hypothesis.



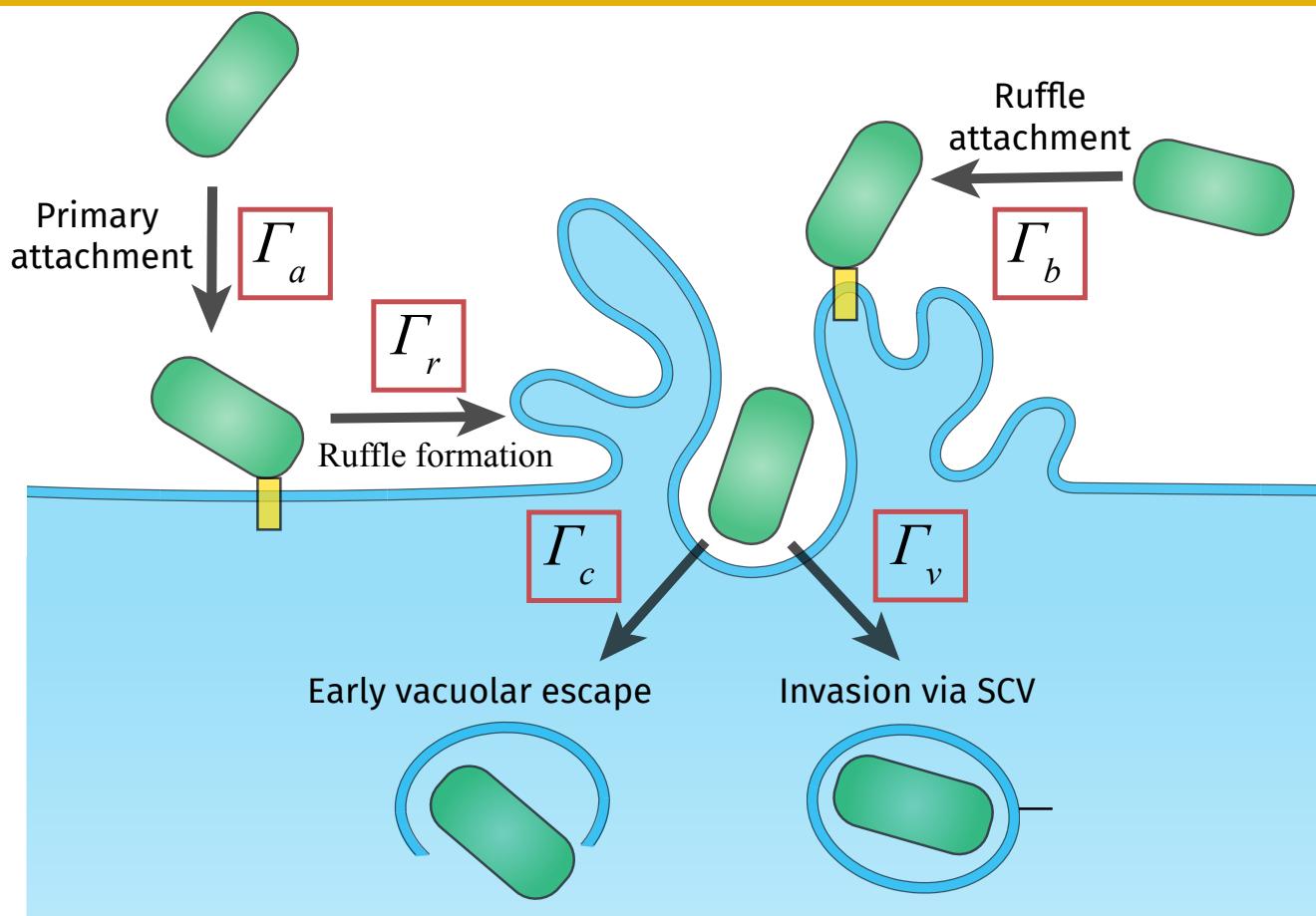
HR appears to depend on **host factors**.



Alternatively, bacteria are making an informed **decision** to hyper replicate.



# Mean field model

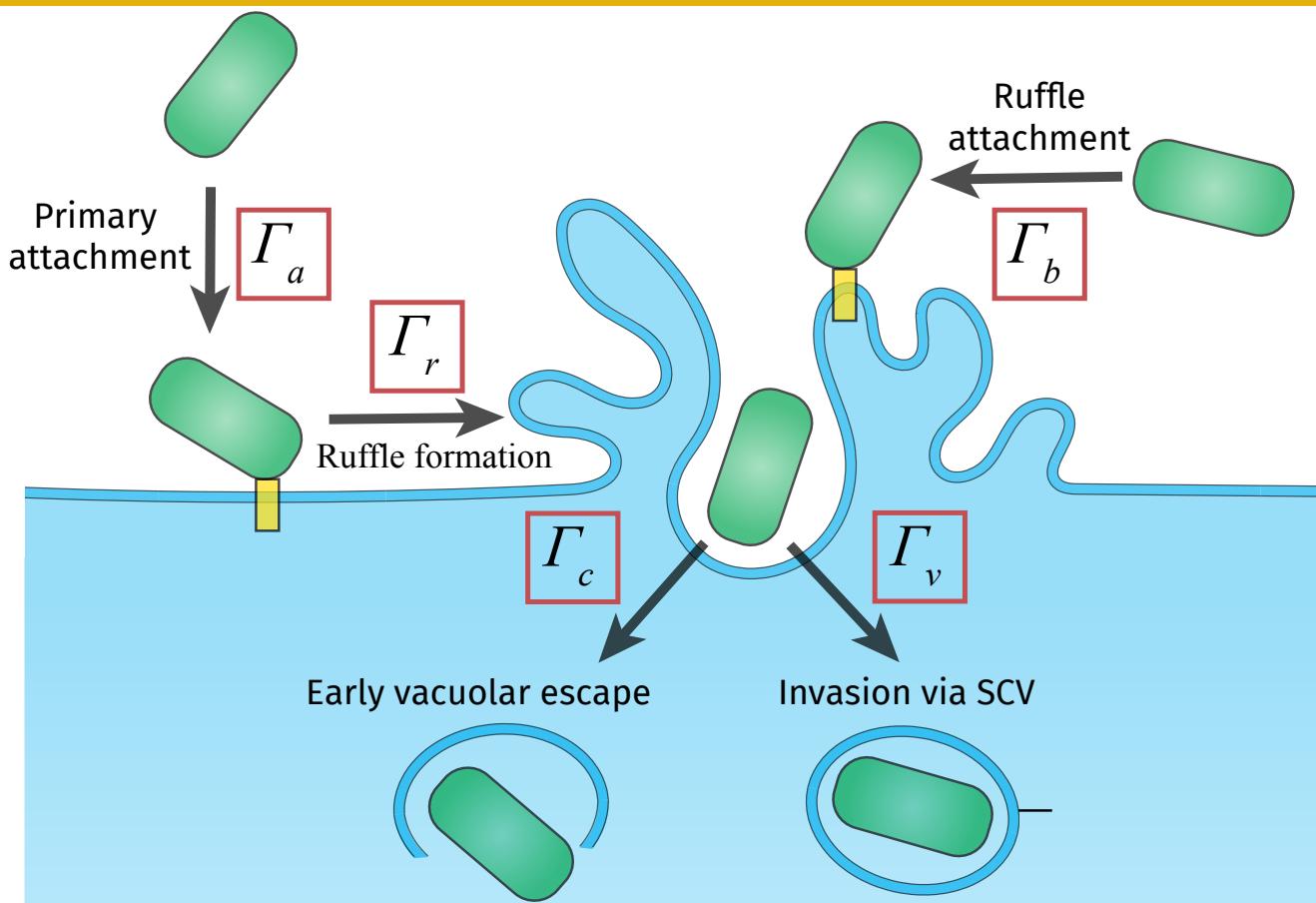


**Model:** *Salmonella Typhimurium* invading HeLa cells in a cell culture well.

**Rates:**  $\Gamma_a, \Gamma_b, \Gamma_r, \Gamma_v, \Gamma_c$  describe host-pathogen interactions.

**Variables:** Multiplicity of infection  $m$ , confluency  $c$ , incubation time  $t_{\max}$ .

# Mean field model



**Example:** Fraction of host HeLa cells with attached bacteria.

$$\dot{H}_a = \left( \begin{array}{l} \text{host cells without} \\ \text{attached bacteria} \end{array} \right) \times \left( \begin{array}{l} \text{bacterial} \\ \text{density} \end{array} \right) \times \left( \begin{array}{l} \text{attachment} \\ \text{rate} \end{array} \right)$$

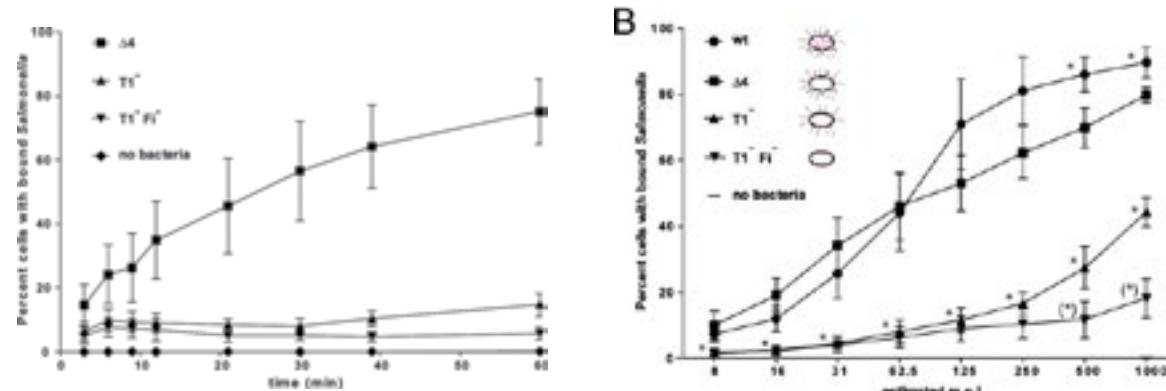
$$\dot{H}_a = (H_{\text{tot}} - H_a) \times \rho_B \times \Gamma_a$$

$$h_a = \frac{\dot{H}_a}{H_{\text{tot}}} = (1 - h_a)\rho_B\Gamma_a$$

**Numerically integrate** coupled differential equations to get time-dependent quantities like  $h_a(t)$  and  $b_x(t)$

# Parameterizing the model

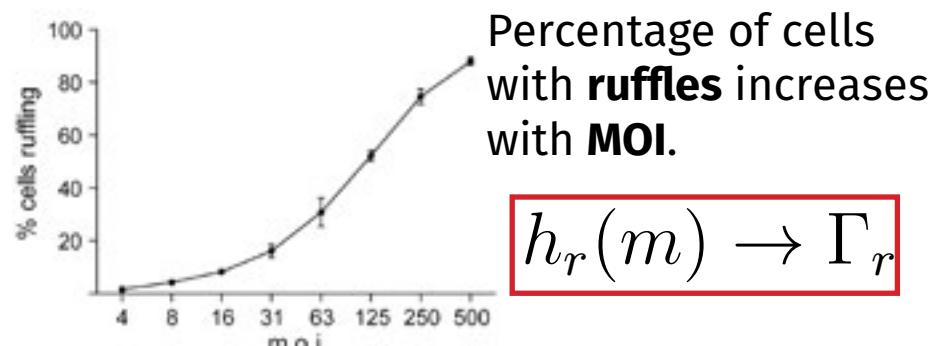
Misselwitz et al. *Infect Immun* (2011).



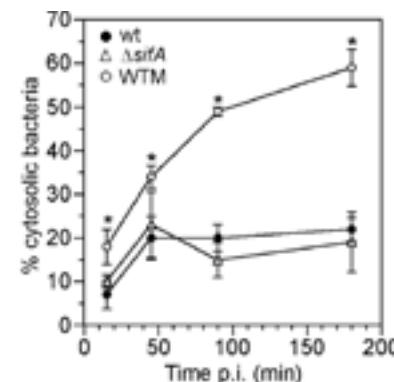
Percentage of cells with **bound *Salmonella*** increases with **MOI** and **time**.

$$h_a(t, m) \rightarrow \Gamma_a$$

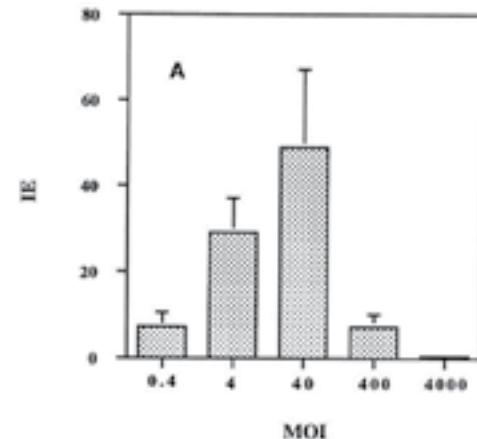
Misselwitz et al. *PLoS Pathogens* (2012).



Knodler et al. *PLoS ONE* (2014).



Huang et al. *Infect Immun* (1998).



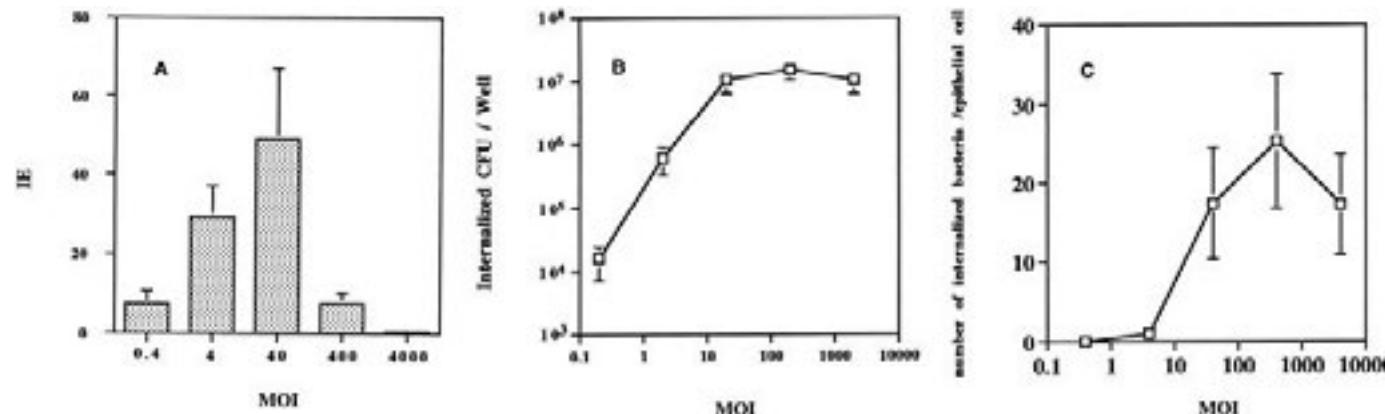
| Time (min) | Plate count assay          |                                          |
|------------|----------------------------|------------------------------------------|
|            | IE (%)<br>(mean $\pm$ SEM) | No. of bacteria/cell<br>(mean $\pm$ SEM) |
| 0          | 0                          | 0                                        |
| 10         | 1.2 $\pm$ 0.3              | 0.3 $\pm$ 0.1                            |
| 20         | 6.5 $\pm$ 2.5              | 1.9 $\pm$ 0.9                            |
| 30         | 13.7 $\pm$ 3.2             | 3.5 $\pm$ 0.9                            |
| 45         | 21.1 $\pm$ 6.2             | 5.9 $\pm$ 1.3                            |
| 60         | 54.9 $\pm$ 15.2            | 14.9 $\pm$ 2.0                           |

**Invasion efficiency** reaches a maximum then decreases with **MOI**.

$$b_x(t, m), h_x(t, m) \rightarrow \Gamma_x^*$$

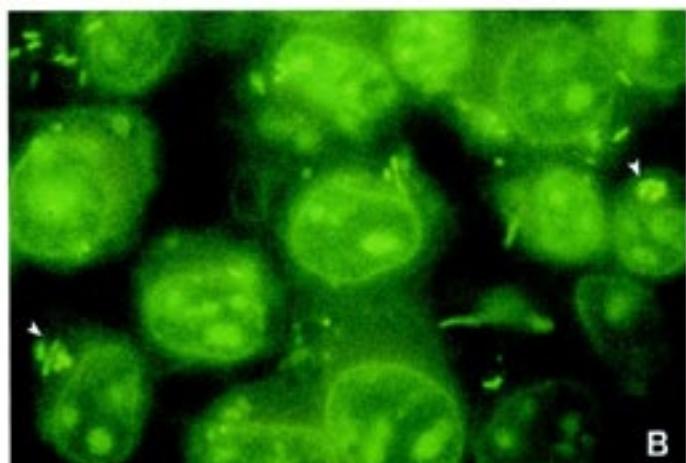
# Parameterizing the model

Huang et al. *Infect Immun* (1998).



Bacterial internalization appears to be limited by  $\tilde{b}_{x,\max} \approx 20$  bacteria.

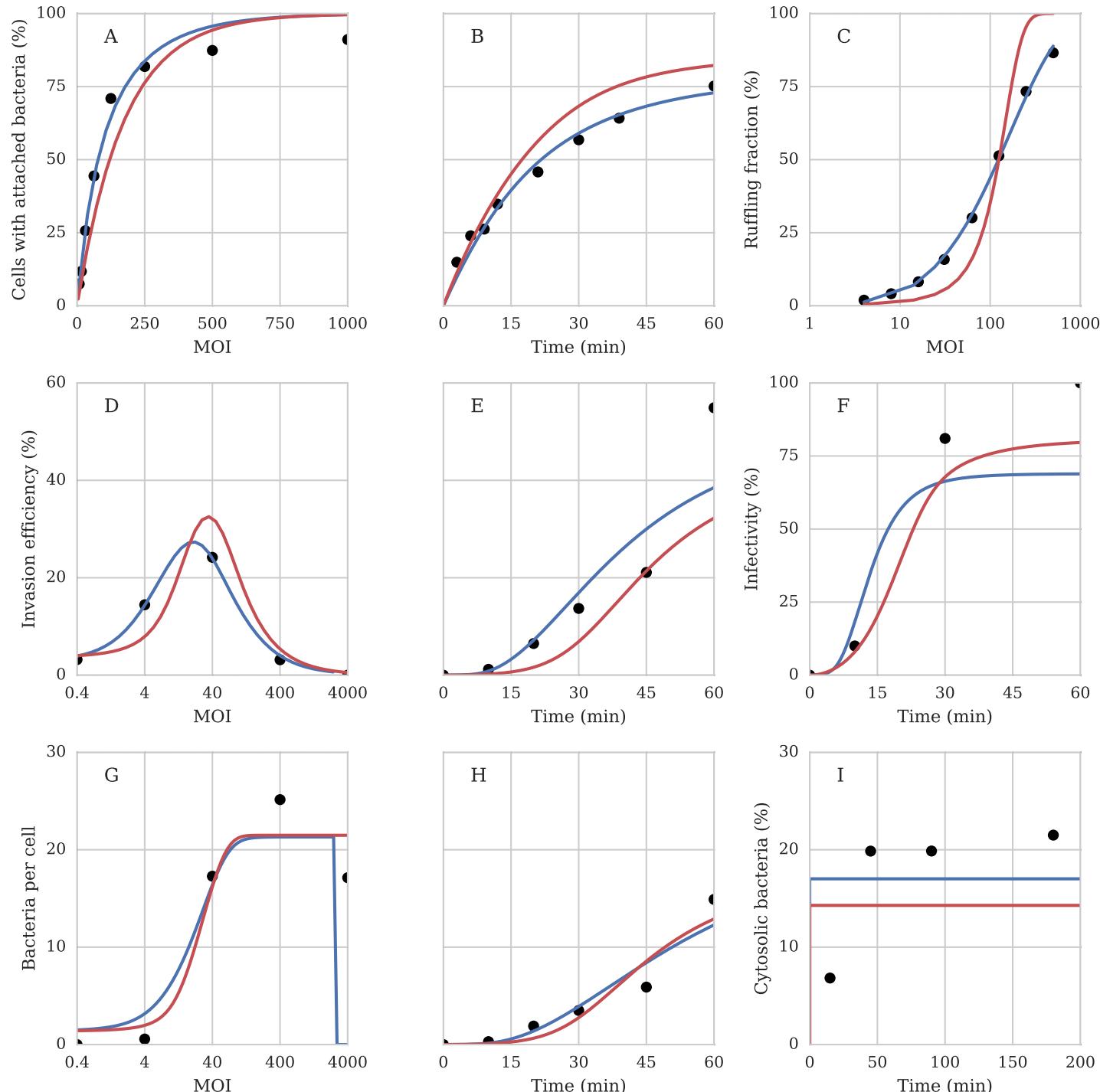
$$\Gamma_x^* = \Gamma_x \left( 1 - \frac{\tilde{b}_x}{\tilde{b}_{x,\max}} \right)$$



Foci of infection (ruffles) per cell appears to be limited to  $\tilde{r}_{\max} \approx 2$  ruffles.

$$\Gamma_r^* = \Gamma_r \left( 1 - \frac{\tilde{r}}{\tilde{r}_{\max}} \right)$$

# Parameterizing the model



Individual fits  
Global fit

# Parameterizing the model

|               | $\Gamma_a$     | $\Gamma_b$    | $\Gamma_r$    | $\Gamma_v$    | $\Gamma_c$     | $\tilde{b}_{x,\max}$ | $\tilde{r}_{\max}$ | $c$          |
|---------------|----------------|---------------|---------------|---------------|----------------|----------------------|--------------------|--------------|
| Initial guess | 0.001          | 0.1           | 0.1           | 0.01          | 0.0025         | 20                   | 2                  | 1            |
| A             | <b>0.00115</b> | 0.1           | 0.1           | 0.01          | 0.0025         | 20                   | 2                  | <b>1.00</b>  |
| B             | <b>0.00279</b> | 0.1           | 0.1           | 0.01          | 0.0025         | 20                   | 2                  | <b>0.199</b> |
| C             | <b>0.00199</b> | <b>0.123</b>  | <b>0.108</b>  | 0.01          | 0.0025         | 20                   | <b>10.0</b>        | <b>0.364</b> |
| D             | 0.00199        | 0.123         | 0.108         | <b>0.0303</b> | <b>0.00473</b> | <b>16.1</b>          | <b>1.57</b>        | 0.420        |
| E             | 0.00199        | 0.123         | 0.108         | <b>0.0516</b> | <b>0.00162</b> | <b>21.3</b>          | <b>2.81</b>        | 0.299        |
| F             | 0.00199        | 0.123         | 0.108         | <b>0.0111</b> | <b>0.00450</b> | <b>30.0</b>          | <b>1.00</b>        | 0.500        |
| G             | 0.00199        | 0.123         | 0.108         | <b>0.0398</b> | <b>0.00694</b> | <b>10.0</b>          | <b>2.06</b>        | 1.00         |
| H             | 0.00199        | 0.123         | 0.108         | <b>0.0246</b> | <b>0.00501</b> | <b>30.0</b>          | <b>1.00</b>        | 0.322        |
| I             | 0.00199        | 0.123         | 0.108         | <b>0.0244</b> | <b>0.00633</b> | 30.0                 | 1.00               | <b>0.999</b> |
| All data      | <b>0.00523</b> | <b>0.0941</b> | <b>0.0102</b> | <b>0.0327</b> | <b>0.00490</b> | <b>21.5</b>          | <b>4.89</b>        | -            |

**Manual fit**  
**Individual fits**  
**Global fit**  
**Inconsistencies?**

# Parameterizing the model

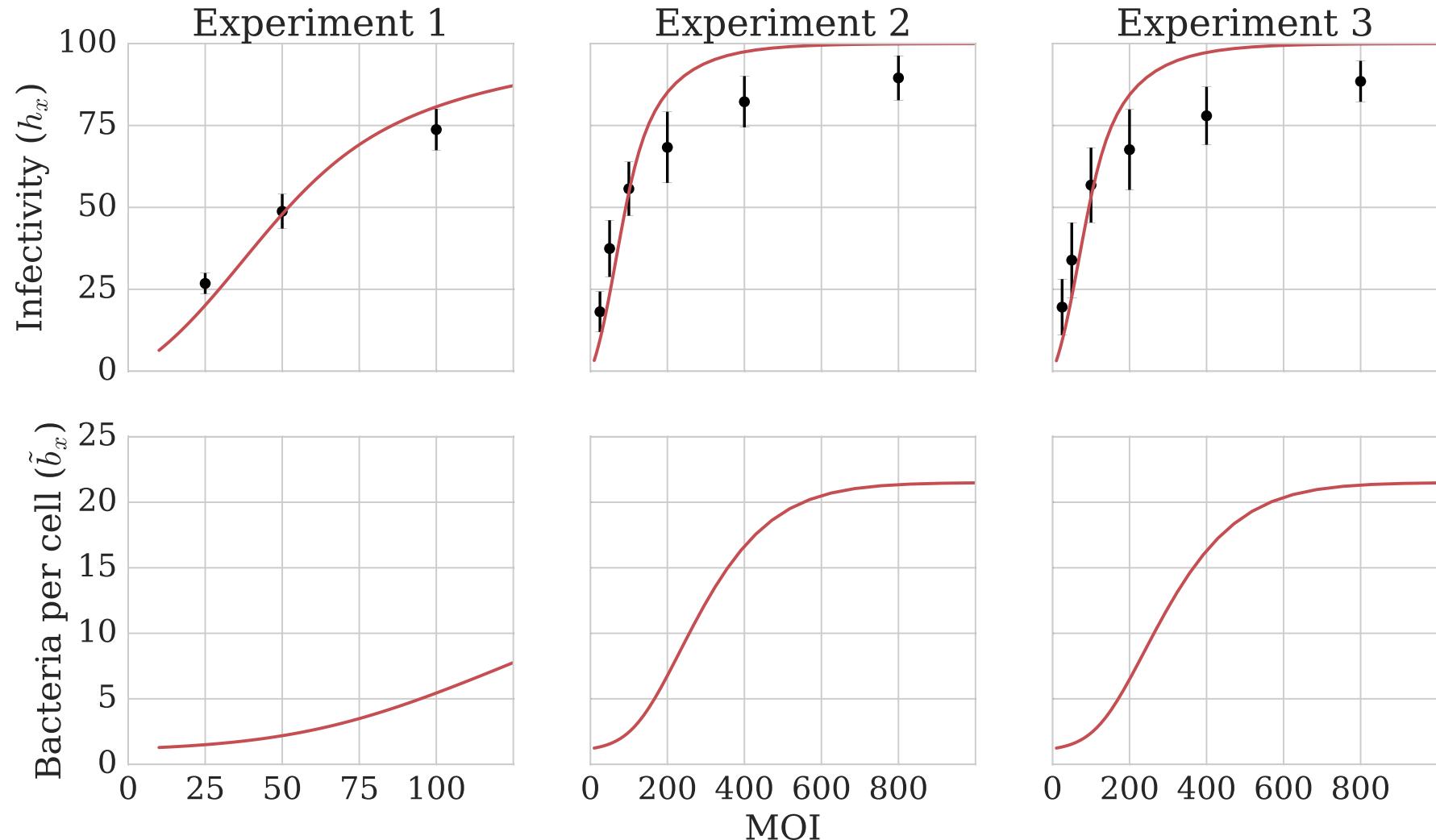
|               | $\Gamma_a$     | $\Gamma_b$    | $\Gamma_r$    | $\Gamma_v$    | $\Gamma_c$     | $\tilde{b}_{x,\max}$ | $\tilde{r}_{\max}$ | $c$          |
|---------------|----------------|---------------|---------------|---------------|----------------|----------------------|--------------------|--------------|
| Initial guess | 0.001          | 0.1           | 0.1           | 0.01          | 0.0025         | 20                   | 2                  | 1            |
| A             | <b>0.00115</b> | 0.1           | 0.1           | 0.01          | 0.0025         | 20                   | 2                  | <b>1.00</b>  |
| B             | <b>0.00279</b> | 0.1           | 0.1           | 0.01          | 0.0025         | 20                   | 2                  | <b>0.199</b> |
| C             | <b>0.00199</b> | <b>0.123</b>  | <b>0.108</b>  | 0.01          | 0.0025         | 20                   | <b>10.0</b>        | <b>0.364</b> |
| D             | 0.00199        | 0.123         | 0.108         | <b>0.0303</b> | <b>0.00473</b> | <b>16.1</b>          | <b>1.57</b>        | 0.420        |
| E             | 0.00199        | 0.123         | 0.108         | <b>0.0516</b> | <b>0.00162</b> | <b>21.3</b>          | <b>2.81</b>        | 0.299        |
| F             | 0.00199        | 0.123         | 0.108         | <b>0.0111</b> | <b>0.00450</b> | <b>30.0</b>          | <b>1.00</b>        | 0.500        |
| G             | 0.00199        | 0.123         | 0.108         | <b>0.0398</b> | <b>0.00694</b> | <b>10.0</b>          | <b>2.06</b>        | 1.00         |
| H             | 0.00199        | 0.123         | 0.108         | <b>0.0246</b> | <b>0.00501</b> | <b>30.0</b>          | <b>1.00</b>        | 0.322        |
| I             | 0.00199        | 0.123         | 0.108         | <b>0.0244</b> | <b>0.00633</b> | 30.0                 | 1.00               | <b>0.999</b> |
| All data      | <b>0.00523</b> | <b>0.0941</b> | <b>0.0102</b> | <b>0.0327</b> | <b>0.00490</b> | <b>21.5</b>          | <b>4.89</b>        | -            |

|   | Source          | Independent variable | MOI    | Incubation time (minutes) | Confluency (cells/well) | HeLa strain | Incubation temperature | Culture plate          | Incubation medium | Growth medium |
|---|-----------------|----------------------|--------|---------------------------|-------------------------|-------------|------------------------|------------------------|-------------------|---------------|
| A | Misselwitz 2011 | MOI                  | -      | 9                         | $6 \cdot 10^3$          | Kyoto       | 37°C                   | 96-well Greiner        | DMEM, 10% FCS     | DMEM          |
| B | Misselwitz 2012 | Time                 | 62.5   | 9                         | $6 \cdot 10^3$          | ?           | 37°C                   | 96-well Greiner        | DMEM, 10% FCS     | LB Broth      |
| C | Misselwitz 2012 | MOI                  | -      | 9                         | $3 \cdot 10^5$          | ?           | 37°C                   | 96-well Greiner        | DMEM, 10% FCS     | LB Broth      |
| D | Huang 1998      | MOI                  | -      | 60                        | $6 \cdot 10^5$          | INT407      | 37°C                   | 24-well Sarstedt       | MEM, 10% FCS      | LB Miller     |
| E | Huang 1998      | Time                 | 40     | 60                        | $6 \cdot 10^5$          | INT407      | 37°C                   | 24-well Sarstedt       | MEM, 10% FCS      | LB Miller     |
| F | Huang 1998      | Time                 | 40     | 60                        | $6 \cdot 10^5$          | INT407      | 37°C                   | 24-well Sarstedt       | MEM, 10% FCS      | LB Miller     |
| G | Huang 1998      | MOI                  | -      | 60                        | $6 \cdot 10^5$          | INT407      | 37°C                   | 24-well Sarstedt       | MEM, 10% FCS      | LB Miller     |
| H | Huang 1998      | Time                 | 40     | 60                        | $6 \cdot 10^5$          | INT407      | 37°C                   | 24-well Sarstedt       | MEM, 10% FCS      | LB Miller     |
| I | Knodler 2014    | Time                 | 50-100 | 10                        | $5 \cdot 10^4$          | ATCC        | 37°C                   | 24-well Corning Costar | EMEM, 10% FCS     | LB Gibco      |

- Q: Are *Salmonella*-HeLa interactions describable by time-independent rates?  
 Q: What effects (model-related or experiment-related) can explain misfits and inconsistencies in parameters?

# Model applications

A mean field model of invasion could be a useful predictive tool when designing invasion assays (e.g. our HR data).



By infecting with up to 800 MOI, invasion saturation ( $\tilde{b}_{x,\max} \approx 20$ ) was likely reached.

# Conclusions and outlook

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HR fraction is **independent** of bacterial load.

**Hypothesis:** Hyper-replication is host cell-dependent. Approximately 8% of infected cells are susceptible to HR.

**Future work:** Further experiments with labelling for subcellular localization (e.g.LAMP1).

A **mean field model** with interaction rates can approximate *Salmonella*-HeLa invasion.

**Future work:** Fitting more data, error analysis.

**Q:** Can other host-pathogen systems be modelled in the same way?

## Index

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### Introduction

- 1-2. Host-pathogen interactions
- 3. Host defense and antibiotics
- 4. Antibiotic resistance
- 5. Lifestyles of pathogens
- 6. Bacterial decision making
- 7. *Salmonella enterica*
- 8. *S. Typhimurium* invasion
- 9. Cooperative invasion via ruffles
- 10. Vacuolar maintenance
- 11-12. Vacuolar escape and HR
- 13. Independent escape hypothesis

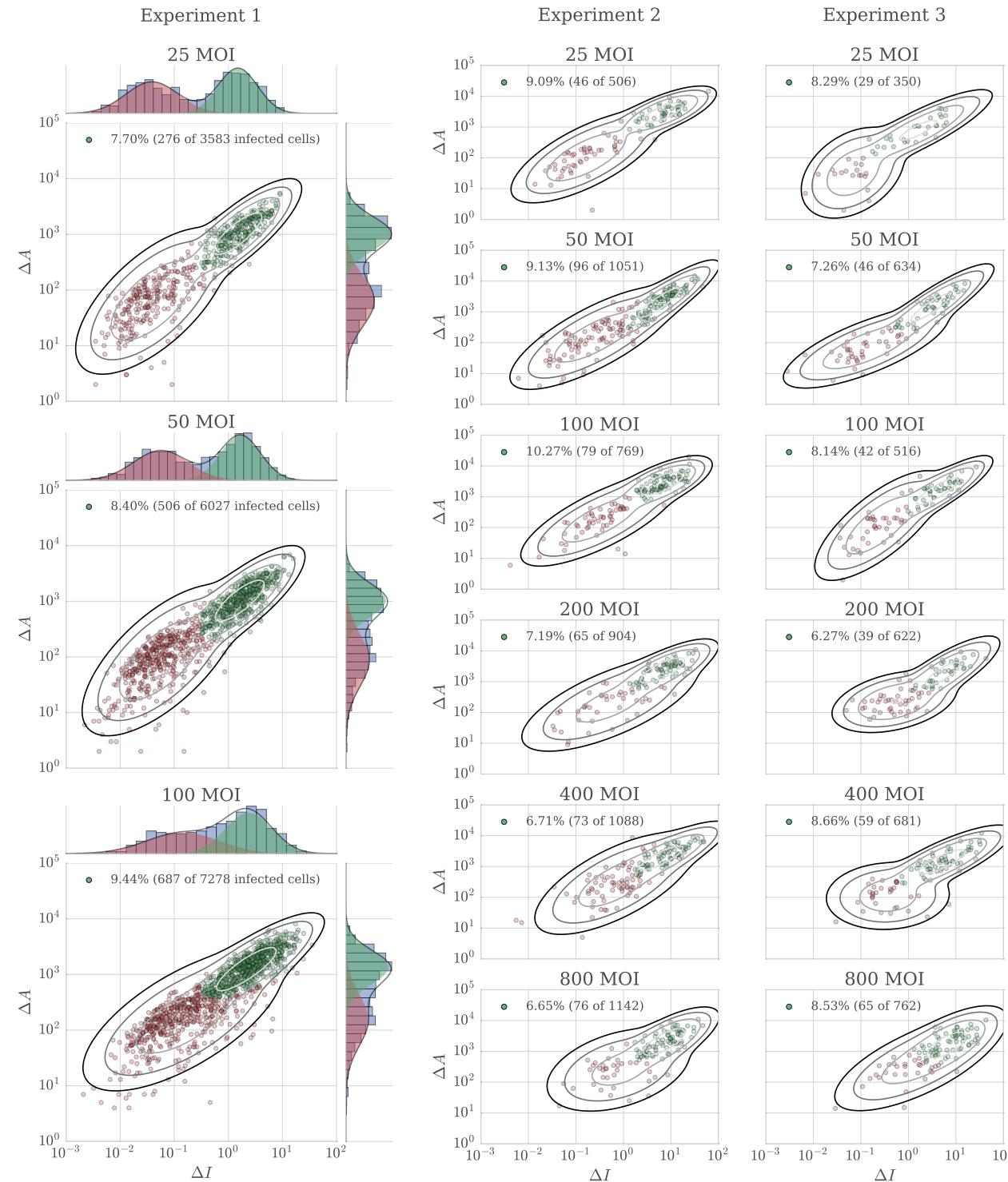
### Image Segmentation and Hyper-Replication

- 14. Timelapse fluorescence microscopy
- 15. Image segmentation
- 16-19. Tracking cells
- 20-21. Single-cell analysis
- 22-24. Bacterial growth distributions
- 25-28. HR fraction vs infectivity
- 29. Bacterial load vs MOI
- 30. Implications

### Mean Field Model of Invasion

- 31-32. Mean field model
- 33-37. Parameterizing the model
- 38. Model applications

# Bacterial growth distributions



# Mean field model variables and rates

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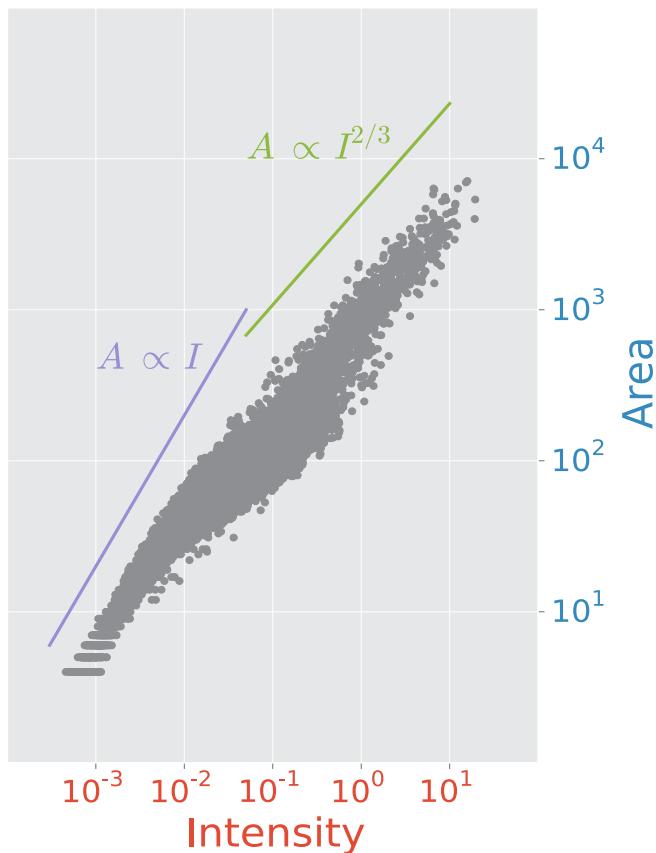
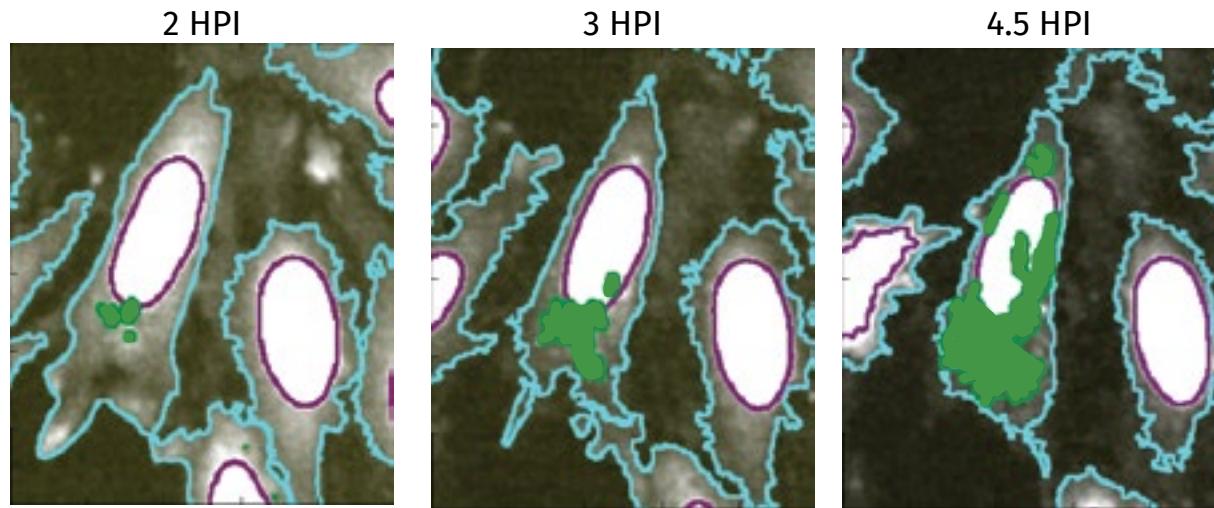
| Variable         | Definition                                     | Equals               |
|------------------|------------------------------------------------|----------------------|
| $h(t)$           | fraction of host cells without bacteria        | $H/H_{\text{tot}}$   |
| $h_a(t)$         | fraction of host cells with attached bacteria  | $H_a/H_{\text{tot}}$ |
| $h_x(t)$         | fraction of host cells with invaded bacteria   | $H_x/H_{\text{tot}}$ |
| $h_v(t)$         | fraction of host cells with vacuolar bacteria  | $H_v/H_{\text{tot}}$ |
| $h_c(t)$         | fraction of host cells with cytosolic bacteria | $H_c/H_{\text{tot}}$ |
| $h_r(t)$         | fraction of host cells with ruffles            | $H_r/H_{\text{tot}}$ |
| $b(t)$           | fraction of swimming bacteria                  | $B/B_{\text{tot}}$   |
| $b_a(t)$         | fraction of attached bacteria                  | $B_a/B_{\text{tot}}$ |
| $b_x(t)$         | fraction of invaded bacteria                   | $b_c + b_v$          |
| $b_v(t)$         | fraction of vacuolar bacteria                  | $B_v/B_{\text{tot}}$ |
| $b_c(t)$         | fraction of cytosolic bacteria                 | $B_c/B_{\text{tot}}$ |
| $\tilde{r}(t)$   | ruffles per host                               | $R/H_r$              |
| $\tilde{b}_a(t)$ | attached bacteria per host                     | $B_a/H_a = mb_a/h_a$ |
| $\tilde{b}_x(t)$ | invaded bacteria per host                      | $B_x/H_x = mb_x/h_x$ |

List of the time-dependent model quantities and their definitions.

| Variable     | Definition                                                      |
|--------------|-----------------------------------------------------------------|
| $\Gamma_a$   | primary attachment rate per bacterial density, per host         |
| $\Gamma_b$   | ruffle recruitment rate per bacterial density, per ruffle       |
| $\Gamma_r$   | ruffle formation rate per attached bacteria, per host           |
| $\Gamma_v$   | vacuolar invasion rate per attached bacteria, per host          |
| $\Gamma_c$   | cytosolic invasion rate per attached bacteria, per host         |
| $\Gamma_x$   | combined invasion rate per attached bacteria, per host          |
| $\Gamma_x^*$ | effective invasion rate per attached bacteria, per host         |
| $\Gamma_r^*$ | effective ruffle formation rate per attached bacteria, per host |

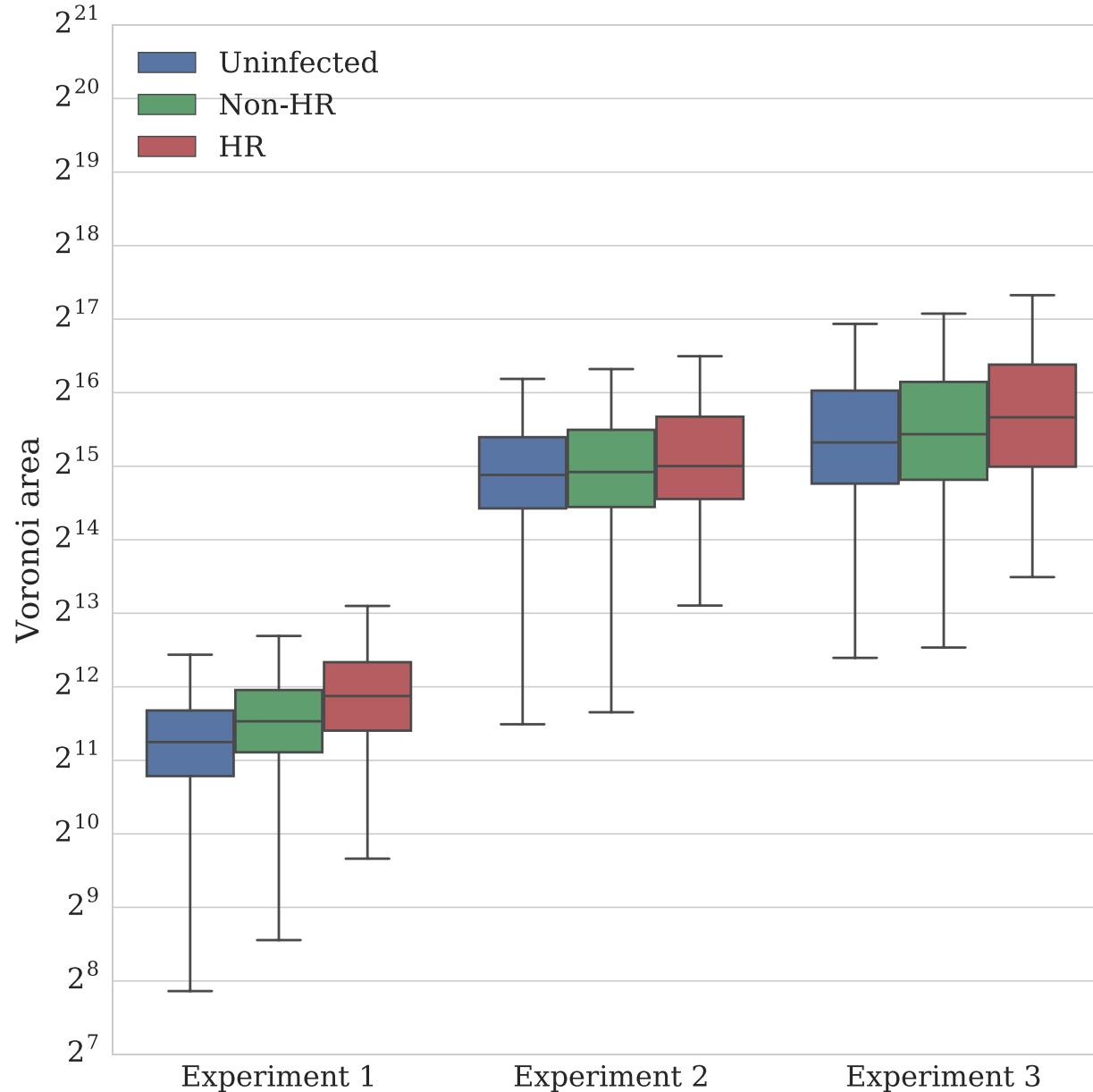
List of the rates governing the mean field invasion model.

# Bacteria intensity versus area

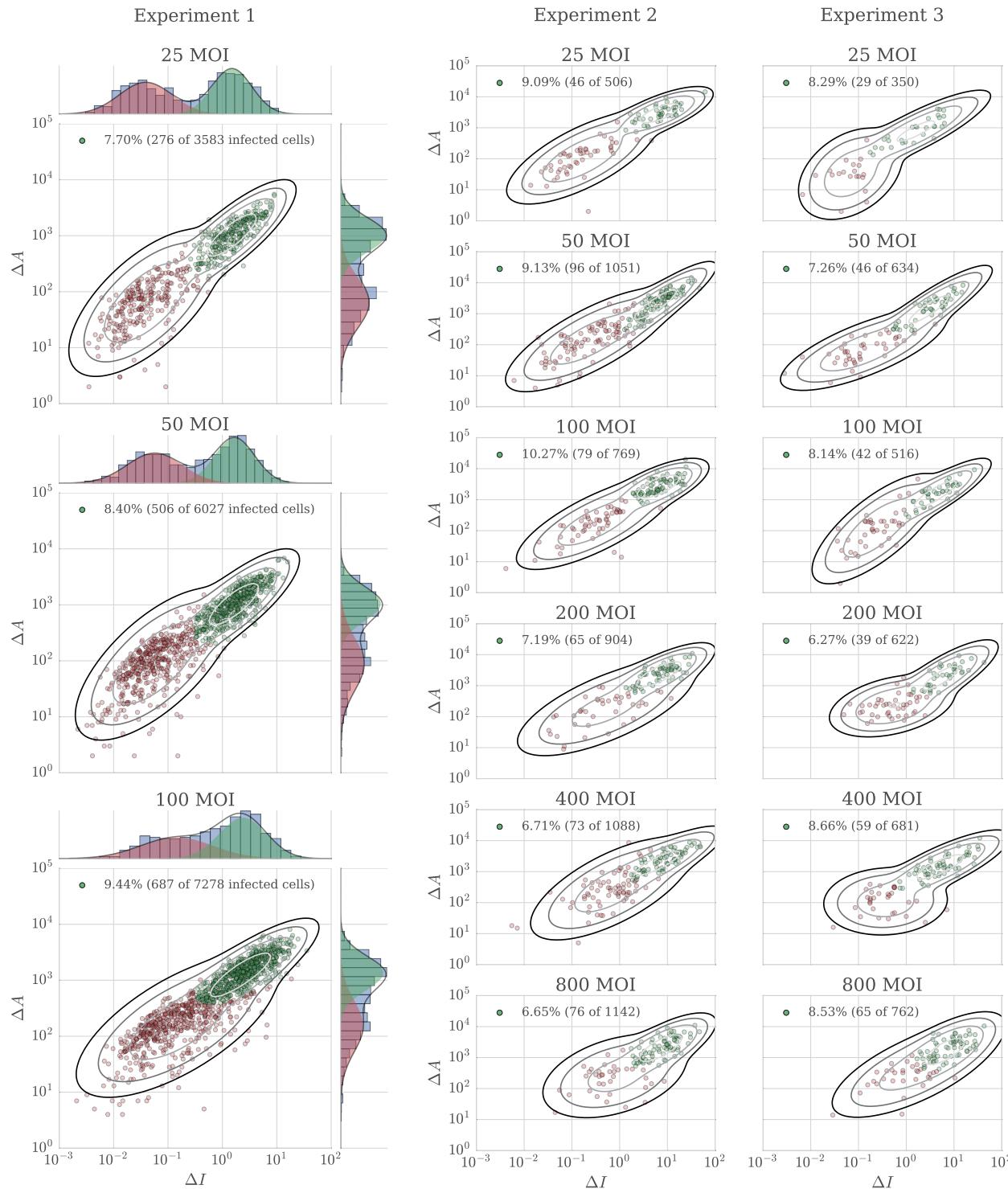


# Cell context effects

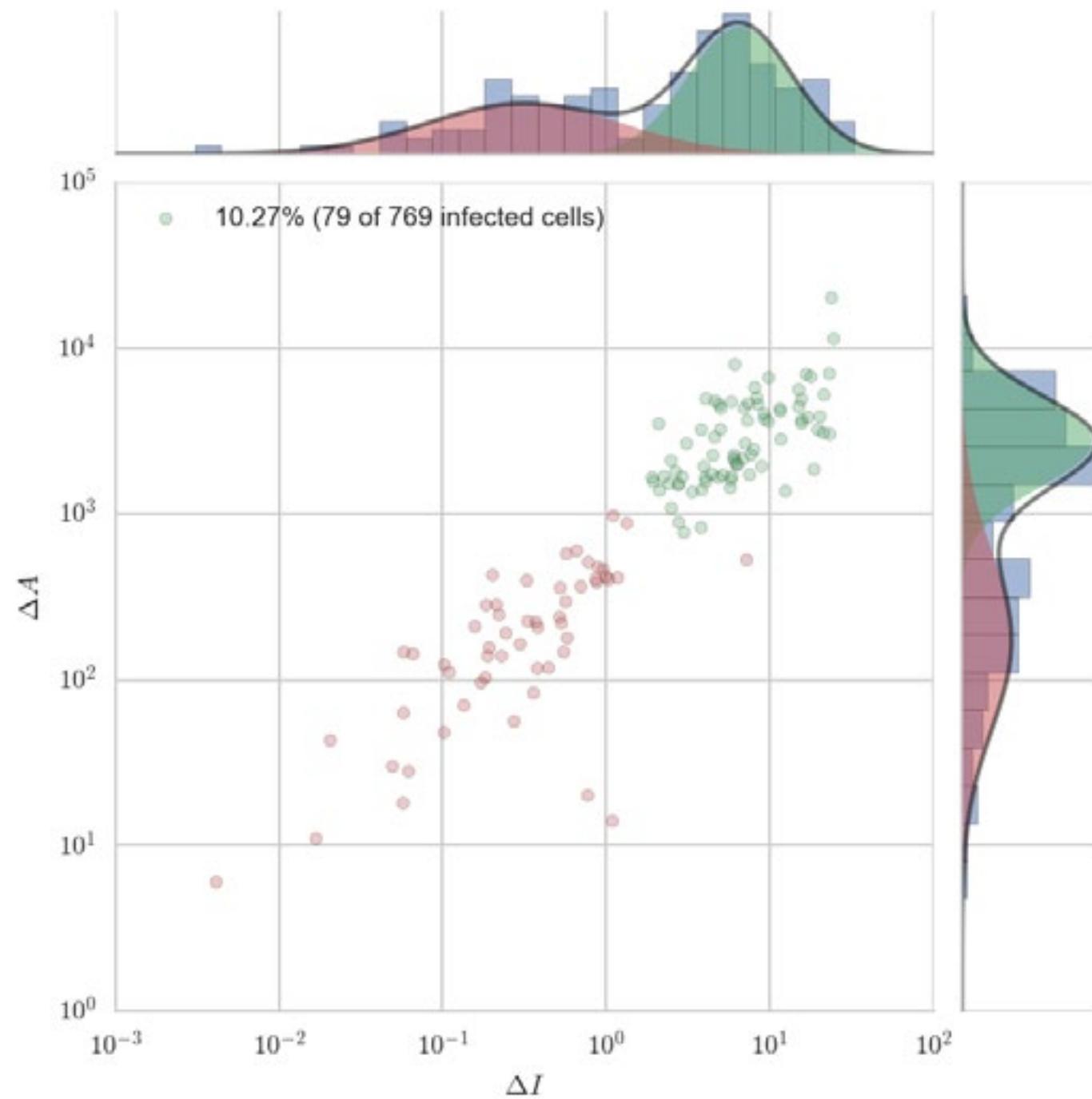
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# Fit example



# More bacterial growth distributions



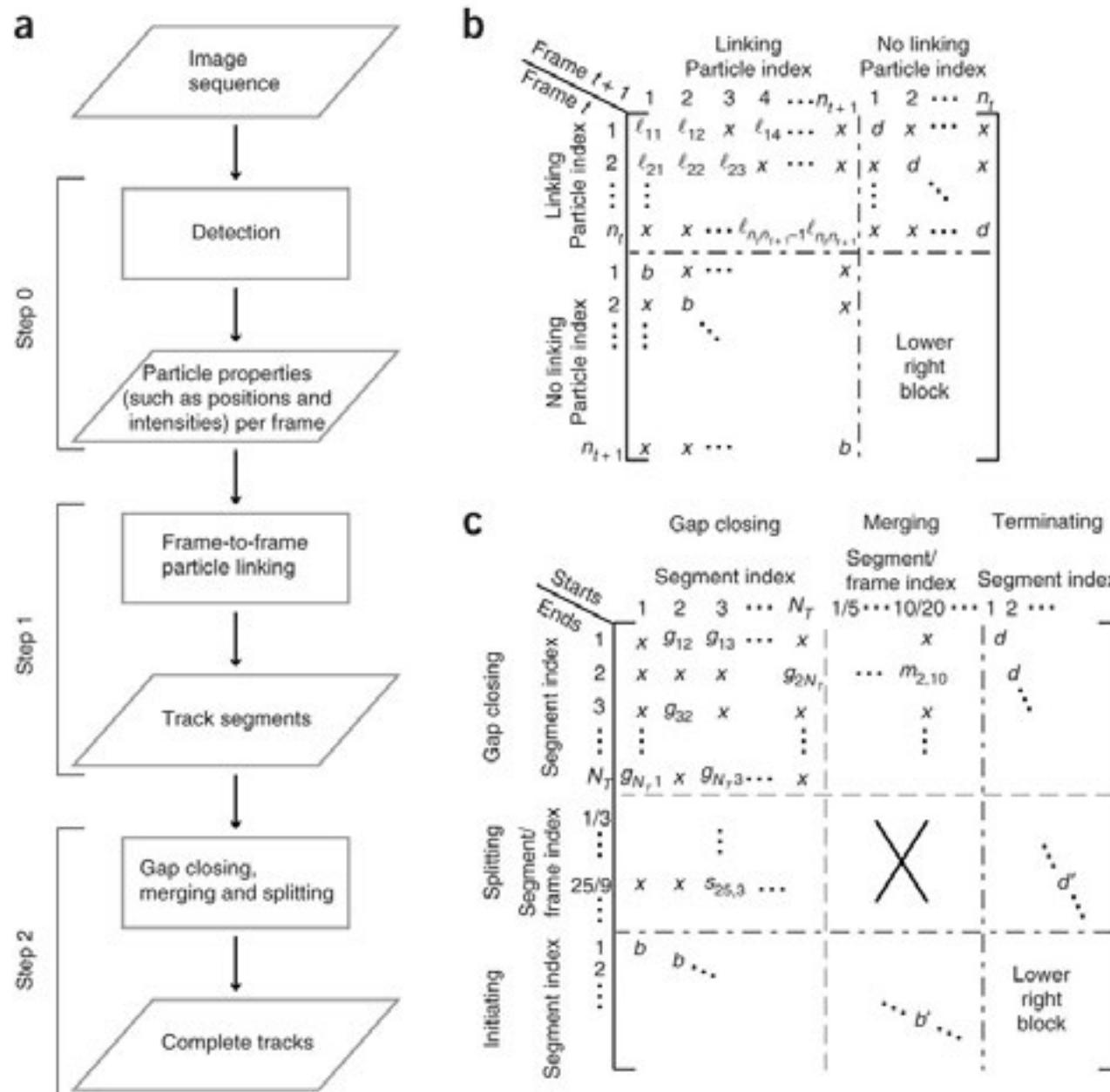
# Timelapse fluorescence microscopy

- Images taken by our experimental collaborator **Jennifer Fredlund**, in the lab of **Jost Eninga** at the **Institut Pasteur**.
- Images taken, at various time points post infection, of **DsRed wildtype *Salmonella Typhimurium*** infecting **Draq5 HeLa** in cell culture plates.

|                   | Experiment 1                  | Experiment 2                  | Experiment 3                  |
|-------------------|-------------------------------|-------------------------------|-------------------------------|
| Confluency        | $2 \cdot 10^5$                | $2 \cdot 10^4$                | $1.5 \cdot 10^4$              |
| Scope objective   | 10x                           | 40x                           | 40x                           |
| MOI               | 25, 50, 100                   | 25, 50, 100,<br>200, 400, 800 | 25, 50, 100,<br>200, 400, 800 |
| Wells per MOI     | 3                             | 2-4                           | 2-4                           |
| Fields per well   | 9                             | 17                            | 17                            |
| Cells per well    | $3960 \pm 183$                | $682 \pm 10$                  | $459 \pm 8$                   |
| Time points (HPI) | 1.5, 2, 2.5, 3, 3.5, 4.5, 5.5 | 1.5, 3, 5, 5.5                | 1.5, 3, 4, 5, 5.5             |

Table 2.1: Differences between the independent invasion experiments for this study. Confluency is the number of HeLa cells seeded per well. Cells per well is the mean  $\pm$  standard error of the numbers of cells segmented and tracked throughout the timelapse.

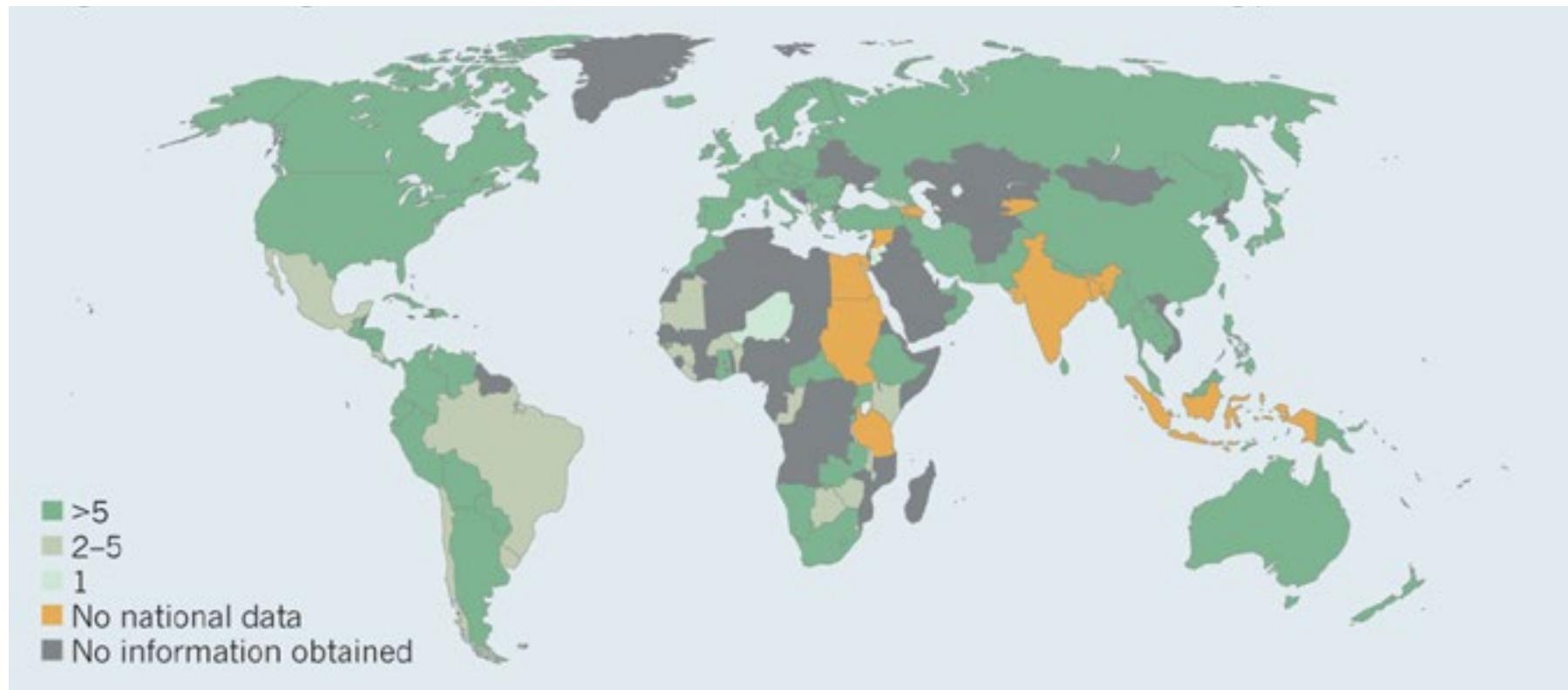
# Tracking cells



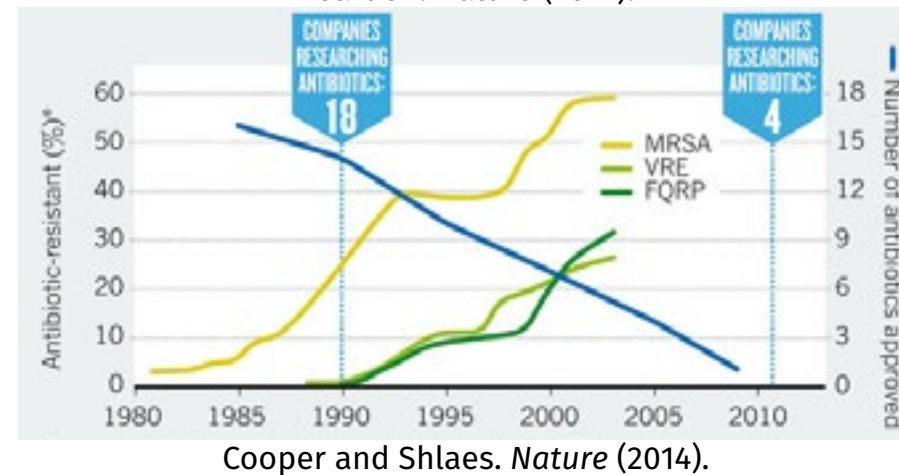
Jaqaman et al. *Nature Methods* (2008).

# Antibiotic resistance trends

Resistant strains of deadly bacteria are a growing threat worldwide.



Reardon. *Nature* (2014).



# Microplates

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