

# **Stochastic Simulation of Salmonella Infection**

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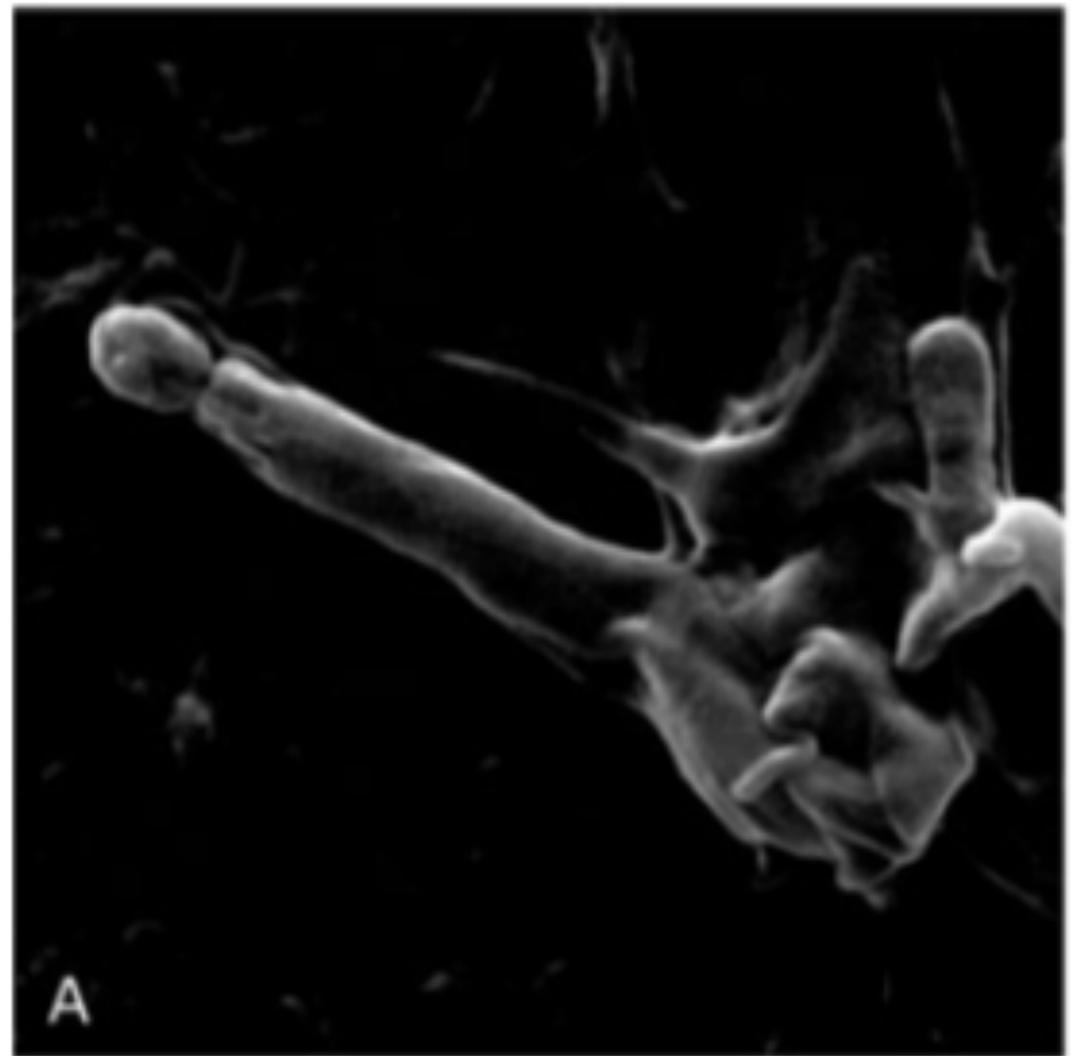
Supervised by Andrew Rutenberg

# Salmonella

- Bacterium that infects vertebrates through ingestion of contaminated food or water
- Most common strain is *S. Typhimurium*
- Bacteria infect the epithelial cell lining of the small intestine (a 2-dimensional surface of cells)
- Once attached to cells, secretes effector proteins that cause the formation of cell membrane ruffles.

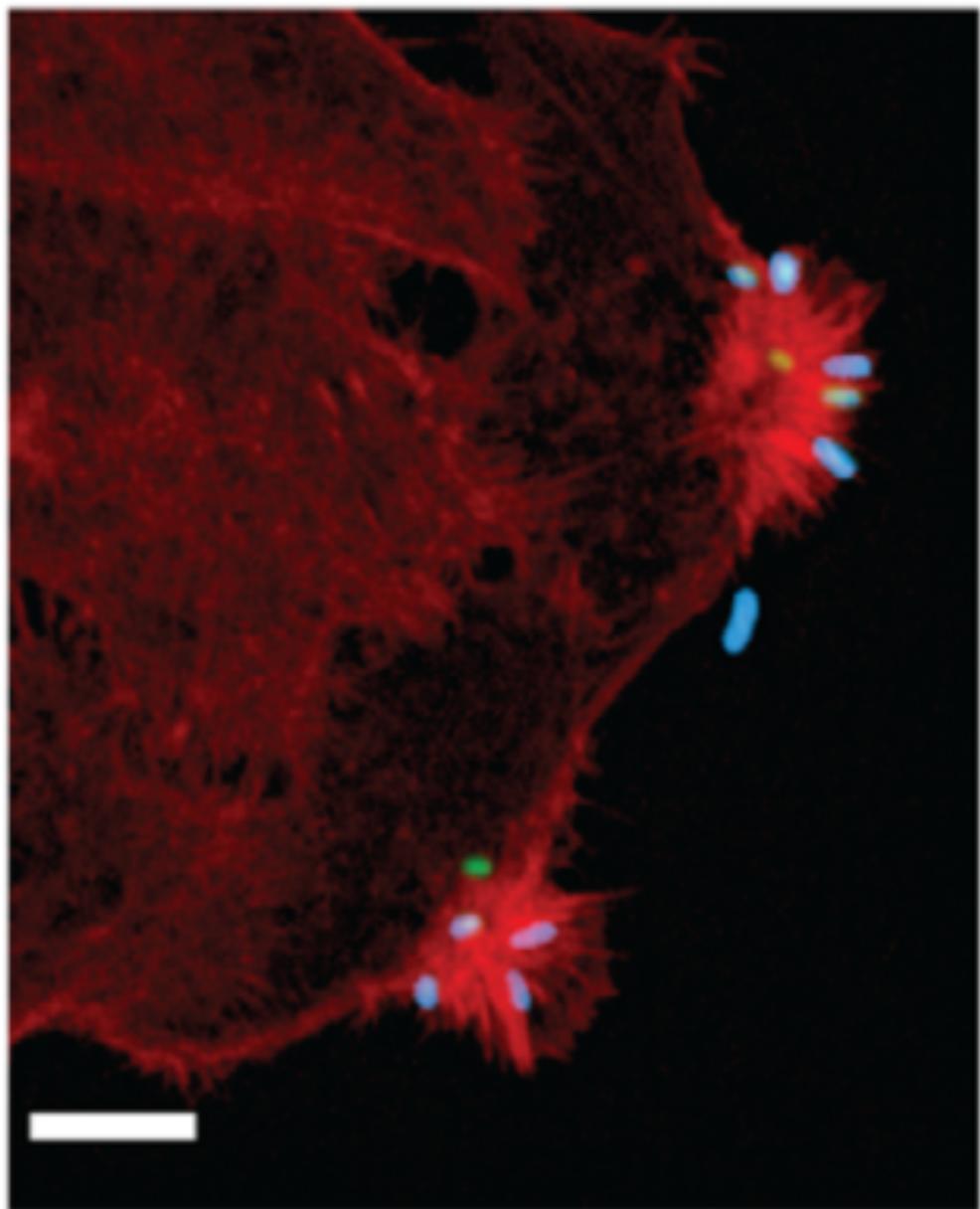
# Ruffles

- Ruffles are topographical features of the cell membrane comprised of many finger-like projections
- Significantly increase membrane surface area, likelihood of salmonella attachment
- Nonlinear mechanism for cooperativity of infection



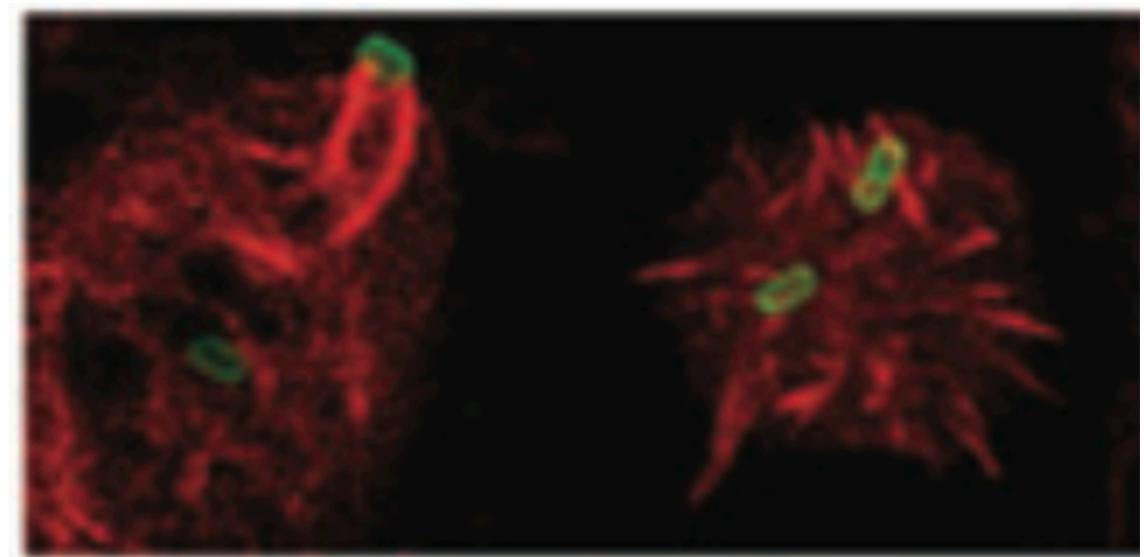
An SEM image of a cell membrane ruffle  
[C.A. Perrett et al., Cellular Microbiology (2009)]

# More Images of Ruffles



**CLSM image of ruffling cell (bacteria in blue)**

[Misselwitz et al, PLoS Pathogens, (2012)]

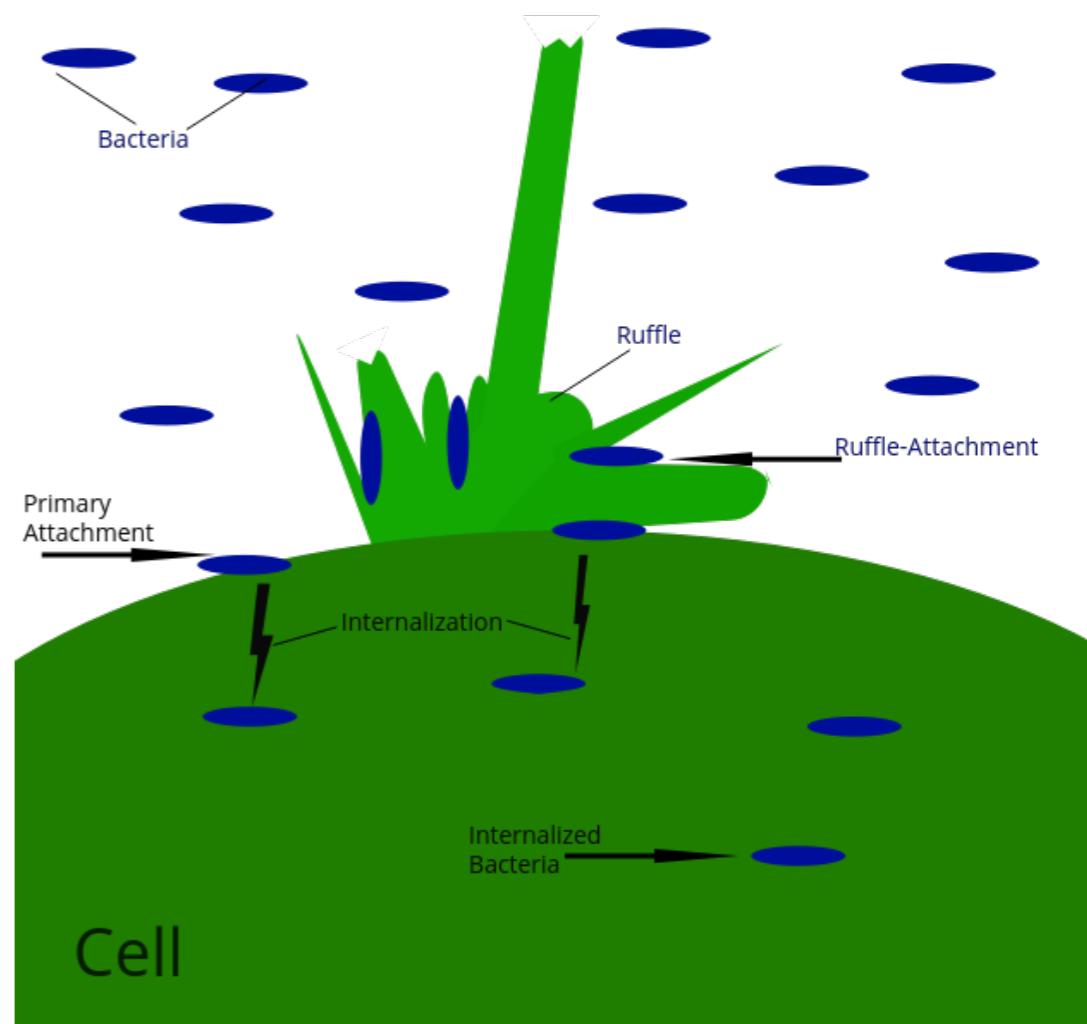


**CLSM images of ruffling cells (bacteria in green)**

[C.A. Perrett et al., Cellular Microbiology (2009)]

# Simplified System

- Well-mixed population of  $H$  cells and  $mH$  bacteria
- $m$ : Multiplicity of infection
- Bacteria can attach to cells, form ruffles, attach to ruffles, and invade cells (internalization)
- We have a maximum number of ruffles per cell, as well as a maximum number of internalized bacteria per cell



# Stochastic Model

- Bacteria in the model can undergo four stochastic processes with the following rates for each cell  $i \in \{1, \dots, H\}$ :

- Primary Attachment:  $\Gamma_a mb$

- Ruffle Formation:  $\Gamma_r a_i \left(1 - \frac{r_i}{r_{max}}\right)$

- Ruffle Attachment:  $\Gamma_{ar} m b r_i$

- Internalization:  $\Gamma_x \left(a_i + a_{r_i}\right) \left(1 - \frac{x_i}{x_{max}}\right)$

$b$	Fraction of bacteria free-swimming
$a$	# of primary attached bacteria for each cell
$a_r$	# of ruffle-attached bacteria for each cell
$x$	# of internalized bacteria for each cell
$r$	Number of ruffles for each cell
$\Gamma_a$	Base rate of primary attachment
$\Gamma_{ar}$	Base rate of ruffle attachment
$\Gamma_r$	Base rate of ruffle formation
$\Gamma_x$	Base rate of bacteria internalization
$x_{max}$	Maximum # of internalized bacteria per cell
$r_{max}$	Maximum # of ruffles per cell

# Gillespie Dynamics/ Kinetic Monte Carlo

- Rates are summed to yield overall rate of events happening:  $\Gamma_{tot} = \sum_i \Gamma_i$ , for  $i \in \{\text{space of possible processes}\}$
- Time-step to next event is selected from an exponential distribution with parameter  $\Gamma_{tot}$ :
$$p(\Delta t) = \Gamma_{tot} e^{-\Gamma_{tot} \Delta t}$$
- A single event from among the possibilities is then chosen to occur (with probabilities  $p(\Gamma_i) = \Gamma_i / \Gamma_{tot}$ ), and the time is increased by  $\Delta t$ .
- This process is repeated until the desired incubation time is reached.

# Mean Field Model

- To identify the stochastic effects in this system, we can compare the stochastic model with an analogous mean field model
- $a, a_r, x, r$  become continuous mean values for all cells
- System can then be integrated numerically

$$\dot{b} = -(\Gamma_a + \Gamma_{ar}r)b$$

$$\dot{a} = \Gamma_a mb - \Gamma_r a \left( 1 - \frac{r}{r_{max}} \right) - \Gamma_x a \left( 1 - \frac{x}{x_{max}} \right)$$

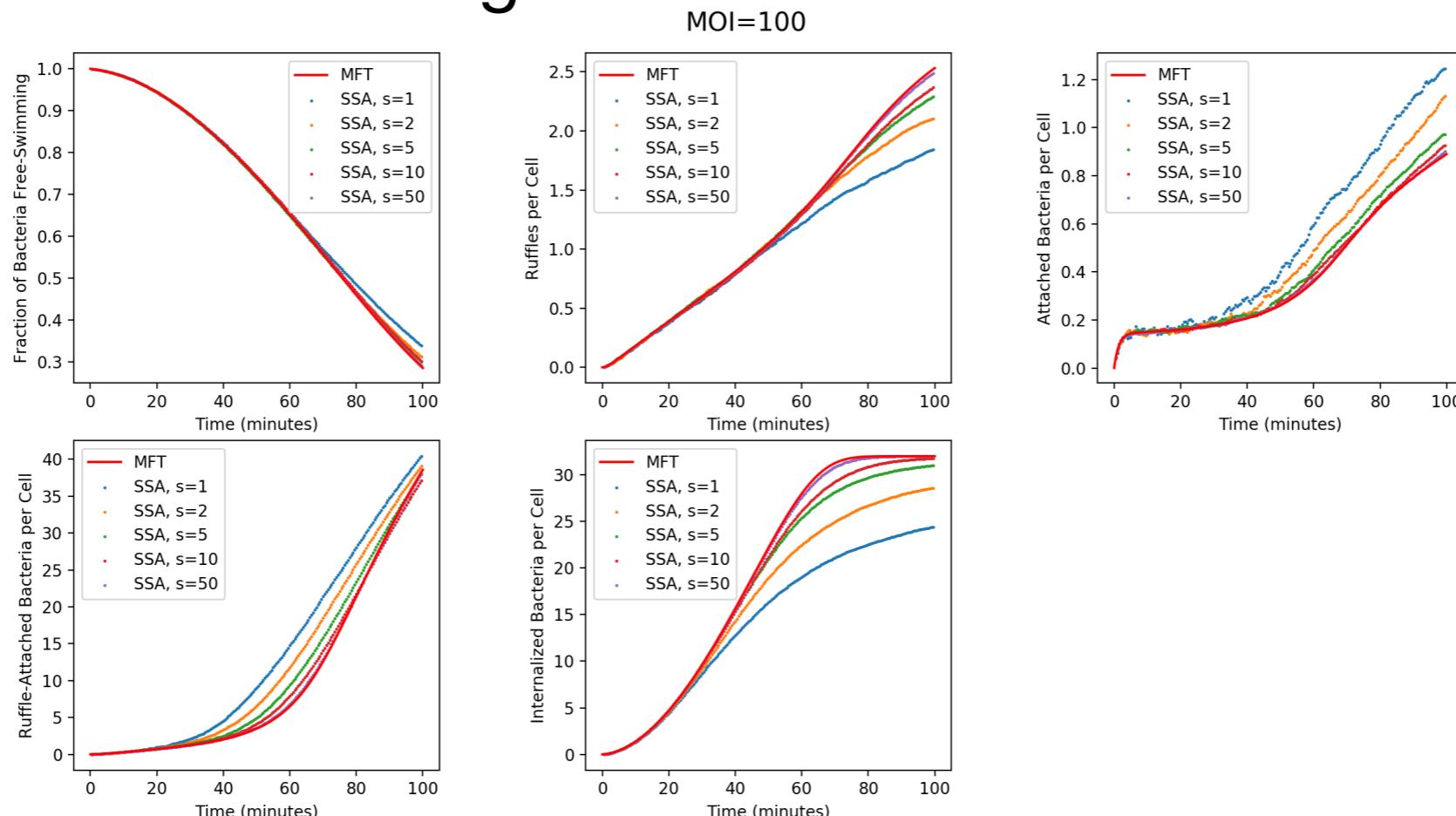
$$\dot{a}_r = \Gamma_{ar} mbr + \Gamma_r a \left( 1 - \frac{r}{r_{max}} \right) - \Gamma_x a_r \left( 1 - \frac{x}{x_{max}} \right)$$

$$\dot{x} = \Gamma_x (a + a_r) \left( 1 - \frac{x}{x_{max}} \right)$$

$$\dot{r} = \Gamma_r a \left( 1 - \frac{r}{r_{max}} \right)$$

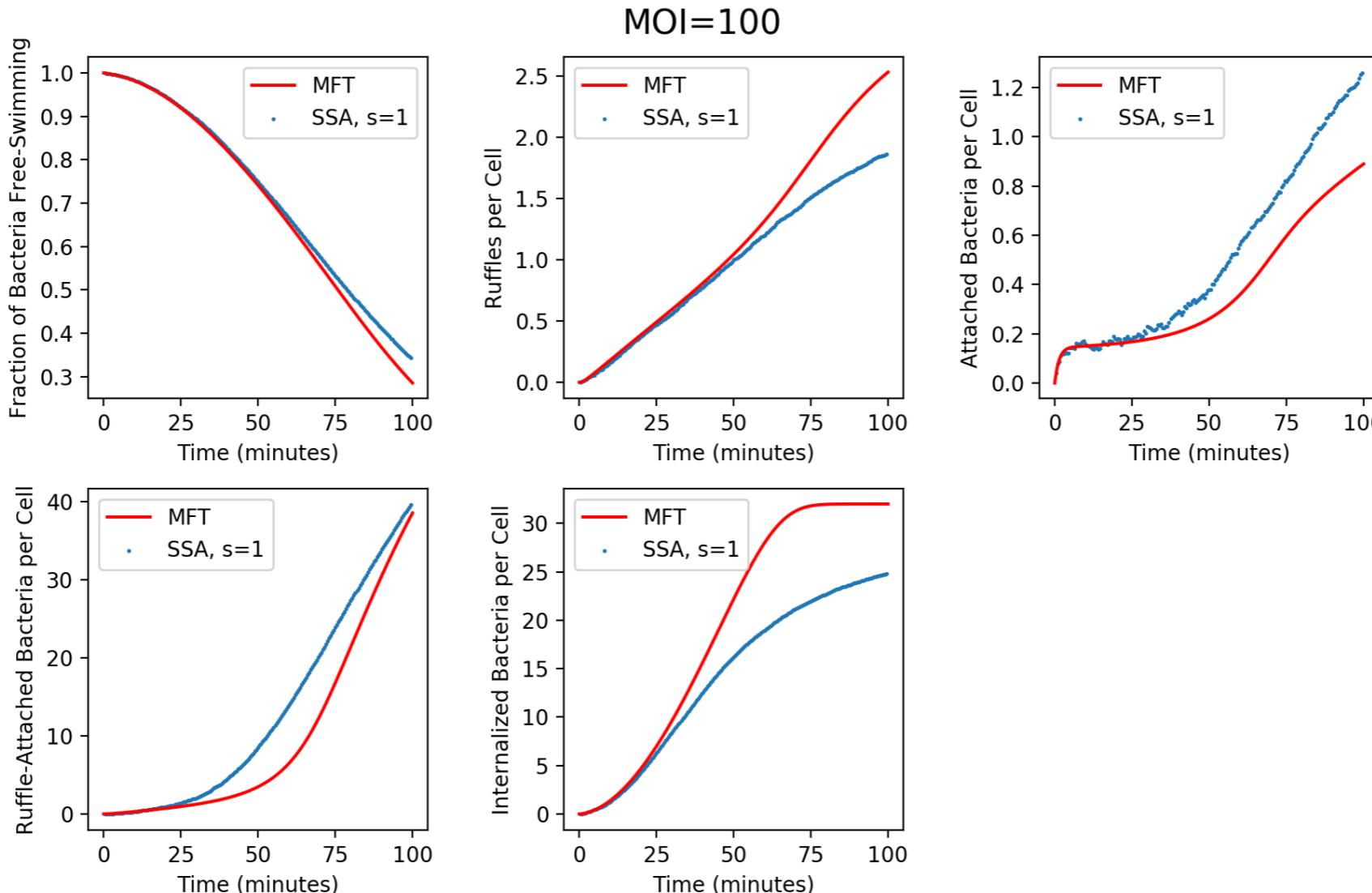
# Convergence to Mean Field Limit

- To verify that the stochastic model converges to the mean field model in some limit, we introduce a parameter 's' to the model.
- Bacteria are split into s 'sub-bacteria', which can form 'sub-ruffles'.
- Stochastic model converges to Mean Field Model for  $s > 50$ .



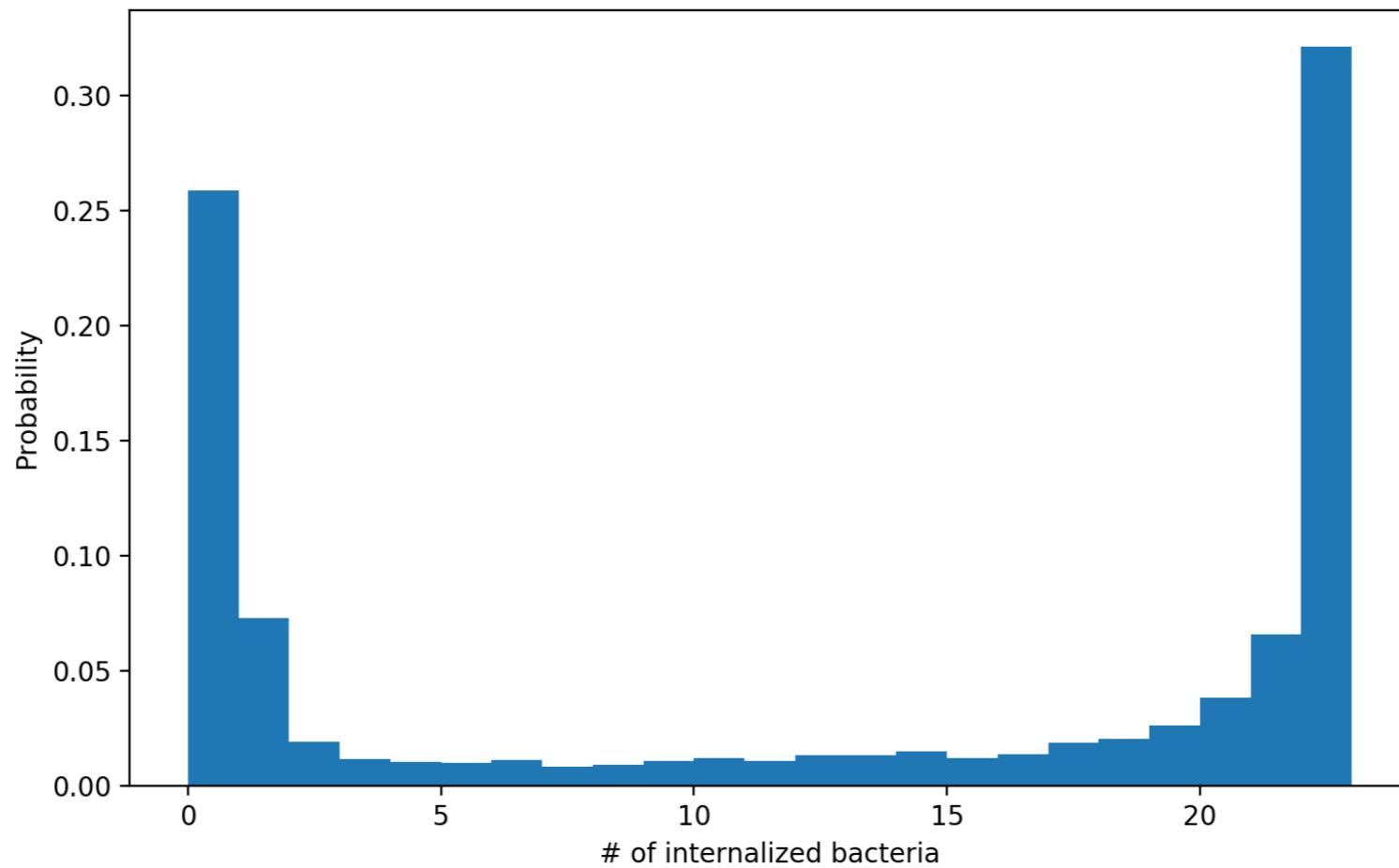
# Stochastic Effects

- By comparing the stochastic model with the mean field limit, we can identify the stochastic effects
- Key effects include fewer bacteria internalized - this is due to cells saturating with internalized bacteria, continuing to attach bacteria due to their high number of ruffles



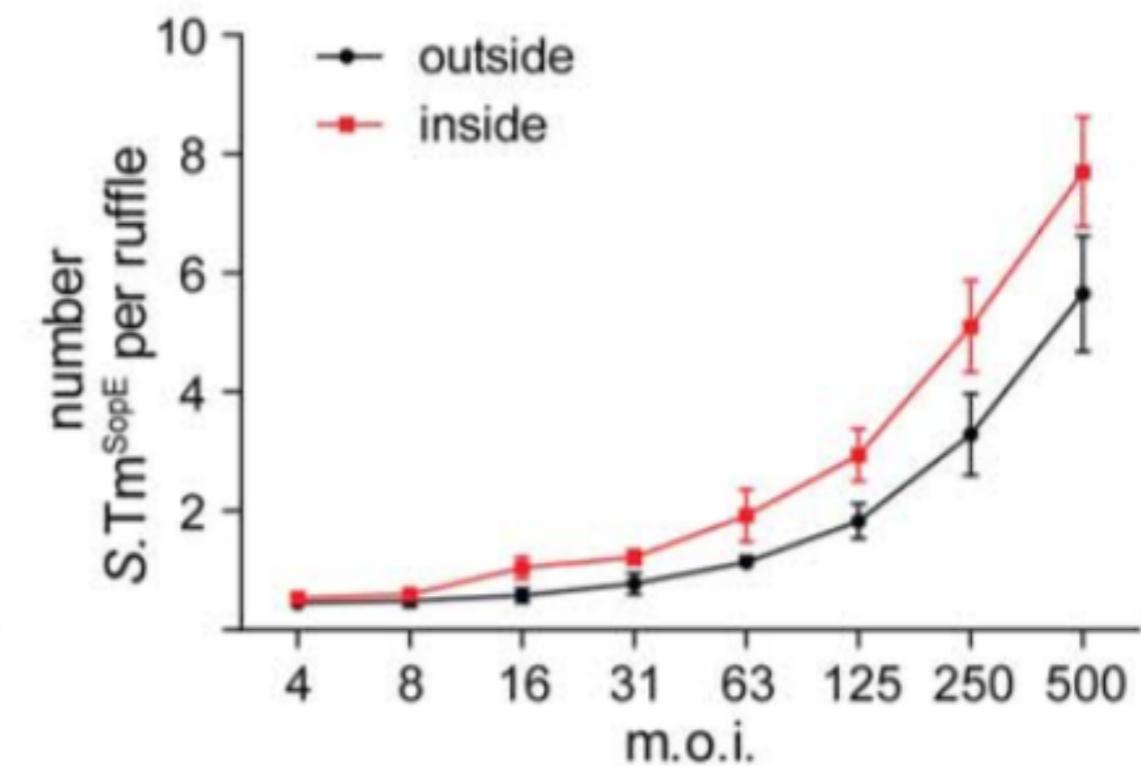
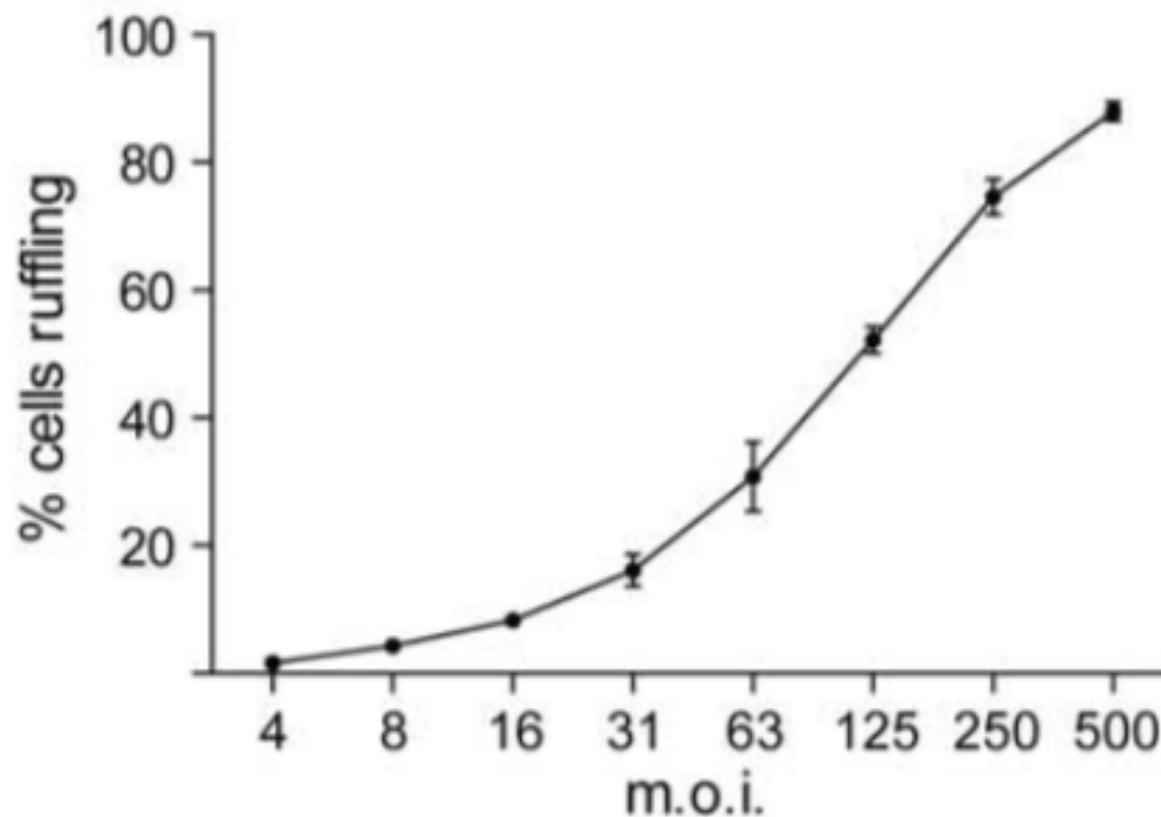
# Bimodal Distributions

- In some areas of parameter space, bimodal probability distributions can be observed for the number of bacteria internalized per cell



# Hardt Lab Experimental Data

- For 8 different MOI:
  - % of cells ruffling (~600 cells)
  - Counts of attached and internalized bacteria for ~100 cells



[Misselwitz et al, PLoS Pathogens, (2012)]

# Hellinger Distance

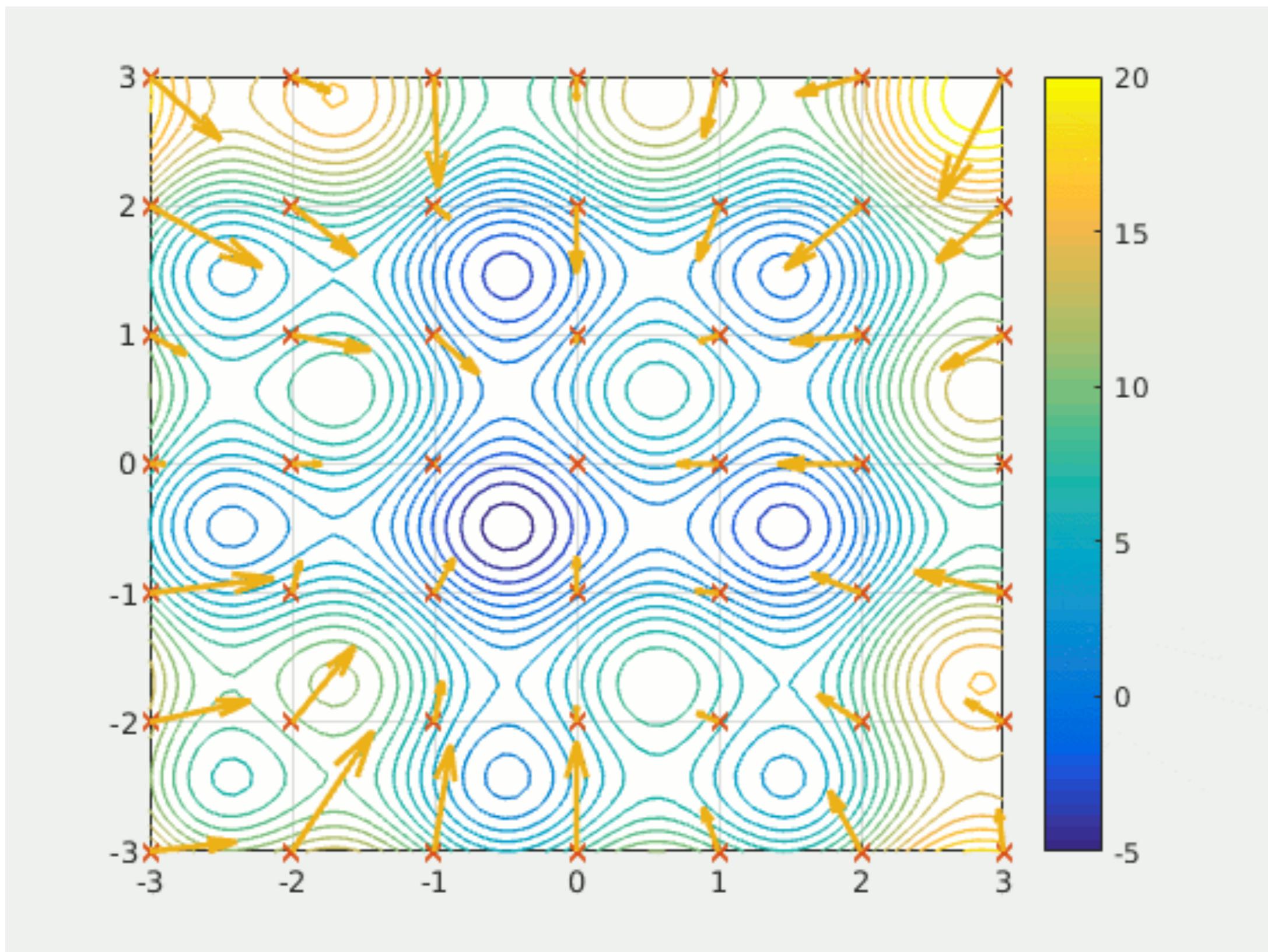
**For two discrete probability distributions  $p(n)$  and  $q(n)$ ,**

**the Hellinger distance is defined as** 
$$H(p, q) = \frac{1}{\sqrt{2}} \sqrt{\sum_{i=1}^N (\sqrt{p_i} - \sqrt{q_i})^2}$$

- The Hellinger distance is a metric on any set of discrete probability distributions (there is a continuous analog). Some key properties include:
- Symmetric (i.e.  $H(p, q) = H(q, p)$ )
- Still defined if  $\exists n$  such that  $p(n) = 0$  and  $q(n) \neq 0$  or vice versa.

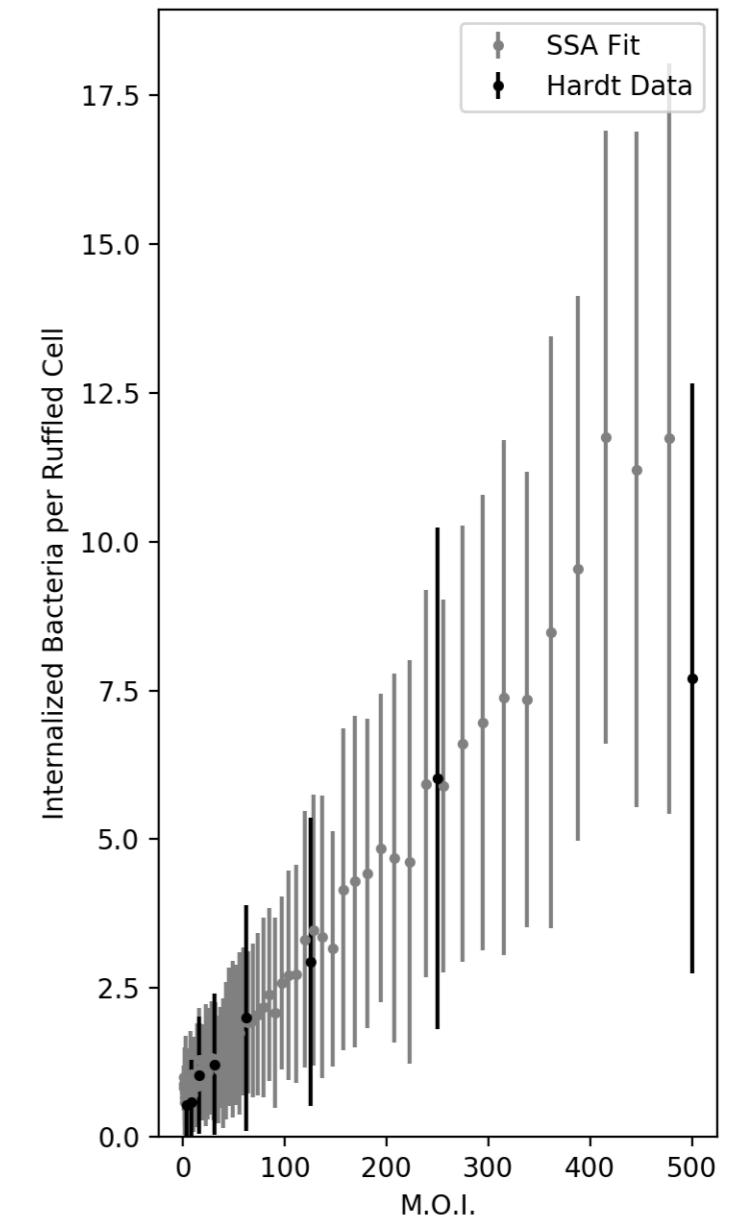
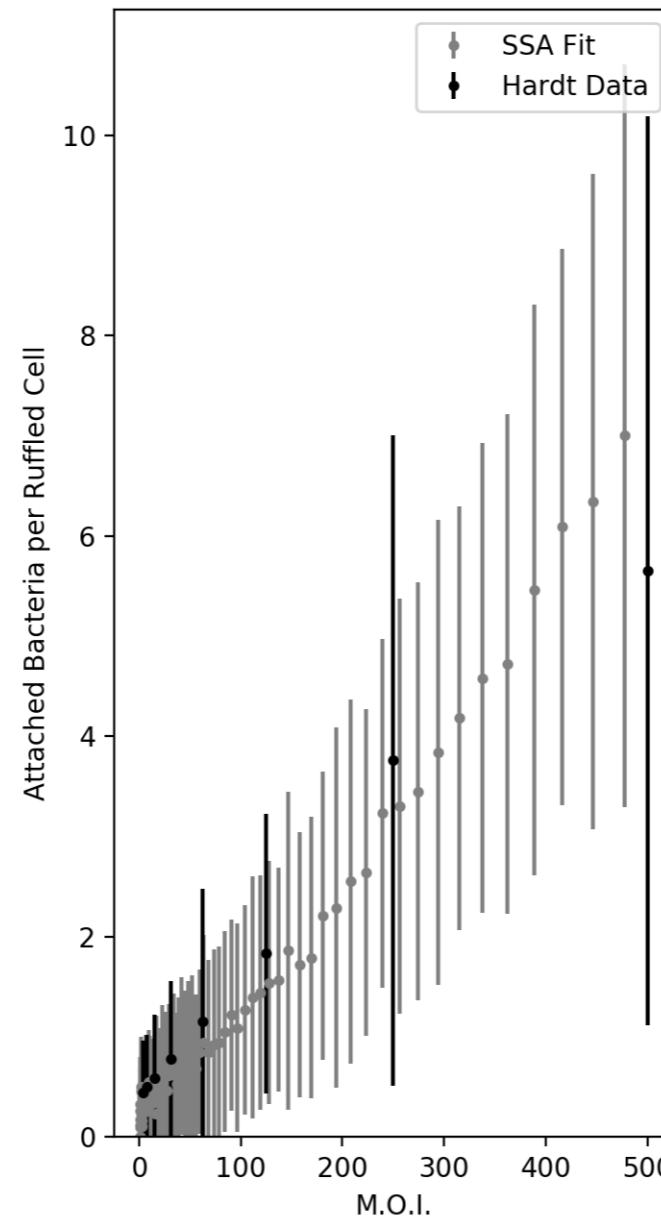
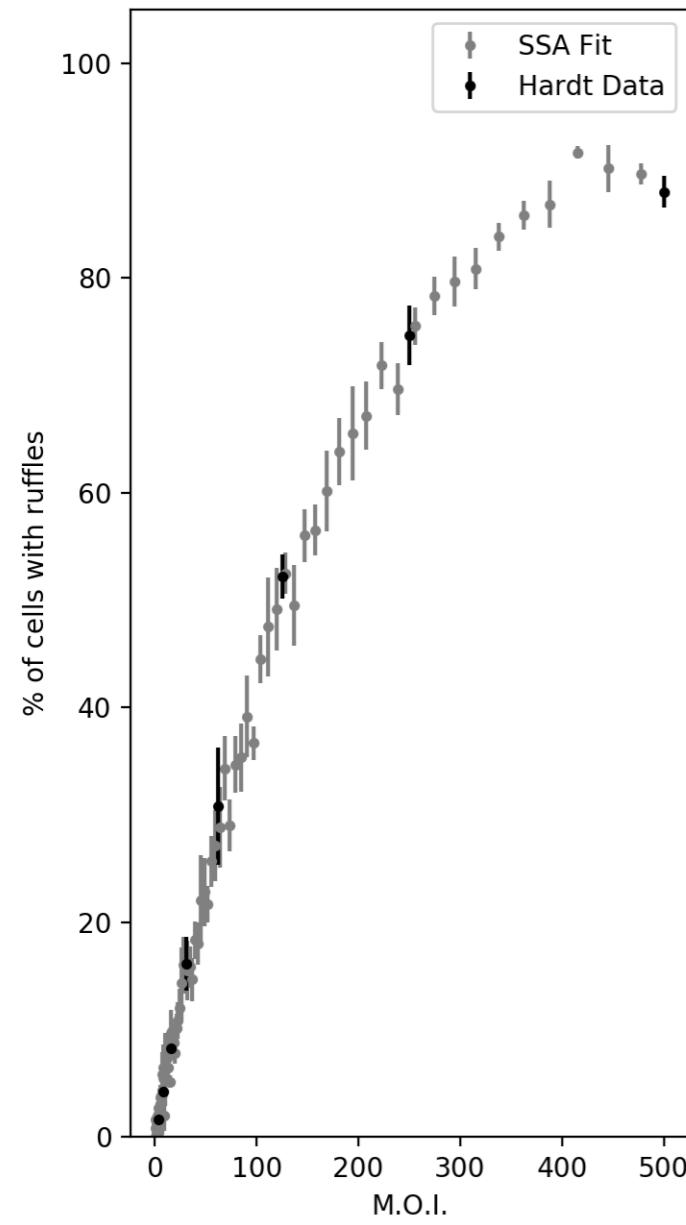
# Particle Swarm Optimization

- Optimization algorithm which can handle stochastic inputs (doesn't need gradient) and rough parameter landscapes
- A number of 'particles' are placed uniformly throughout multi-dimensional parameter space with a random initial velocity
- Particles continuously update their velocity with respect to the positions and results of other particles, with an additional random component to avoid being trapped in local minima
- Swarm will converge to global minimum
- Different networks are possible for communication between the particles (e.g. all inclusive, nearest neighbours only, social groups, etc...)

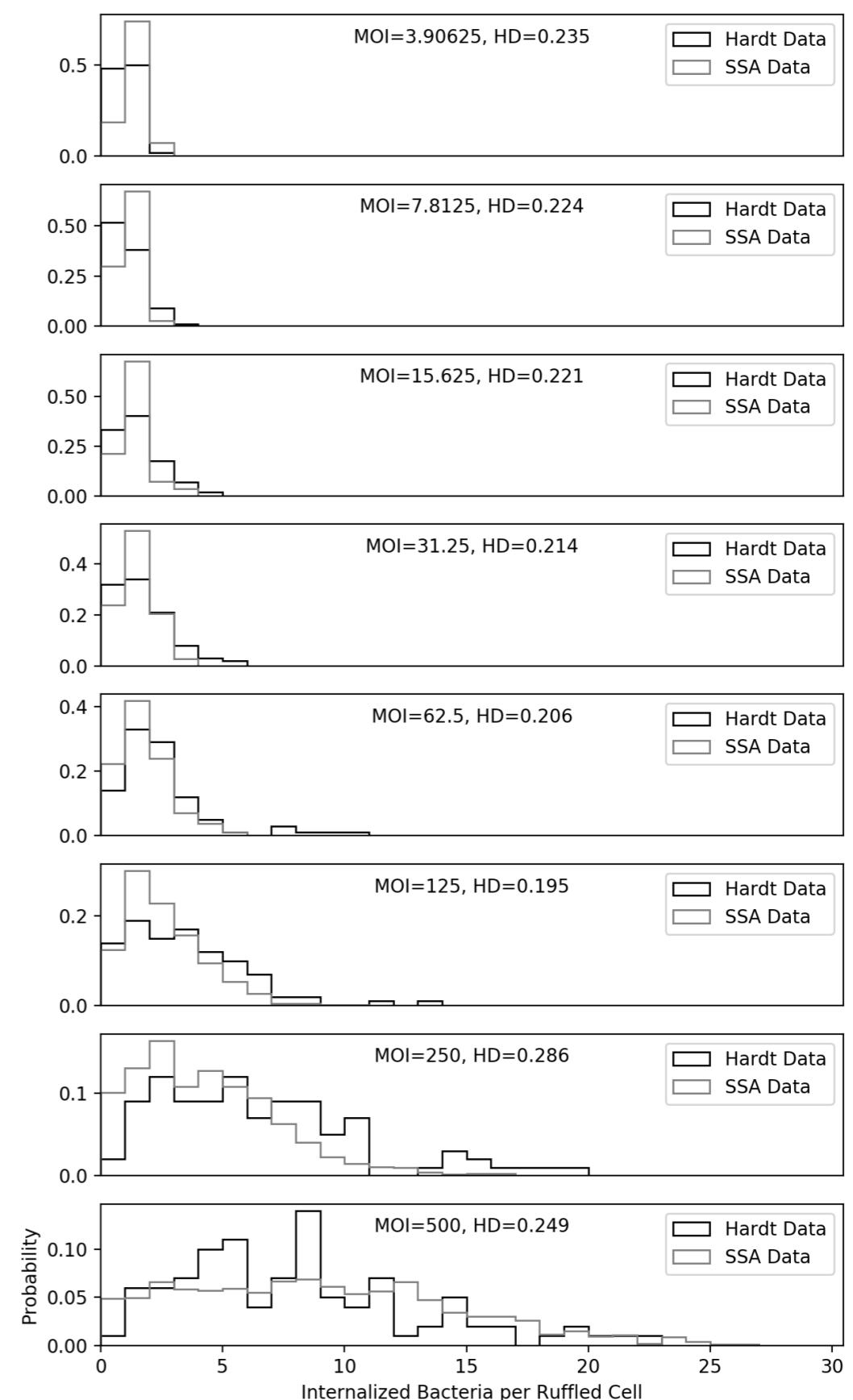
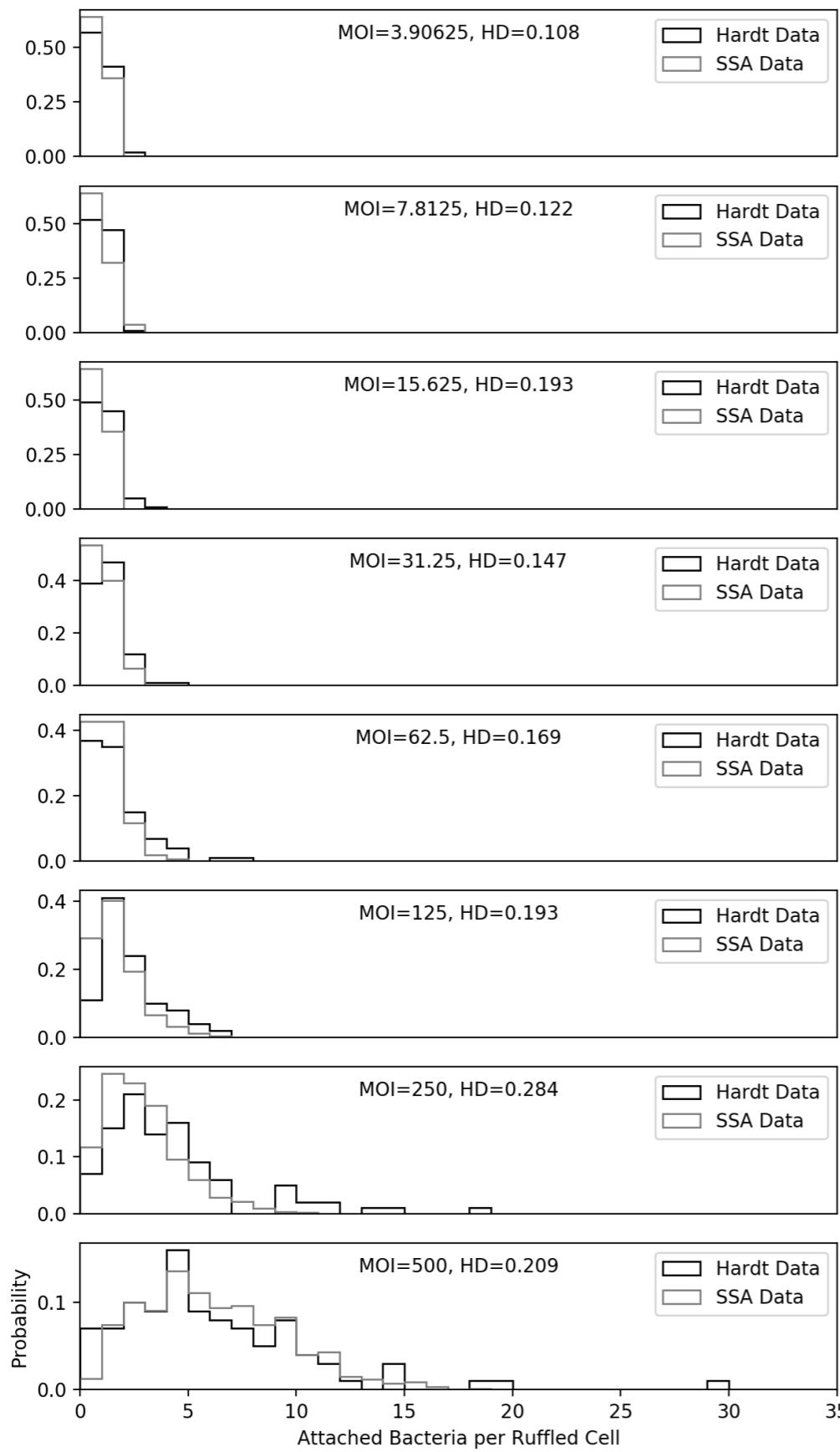


By Ephramac [CC BY-SA 4.0 (<https://creativecommons.org/licenses/by-sa/4.0>)], from Wikimedia Commons

# Hardt Lab Data Fit

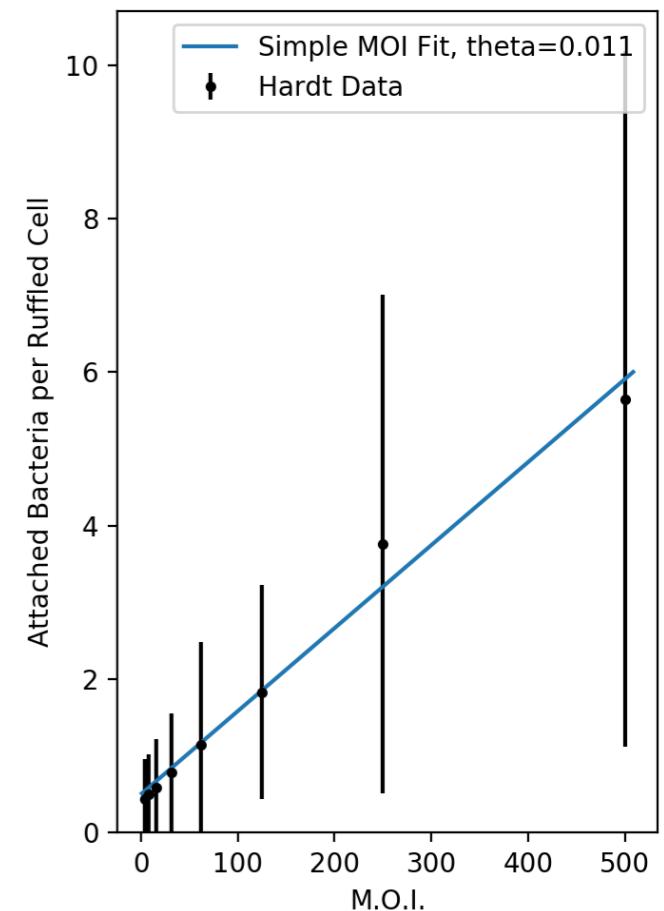
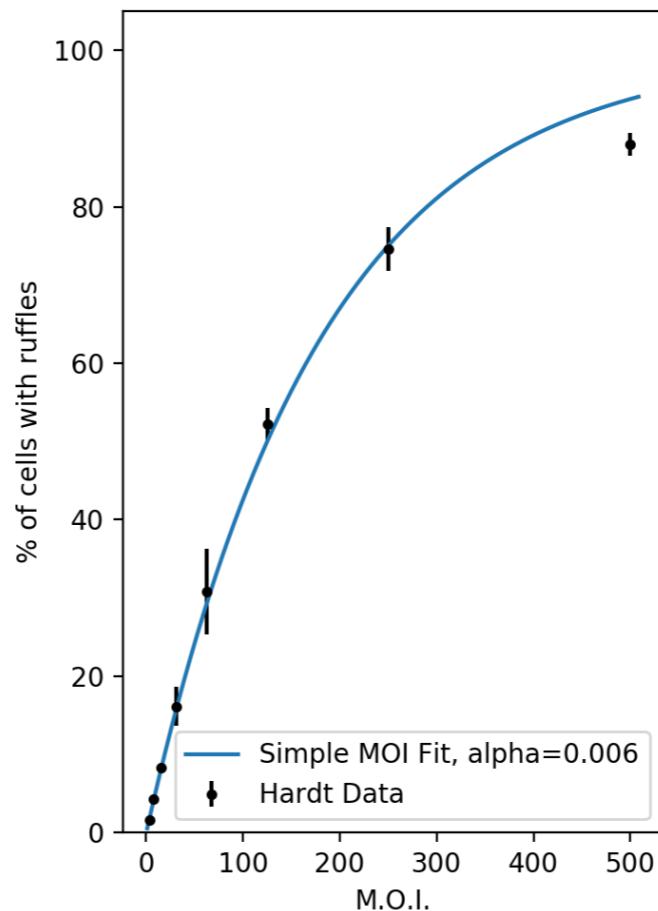


# Distributions



# Simple MOI Fits

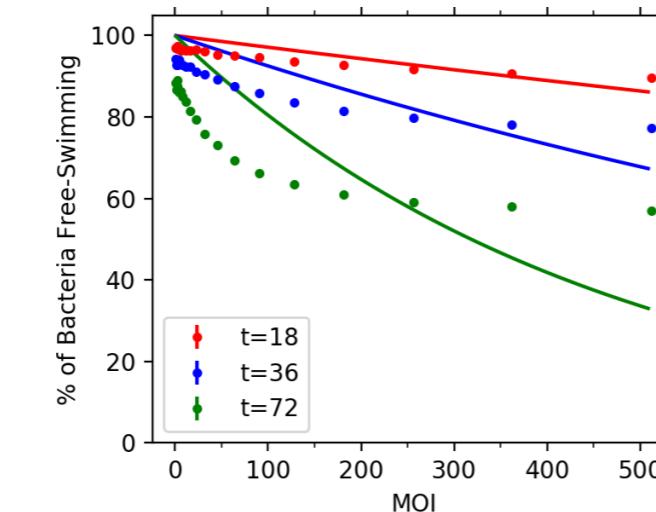
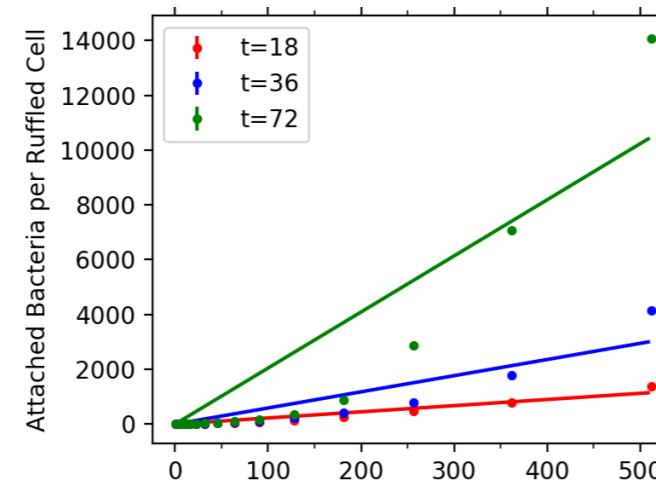
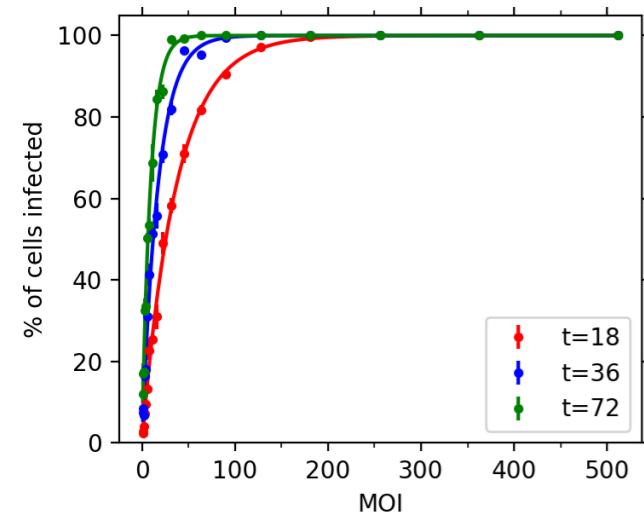
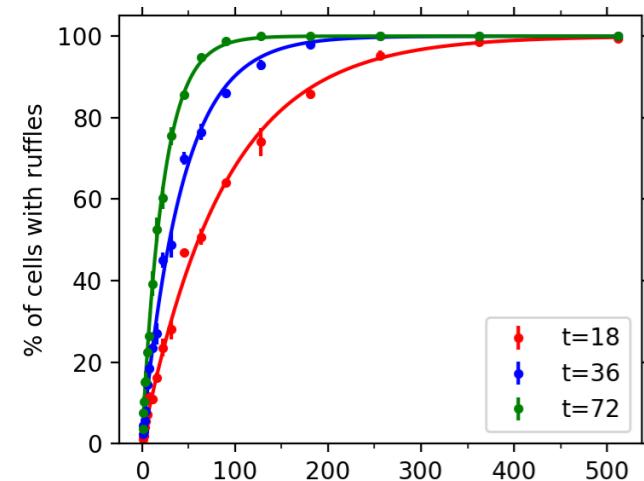
- We observed two simple phenomenological fits to measurements from the experimental data:
- These simple fits accurately describe the experimental data as well as data from the stochastic model for a wide range of parameters
- We have also observed other simple MOI fits to the stochastic model data



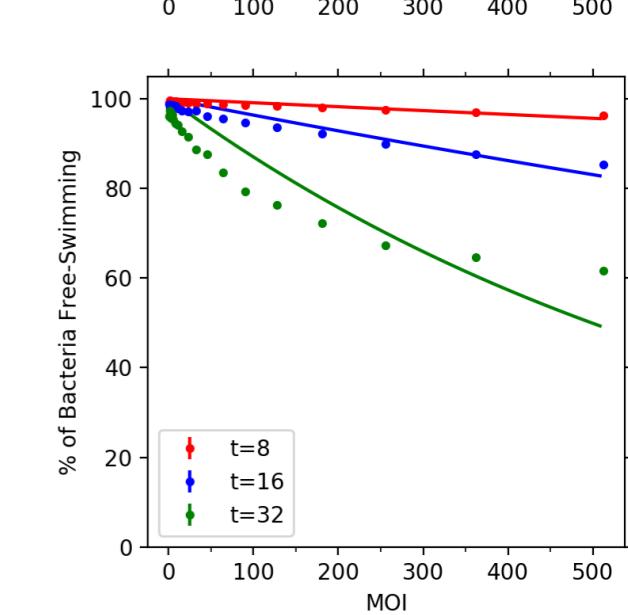
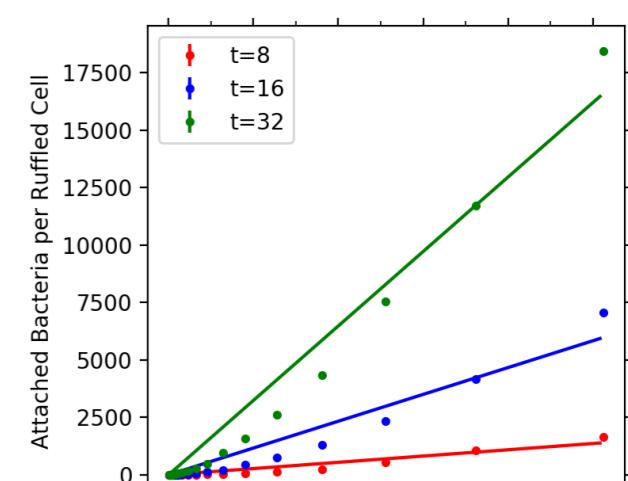
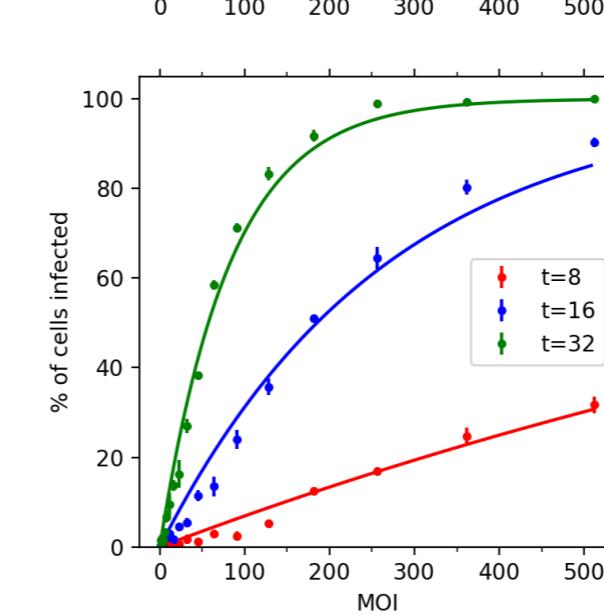
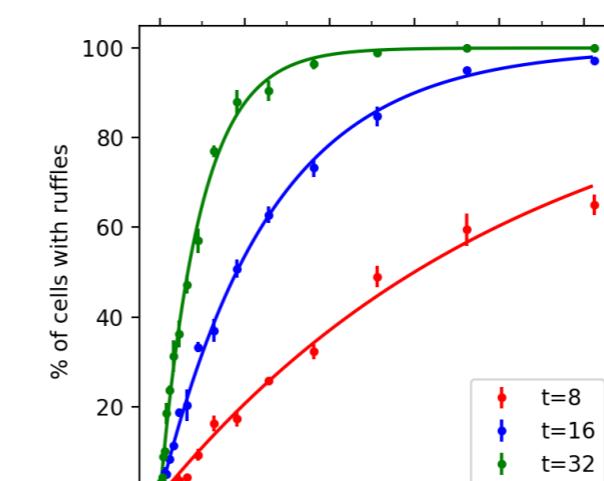
Statistic	Fit to MOI (m)
% of cells ruffling	$100(1 - e^{-\alpha m})$
Attached bacteria per ruffled cell	$0.5 + \theta m$
% of cells infected	$100(1 - e^{-\beta m})$
% of bacteria free-swimming	$100e^{-\phi m}$

# Further Examples of Phenomenological Fits

**Hardt data fit parameters:**



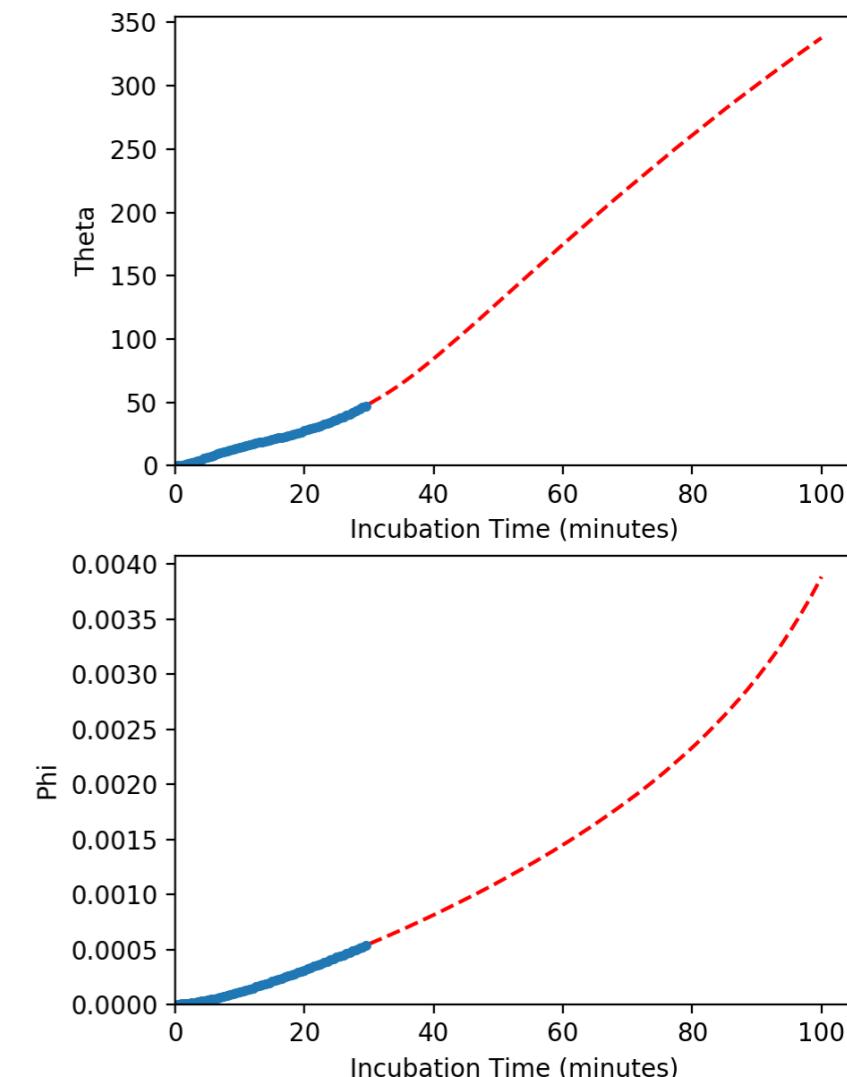
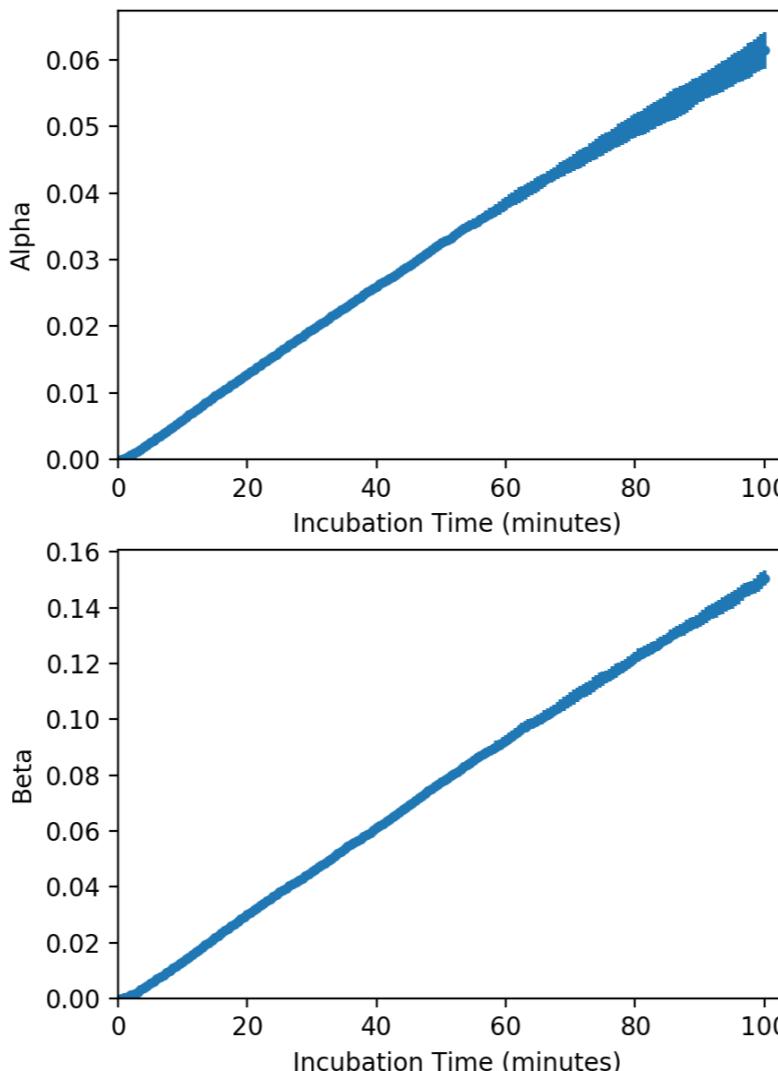
**Alternate parameters:**



Statistic	Fit to MOI (m)
% of cells ruffling	$100(1 - e^{-\alpha m})$
Attached bacteria per ruffled cell	$0.5 + \theta m$
% of cells infected	$100(1 - e^{-\beta m})$
% of bacteria free-swimming	$100e^{-\phi m}$

# Simple Time Dependence

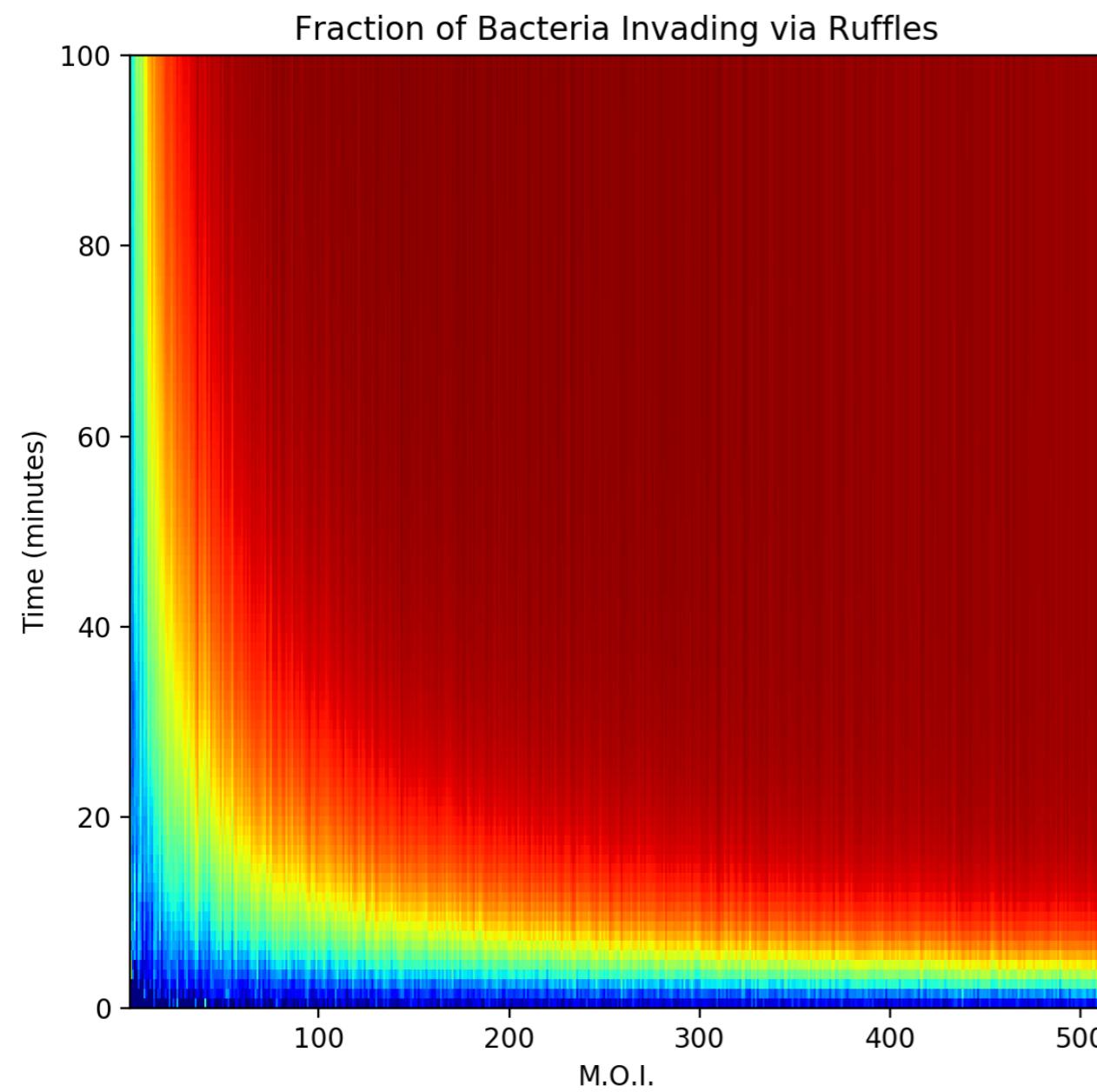
- Fits for ‘attached bacteria per ruffled cell’ and ‘% of bacteria free-swimming become significantly worse for incubation times over 30 minutes
- Alpha and Beta remain good fits for all incubation times, increase linearly
- Shows that ruffling correlates directly with infection



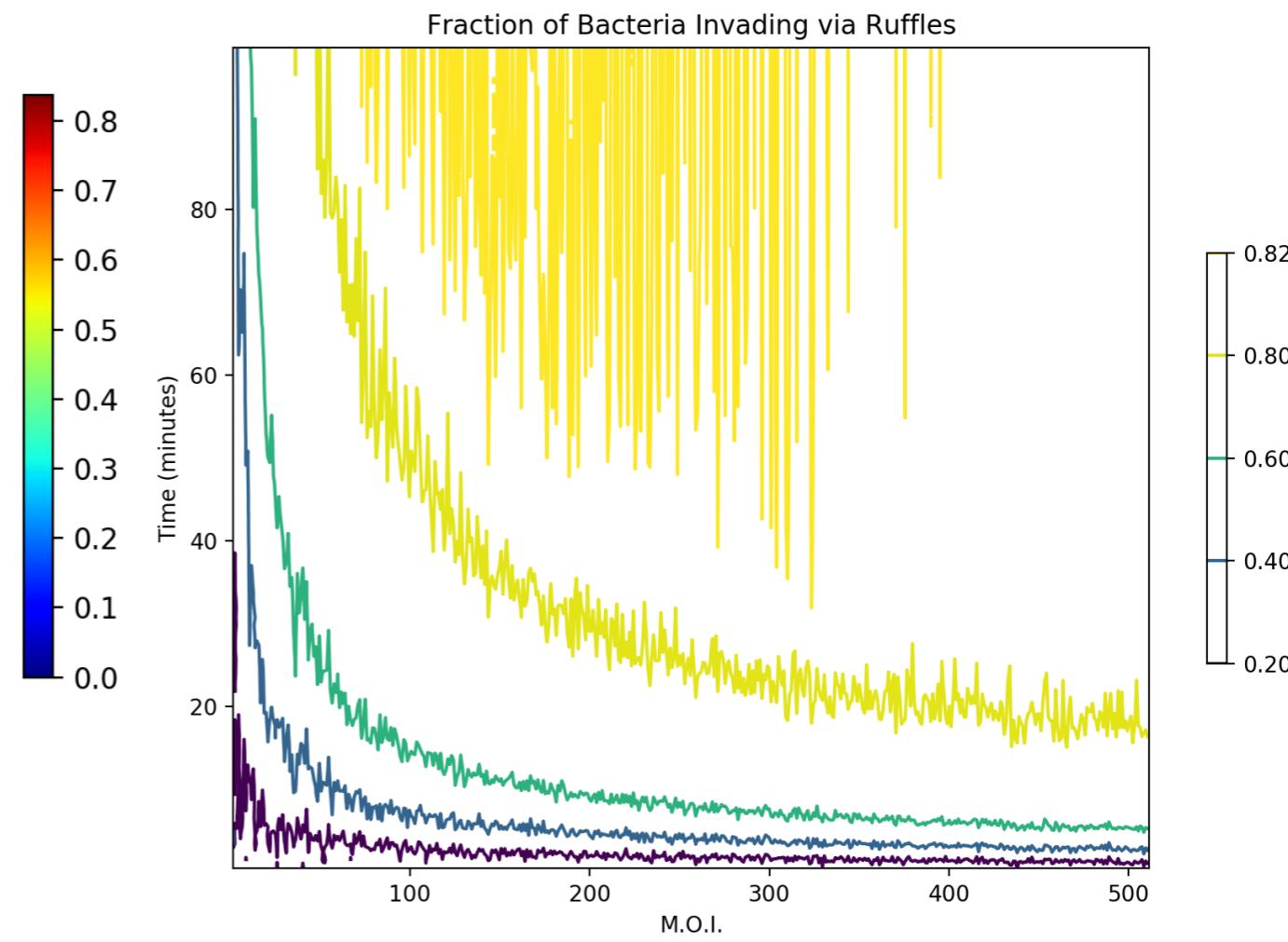
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# Investigating Cooperativity

Fraction of bacteria internalized through ruffles ( $\frac{X_r}{X}$ ):



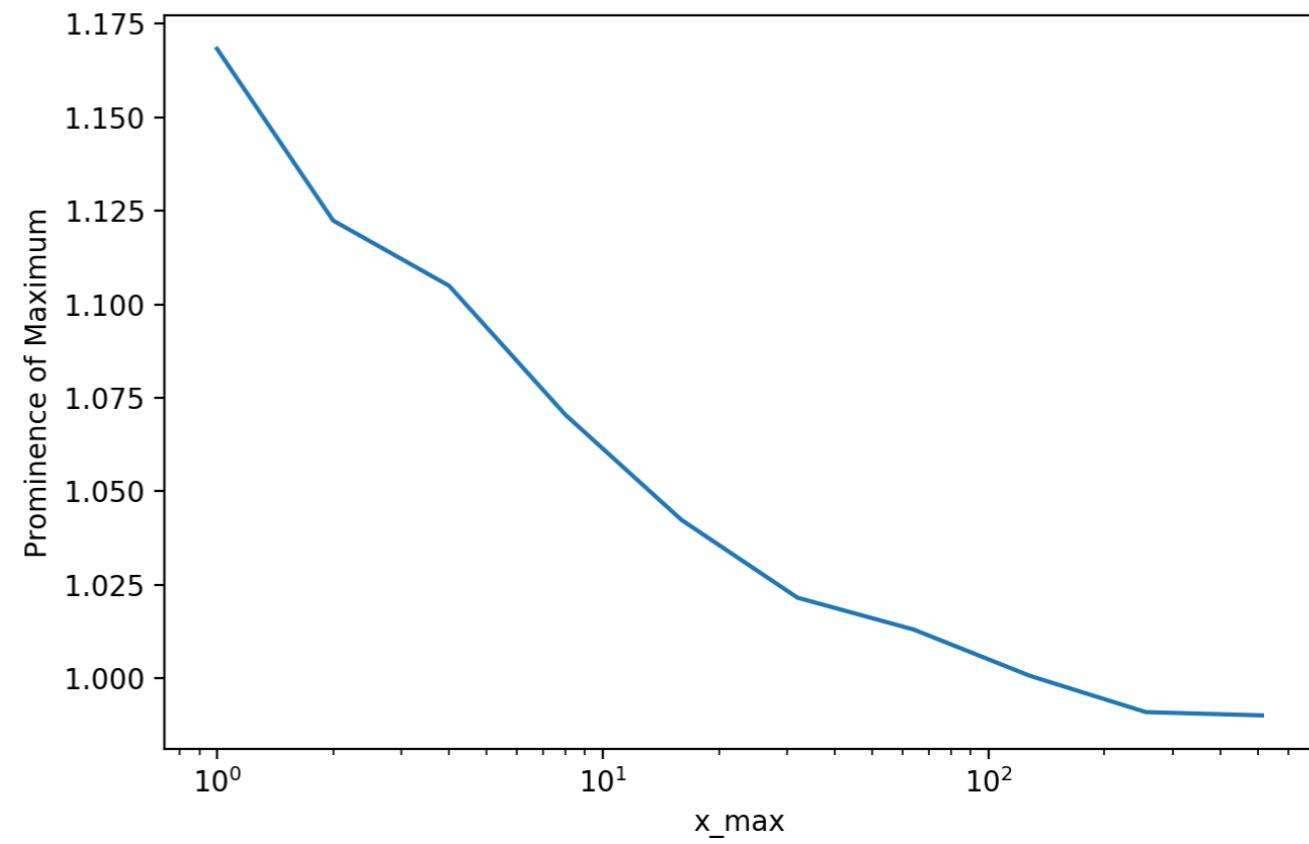
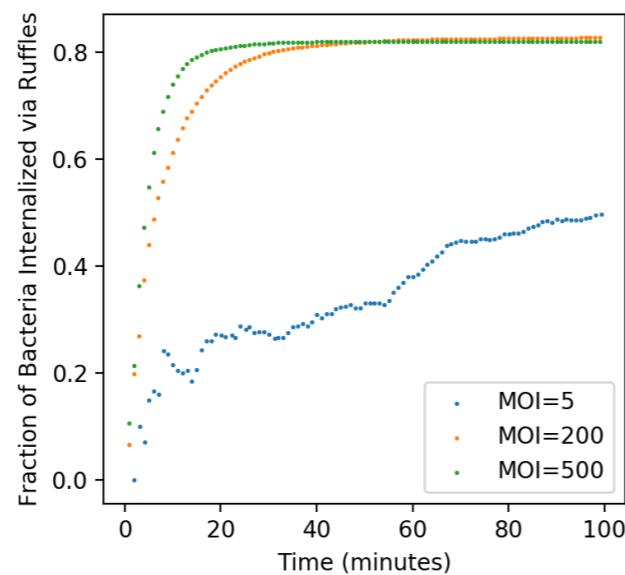
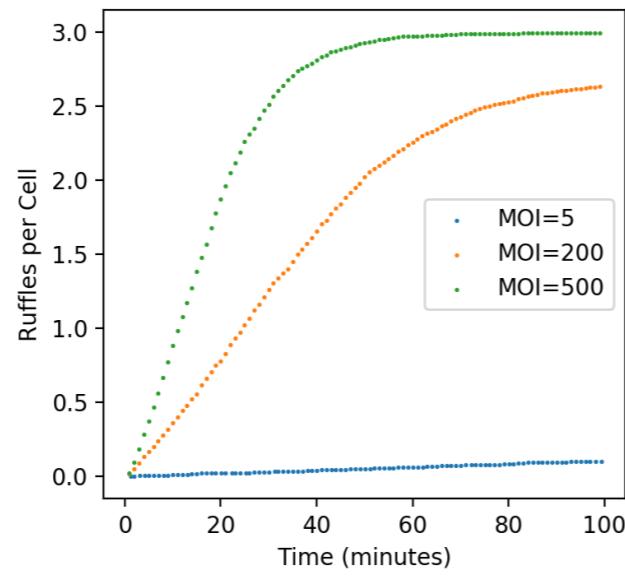
Note unexpected maximum at medium MOI:



# Explanation of Maximum

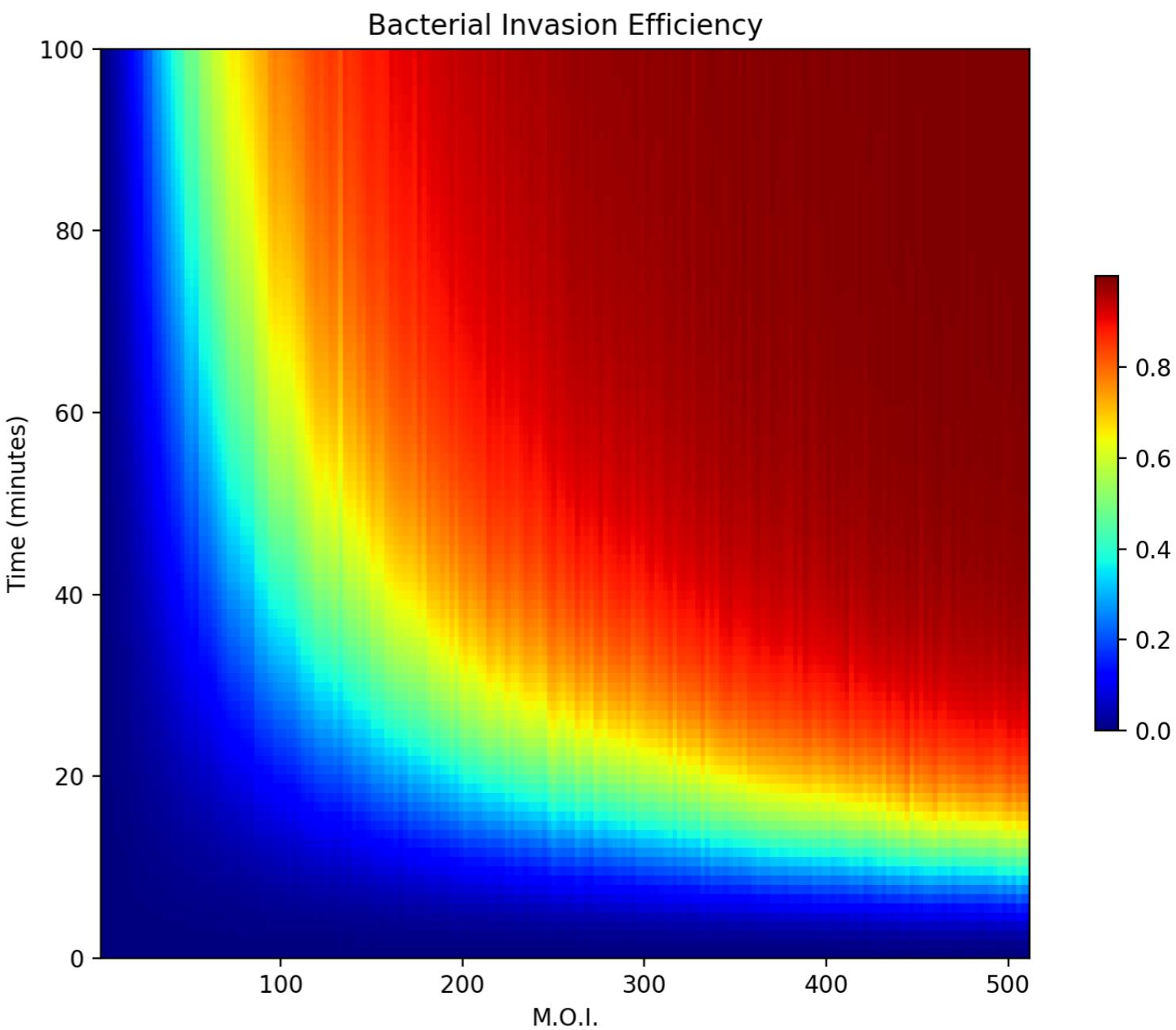
- Maximum is dependent on  $x_{max}$

For  $x_{max} = 32$ :



# Invasion Efficiency

- Max # of bacteria that can be internalized:  $H \cdot x_{max}$
- Measuring  $\frac{\sum_x}{H \cdot x_{max}}$  at different incubation times and MOI
- Looks as expected, monotonic function of both MOI and incubation time



# Advantage of the Ruffling Mechanism

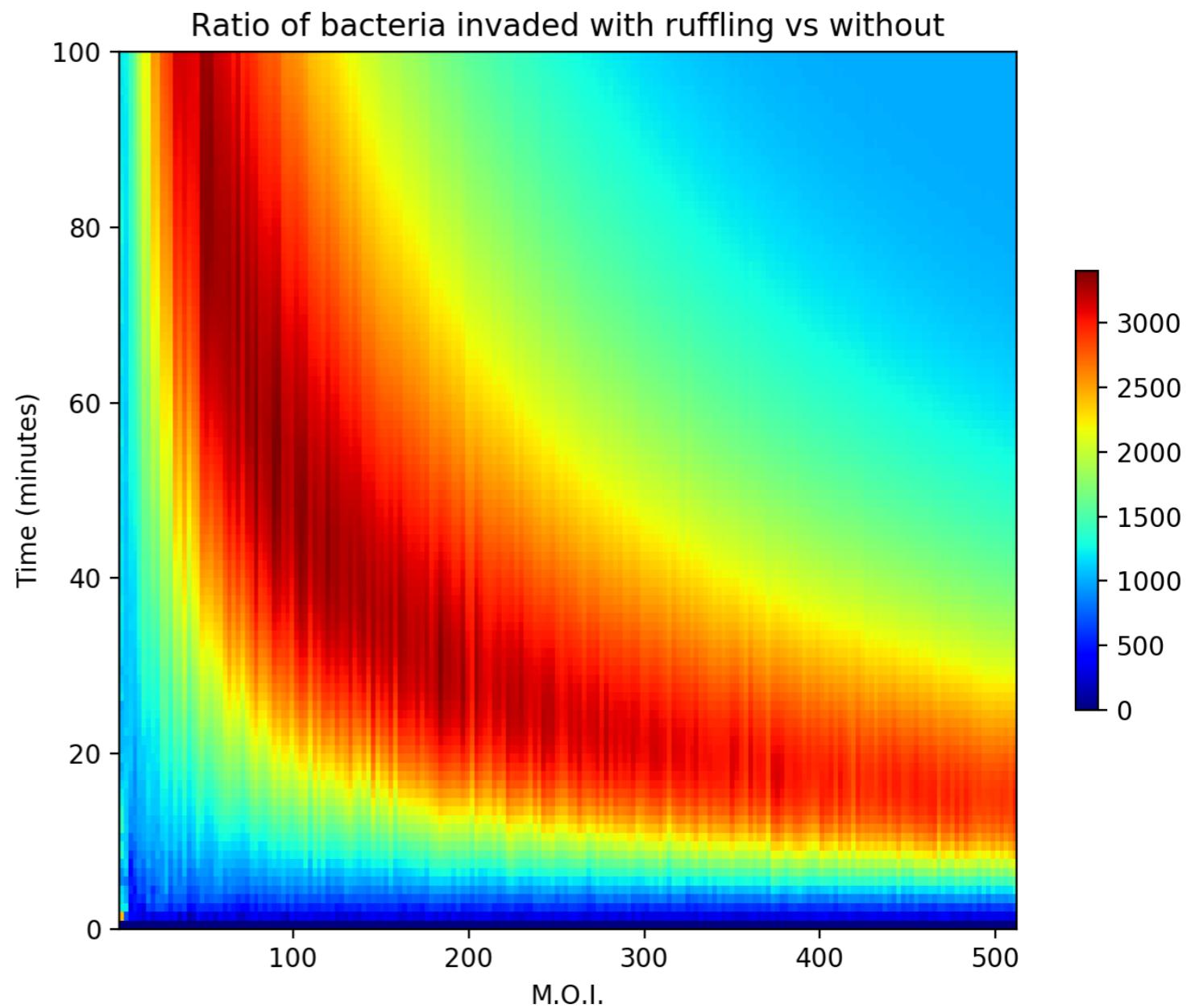
- Without ruffling ( $\Gamma_r \rightarrow 0$ ):

$$\dot{b} = -\Gamma_a c b$$

$$\dot{a} = \Gamma_a m c b - \Gamma_x a \left( 1 - \frac{x}{x_{max}} \right)$$

$$\dot{x} = \Gamma_x a \left( 1 - \frac{x}{x_{max}} \right)$$

- Measuring  $\frac{X_{ruffling}}{X_{no\ ruffling}}$  at different incubation times and MOI
- Optimal regime for evolution of the ruffling mechanism?



# Future Modelling Considerations

- Different rates of internalization for primary-attached vs ruffle-attached bacteria
- Continuous rather than discrete ruffles

**Thanks!**