

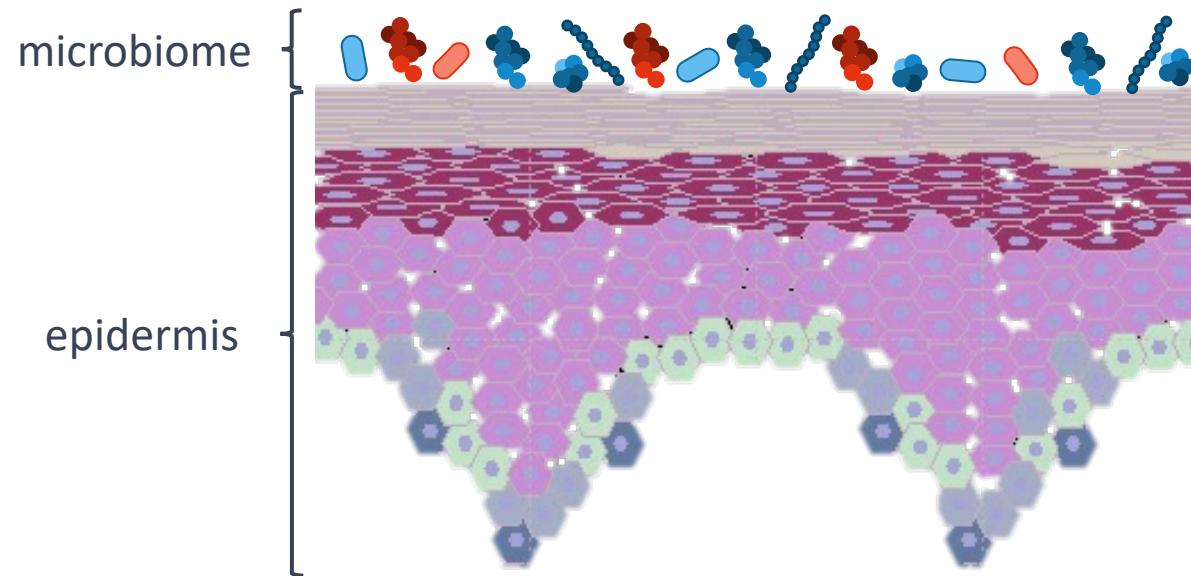
Stability versus Meta-stability in a Skin Microbiome Model

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The skin microbiome

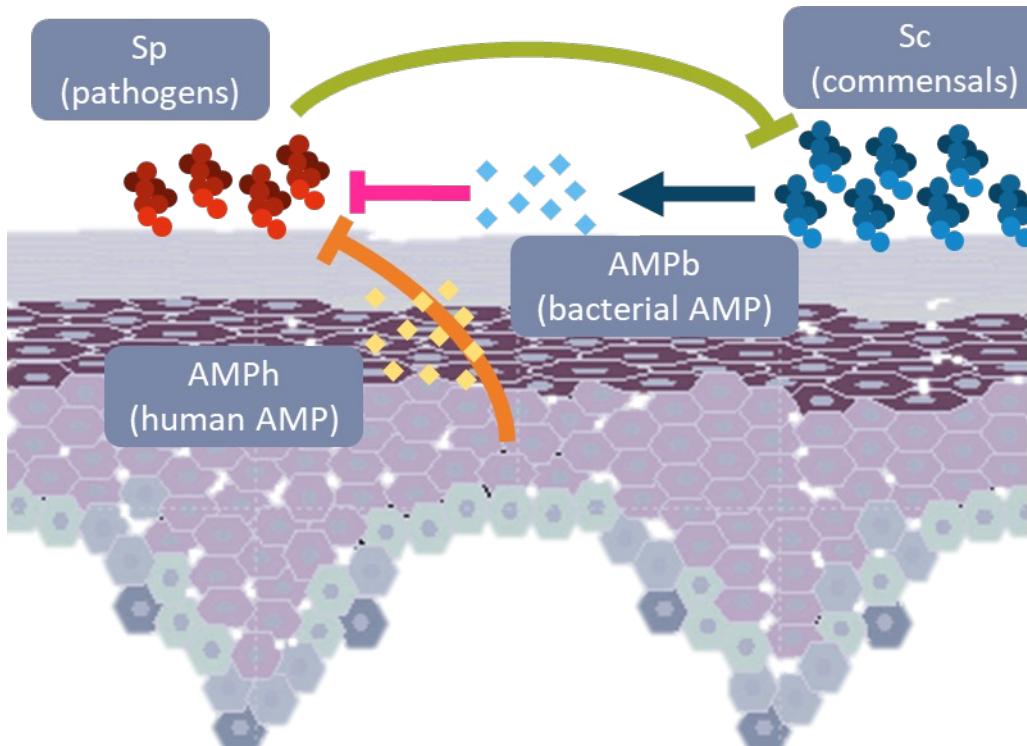


- Diverse
- Close interactions with the skin: defense against pathogen colonization
- Alterations of its composition linked with skin conditions (acne, eczema,...)

Initial ODE skin microbiome model

Objective

Identify factors influencing the dominance of the pathogenic population over the commensals



AMP = Antimicrobial peptides

$$\left\{ \begin{array}{l} \frac{d[S_c]}{dt} = \left(r_{sc} \left(1 - \frac{[S_c]}{K_{sc}} \right) - \frac{d_{sc}[S_p]}{C_1 + [S_p]} \right) [S_c] \\ \frac{d[S_p]}{dt} = \left(r_{sp} \left(1 - \frac{[S_p]}{K_{sp}} \right) - \frac{d_{spb}[Amp_b]}{C_{ab} + [Amp_b]} - \frac{d_{sph}[Amp_h]}{C_{ah} + [Amp_h]} \right) [S_p] \\ \frac{d[Amp_b]}{dt} = k_c [S_c] - d_a [Amp_b] \end{array} \right.$$

Available quantitative data

Kohda et al., 2021 ⁽¹⁾

- *in vitro*
- 3D epidermal equivalent
- **mono-cultures** and **co-cultures** of *Staphylococcus epidermidis* and *Staphylococcus aureus* (*S.aureus*)
- population sizes of the two species measured after 48 hours of incubation

Nakatsuji et al., 2017 ⁽²⁾

- *in vitro*
- culture media
- Influence of mammalian (LL-37) and bacterial (Sh-lantibiotics- α and β) **AMPs** on *S.aureus* growth.
- *S.aureus* population size measured after 24 hours of incubation
- Several concentrations of LL-37 and Sh-lantibiotics tested

1) Kohda K. et al. "An In Vitro Mixed Infection Model With Commensal and Pathogenic Staphylococci for the Exploration of Interspecific Interactions and Their Impacts on Skin Physiology." *Frontiers in cellular and infection microbiology* vol. 11 712360. (2021)

2) Nakatsuji, T. et al. "Antimicrobials from human skin commensal bacteria protect against *Staphylococcus aureus* and are deficient in atopic dermatitis." *Science translational medicine* vol. 9,378 (2017)

Parameter identification by steady state reasoning

Experimental data [Nakatsuji 2021]				
[AMPh] (LL-37)	4	8	0	0
[AMPb] (<i>Sh</i> -lantibiotics)	0	0	0.32	0.64
→ Sp (<i>S. aureus</i>)	10^9	$6 \cdot 10^5$	$5 \cdot 10^8$	$3 \cdot 10^3$

Corresponding submodel

$$\begin{cases} \frac{d[S_c]}{dt} = \left(r_{sc} \left(1 - \frac{[S_c]}{K_{sc}} \right) - \frac{d_{sc}[S_p]}{C_1 + [S_p]} \right) [S_c] \\ \frac{d[S_p]}{dt} = \left(r_{sp} \left(1 - \frac{[S_p]}{K_{sp}} \right) - \frac{d_{spb}[Amp_b]}{C_{ab} + [Amp_b]} - \frac{d_{sph}[Amp_h]}{C_{ah} + [Amp_h]} \right) [S_p] \\ \frac{d[Amp_b]}{dt} = k_c [S_c] - d_a [Amp_b] \end{cases}$$

Substitution

steady state

Parameter identification

$$\begin{aligned} d_{sph} &= 2 r_{sp} & C_{ah} &= 8 \\ d_{spb} &= \frac{5}{4} r_{sp} & C_{ab} &= 0.16 \end{aligned}$$

$$\begin{aligned} [S_p]^* &= K_{sp} \left(1 - \frac{d_{spb}[Amp_b]}{r_{sp}(C_{ab} + [Amp_b])} - \frac{d_{sph}[Amp_h]}{r_{sp}(C_{ah} + [Amp_h])} \right) \\ &\text{or} \\ [S_p]^* &= 0 \end{aligned}$$

Parameter identification by steady state reasoning

Co-culture [Kohda 2021]

$$\begin{cases} \frac{d[S_c]}{dt} = \left(r_{sc} \left(1 - \frac{[S_c]}{K_{sc}} \right) - \frac{d_{sc}[S_p]}{C_1 + [S_p]} \right) [S_c] \\ \frac{d[S_p]}{dt} = \left(r_{sp} \left(1 - \frac{[S_p]}{K_{sp}} \right) - \frac{d_{spb}[Amp_b]}{C_{ab} + [Amp_b]} - \frac{d_{sph}[Amp_h]}{C_{ah} + [Amp_h]} \right) [S_p] \\ \frac{d[Amp_b]}{dt} = k_c [S_c] - d_a [Amp_b] \end{cases}$$



$$\begin{cases} [S_c]^* = K_{sc} \left(1 - \frac{d_{sc}[S_p]^*}{r_{sc}(C_1 + [S_p]^*)} \right) \quad \text{or} \quad [S_c]^* = 0 \\ [S_p]^* = K_{sp} \left(1 - \frac{d_{spb}[Amp_b]}{r_{sp}(C_{ab} + [Amp_b])} - \frac{d_{sph}[Amp_h]}{r_{sp}(C_{ah} + [Amp_h])} \right) \quad \text{or} \quad [S_p]^* = 0 \\ [Amp_b]^* = \frac{k_c [S_c]^*}{d_a} \end{cases}$$



Steady State

Substitution of experimental data

$$d_{sc} = r_{sc} \left(\frac{3}{4 \cdot 10^9} C_1 + \frac{3}{4} \right)$$

Parameter identification

$$d_a = 10^8 k_c \frac{56 + 31[Amp_h]}{2.56(4 - [Amp_h])} \quad \text{with} \quad [Amp_h] < 4$$

Mono-cultures [Kohda 2021]

$$\begin{cases} \frac{d[S_c]}{dt} = \left(r_{sc} \left(1 - \frac{[S_c]}{K_{sc}} \right) - \frac{d_{sc}[S_p]}{C_1 + [S_p]} \right) [S_c] \\ \frac{d[S_p]}{dt} = \left(r_{sp} \left(1 - \frac{[S_p]}{K_{sp}} \right) - \frac{d_{spb}[Amp_b]}{C_{ab} + [Amp_b]} - \frac{d_{sph}[Amp_h]}{C_{ah} + [Amp_h]} \right) [S_p] \\ \frac{d[Amp_b]}{dt} = k_c [S_c] - d_a [Amp_b] \end{cases}$$



$$\begin{cases} [S_c]^* = K_{sc} \quad \text{or} \quad [S_c]^* = 0 \\ [S_p]^* = K_{sp} \quad \text{or} \quad [S_p]^* = 0 \end{cases}$$



$$K_{sc} = 4 \cdot 10^8 \text{ CFU.ASU}^{-1}$$

$$K_{sp} = 3 \cdot 10^9 \text{ CFU.ASU}^{-1}$$

Reduced model with 5 parameters

Parameters identified

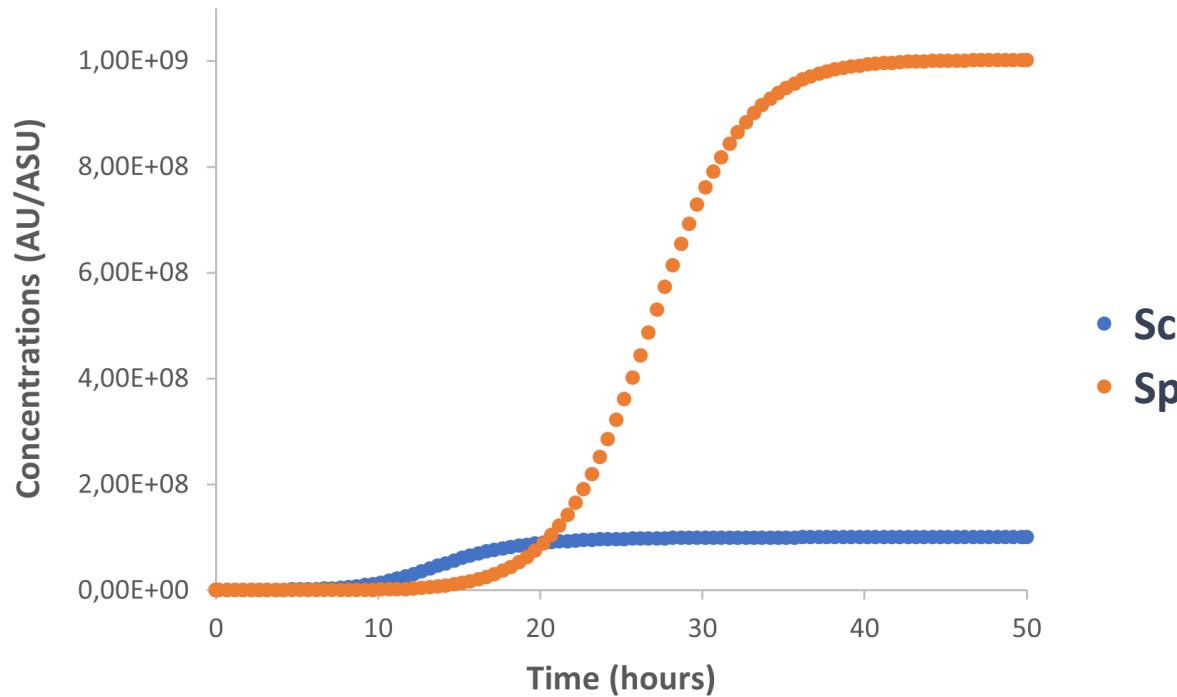
Parameter	Value or relation to other parameters
K_{sc}	4.10^8
K_{sp}	3.10^9
C_{ah}	8
C_{ab}	0.16
d_{sph}	$2 r_{sp}$
d_{spb}	$\frac{5}{4} r_{sp}$
d_{sc}	$r_{sc} \left(\frac{3}{4.10^9} C_1 + \frac{3}{4} \right)$
d_a	$10^8 k_c \frac{56 + 31[Amp_h]}{2.56 (4 - [Amp_h])}$ with $[Amp_h] < 4$

Reduced model

$$\begin{cases} \frac{d[S_c]}{dt} = \mathbf{r}_{sc}[S_c] \left(1 - \frac{[S_c]}{4.10^8} - \frac{3}{4} \frac{(10^{-9} \mathbf{C}_1 + 1)[S_p]}{\mathbf{C}_1 + [S_p]} \right) \\ \frac{d[S_p]}{dt} = \mathbf{r}_{sp}[S_p] \left(1 - \frac{[S_p]}{3.10^9} - \frac{5 [Amp_b]}{0.64 + 4[Amp_b]} - \frac{2 [Amp_h]}{8 + [Amp_h]} \right) \\ \frac{d[Amp_b]}{dt} = \mathbf{k}_c \left([S_c] - 10^8 \frac{56 + 31[Amp_h]}{2.56 (4 - [Amp_h])} [Amp_b] \right) \end{cases}$$

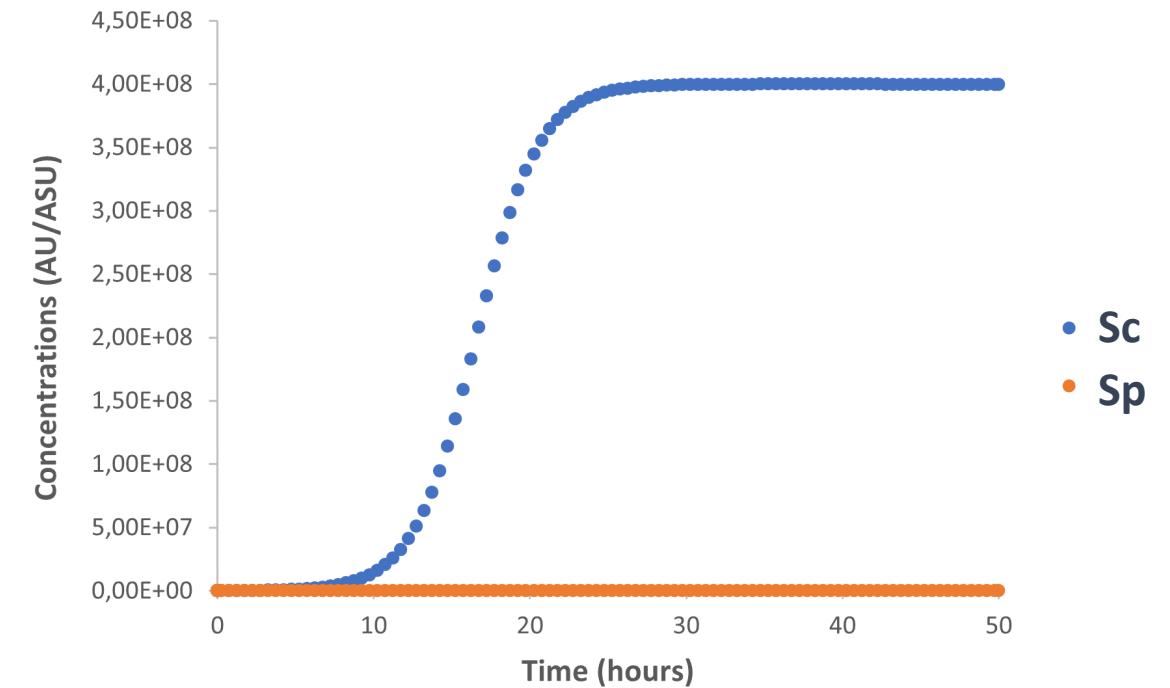
Simulations at the time scale of the experiments

Reproducing co-culture data [Kohda 2017]



$$[S_c](0) = 10^5, [S_p](0) = 10^3, [Amp_b](0) = 0 \\ [Amp_h] = 1.5, r_{sc} = 0.5, r_{sp} = 1, C_1 = 5 \cdot 10^6, k_c = 0.01$$

Commensals dominance with reduced Sp aggressiveness and increased skin defense



$$[S_c](0) = 10^5, [S_p](0) = 10^3, [Amp_b](0) = 0 \\ [Amp_h] = 3, r_{sc} = r_{sp} = 0.5, C_1 = 2 \cdot 10^8, k_c = 0.01.$$

Robustness analysis of steady state

Property of interest:

System stabilization at the time scale of the experiments: expected steady state reached around Time = 40 hours.



Temporal logic specification with Biocham⁽¹⁾

$$F \left(Time == 40 \wedge NSc = x1 \wedge NSp = y1 \wedge F(G(NSc = x2 \wedge NSp = y2)) \right)$$

Objective values: $x1 \rightarrow 1 ; x2 \rightarrow 1 ; y1 \rightarrow 1 ; y2 \rightarrow 1$

Normalization: $NSc = \frac{[S_c]}{[\widehat{S_c}]} \quad NSp = \frac{[S_p]}{[\widehat{S_p}]}$ $[\widehat{S_c}], [\widehat{S_p}]$: expected values at steady state based on previous simulation

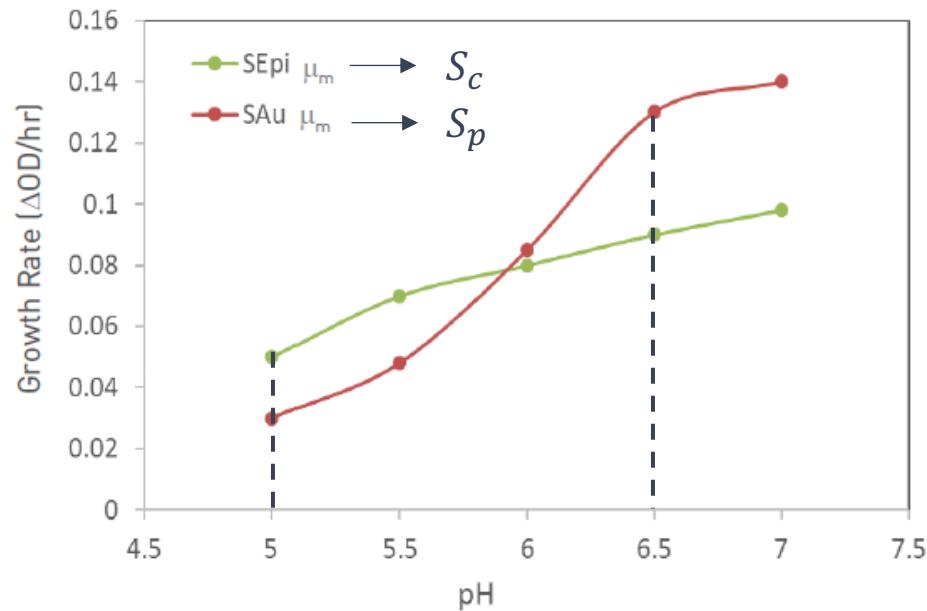
1) Rizk A. et al, "Continuous valuations of temporal logic specifications with applications to parameter optimization and robustness measures", Theoretical Computer Science, Volume 412, Issue 26, 2011, Pages 2827-2839

High sensitivity to variations of the pathogen initial concentration

Parameter	Coefficient of variation	Robustness degree
r_{sc}	0.1	0.74
r_{sp}	0.1	0.67
C_1	10	0.95
k_c	0.1	0.95
$[Amp_h]$	0.1	0.62
$[S_p](0)$	10	0.16
$[S_c](0)$	10	0.57
(r_{sc}, r_{sp})	0.1	0.64
$([S_c](0), [S_p](0))$	10	0.34

Skin pH elevation influences bacterial growth

Growth rates (μ_m) of *S. aureus* and *S. epidermidis* as a function of pH



skin surface pH of 5 (healthy skin):

$$r_{sc} = 0.5, r_{sp} = 0.3$$

skin surface pH of 6.5 (compromised skin):

$$r_{sc} = 0.9, r_{sp} = 1.3$$

Dasgupta A. et al., 16502 Effect of pH on growth of skin commensals and pathogens, JAAD, Volume 83, Issue 6, Supplement, AB180, 2020

Does skin pH elevation influence the skin resistance to pathogen colonization?

Property of interest:

Dominance of the commensal population by at least 10-fold over the pathogenic one, after around 40 hours of simulation and at the end.

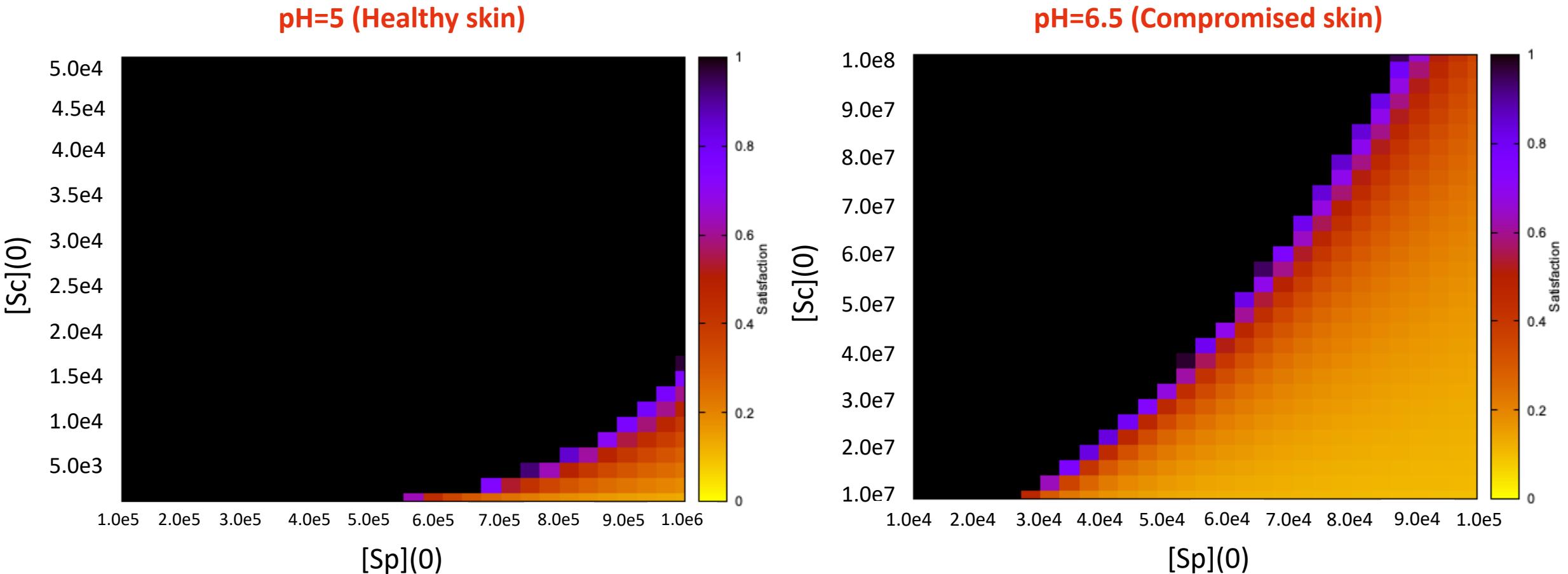
Corresponding temporal logic formula in Biocham formalism:

$$F \left(Time == 40 \wedge ([S_c] > u1 [S_p]) \wedge F \left(G([S_c] > u2 [S_p]) \right) \right)$$

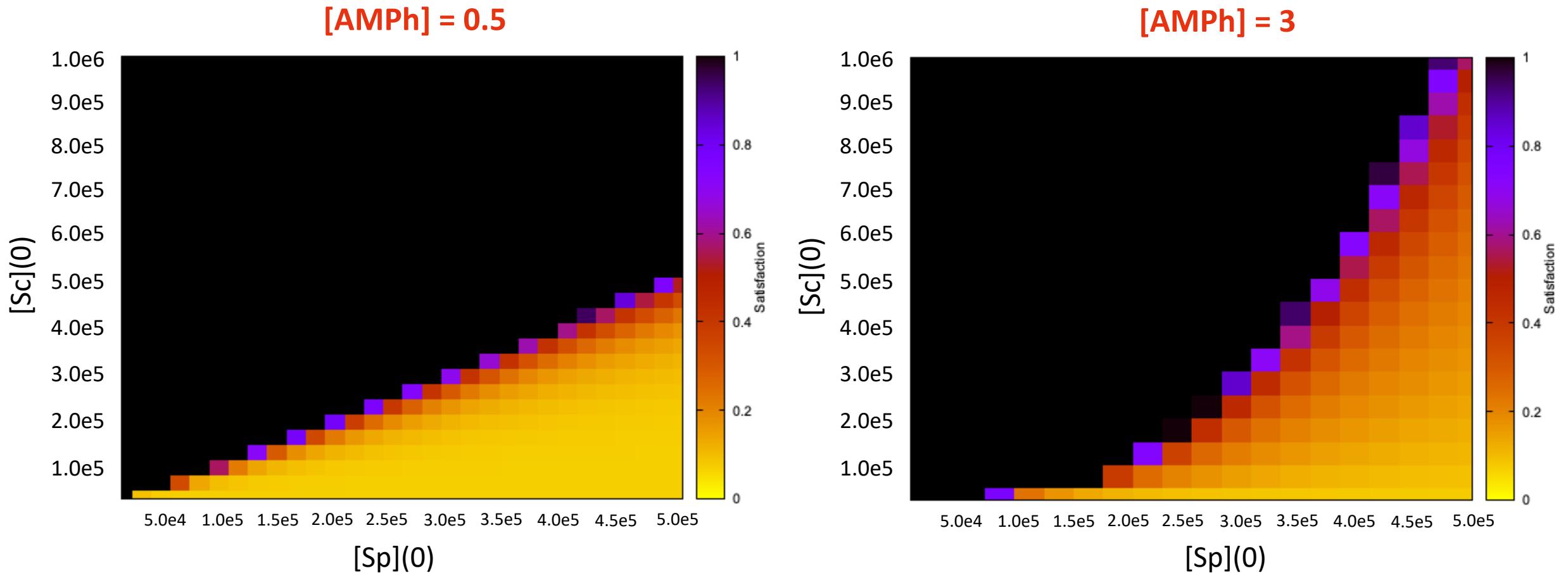
Objective values: $u1 \rightarrow 10 ; u2 \rightarrow 10$

→ Satisfaction degree with respect to variations of $[S_c](0)$ and $[S_p](0)$.

Skin pH elevation creates favorable conditions for the pathogen population



Contradictory effect of [AMPh] increase depending on the initial concentration of pathogen



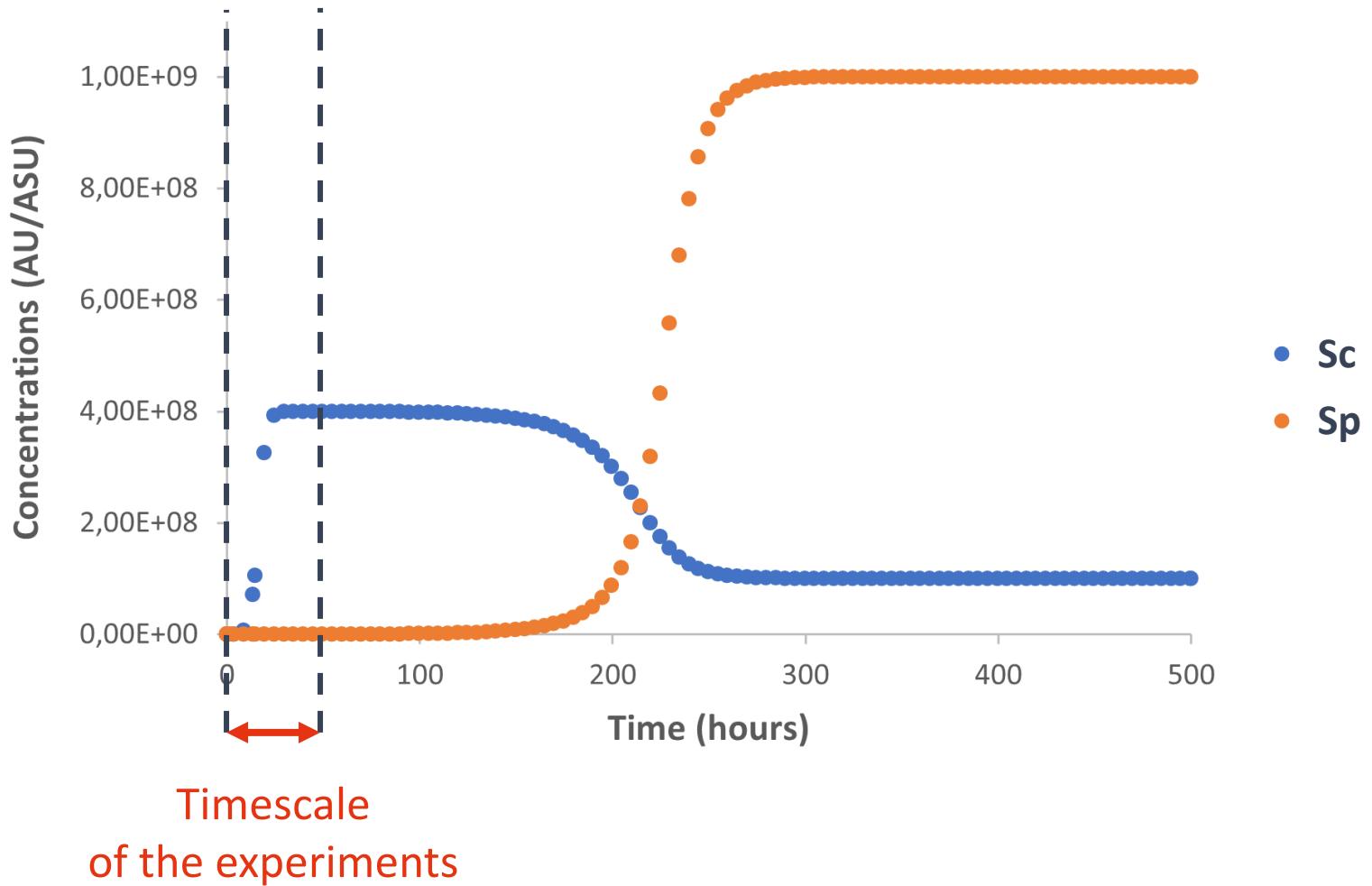
Meta-stability revealed by simulation on a long timescale!

Parameter values used to get commensal dominance on the timescale of the experiments:

$$[S_c](0) = 10^5$$
$$[S_p](0) = 10^3$$

$$[Amp_b](0) = 0$$
$$[Amp_h] = 3$$

$$r_{sc} = r_{sp} = 0.5$$
$$C_1 = 2.10^8$$
$$k_c = 0.01.$$



Stability versus Metastability Assumptions in Mathematical Modelling

Classical hypothesis in biomathematics:

- The **stable states observed in experiments** are **stable states of the model**
- *Used here* to determine parameter values and parameter relations

Yet **slowly varying metastable states** could fit the experiments as well

- *Observed here* in our resulting model:
metastable state switching to a stable state
- **Slow dynamics** hidden
at the timescale of the experiments

$$\begin{cases} \frac{d[S_c]}{dt} = r_{sc}[S_c] \left(1 - \frac{[S_c]}{4 \cdot 10^8} - \frac{3}{4} \frac{(10^{-9} C_1 + 1)[S_p]}{C_1 + [S_p]}\right) \\ \frac{d[S_p]}{dt} = r_{sp}[S_p] \left(1 - \frac{[S_p]}{3 \cdot 10^9} - \frac{5[Amp_b]}{0.64 + 4[Amp_b]} - \frac{2[Amp_h]}{8 + [Amp_h]}\right) \\ \frac{d[Amp_b]}{dt} = k_c \left([S_c] - 10^8 \frac{56 + 31[Amp_h]}{2.56(4 - [Amp_h])} [Amp_b]\right) \end{cases}$$

Question for “real” biological systems:

Stable state switches ?

or

Metastable state switches ?

Conclusion

ODE model of skin microbiome with 3 variables and 13 parameters.

- Reduction of 13 to 5 parameters from published experimental data
 - Using stable steady state assumption for the experimental observations
 - Robustness analysis of the model with respect to parameter variations
- Prediction of skin pH elevation as favoring the pathogenic population.
- Prediction of contradictory effects of human AMPs

Enables quick hypothesis testing and increases knowledge on biological system

Meta-stability phenomenon observed at long timescale

- Slow/fast decomposition of the model ?
- Biocham tropical analyzer does not apply to non-polynomial ODEs

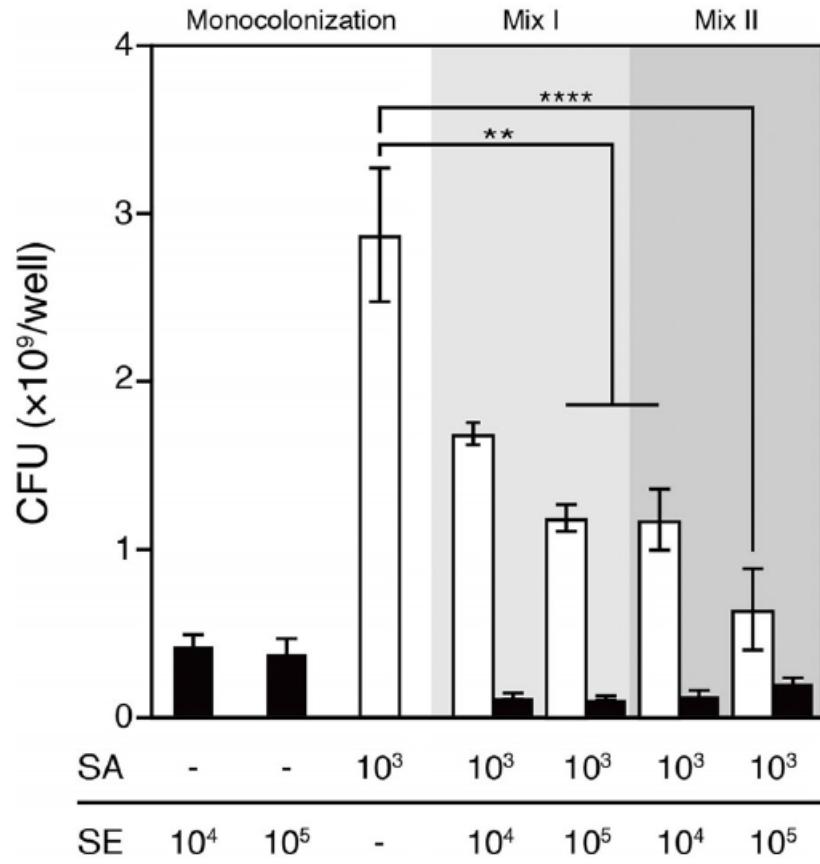
Thank you for your attention!

What is next?

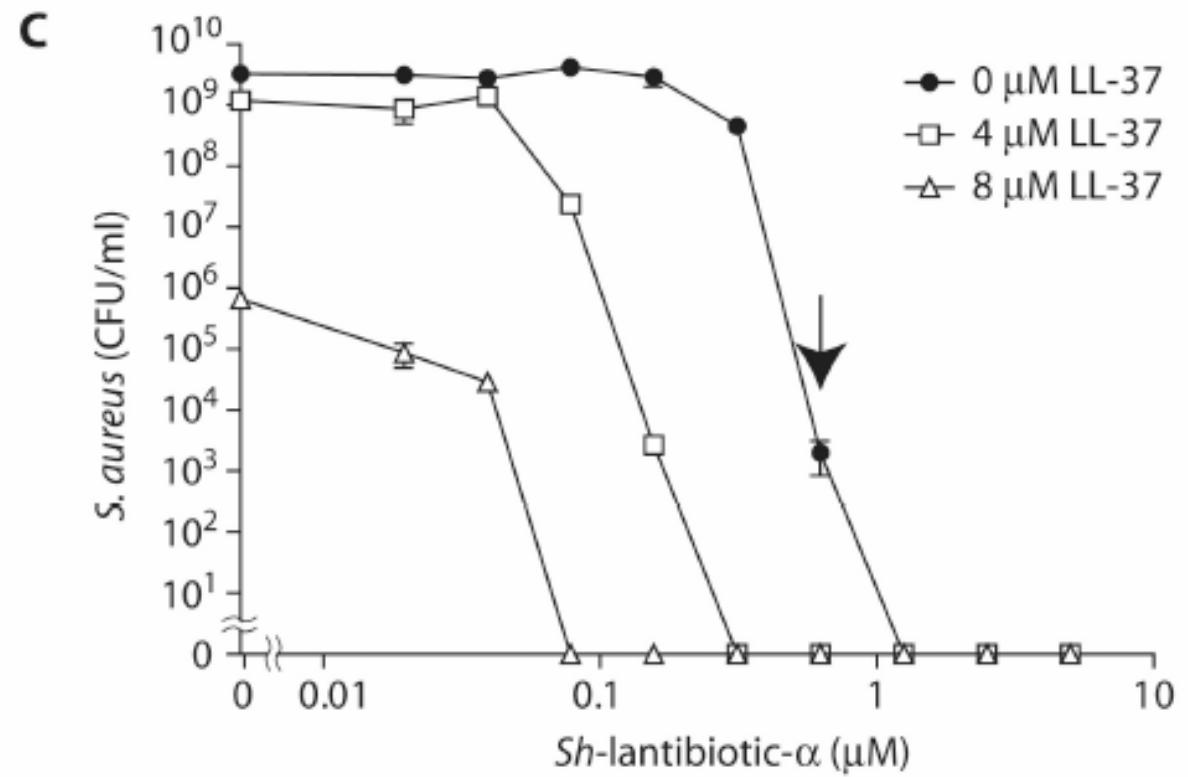
- Reconcile the steady-state assumptions with the resulting system's behavior on the timescale of this experiment
- Investigate the cause of this metastability phenomenon
- Further model calibration with optimization algorithms on the sub-models representative of experimental conditions

Experimental data

Kohda et al., 2021



Nakatsuji et al., 2017



Model reduction by steady state reasoning

Parameter relations inferred from co-culture data

Steady state values

$$[S_c]^* = 0 \quad \text{or} \quad [S_c]^* = K_{sc} \left(1 - \frac{d_{sc}[S_p]^*}{r_{sc}(C_1 + [S_p]^*)} \right)$$

$$[S_p]^* = 0 \quad \text{or} \quad [S_p]^* = K_{sp} \left(1 - \frac{d_{spb}[Amp_b]}{r_{sp}(C_{ab} + [Amp_b])} - \frac{d_{sph}[Amp_h]}{r_{sp}(C_{ah} + [Amp_h])} \right)$$

$$[Amp_b]^* = \frac{k_c[S_c]^*}{d_a}$$

Parameter relation inferred

$$\frac{d_{sc}}{r_{sc}} = \frac{3}{4 \cdot 10^9} C_1 + \frac{3}{4}$$

$$\frac{2}{3} r_{sp} = \frac{d_{sph}[Amp_h]}{C_{ah} + [Amp_h]} + \frac{10^8 d_{spb} k_c}{d_a C_{ab} + 10^8 k_c}$$

Model reduction by steady state reasoning

Parameter relations inferred from co-culture data

$$\frac{2}{3}r_{sp} = \frac{d_{sph}[Amp_h]}{C_{ah} + [Amp_h]} + \frac{10^8 d_{spb} k_c}{d_a C_{ab} + 10^8 k_c}$$

$$C_{ah} = 8$$

$$C_{ab} = 0.16$$



$$\frac{d_{sph}}{r_{sp}} = 2$$

$$\frac{d_{spb}}{r_{sp}} = \frac{5}{4}$$

$$d_a = 10^8 k_c \frac{56 + 31[Amp_h]}{2.56(4 - [Amp_h])} \quad \text{with } [Amp_h] < 4$$

Model parameters

Variables	Interpretation (unit)
$[S_c]$	Surface apparent concentration of S_c ($CFU.ASU^{-1}$)
$[S_p]$	Surface apparent concentration of S_p ($CFU.ASU^{-1}$)
$[Amp_b]$	Concentration of Amp_b ($AU.ASU^{-1}$)
Parameter	Interpretation (unit)
r_{sc}	Growth rate of S_c (h^{-1})
r_{sp}	Growth rate of S_p , (h^{-1})
K_{sc}	Optimum concentration of S_c ($CFU.ASU^{-1}$)
K_{sp}	Optimum concentration of S_p ($CFU.ASU^{-1}$)
d_{sc}	Maximal killing rate of S_c by S_p (h^{-1})
C_1	Concentration of S_p inducing half the maximum killing rate d_{sc} ($CFU.ASU^{-1}$)
d_{spb}	Maximal killing rate of S_p by Amp_b , (h^{-1})
C_{ab}	Concentration of Amp_b inducing half the maximum killing rate d_{spb} ($AU.ASU^{-1}$)
d_{sph}	Maximal killing rate of S_p by Amp_h , (h^{-1})
C_{ah}	Concentration of Amp_h inducing half the maximum killing rate d_{sph} ($AU.ASU^{-1}$)
$[Amp_h]$	Concentration of AMPs produced by the skin cells ($AU.ASU^{-1}$)
k_c	Production rate of Amp_b by S_c ($AU.h^{-1}.CFU^{-1}$)
d_a	Degradation rate of Amp_b ($AU.h^{-1}$)