Developing a mathematical model to understand mechanisms governing antibiotic-persistent MRSA infection

Tsuyoshi Mikkaichi, Ph.D.
Alexander Hoffmann, Ph.D.
Signaling Systems Lab
University of California, Los Angeles

Jan. 31, 2018
U01 Site visit

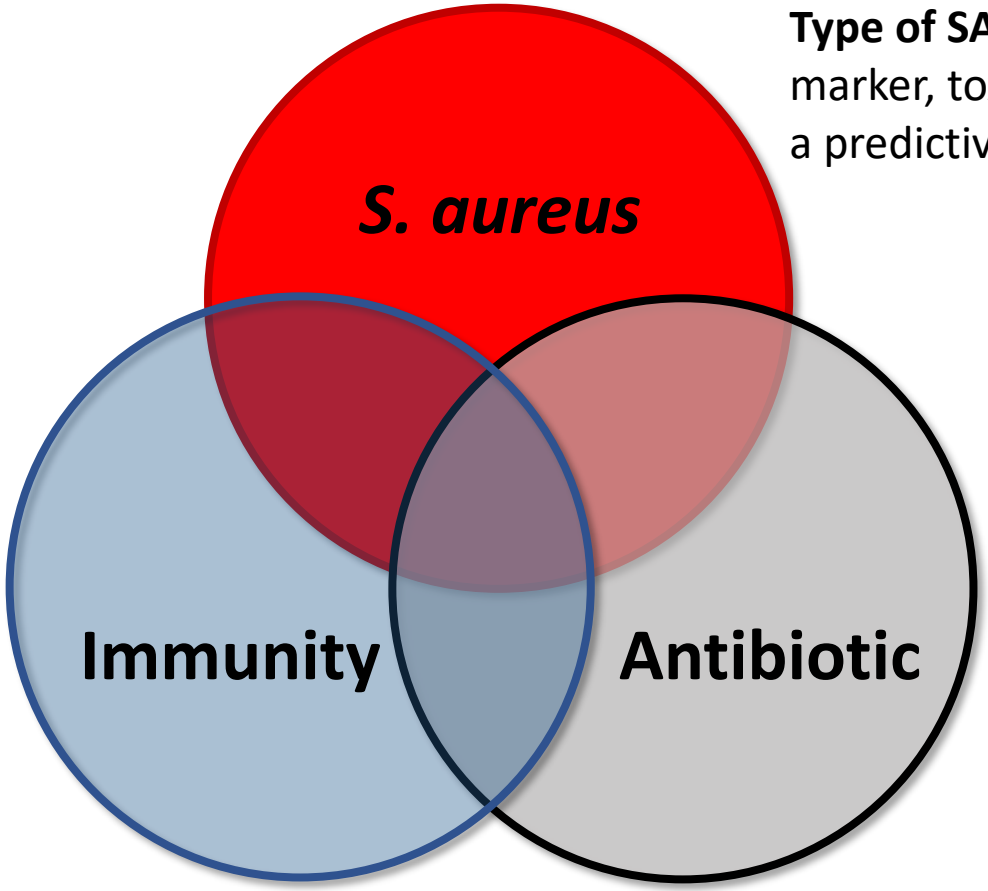
What is antibiotic-persistent MRSA bacteremia (APMB)?

- Staphylococcus aureus (SA) bacteremia is a common, potentially life-threatening bloodstream infection.
- Many of the infectious strains are methicillin-resistant (MRSA).
- For up to 30% of patients infected by MRSA, even gold-standard anti-MRSA antibiotics, such as vancomycin or daptomycin, also fail.
- Hence MRSA infectious are classified as “Resolving” or “Persistent”
  - Antibiotic-Persistent MRSA Bacteremia (APMB): positive blood culture after 5 days of therapy
  - Antibiotic-Resolving MRSA Bacteremia (ARMB): negative blood cultures within 4 days of therapy

Modified from Dr. Yeaman's slide

MRSA Persistence: Intersection of Systems

Type of SA could play a role, but no marker, toxin, surface protein, etc is a predictive biomarker.



Innate immune systems could play a role, as macrophages and neutrophils are key to eliminate SA, but even APMB patients have high titers of specific antibody and circulating T cells, and vaccine efforts have been unsuccessful.

Antibiotics availability could play a role, but there is no difference in antibiotics plasma concentrations between patient with "persistent" and "resolving" MRSA.

Project Goal:
Develop a mathematical model of MRSA infection to identify mechanisms that may determine "persistent" and "resolving" pathogeneses.

Aim 1: Develop a model to explore the MRSA – antibiotic interaction: *in vitro* studies

Aim 2: Develop a model to explore the MRSA – antibiotic – immunity interaction: *in vivo* studies

Aim 3: Develop a multi-compartment model of MRSA infection

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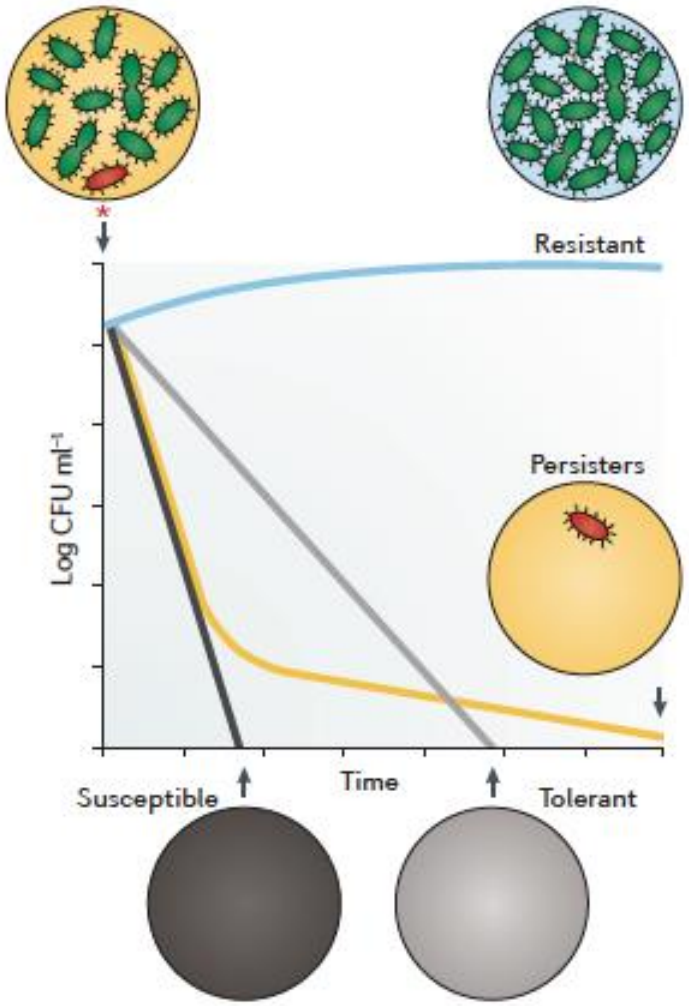
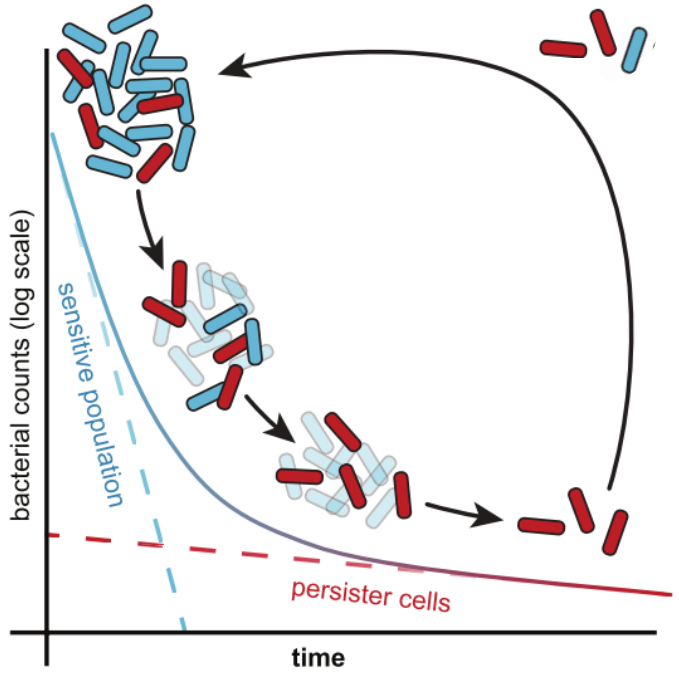
Even *in vitro*: Persistence is distinct from tolerance and resistance



Nat Rev Microbiol. 2017,15(8):453-464.

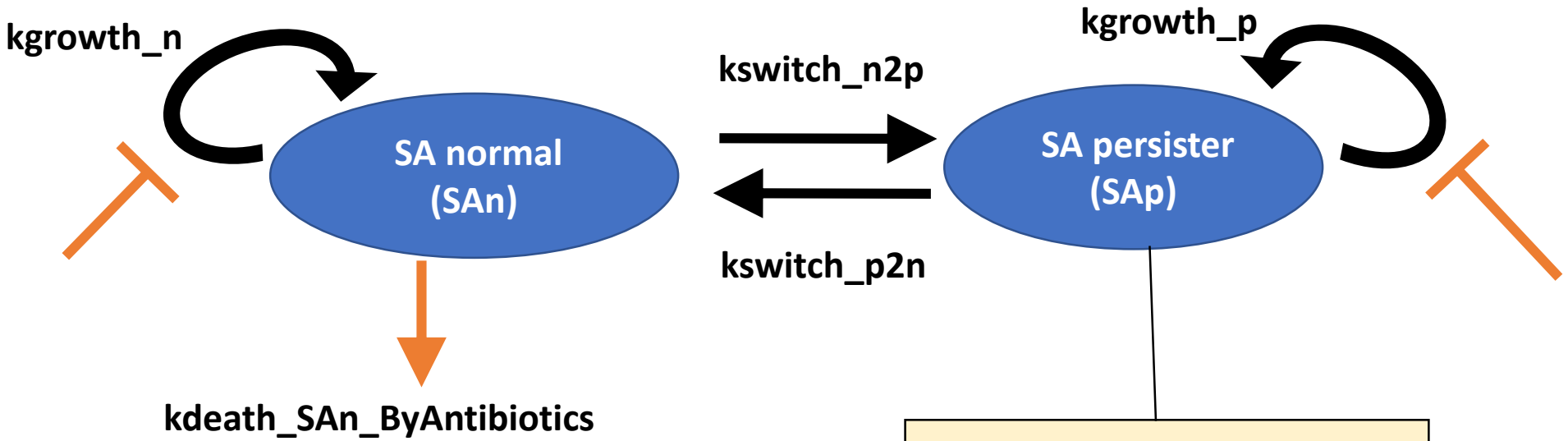
In contrast to resistance and tolerance, which are attributes of whole bacterial populations, persistence is due to an inherent heterogeneity in bacterial populations; normal and persister cells.

Persister cells:
a subpopulation of slow-growing and decreased susceptibility to killing by antibiotics



Harms, A. Science (80). 354, aaf4268 (2016).

Simple model (ver 1) to recapitulate *in vitro* data

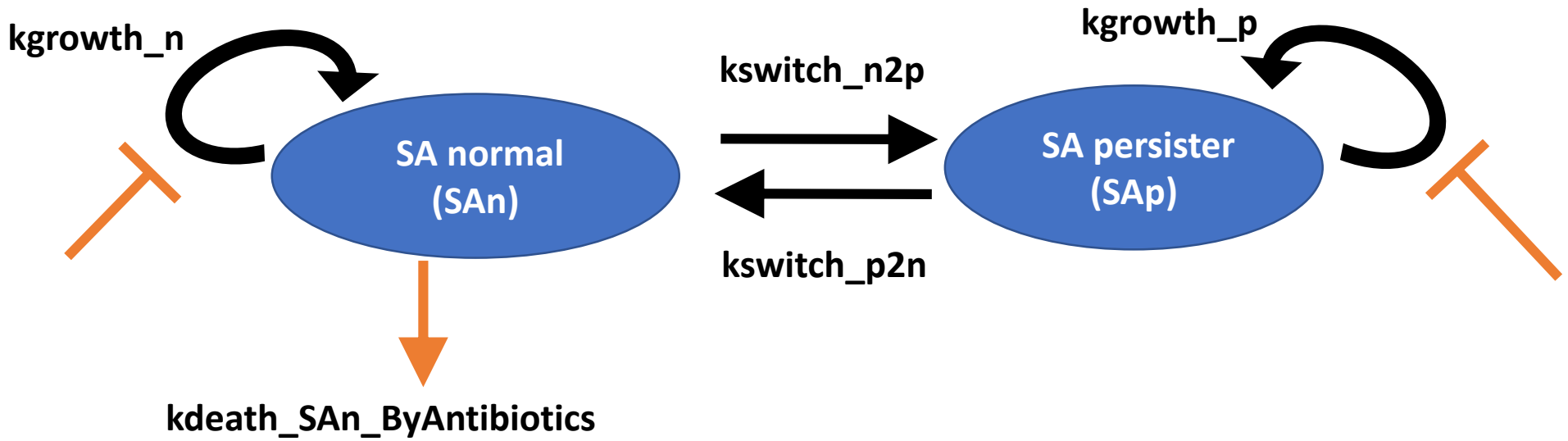


Persisters have following features:

- slower growth
- decreased susceptibility to antibiotics

Balaban, N. Science. 305, 1622–1625 (2004).

Simple model (ver 1) to recapitulate *in vitro* data



$$\frac{dN}{dt} = -sw_{N2P}\,N + sw_{P2N}\,P + g_N\,(1 - 0.95\,Van)\left(1 - \frac{N+P}{SAmax}\right)N - d_{Van}\,Van\,N$$

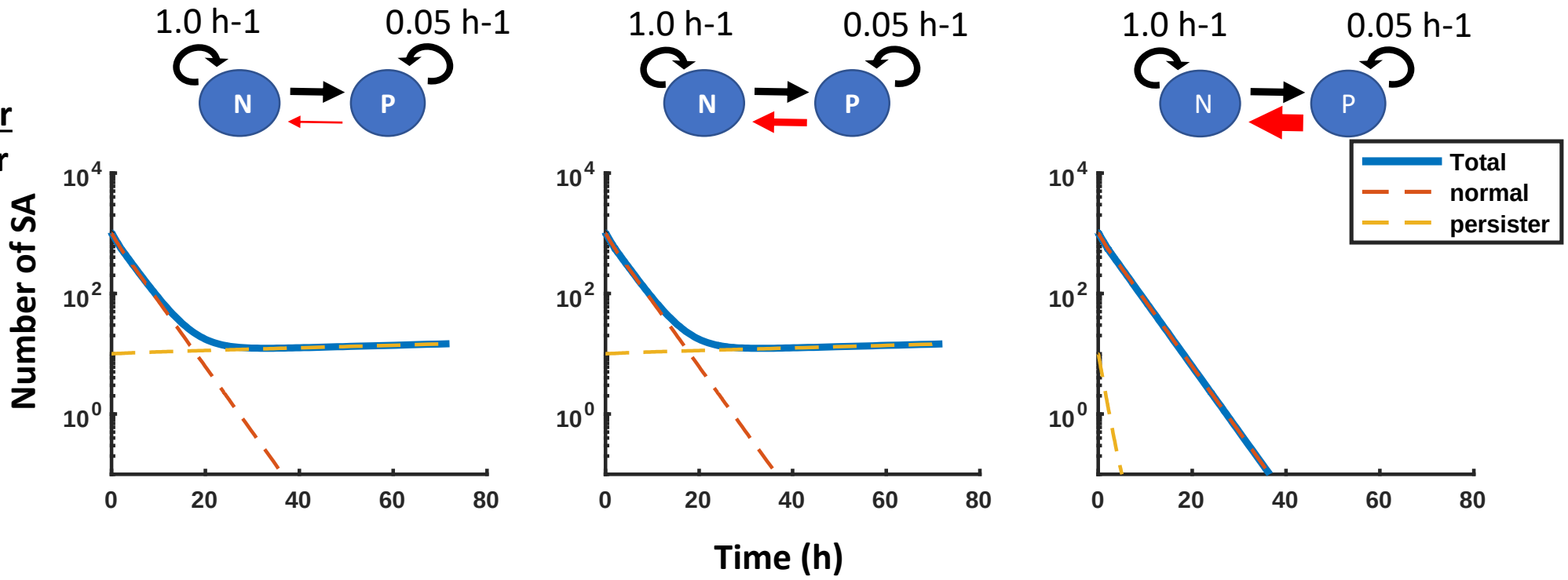
$$\frac{dP}{dt} = sw_{N2P}\,N - sw_{P2N}\,P + g_P\,(1 - 0.95\,Van)\left(1 - \frac{N+P}{SAmax}\right)P$$

Balaban, N. Science. 305, 1622–1625 (2004).

Simulated *in vitro* SA killing by vancomycin

Initial cell number
normal : persister

1000 : 10



Conclusion: the switch rate between persister to normal MRSA is critical in determining persistence *in vitro*.

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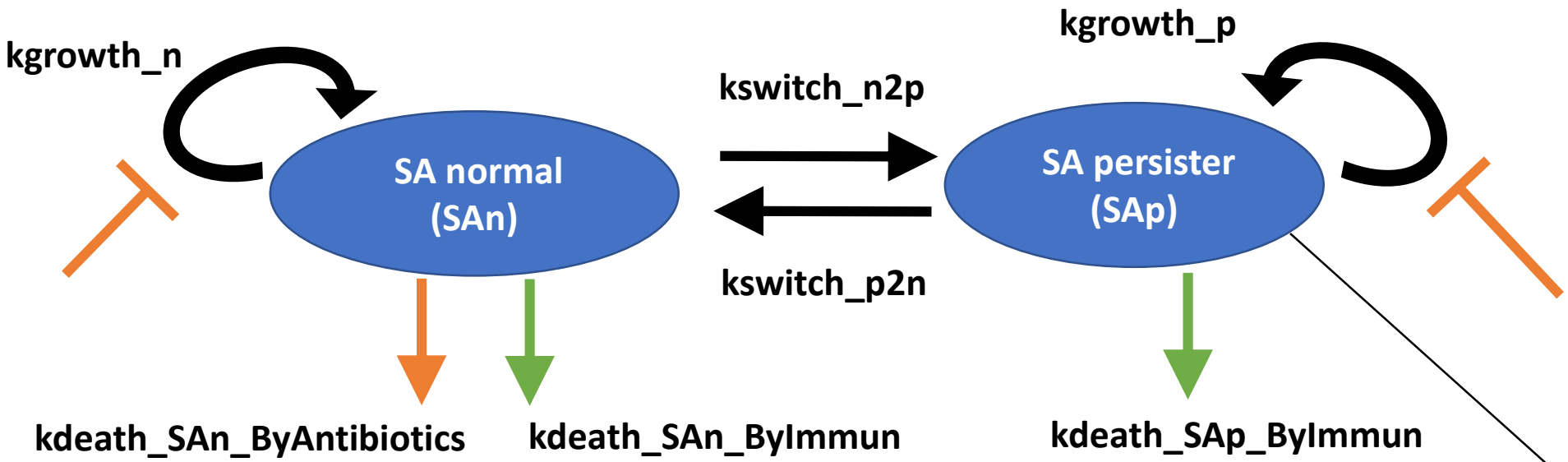
Aim 1: Develop a model to explore the MRSA – antibiotic interaction: *in vitro* studies

Aim 2: Develop a model to explore the MRSA – antibiotic – immunity interaction: *in vivo* studies

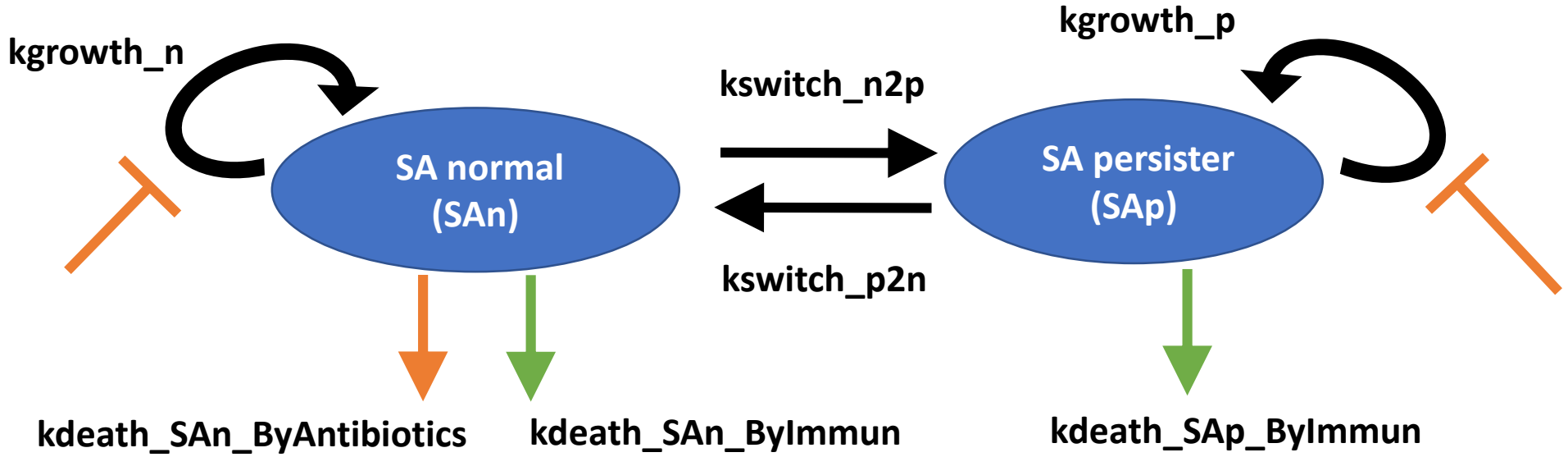
Aim 3: Develop a multi-compartment model of MRSA infection

Simple model (ver 2) to recapitulate *in vivo* data



Persisters have following features:

- slower growth
- decreased susceptibility to antibiotics
- decreased susceptibility to immune killing



$$\frac{dN}{dt} = -sw_{N2P}\,N + sw_{P2N}\,P + g_N\,(1 - 0.95\,Van)\left(1 - \frac{N+P}{SAmax}\right)N - d_{Van}\,Van\,N - c_N\left(\frac{1}{1+a\,T^h}\right)Im\,N$$

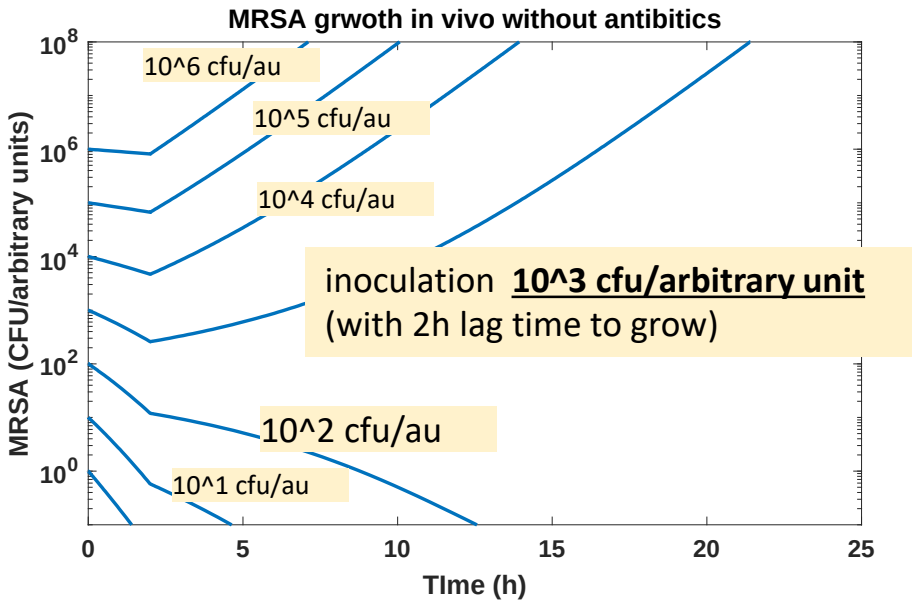
$$\frac{dP}{dt} = sw_{N2P}\,N - sw_{P2N}\,P + g_P\,(1 - 0.95\,Van)\left(1 - \frac{N+P}{SAmax}\right)P - c_P\left(\frac{1}{1+a\,T^h}\right)Im\,P$$

Features of the mathematical model ver 2

The inference is that the immune system is effective when presented with small numbers but it can be saturated.

$$Kill\ of\ SAn\ By\ Immune = kdeath\_SAn\_ByImmune \cdot \frac{1}{1 + a \cdot SAtotal^{hill}} \cdot ImmuneCell \cdot SAn$$

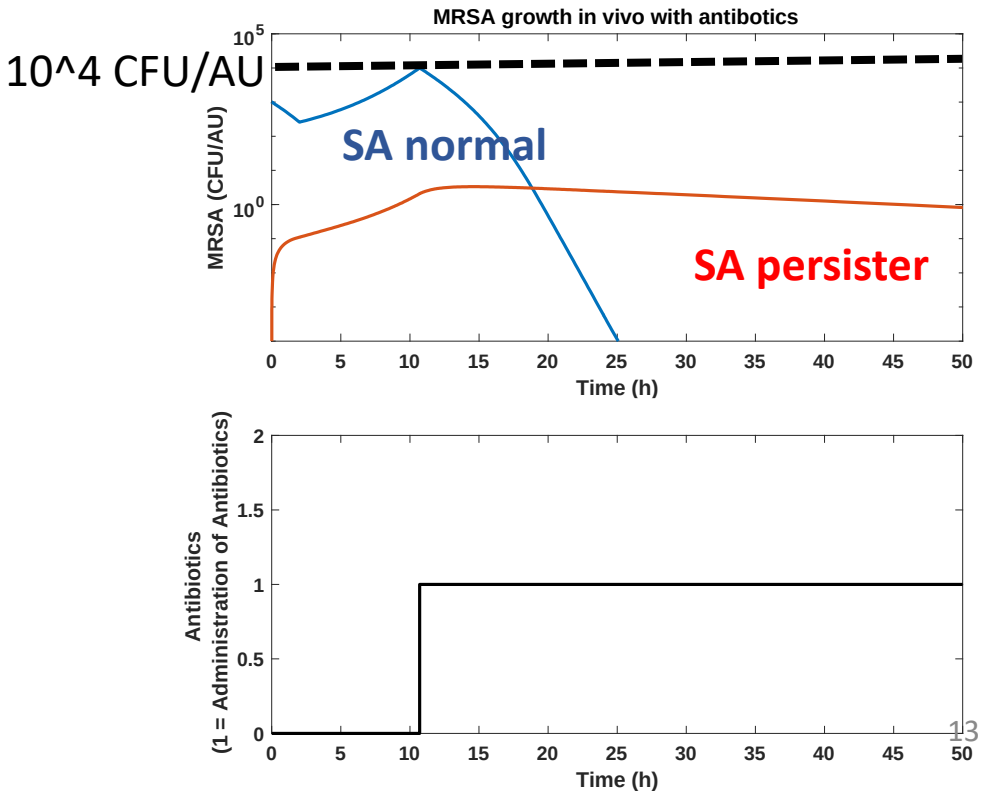
A minimum Inoculum dose is required to establish an infection
(even in absence of antibiotics)



Simulating MRSA Infection and Antibiotic Treatment with model ver 2

Event functions:

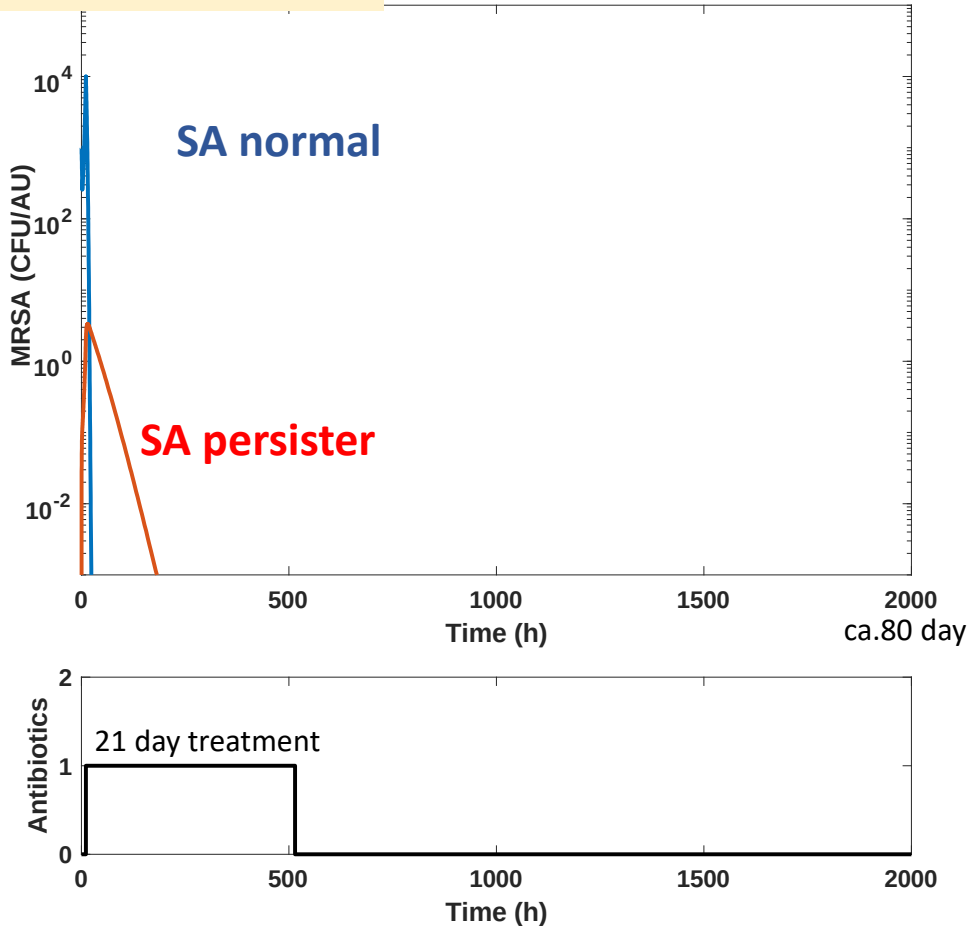
- MRSA start to grow at 2h post-inoculation.
- When SAnormal reach 10^4 CFU/AU, antibiotic is administered for 21 days.
- 24h after final antibiotic administration, SAnormal starts to grow again



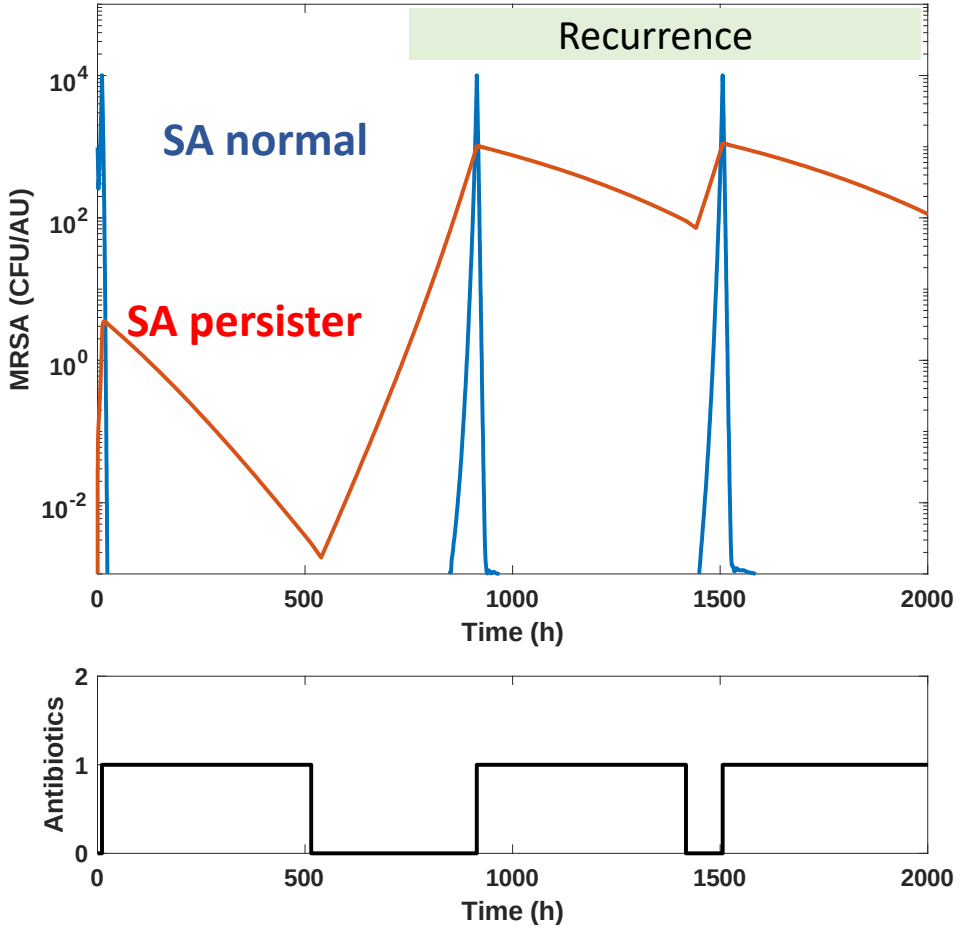
With a picked set of parameter values the model recapitulates resolving and persistent MRSA

Inoculum dose = 10^3 CFU/AU

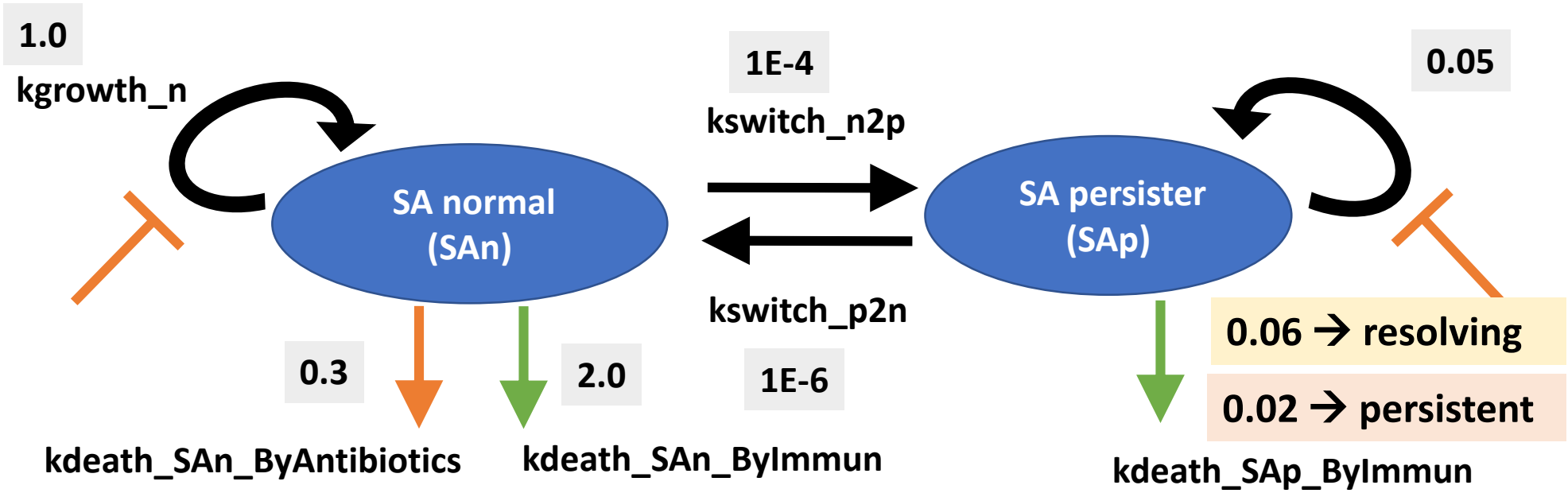
Resolving MRSA



Persistent MRSA

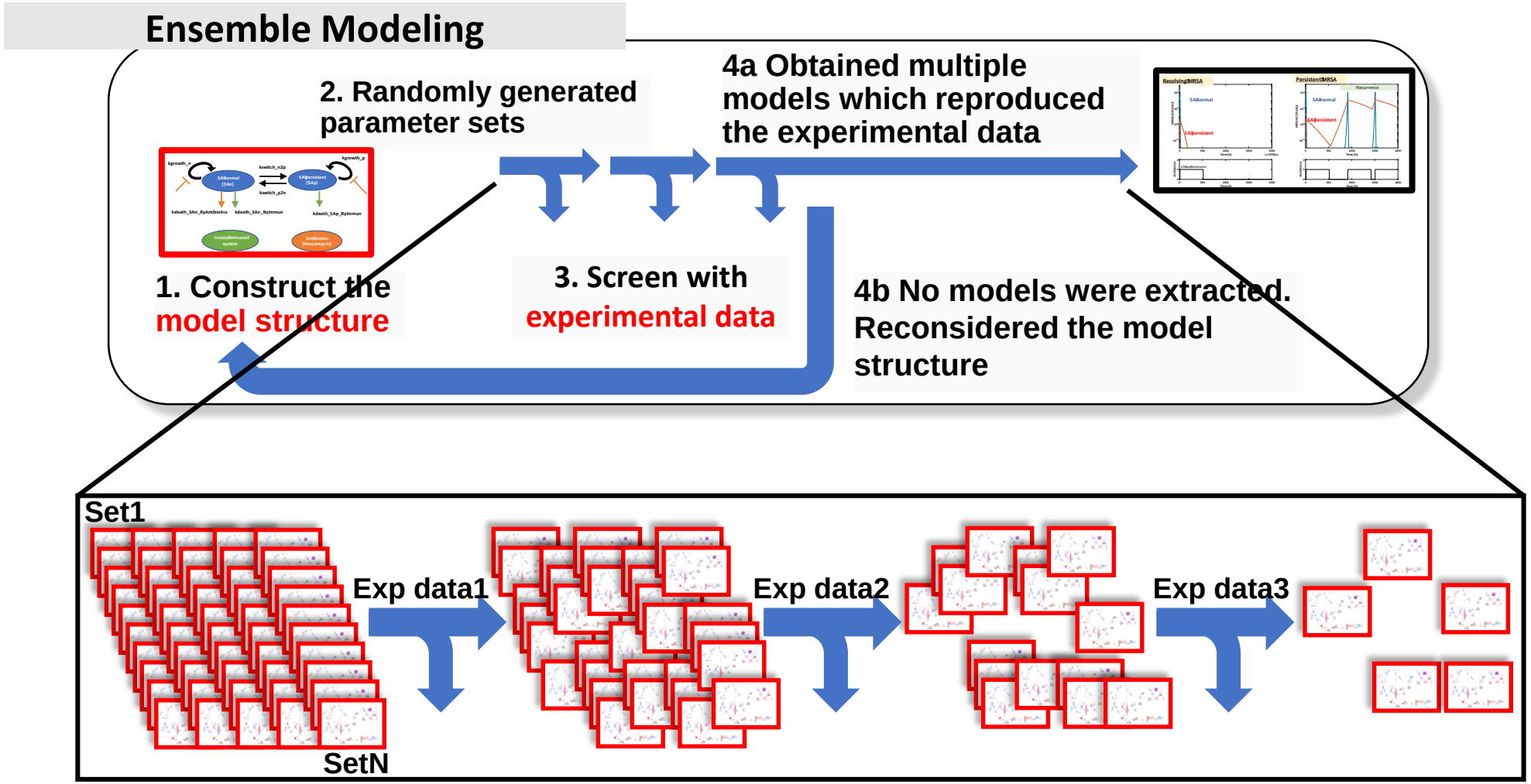


Parameter values picked for simulating resolving and persistent MRSA

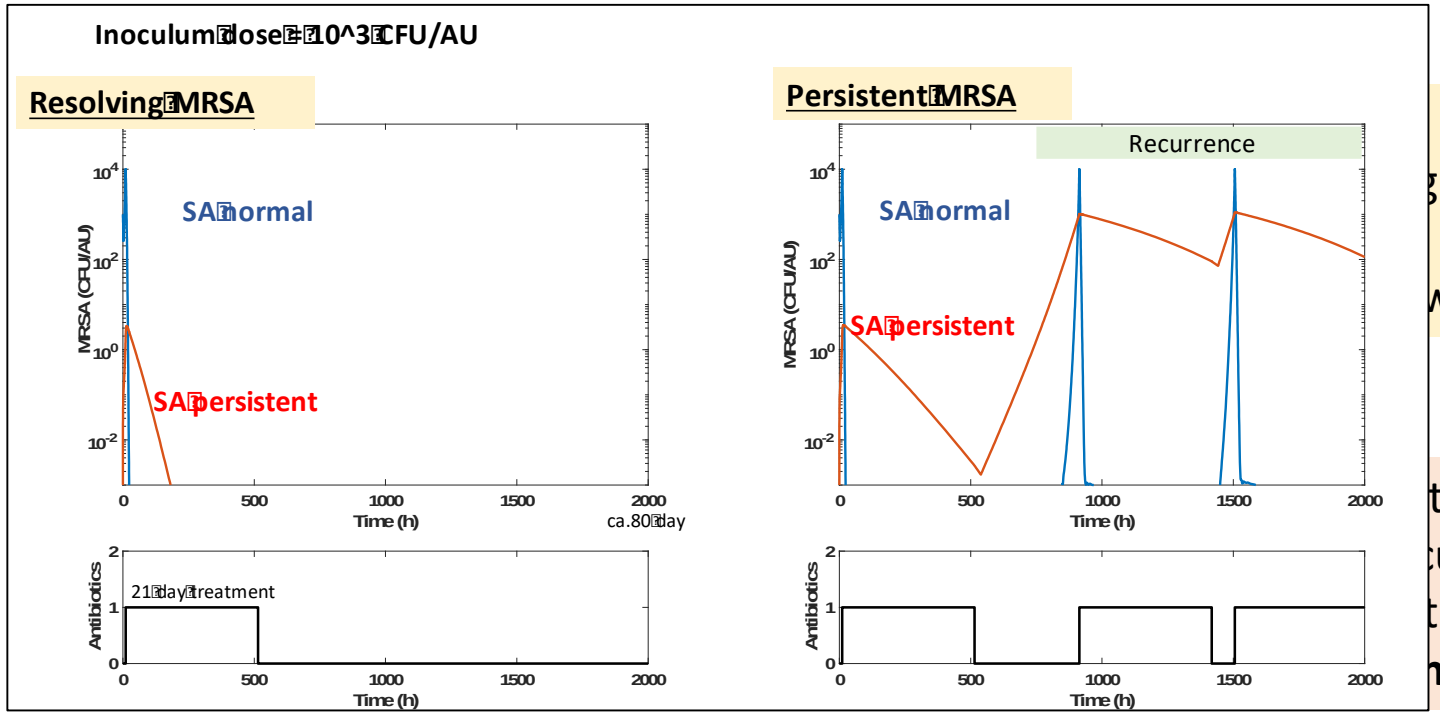


We picked parameters that imply that persistent MRSA is less susceptible to immune killing.

Identify parameter sets that recapitulate resolving vs persistent MRSA: Ensemble simulation



Ensemble Modeling



growth

witch rate

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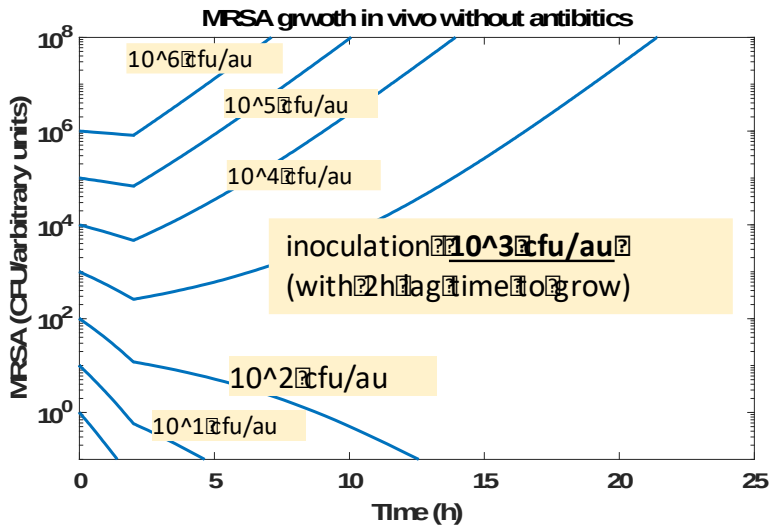
n more than 10^4 cfu/au

Inoculum dose dependency in vivo growth of MRSA without antibiotics



3. **Extract parameter sets which meet criteria 2**

MRSA should NOT growth under conditions where:
1. SAn= 10^2 cfu/au
2. Without Antibiotic treatment

Criteria: SAn at 72h less than 0.1 cfu/au

4. **Divide parameter sets into "Resolving" and "Persistent"**

Number of antibiotics administrations for 4000h (=ca.170 days)

1: Resolving

>2: Persistent

Ensemble Modeling

<Fixed parameters>
Kgrowth_n
Antibiotics: growth inhibition and kill

<SA related>
f_growth: ratio of growth
ksw_n2p
f_switch: ratio of switch rate

<Immune system related>
kdeath_SAn_ByImmune
a
hill
f_deathByImmune: ratio of killing rate

1. **Generate 10,000 parameter sets**
   1. log-uniformly distribute except for “hill”

10,000

2. **Extract parameter sets which meet criteria 1**

8,537

MRSA should growth under conditions where:
1. SAn = 10^3 (Inoculum dose = 10^3 CFU/AU)
2. Without Antibiotic treatment
Criteria: SAn at 72h more than 10^4 cfu/au

3. **Extract parameter sets which meet criteria 2**

671

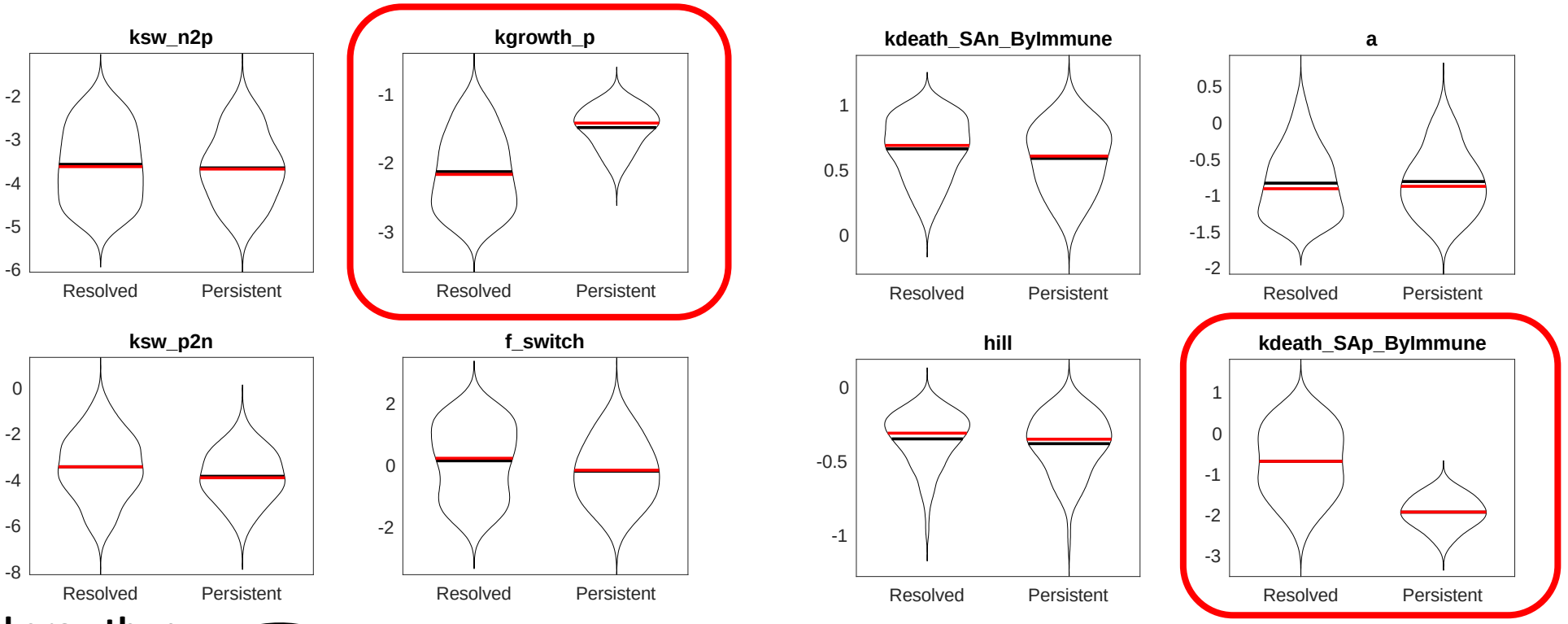
MRSA should NOT growth under conditions where:
1. SAn= 10^2 cfu/au
2. Without Antibiotic treatment
Criteria: SAn at 72h less than 0.1 cfu/au

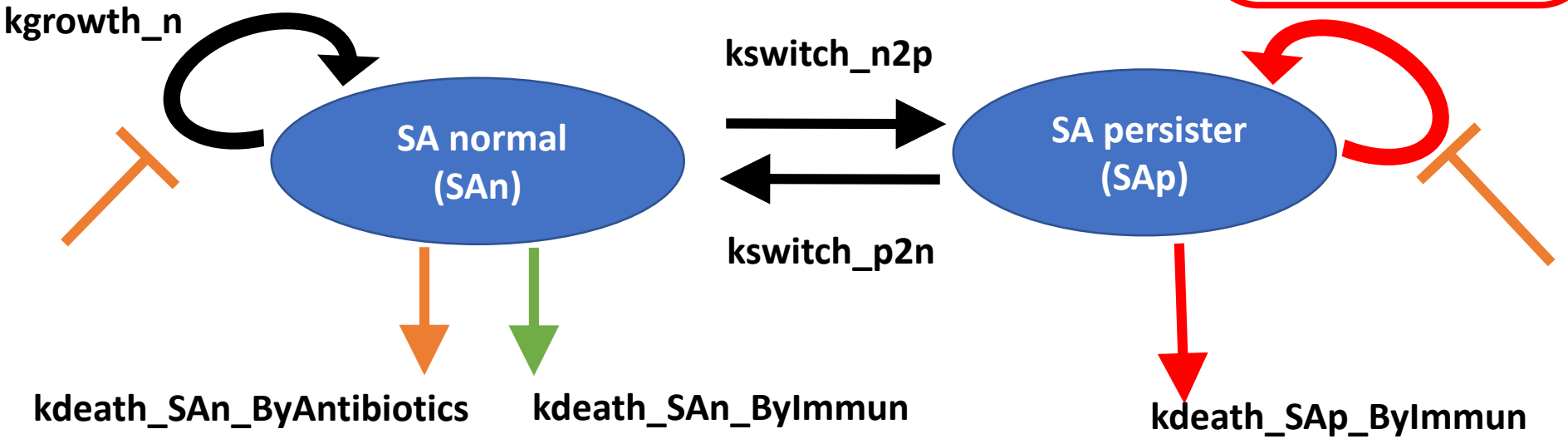
4. **Divide parameter sets into “Resolving” and “Persistent”**

R: 590
P: 79

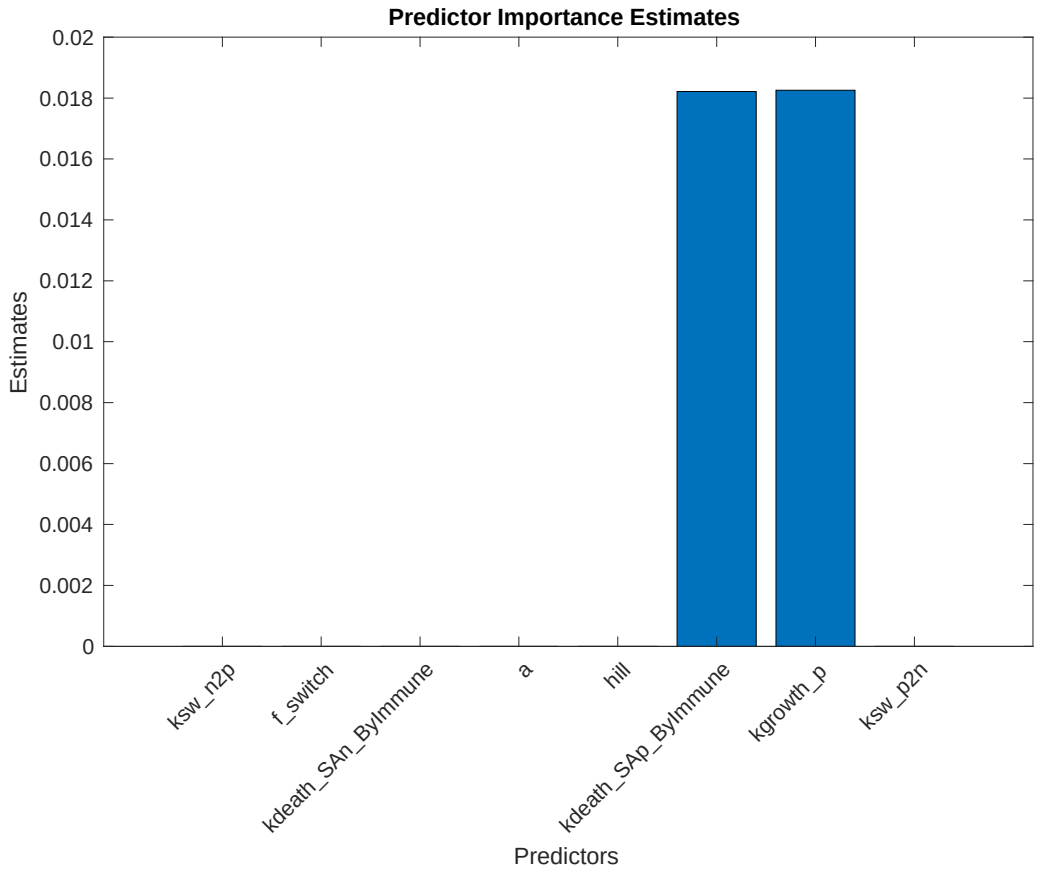
Number of antibiotics administrations for 4000h (=ca.170 days)
1: Resolving
>2: Persistent

Parameter distributions

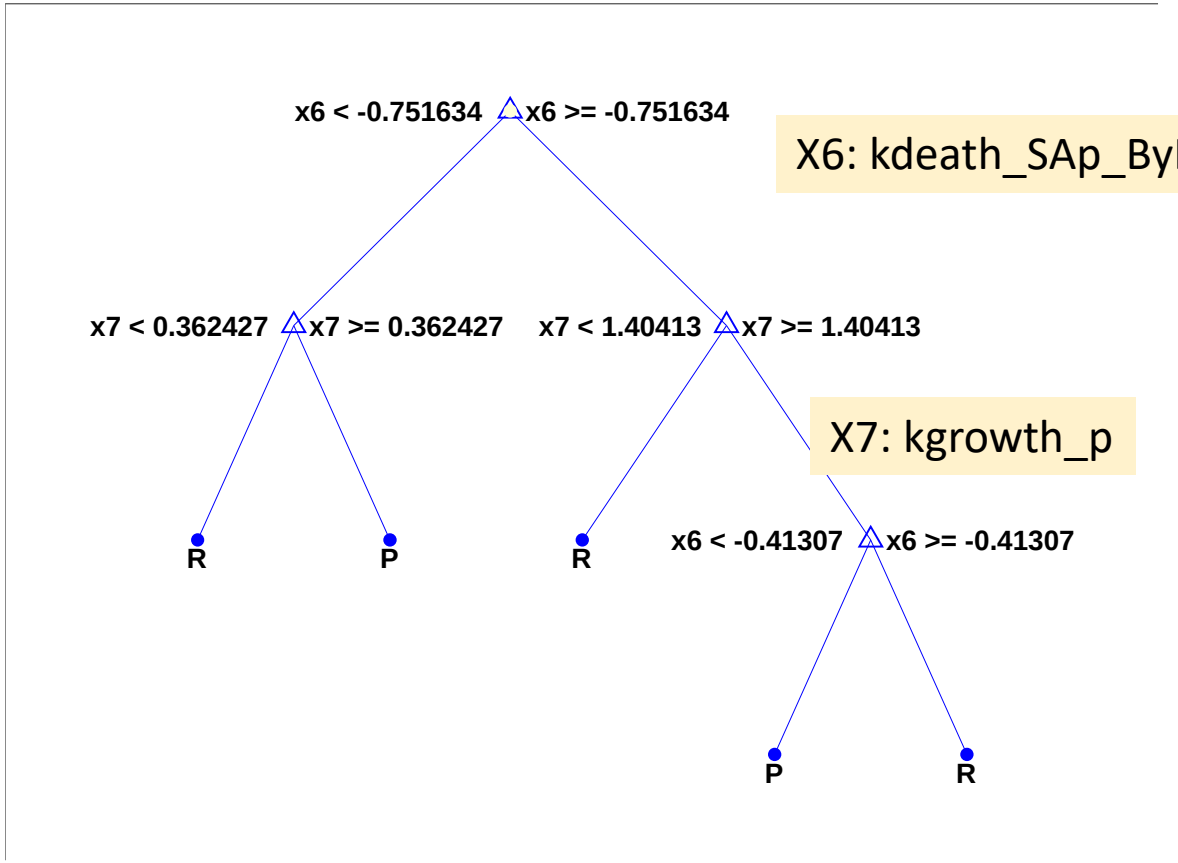




Classification tree: “Resolving” and “Persistent”



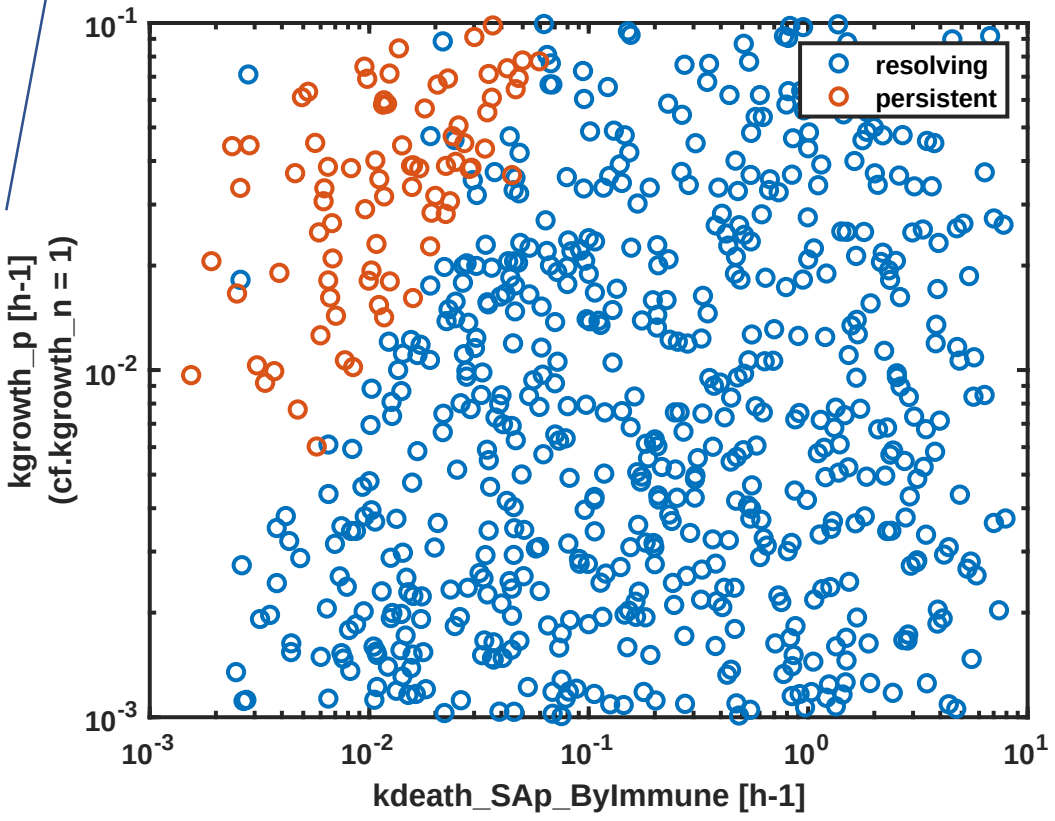
True Positive ”Resolving” = 97.8%
True Positive “Persistent” = 86.1%
(same dataset)



zscore(log(parameter value))

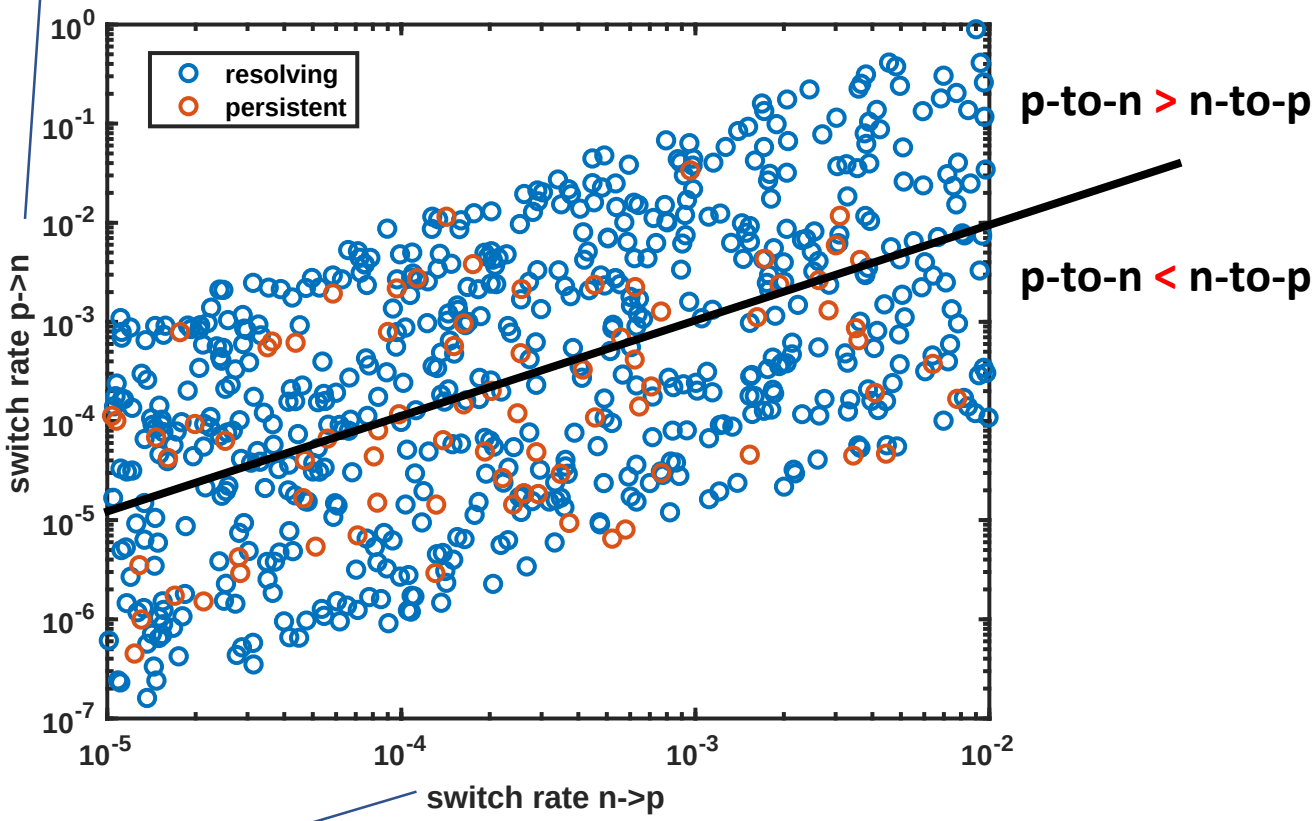
Distinguishing parameters between resolving and persistent: “growth rate” and “death rate” of persister cells

growth rate of persister cells



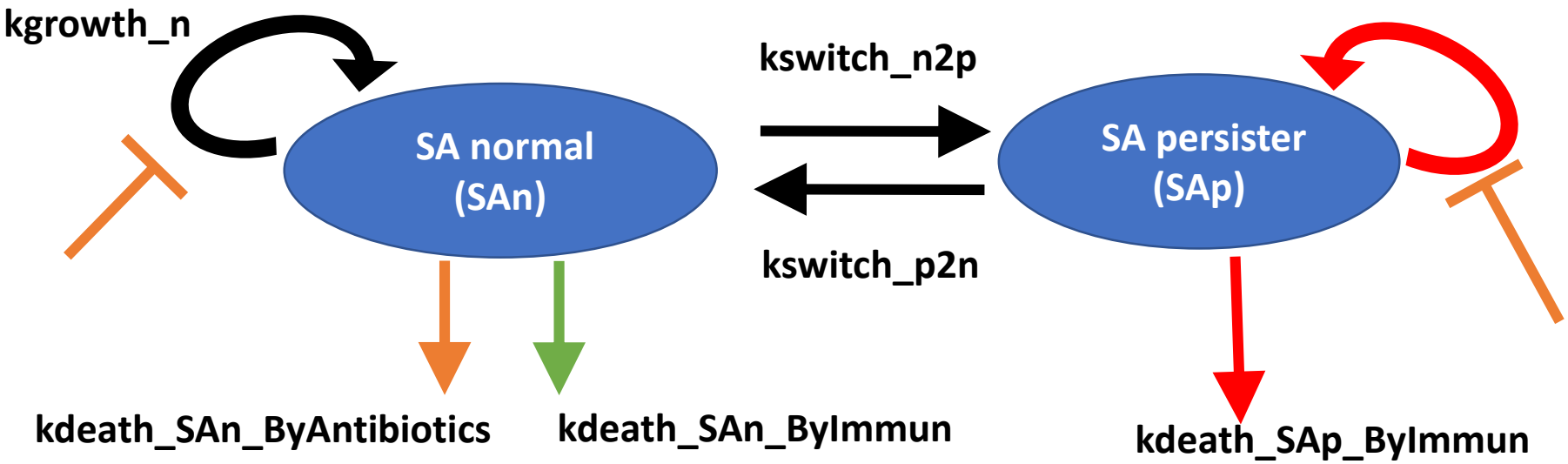
death rate of persister cells by immune systems

switch rate
from persister to normal cells



switch rate
from normal to persister cells

What did we learn about the potential mechanisms of persistence?



Persisters in “persistent/nonresolving” infections (APMB) have
- Higher growth and
- decreased susceptibility to immune elimination

than in “resolving” infections (ARMB).

How might this be possible?

Persisters in “persistent/nonresolving” infections (APMB) have
- Higher growth and
- decreased susceptibility to immune elimination

than in “resolving” infections (ARMB).

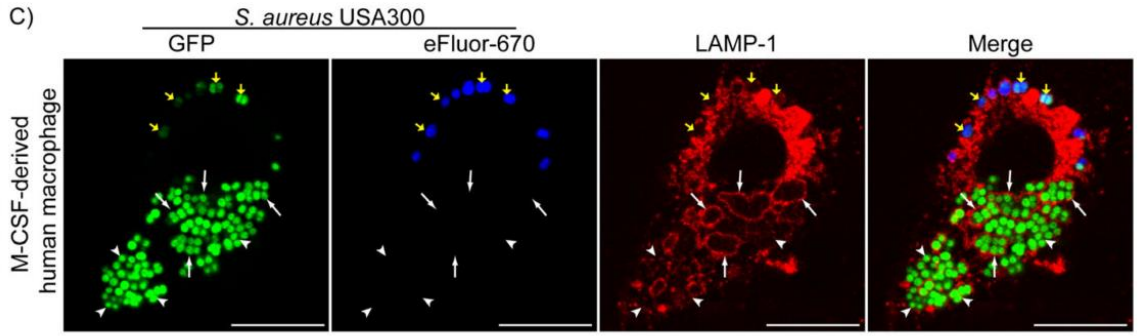
Hypothesis:

1. The bug, specifically the persistors, found in APMB patients, have higher growth and immune evasion mechanisms.

2. In APMB patients the persistors are more likely to grow intra-cellularly, i.e. are evading immune surveillance and grow better.
This might mean that APMB patients’ host cells are better at supporting intra-cellular growth.

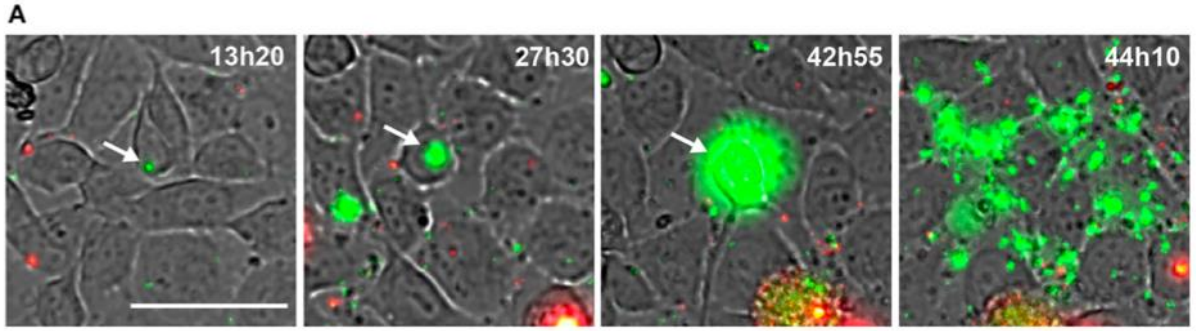
SA is able to live and replicate in immune and non-immune cells

SA is able to live and replicated in primary human M-CSF-derived macrophages.



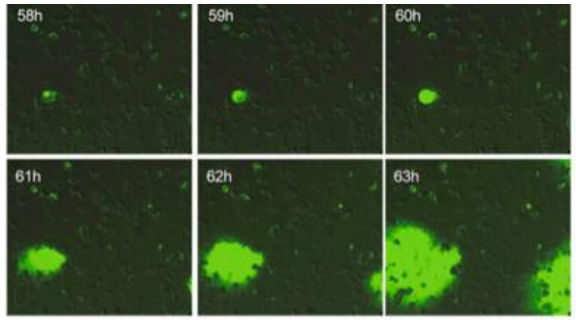
Flannagan, R. S., Heit, B. & Heinrichs, D. E. Intracellular replication of Staphylococcus aureus in mature phagolysosomes in macrophages precedes host cell death, and bacterial escape and dissemination. Cell. Microbiol. 18, 514–535 (2016).

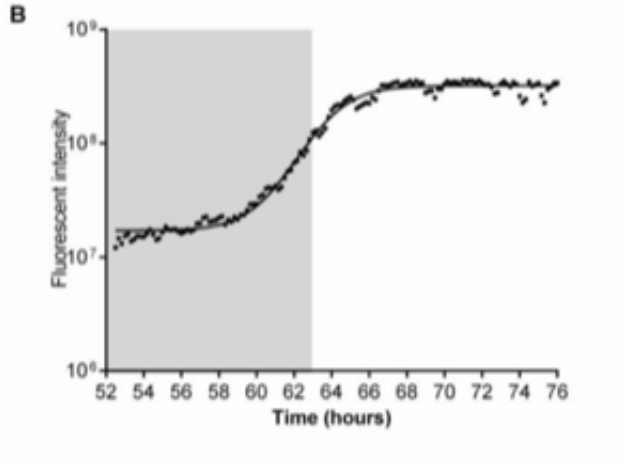
SA in endothelial cells (EA-hy296)



Rollin, G. et al. Intracellular survival of Staphylococcus aureus in endothelial cells: A matter of growth or persistence. Front. Microbiol. 8, 1354 (2017).

SA is able to escape from macrophage (THP-1)





Jubrail, J. et al. Inability to sustain intraphagolysoso bacterial persistence in macrophages. Cell. Microbiol. 18, 80–96 (2016).

Target cells of intracellular SA

- macrophage
- neutrophils
- dendric cells
- epithelial cells
- endothelial cells

etc.

How might this be possible?

Persisters in “persistent/nonresolving” infections (APMB) have
- Higher growth and
- decreased susceptibility to immune elimination

than in “resolving” infections (ARMB).

Hypothesis:

2. In APMB patients the persistors are more likely to grow intra-cellularly, i.e. are evading immune surveillance and grow better.
This might mean that APMB patients’ host cells are better at supporting intra-cellular growth.

How?
- **Lower resistance to bacterial invasion**
- **Enhanced resistance to cell death**
- **Provide conditions for growth (nutrients)**

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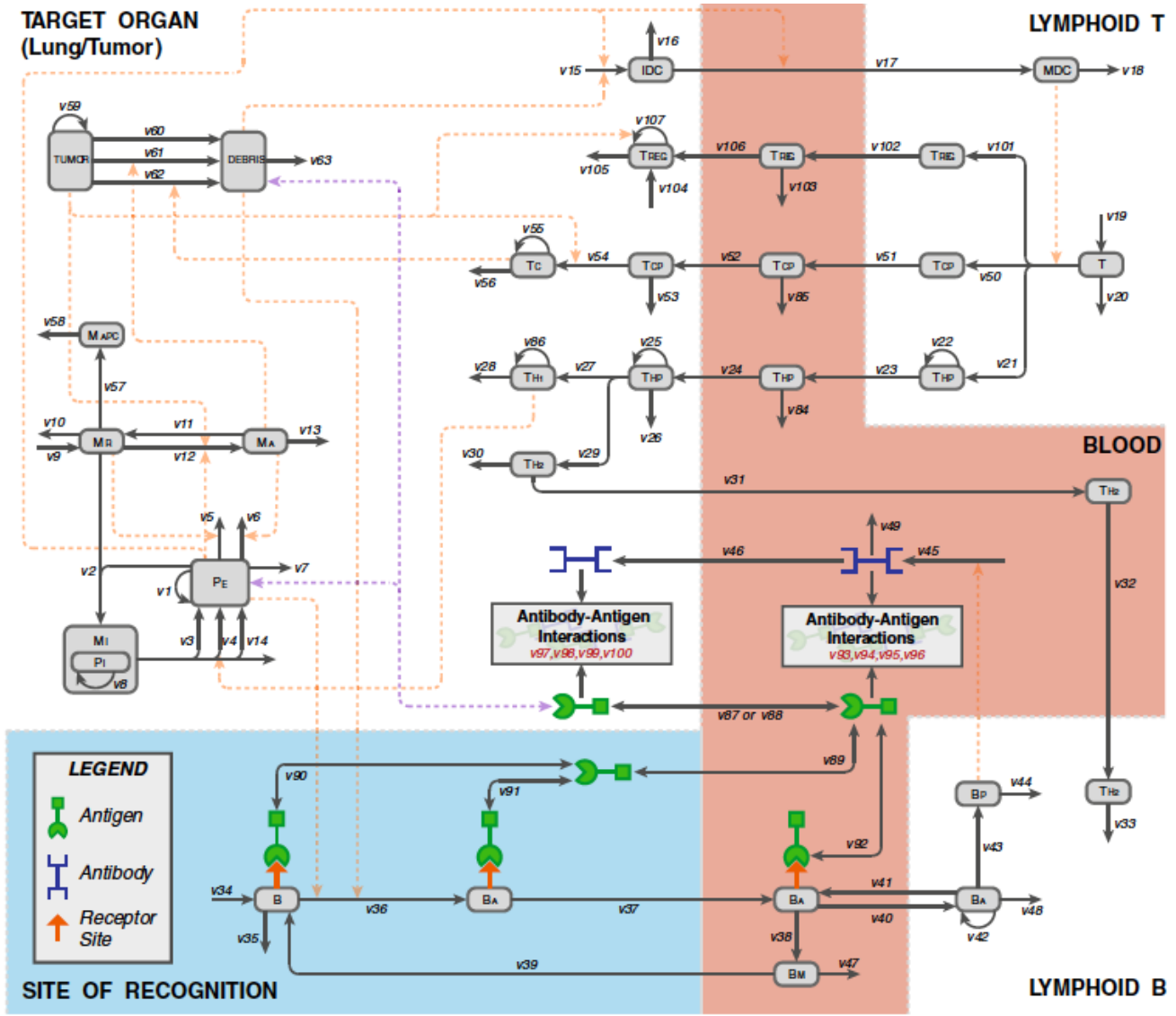
A multi-compartment Model

Based on the current results, I will add more mechanistic features to the model with blood compartment to capture the persistent bacteremia.

- Mathematical model for host-tuberculosis immune response model will be helpful.
  - Tuberculosis is a typical intracellular bacteria



Palsson, S. et al. The development of a fully-integrated immune response model (FIRM) simulator of the immune response through integration of multiple subset models. BMC Syst. Biol. 7, 95 (2013).

acknowledgement