Predictive biology: understanding and reversing the evolution of antibiotic resistance

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Predictive technologies are everywhere

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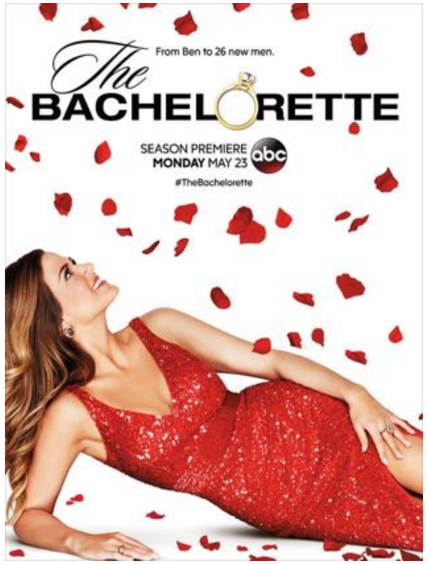
Good predictions depend on context

Good recommendation

Incorrect

Or worse…



Unique microbiomes exist all over the world

Predicting microbial community dynamics is still a big challenge!!!

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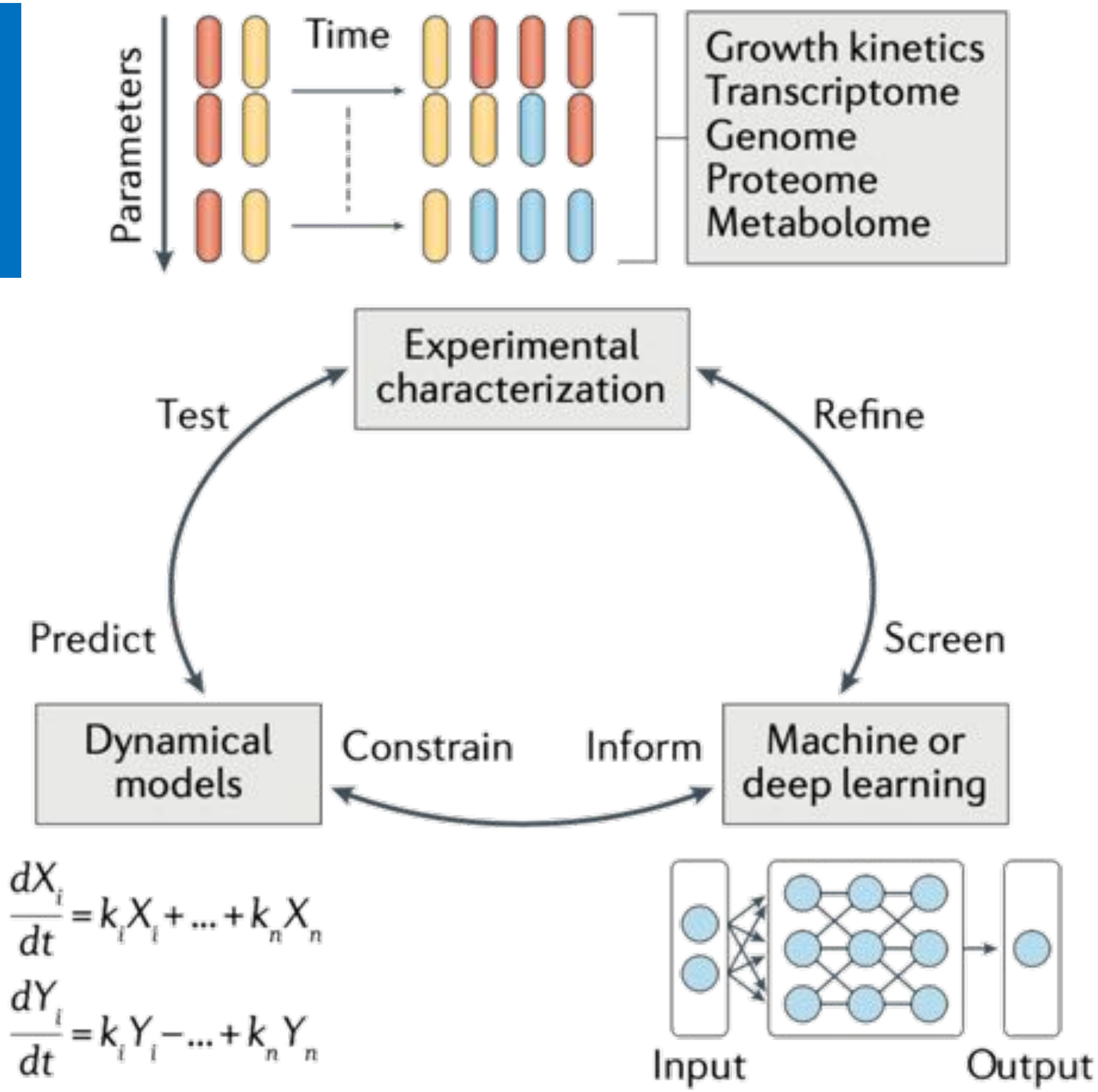
Paradigm of predictive microbiology

1. Well-designed, thoughtful, and precise experimental measurements

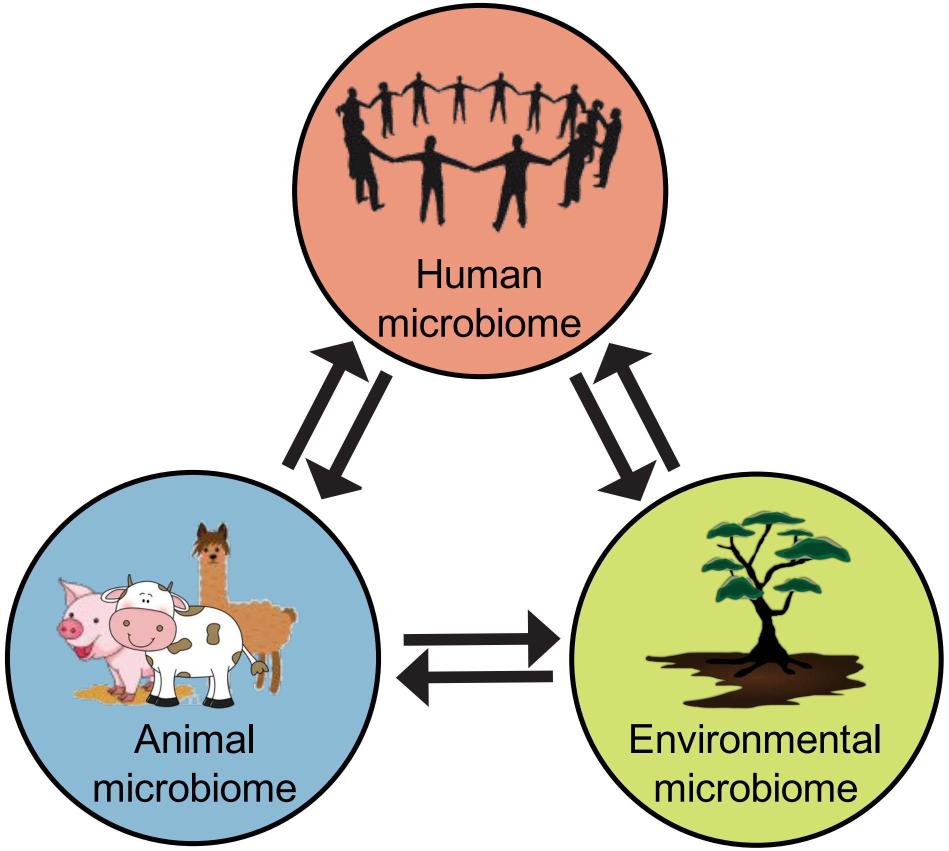


2. Mechanistic models describing how bacteria change over time

3. Predictive machine learning algorithms to tease out underlying relationship

Lopatkin AJ & Collins J, Nature Reviews Microbiology (2020)

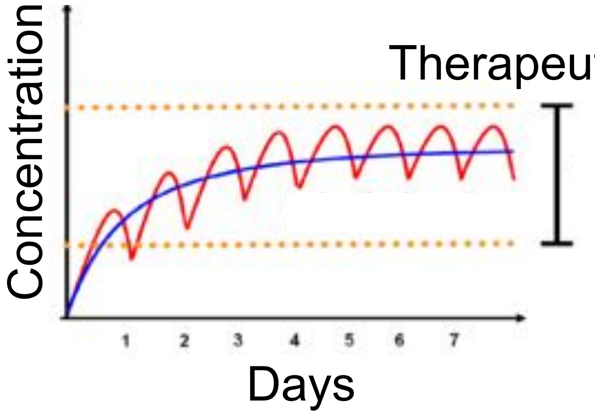
A prime case for predictive biology: antibiotic resistance



How do we prevent the emergence of antibiotic resistance?

1. Develop new antimicrobial strategies

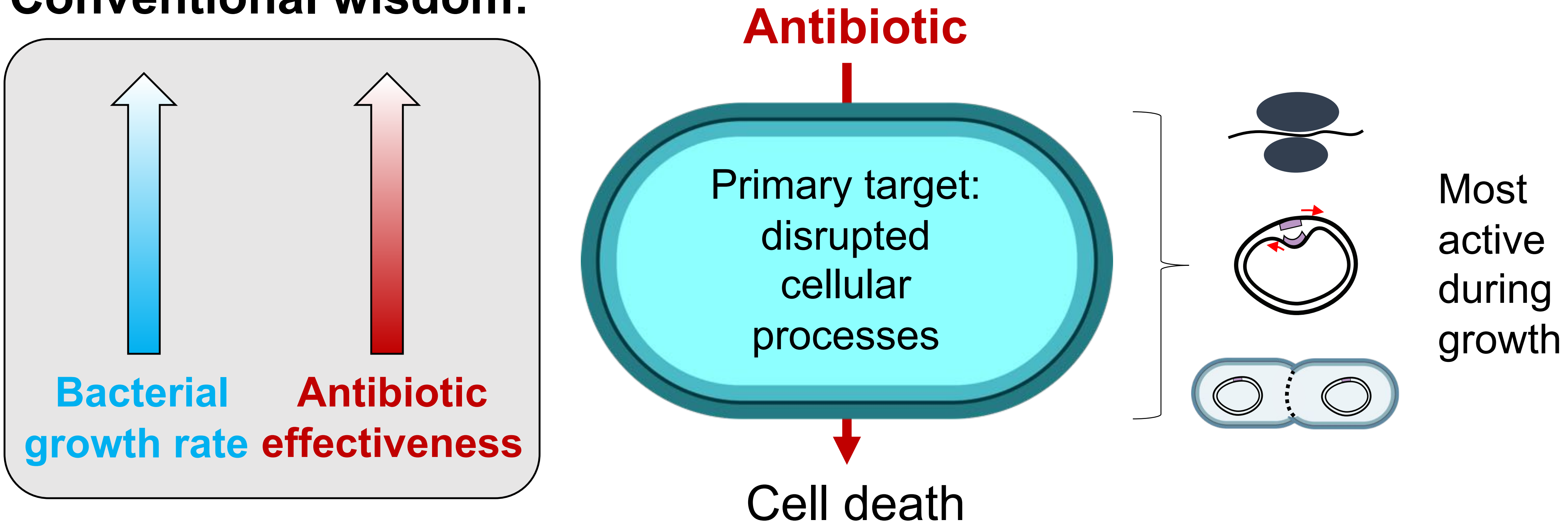
2. Better utilize existing drugs to prolong shelf life



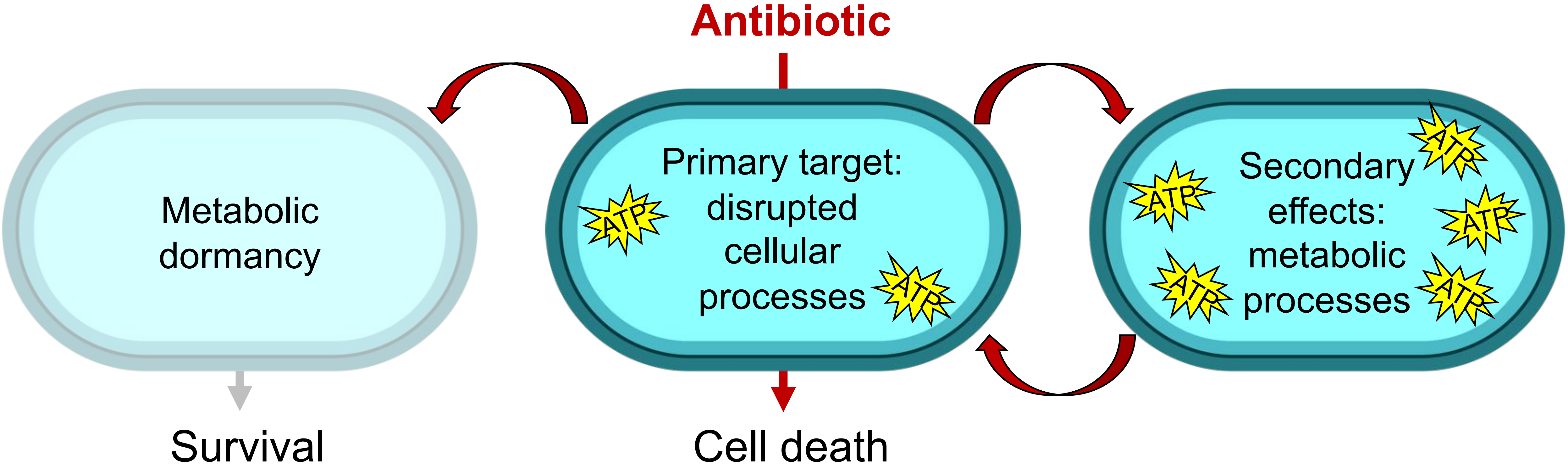
To do so requires better understanding of antibiotic mechanism!

Growth rate correlates with antibiotic efficacy

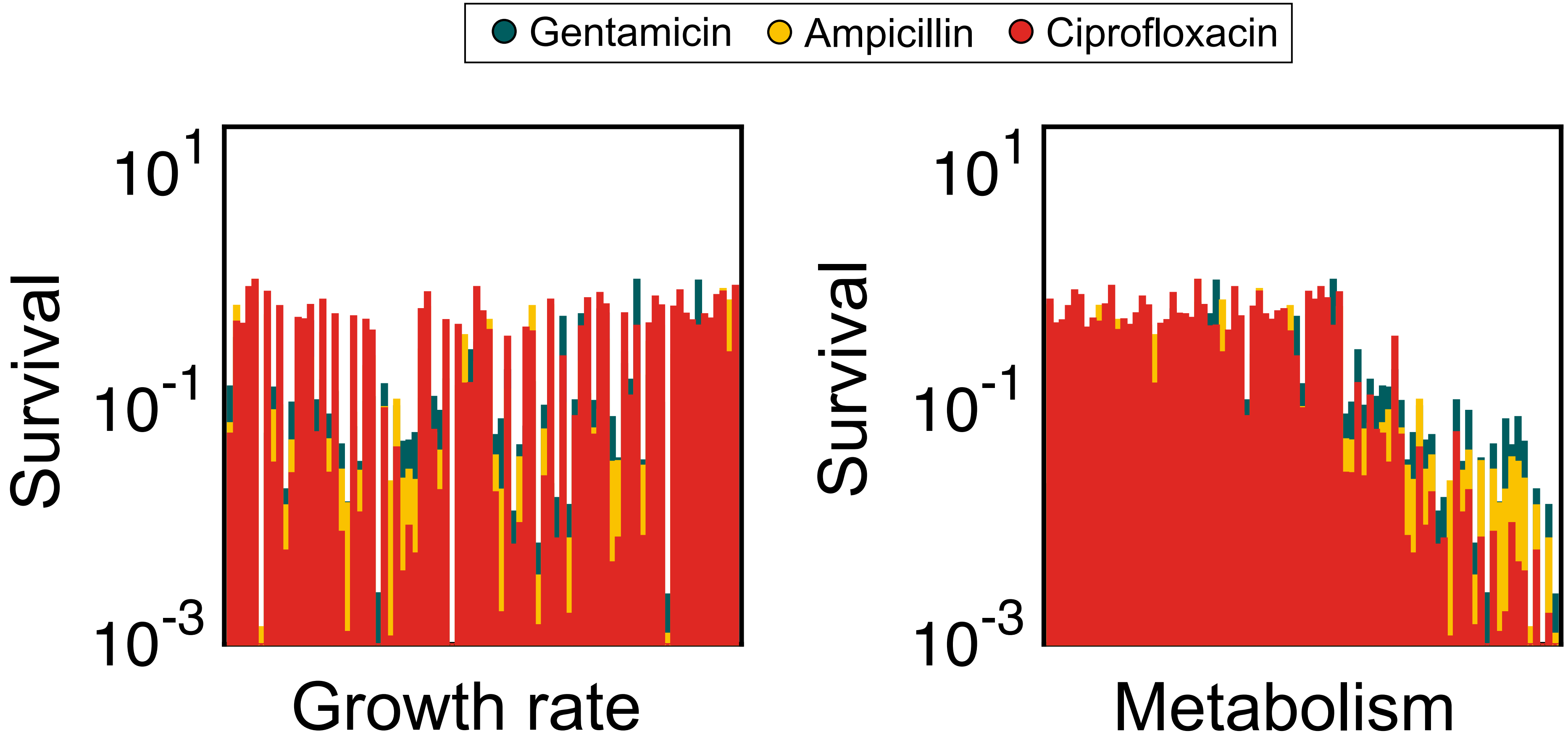
Conventional wisdom:



Metabolism also plays a role in antibiotic efficacy



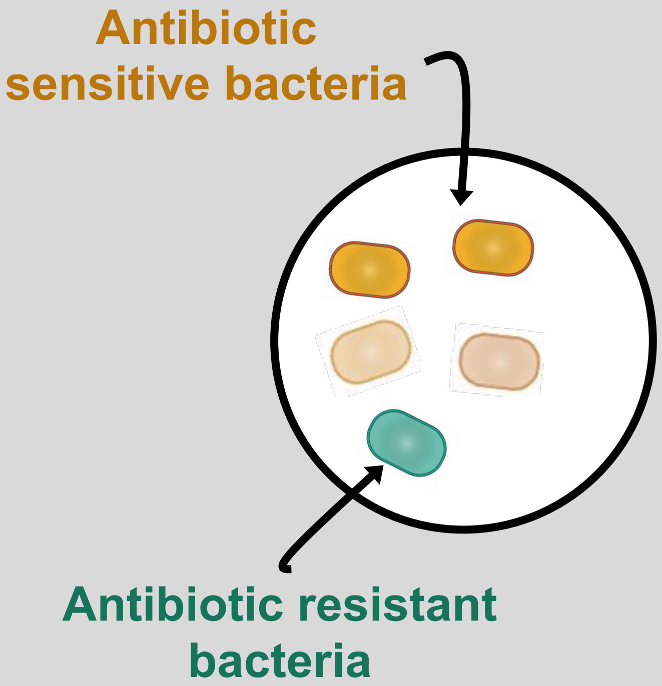
ATP predicts survival better than growth rate



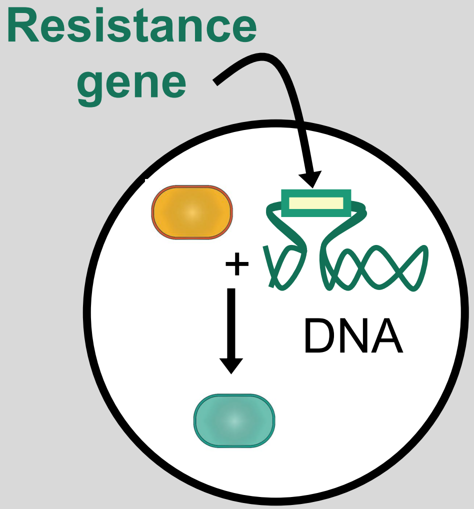
How does metabolism contribute to emergence of resistance?

Modes of resistance acquisition

1.Spontaneous genetic mutations



2. Horizontal gene transfer (HGT)

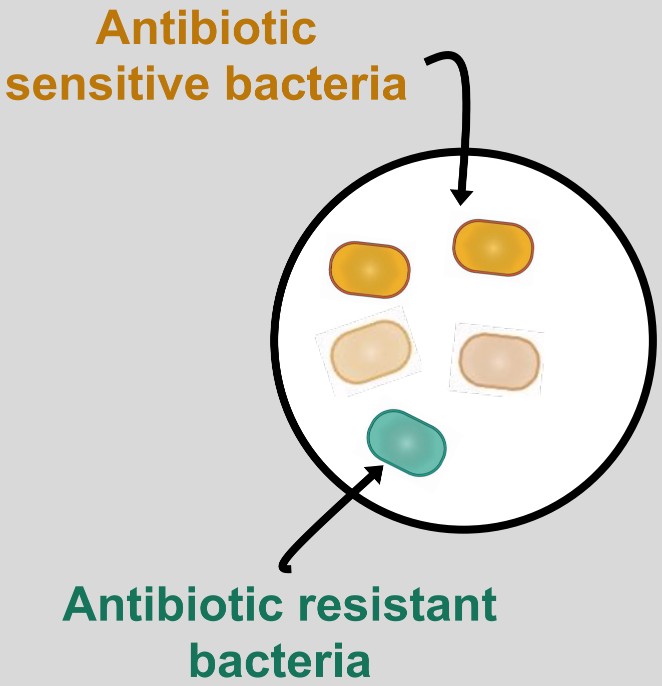


Primary mode for resistance dissemination

How does metabolism contribute to emergence of resistance?

Predicting resistance by incorporating metabolism

1. Model → experiment

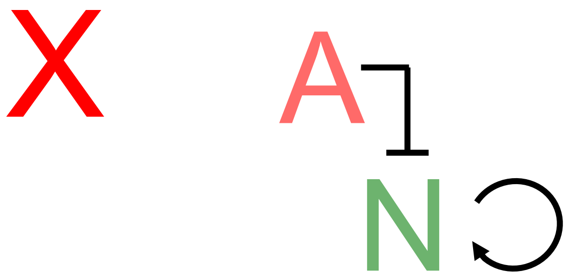


Do cells evolve metabolic-specific resistance mutations?

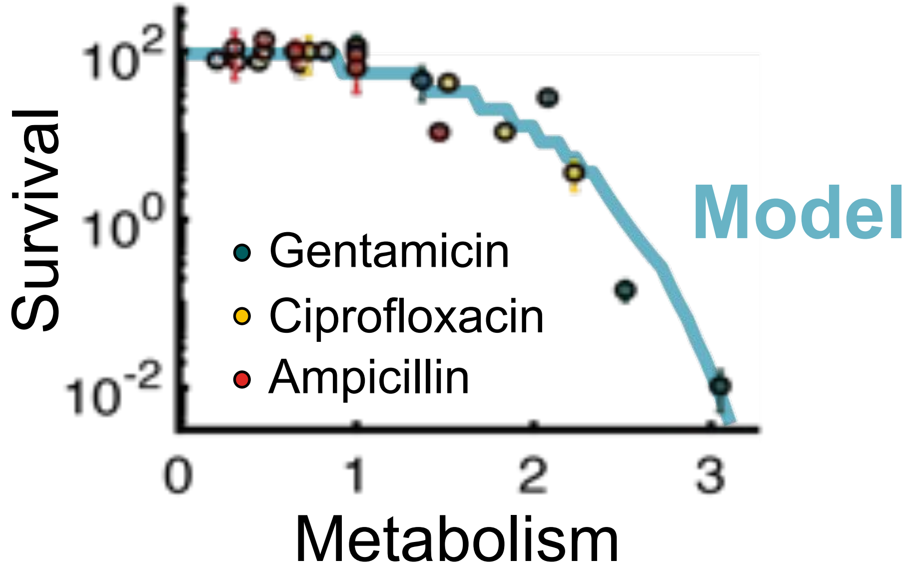
2. Experiment → model

How does metabolism impact resistance dissemination?

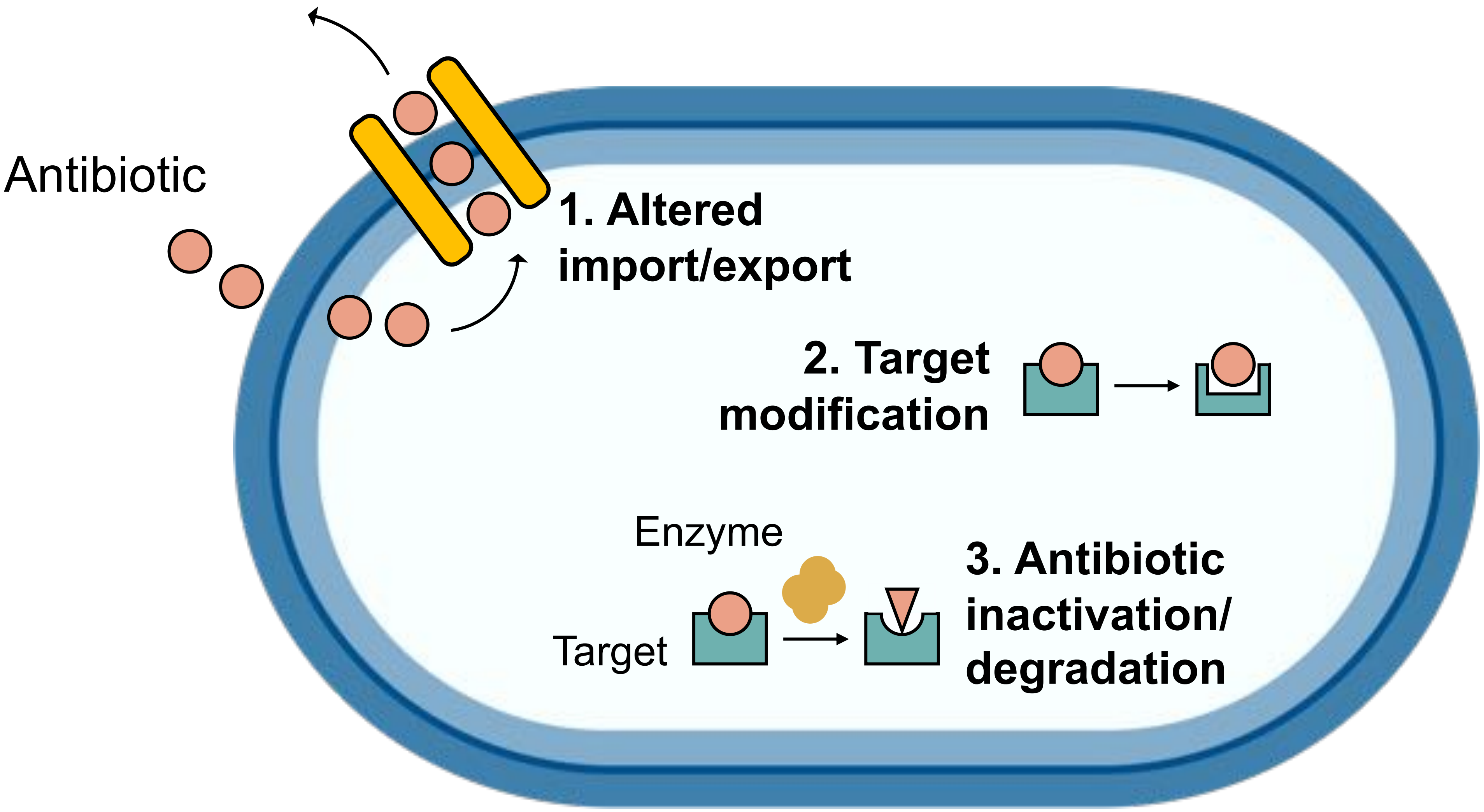
Dynamic model of metabolic-dependent antibiotic lethality



A: Antibiotic
N: Cell density

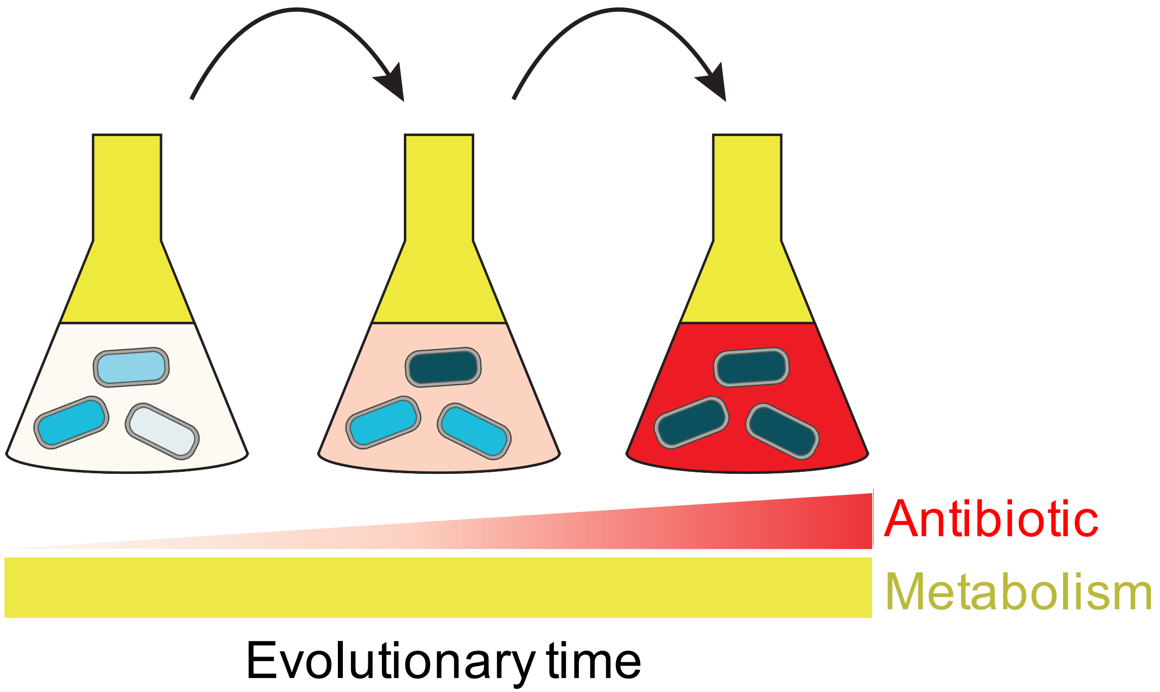


Why is metabolism not a prominent mechanism of resistance?

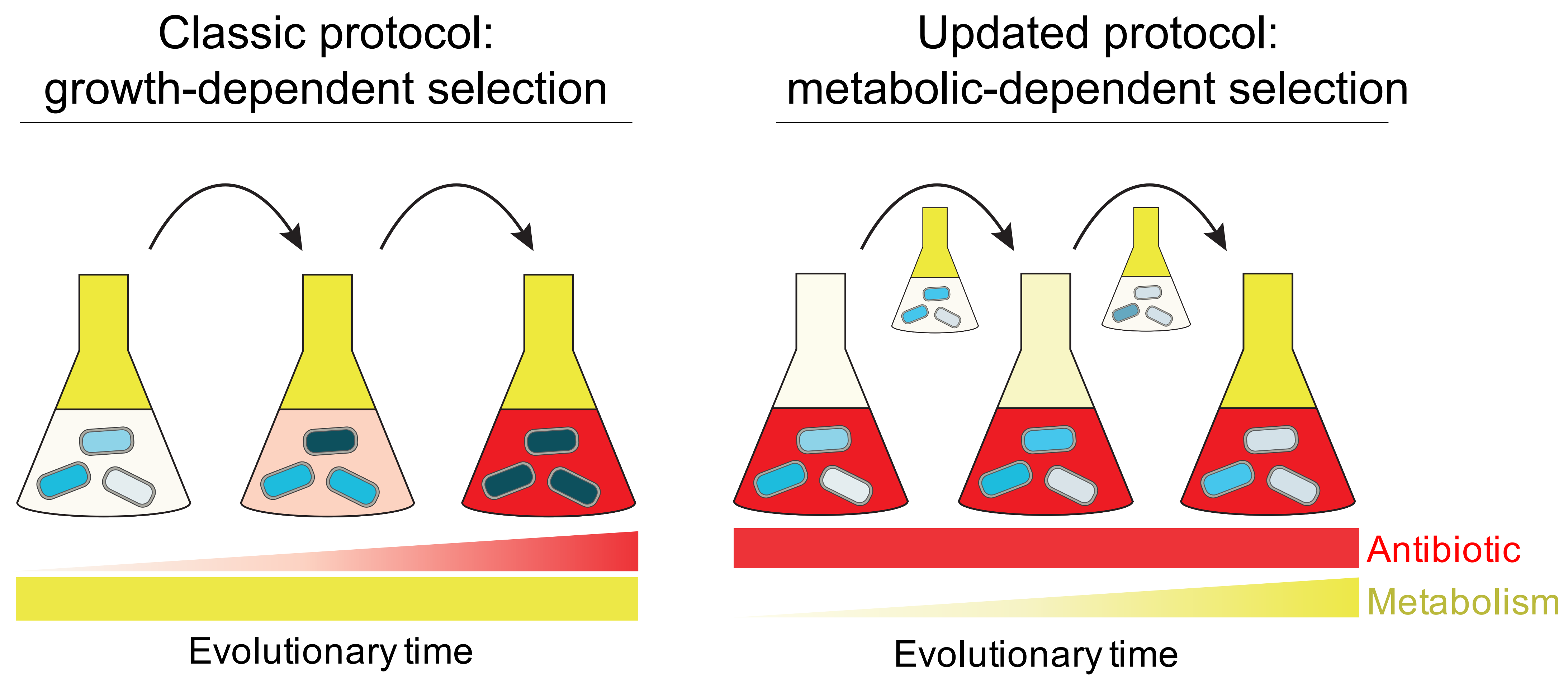


Classic evolution: growth adaptation

Classic protocol:
growth-dependent selection

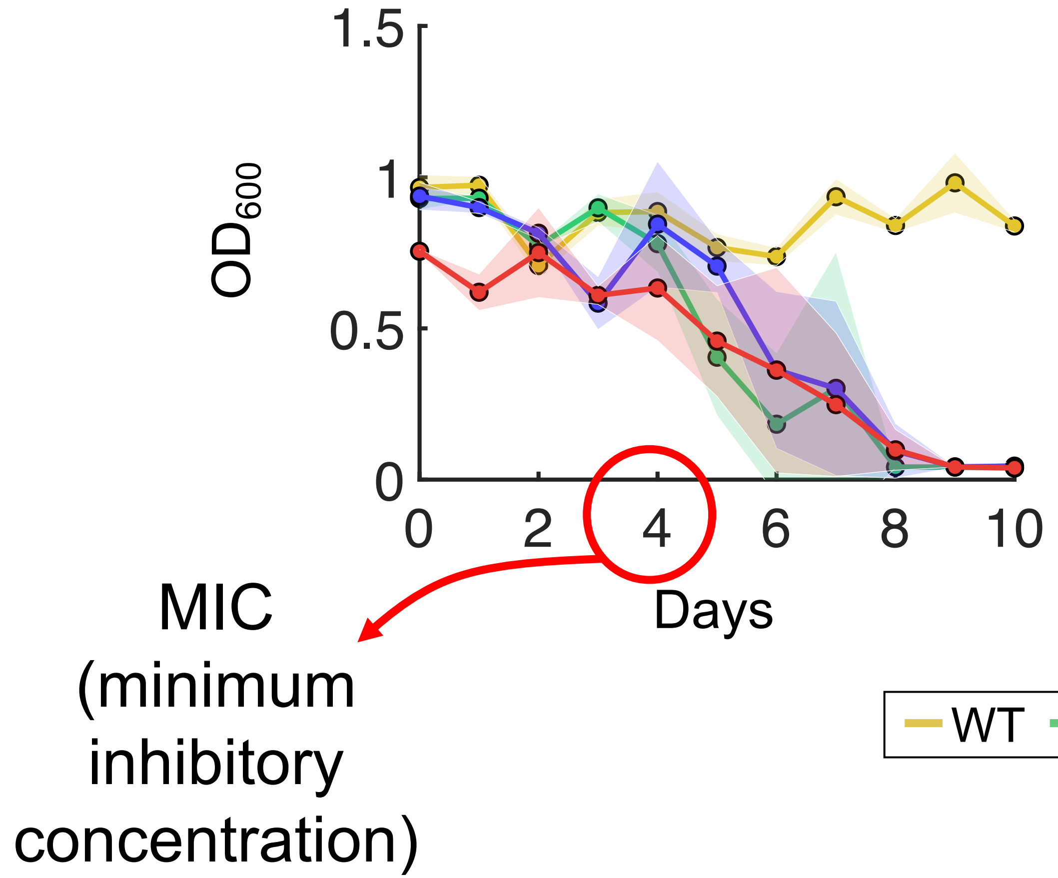


Alternative evolution: metabolic adaptation

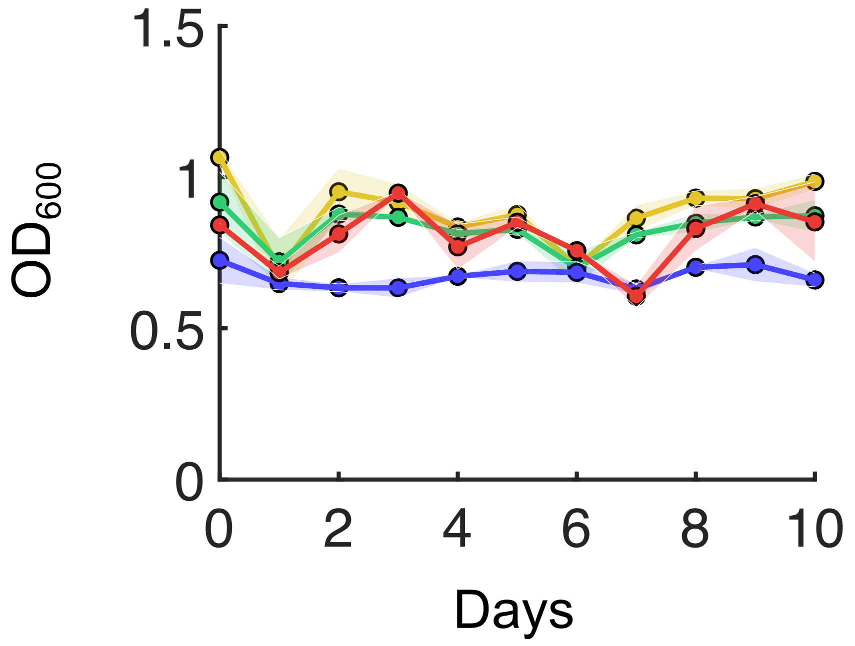


Evolution protocol comparison

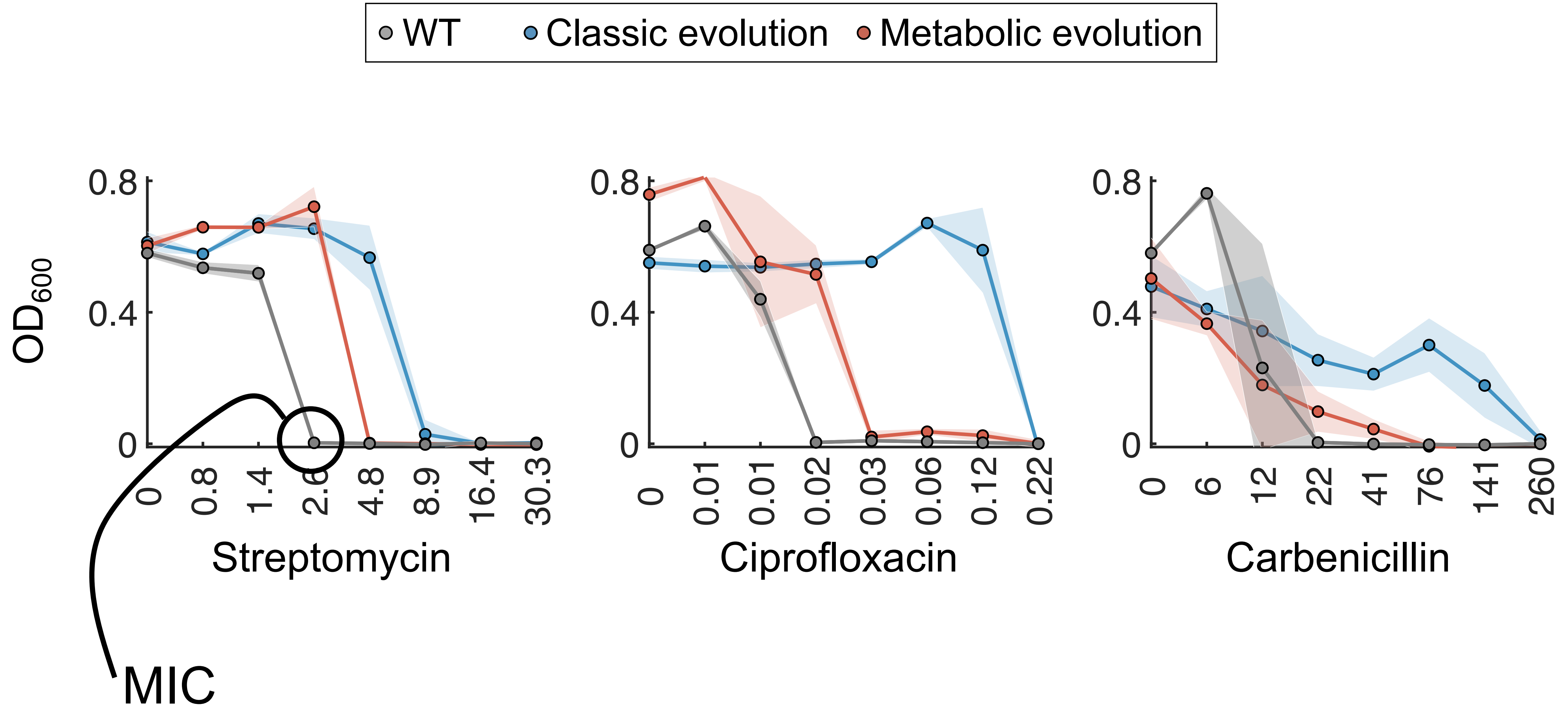
Classic protocol:
growth-dependent selection



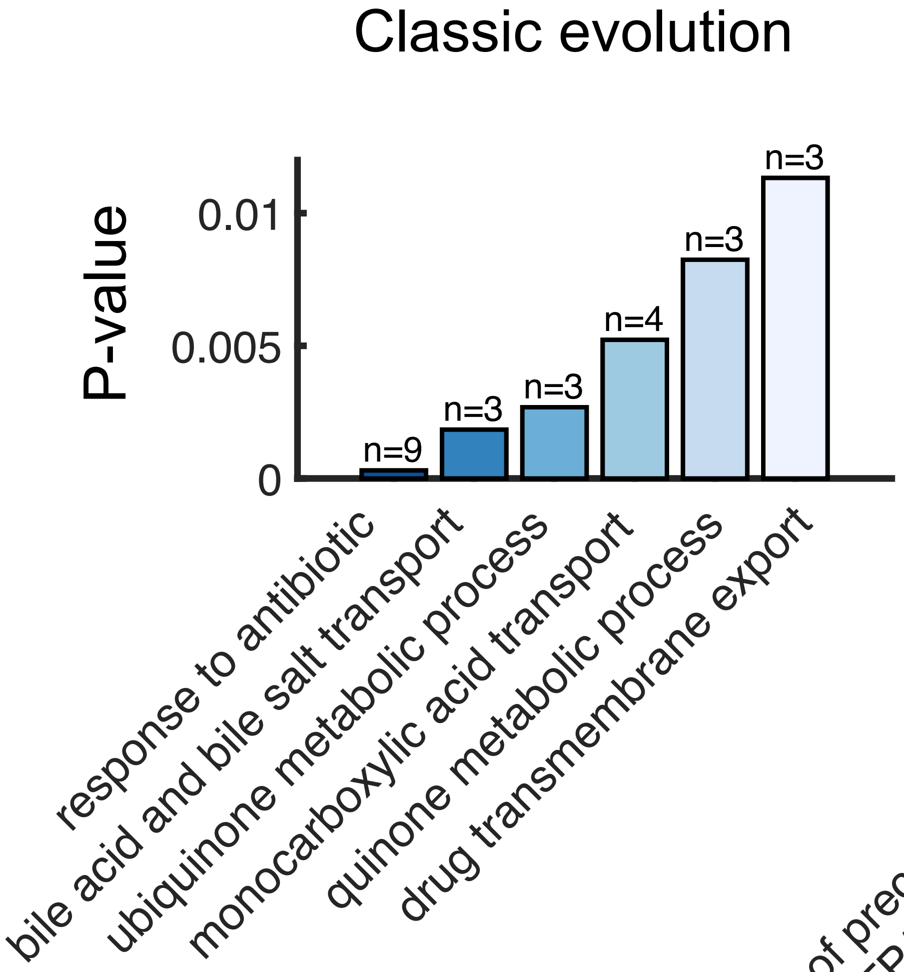
Updated protocol:
metabolic-dependent selection

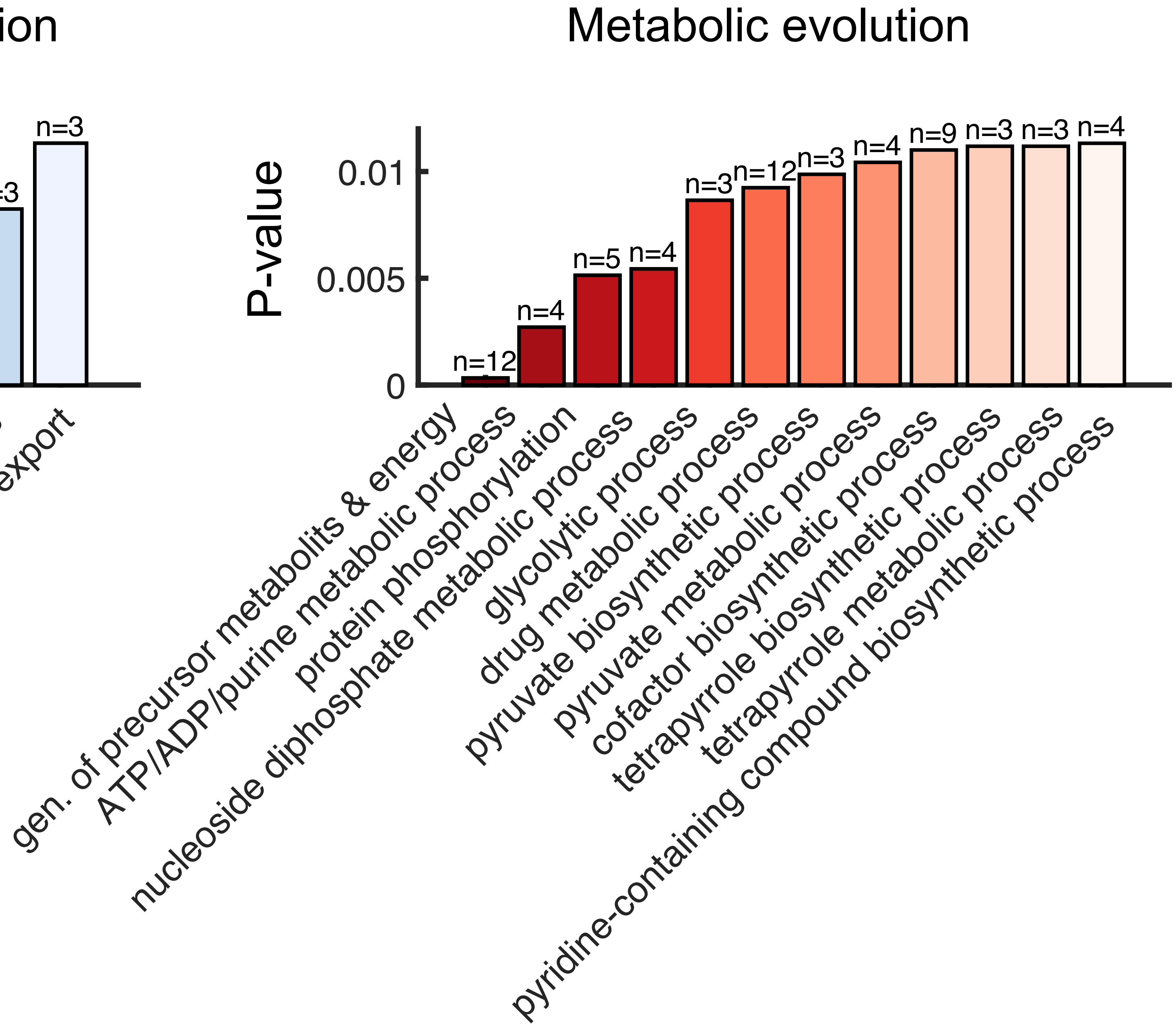


Both evolutions lead to acquired resistance

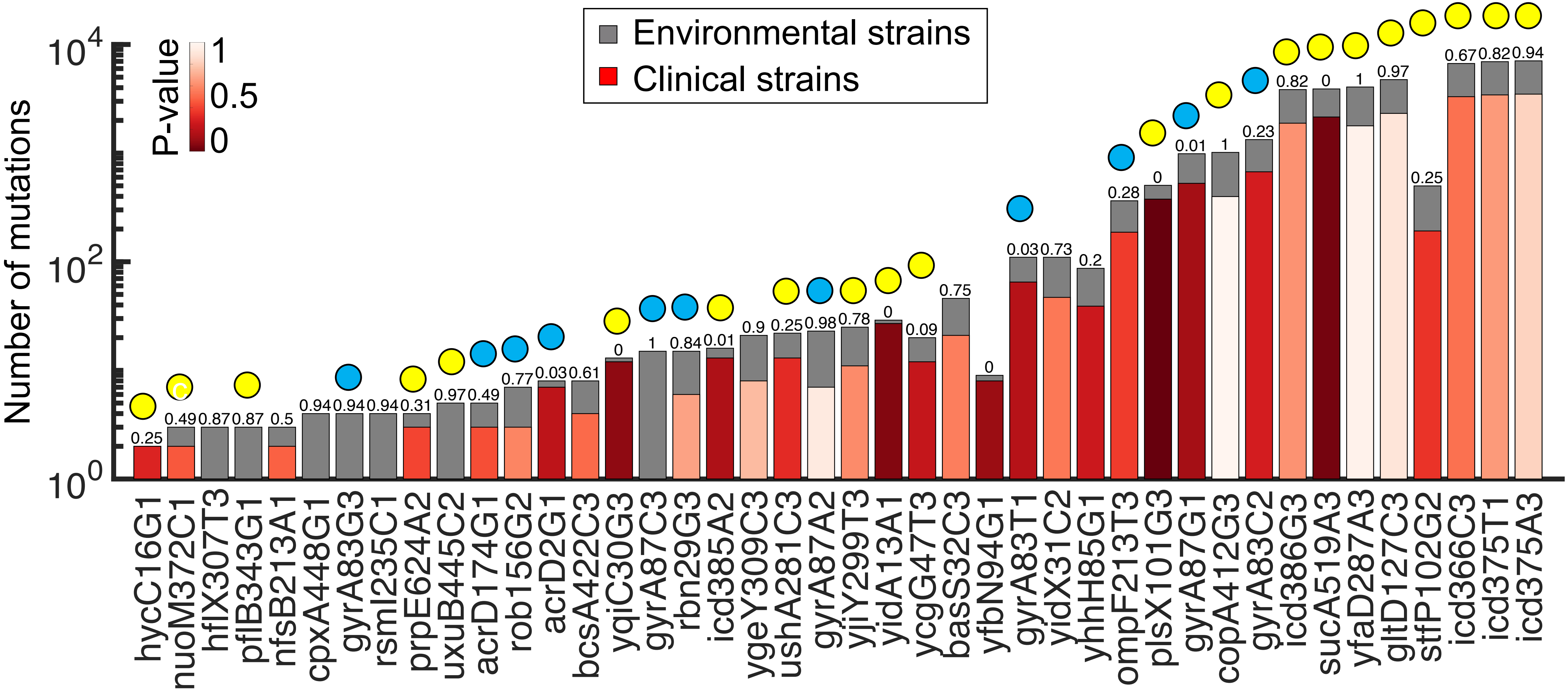


Alternative evolution reveals novel metabolic mutations

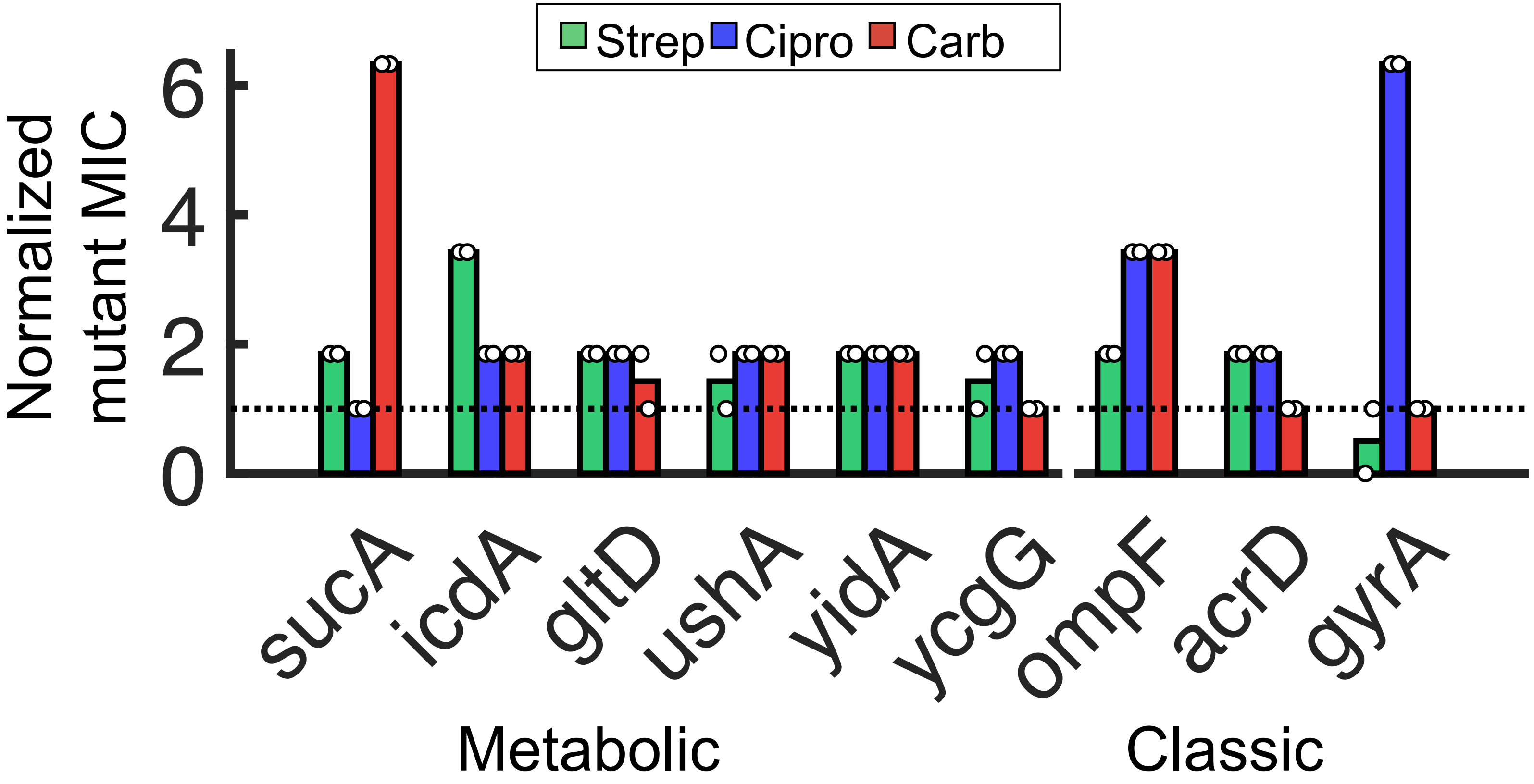




Metabolic mutations are clinically and environmentally relevant

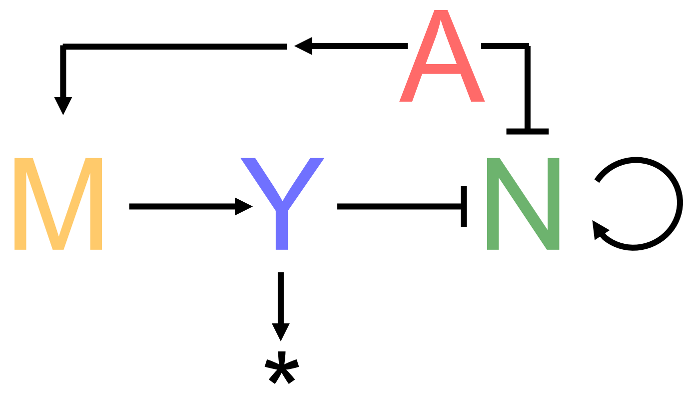


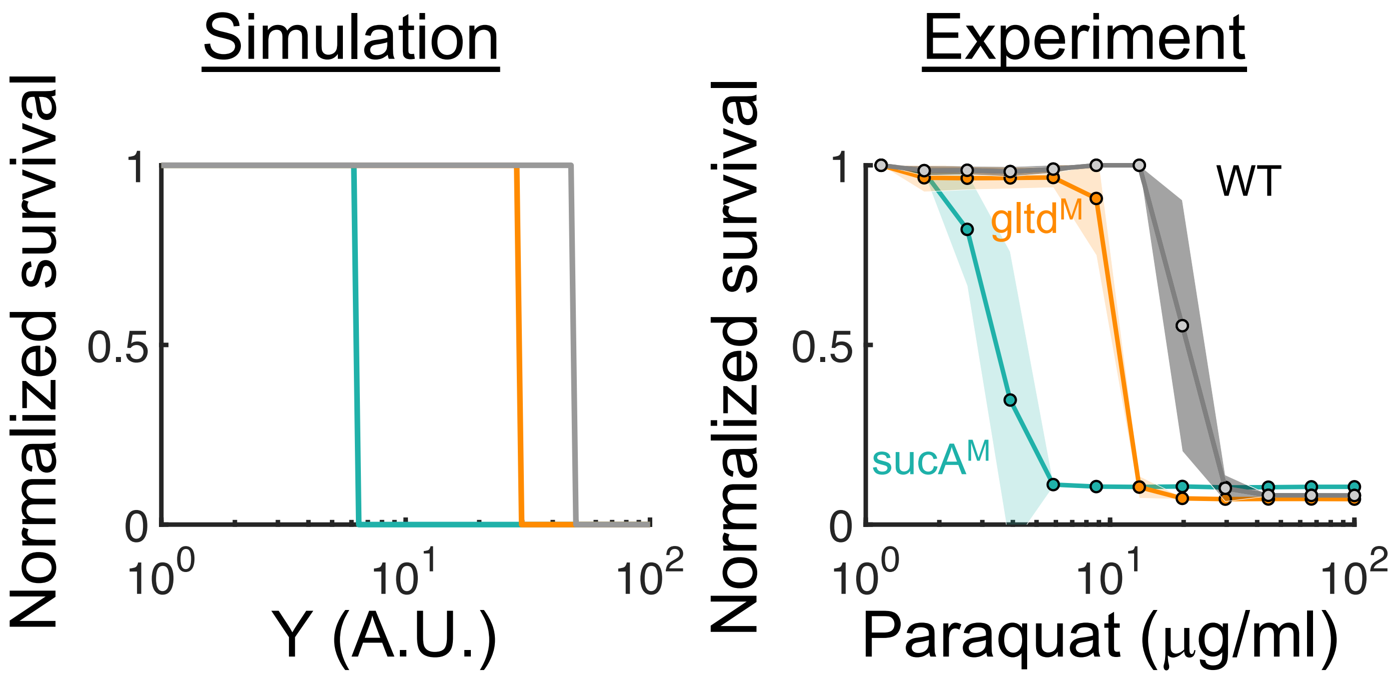
Metabolic mutations independently confer resistance



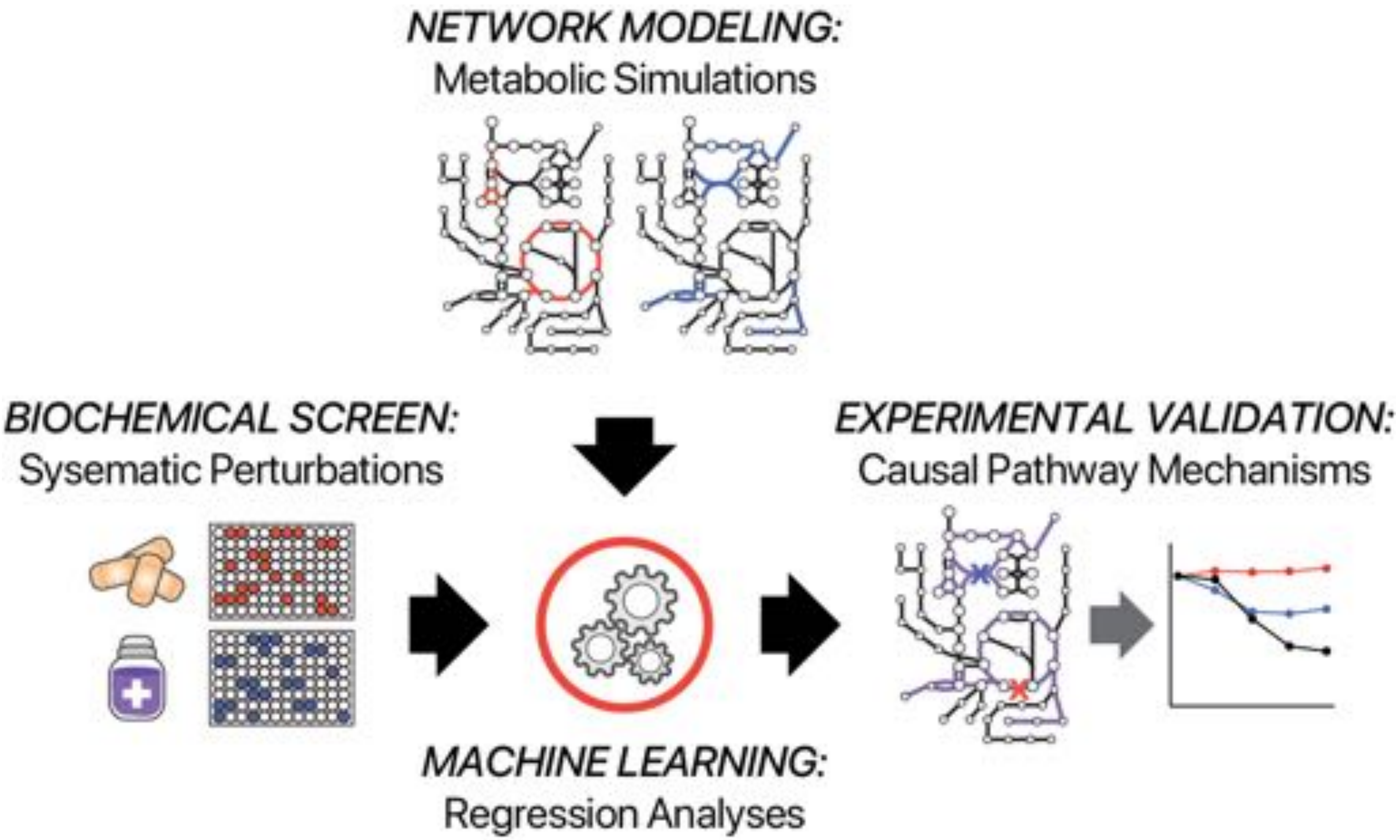
Lopatkin, AJ, et al. Accepted, *Science* (2021)

Model predicts mutant dynamics



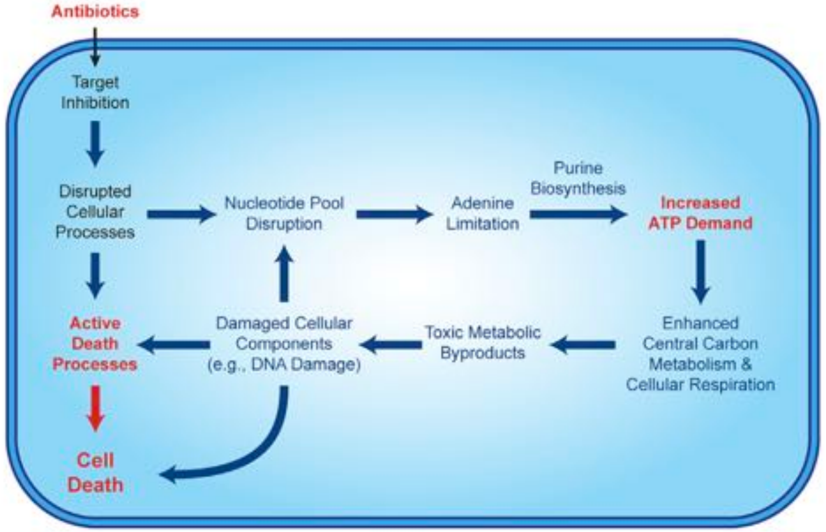


A "WHITE-BOX" APPROACH FOR INTERPRETABLE MACHINE LEARNING

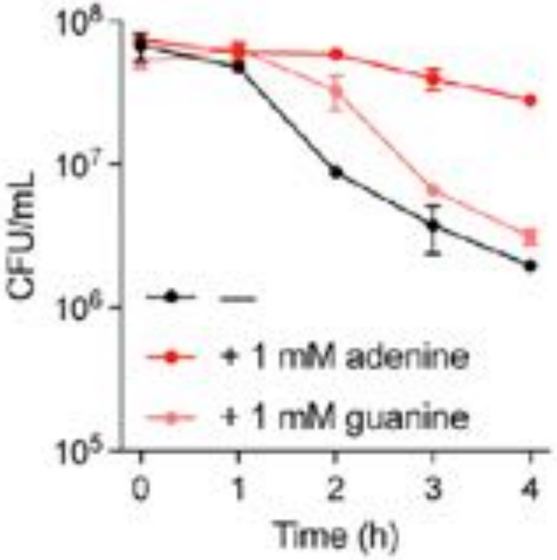


YANG JH, CELL 2019

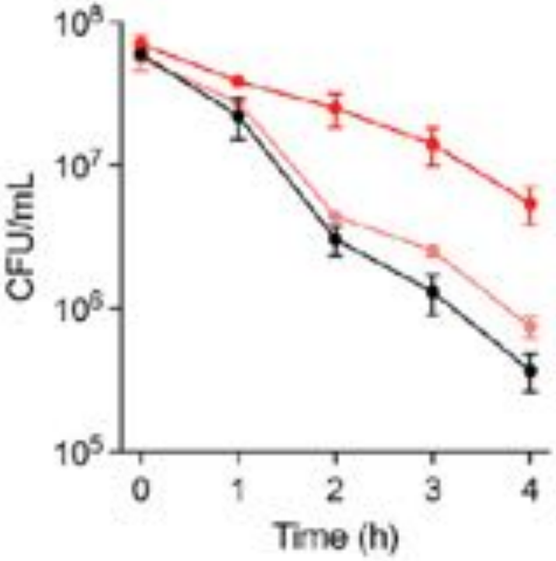
Purine Biosynthesis Contributes to Antibiotic Lethality

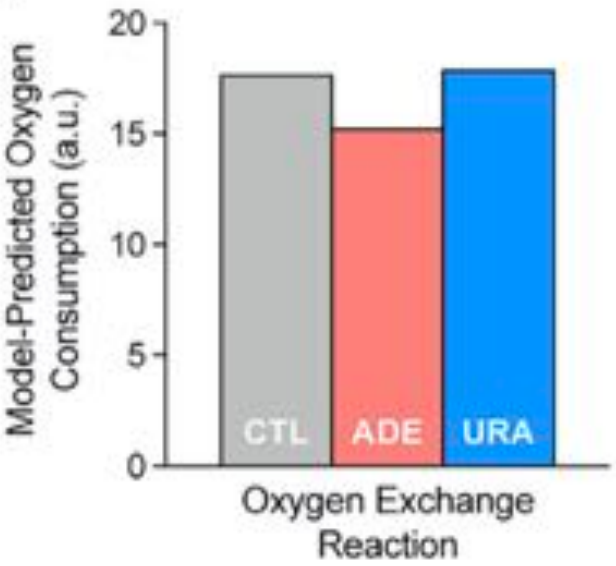


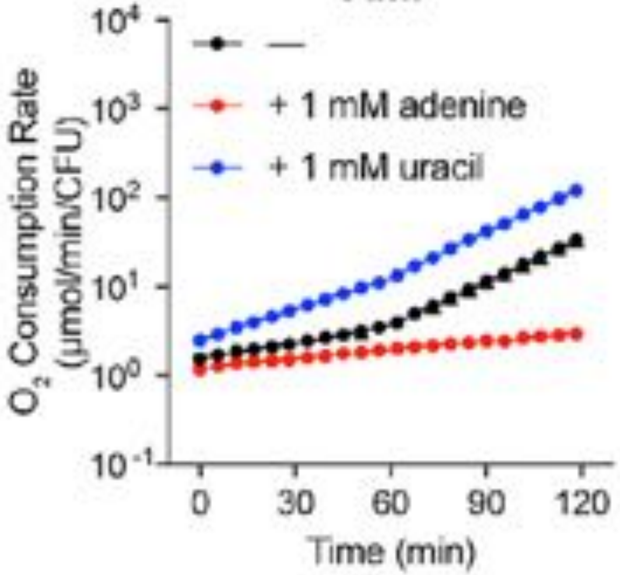
AMPICILLIN

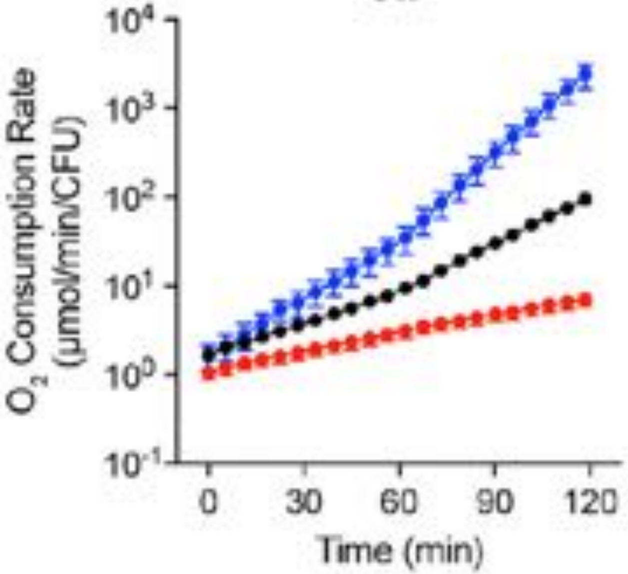


CIPROFLOXACIN





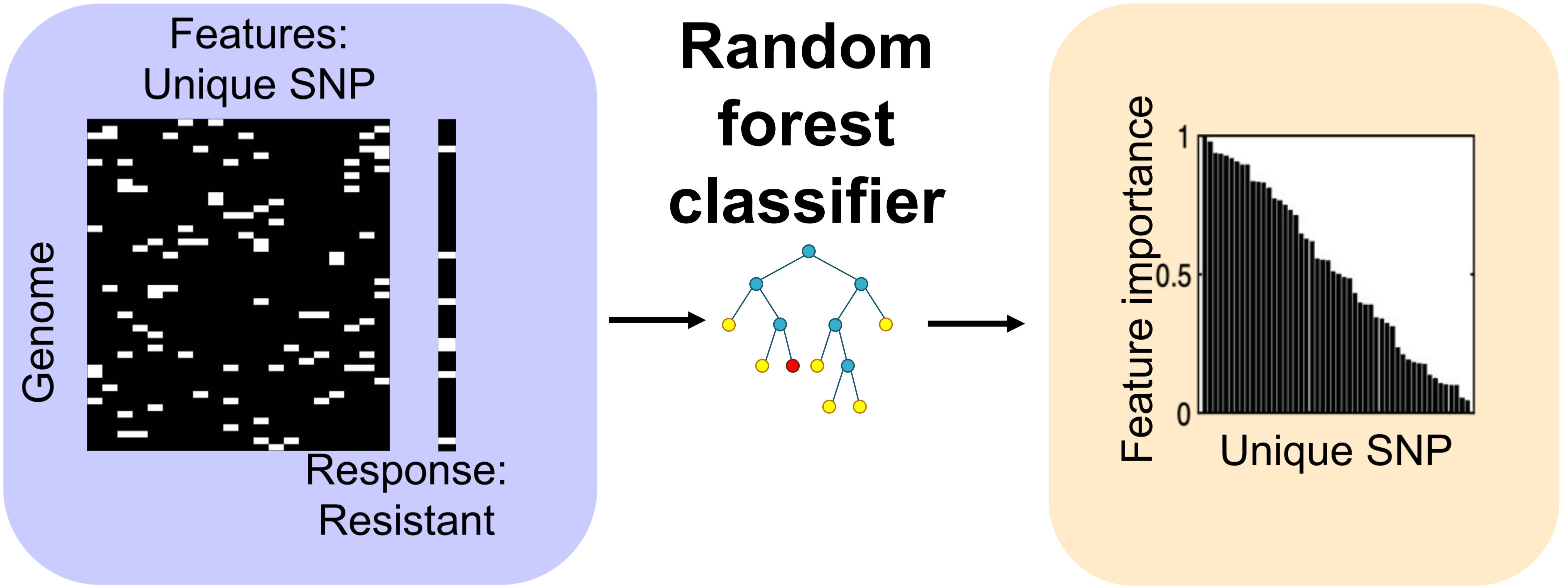




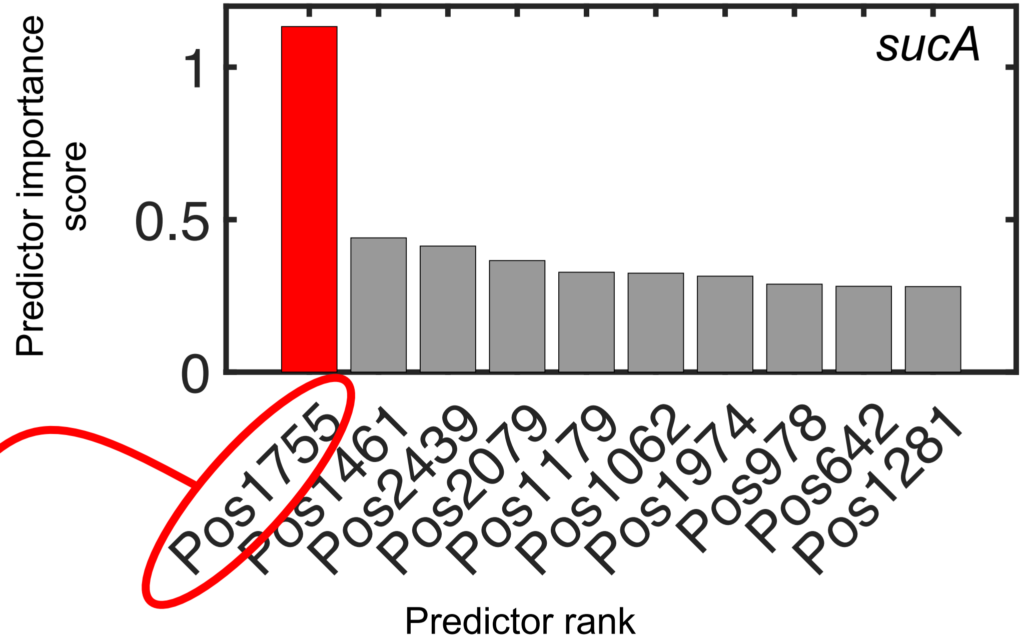
Yang JH, Cell 2019

ML identifies metabolic mutants that confer resistance

Input



Output



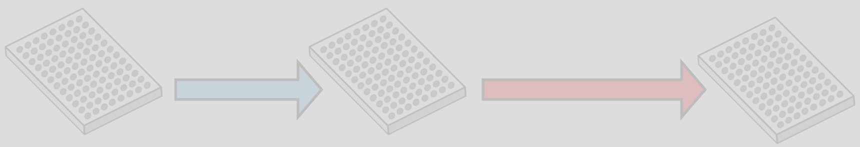
Same mutant from evolution!

Metabolism and antibiotic resistance

1. Model → experiment

- Predictive models inform experimental design to identify metabolic mutants
- Metabolic mutations highly prevalent in pathogens
- Mutations independently confer resistance

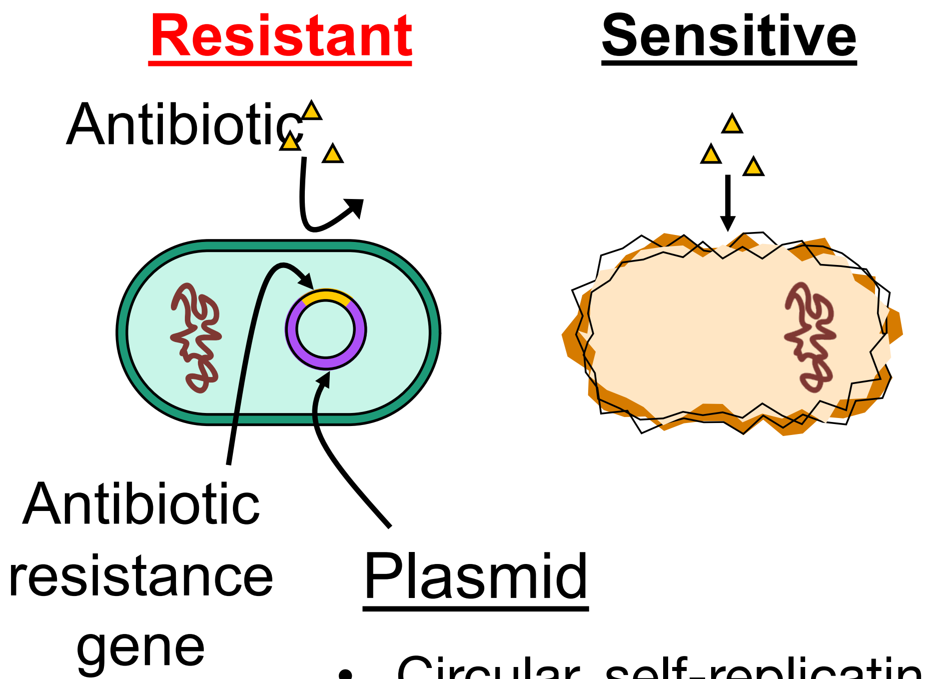
Metabolic-dependent selection



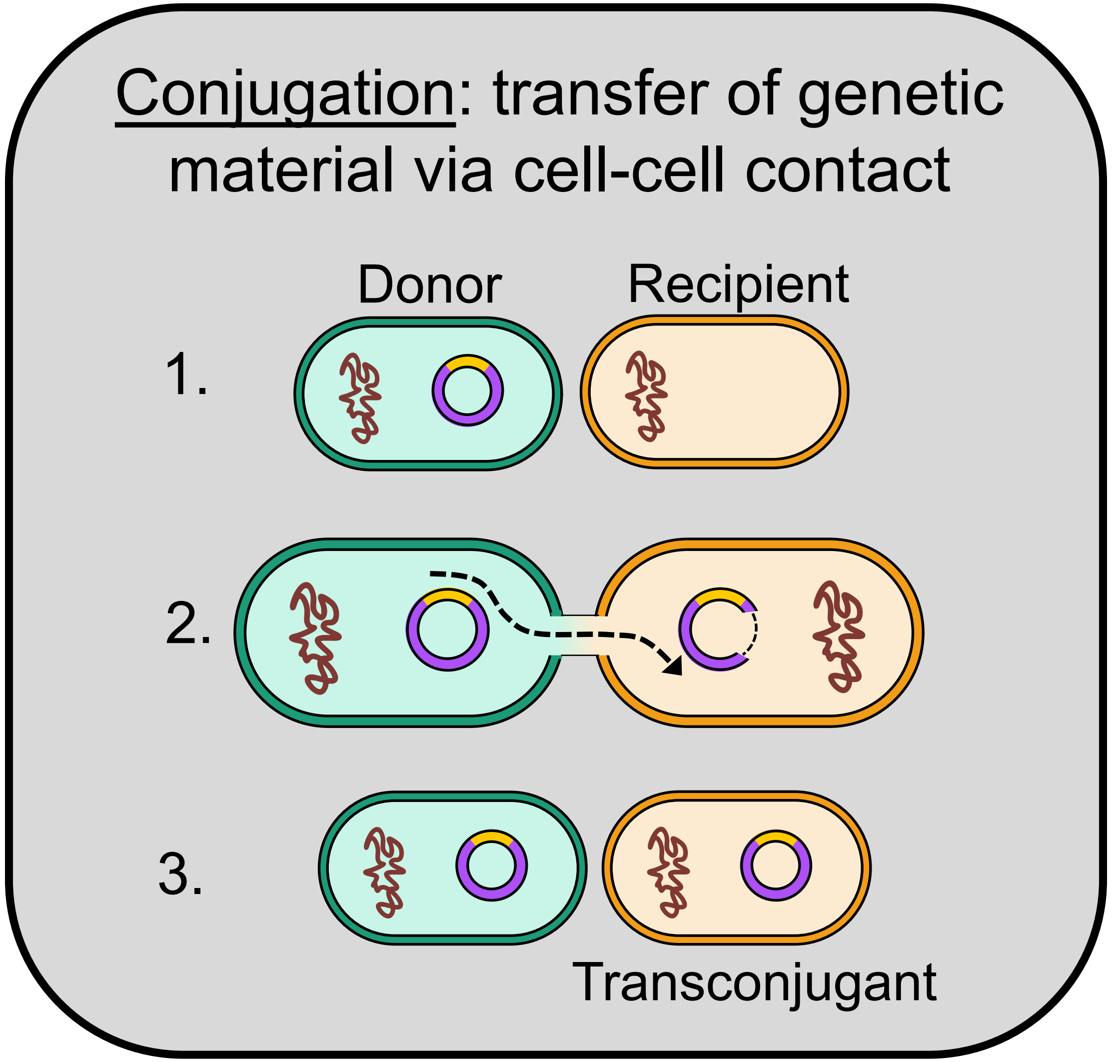
2. Experiment → model

How does metabolism impact resistance dissemination?

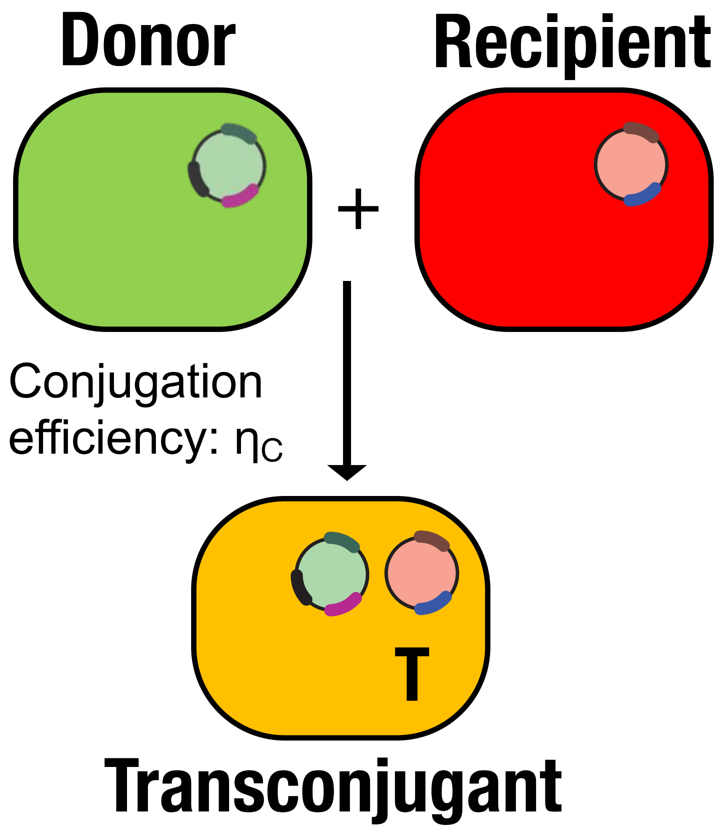
Conjugation: primary mode for resistance dissemination



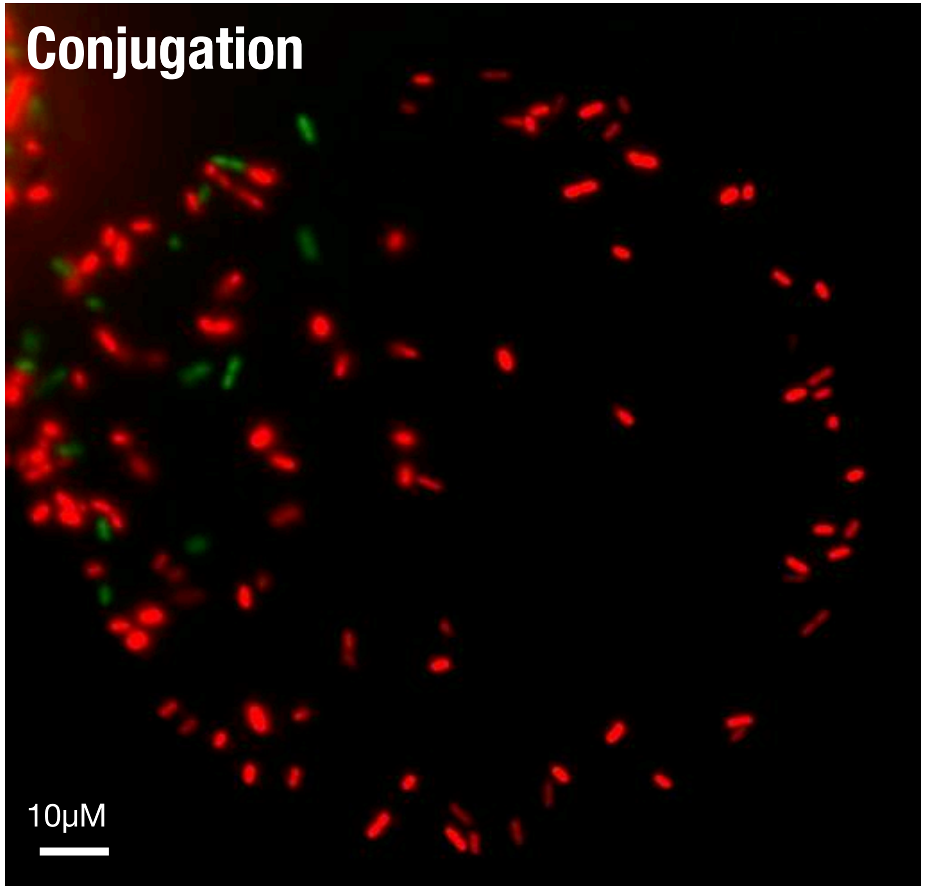
- Circular, self-replicating DNA
- High prevalence
- Carry antibiotic resistance and catabolic genes
- Stable in diverse species



Conjugation: primary mode for resistance dissemination



Population	Resistance	Fluorescence
D	Kan	GFP
R	Cm	mCherry
T	Kan + Cm	GFP + mCherry



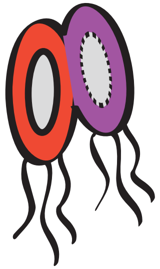
4 Hours pre-growth:
selection with Kanamycin (Kan) + Chloramphenicol (Cm)

Lopatkin, A.J., et al. Nature Microbiology. (2016)

Conjugation dynamics are governed by two processes

1. Conjugation efficiency

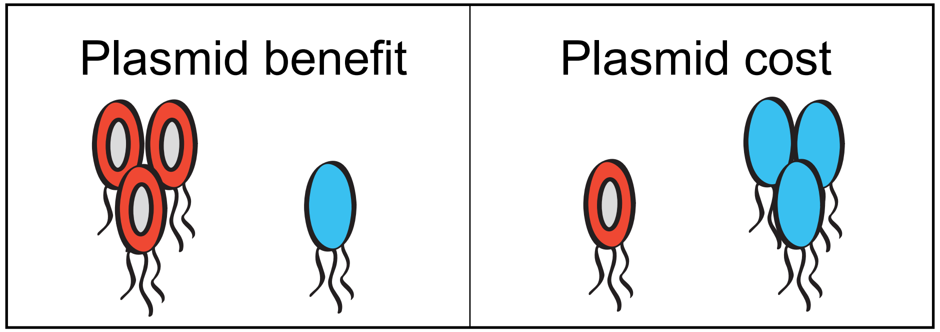
Kinetic rate of plasmid transfer from donor to recipient



η_C

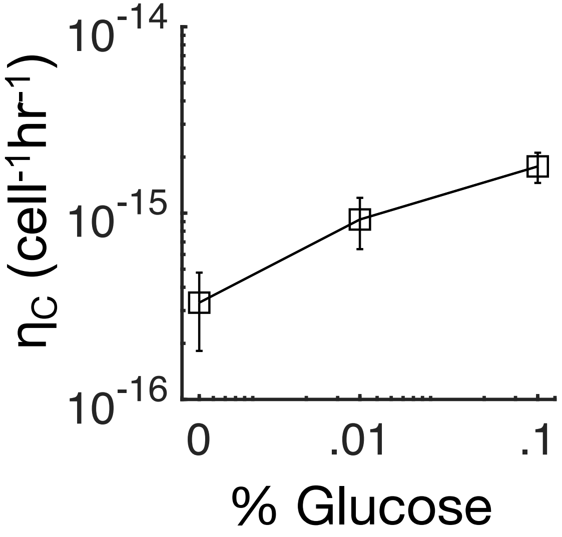
2. Growth dynamics

Relative growth rates of plasmid-free cells compared to plasmid-carrying cells

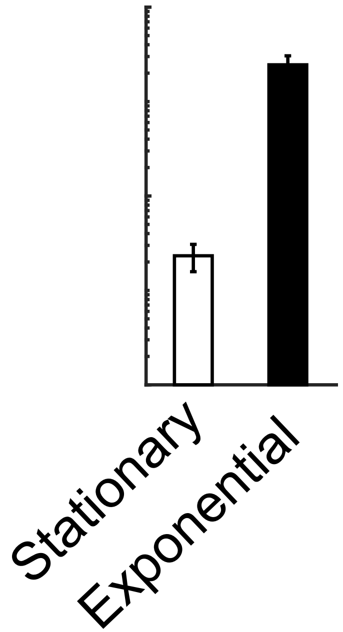


Metabolism impacts on conjugation efficiency

Nutrient composition



Physiological state

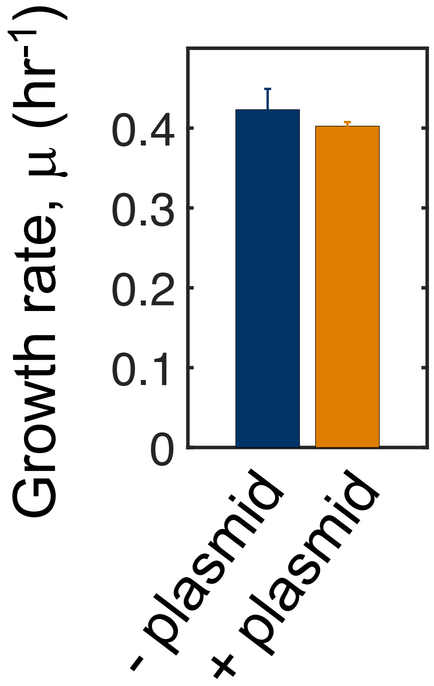


Genetic background

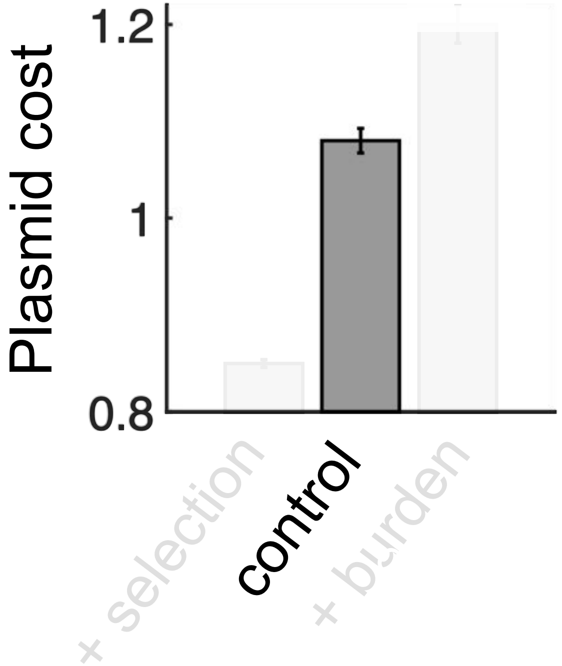
Lopatkin, A.J., et al. Nature Microbiology. (2016)

Metabolism impacts on growth dynamics

Intrinsic plasmid burden



Environment-mediated burden

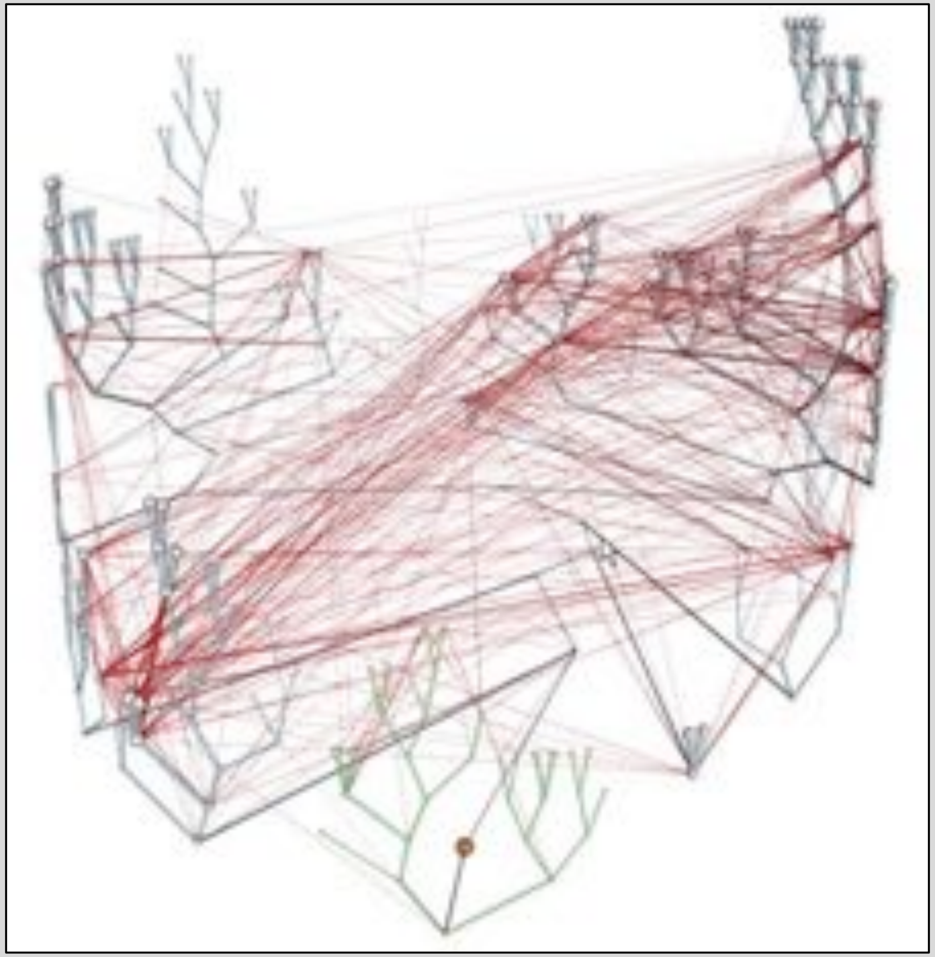


Cost = $\mu/\mu_{plasmid}$

Genetic background

Lopatkin, A.J., et al. Nature Microbiology. (2016)

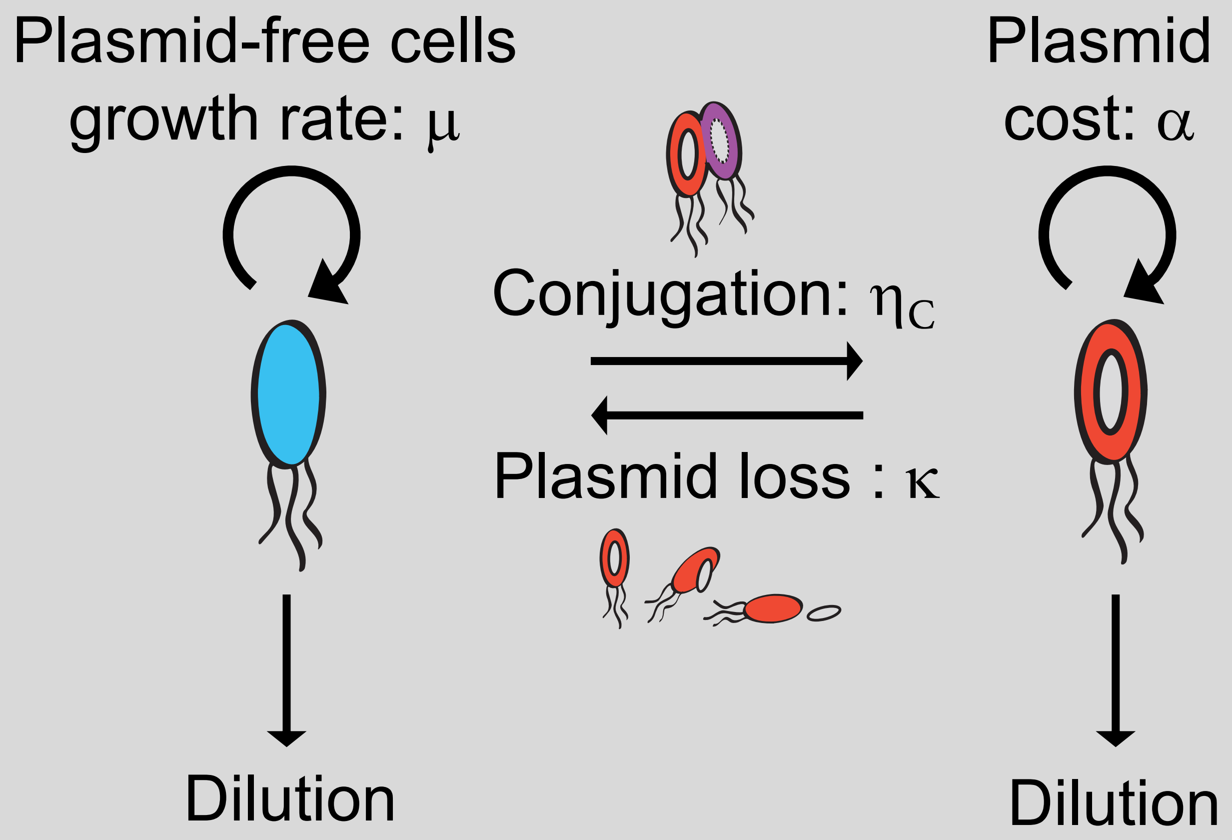
Microbial communities are "hot spots" for HGT!



Kunin, V., et al. European Bioinformatics Institute. (2005)

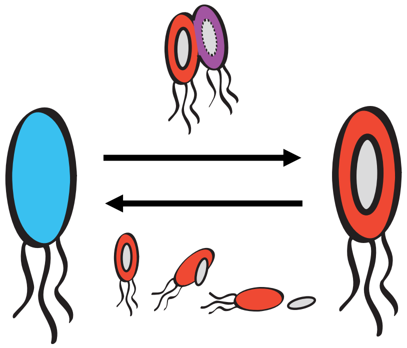
How do these parameters contribute to resistance dissemination?

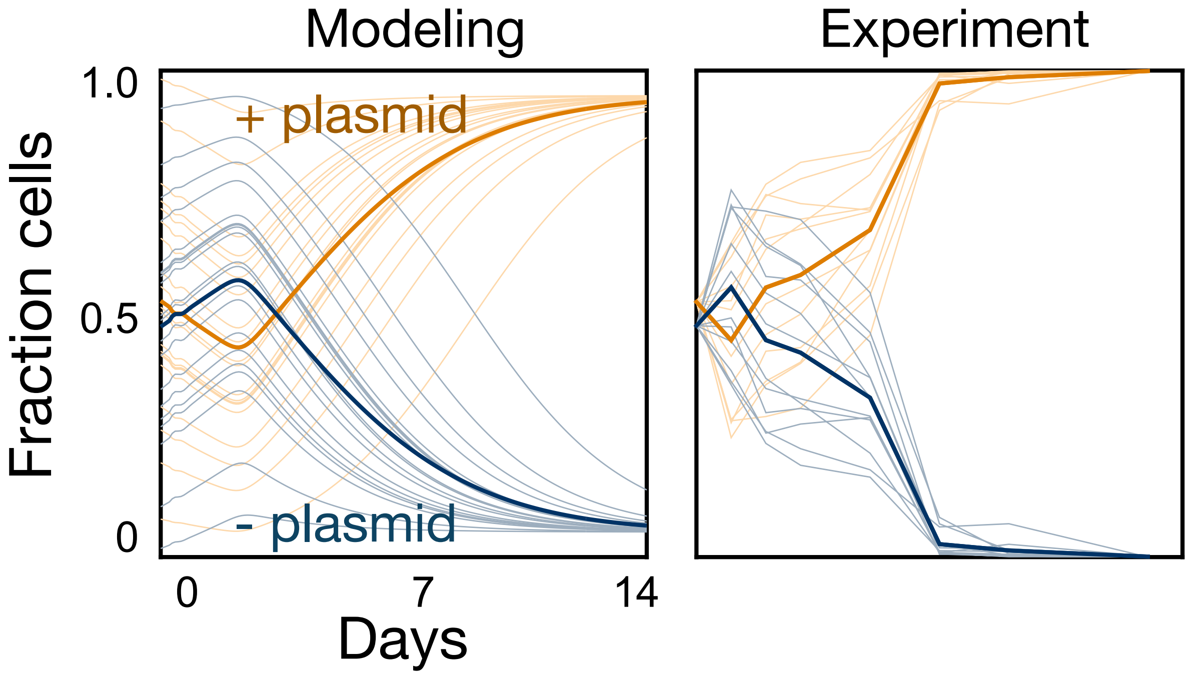
Mathematical model for conjugation dynamics



How do these parameters contribute to resistance dissemination?

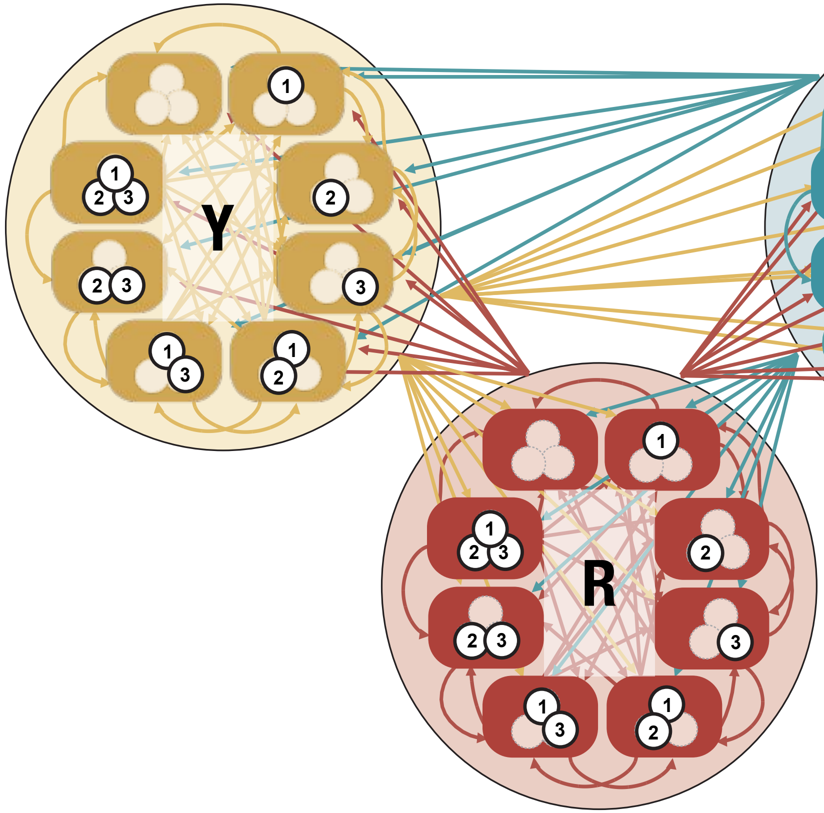
Plasmid persistence in simple bacterial communities

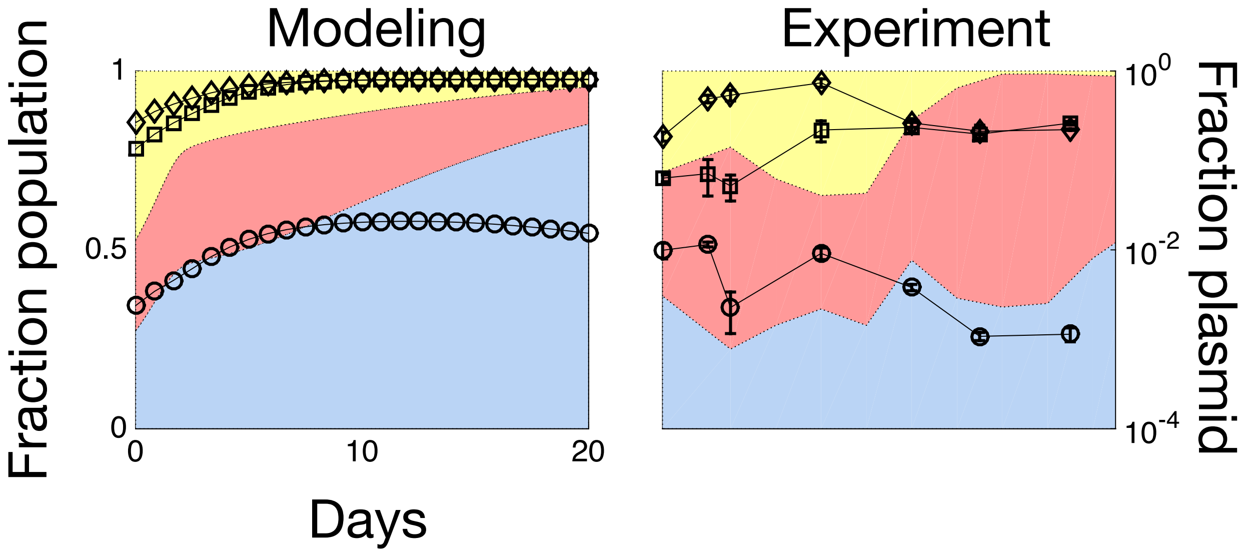




Lopatkin, A.J., et al. Nature Communications. (2017)

Plasmid persistence in complex bacterial communities





Lopatkin, A.J., et al. Nature Communications. (2017)

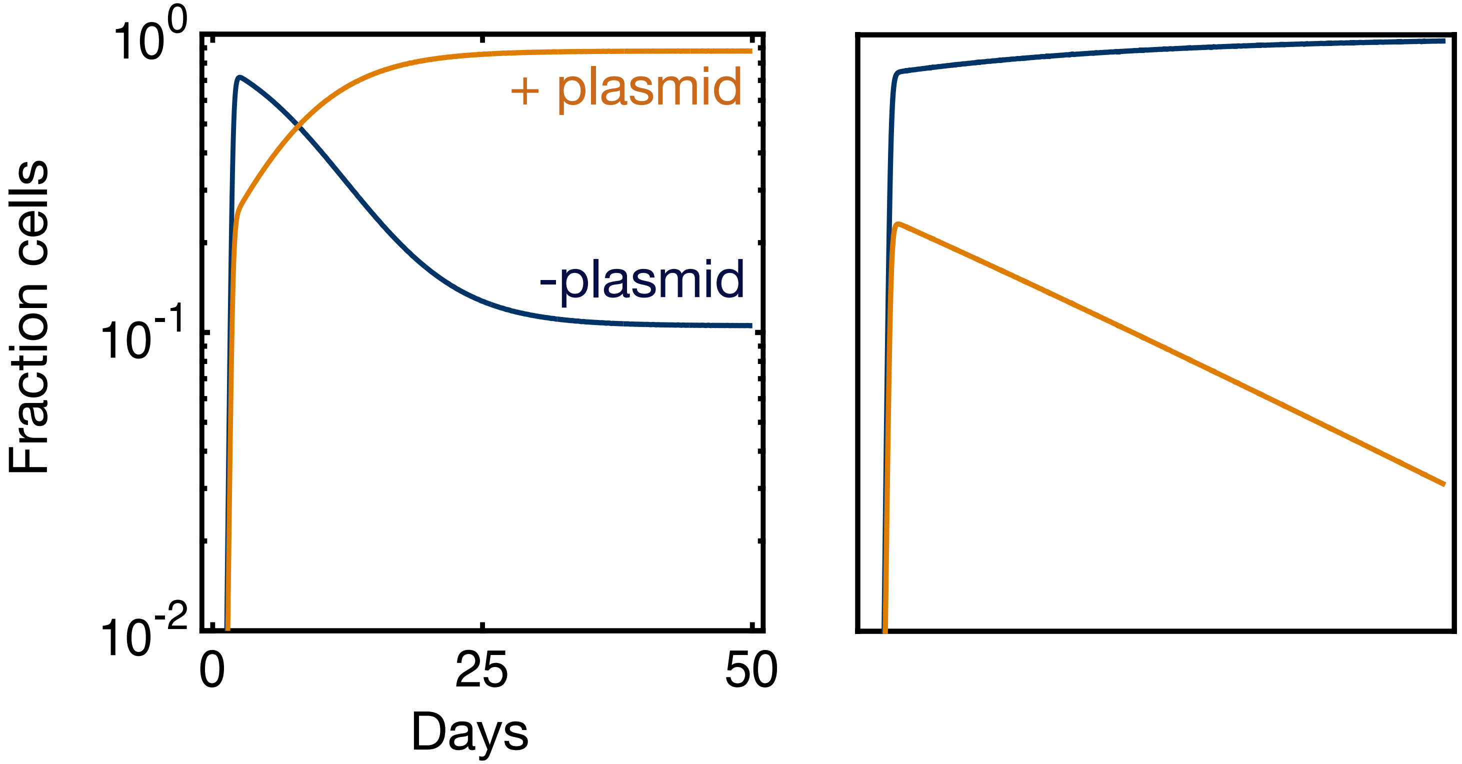
Analytical solution of plasmid persistence: sufficiently high η_C

Plasmid stability criterion

$$\eta_{Crit} = f(\text{plasmid cost, plasmid loss rate})$$

Plasmid persistence ($\eta_C > \eta_{Crit}$)

Plasmid elimination ($\eta_C < \eta_{Crit}$)

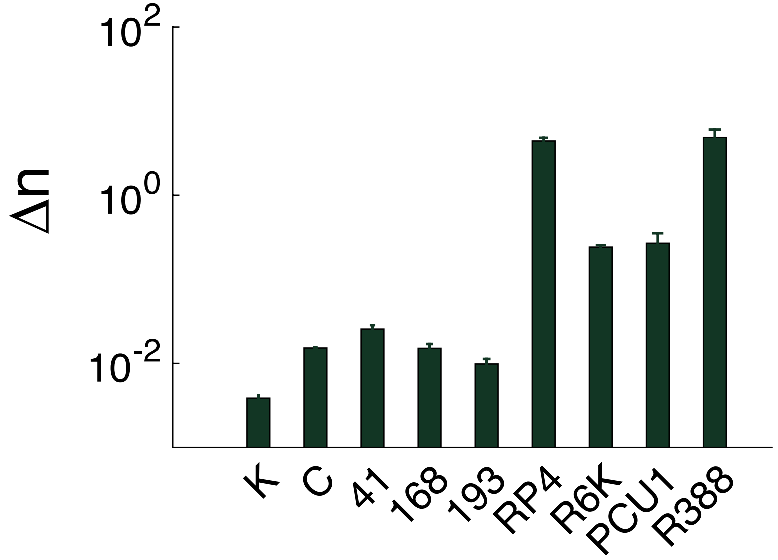


Lopatkin, A.J., et al. Nature Communications. (2017)

Model predicts persistence of native plasmids

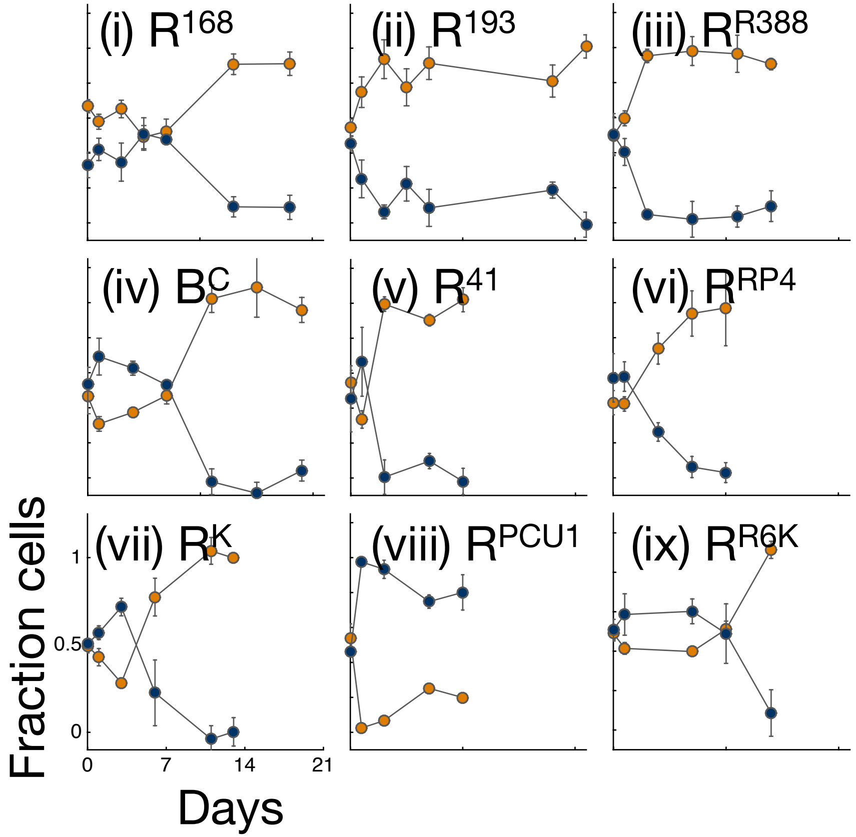
Quantitative prediction

$\Delta n > 0$ → **Persistence**
$\Delta n < 0$ → **Reversal**



Validation

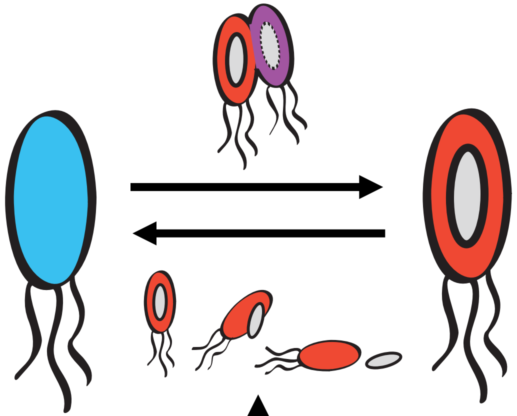
+ plasmid
- plasmid



Lopatkin, A.J., et al. Nature Communications. (2017)

Model insights: reversing resistance

Inhibiting conjugation (Lin)

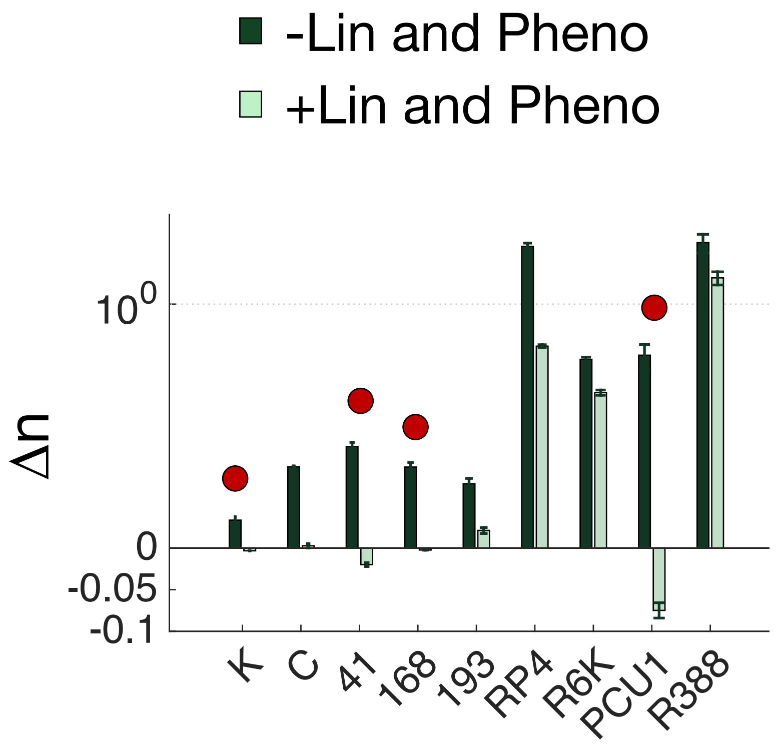


Leveraging metabolic burden (Pheno)

$$\eta_C \gtrless \eta_{Crit}$$

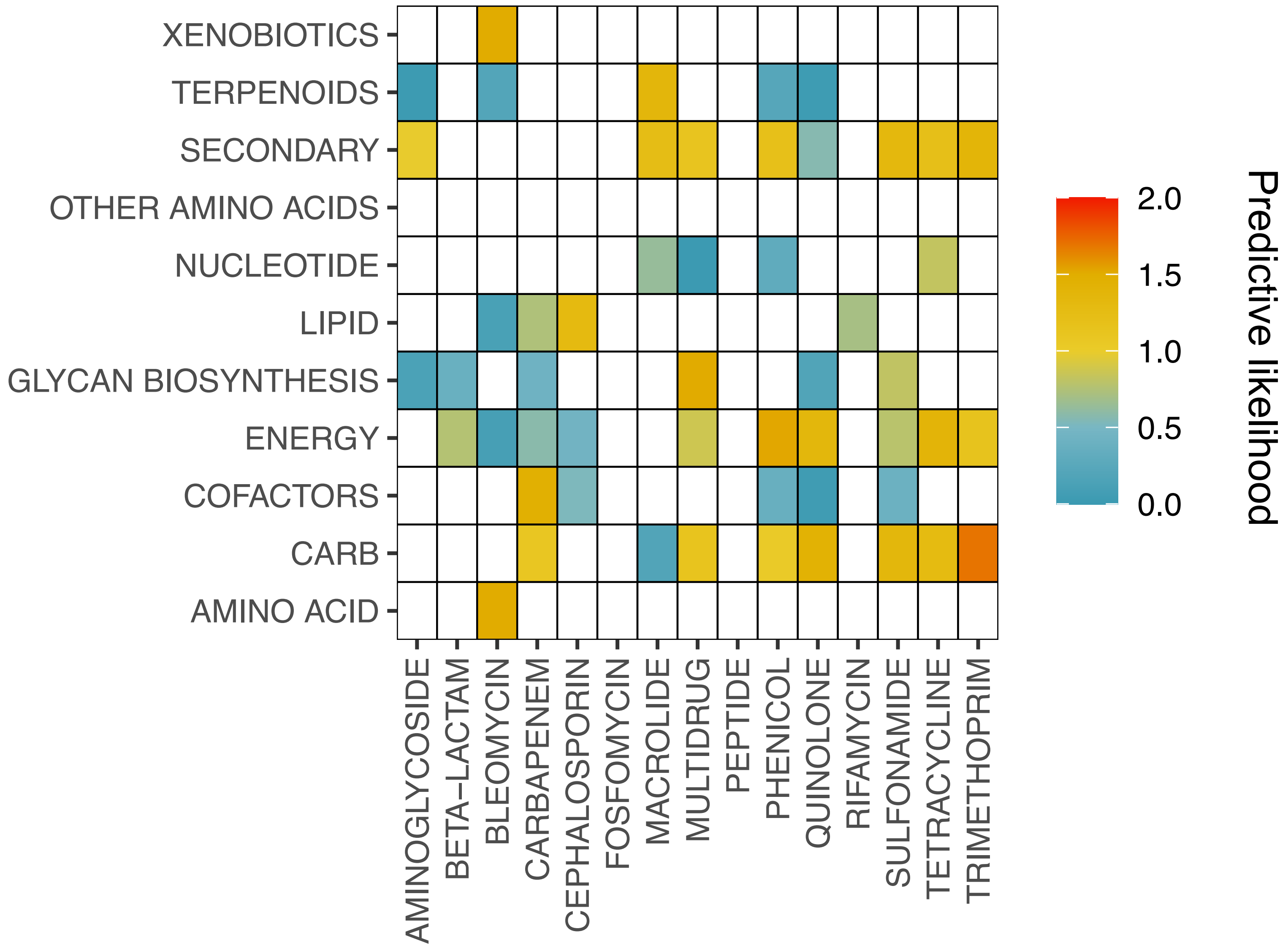
…and it works!

Quantitative prediction



Lopatkin, A.J., et al. Nature Communications. (2017)

Building ML model of metabolic genes on conjugative plasmids

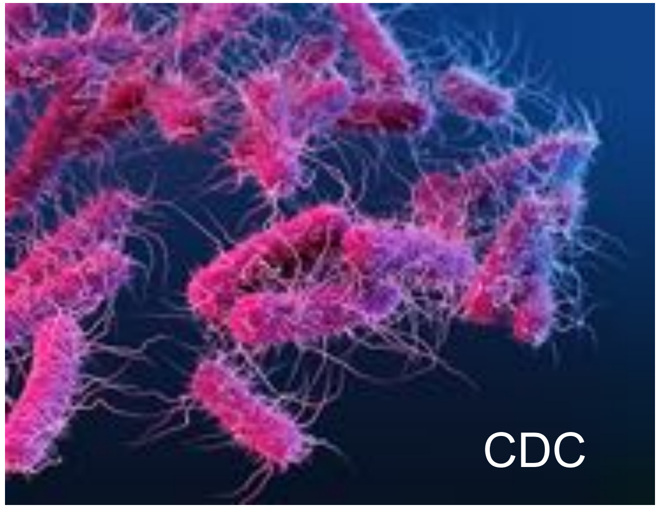


Integrating HGT model with QMRA for environmental applications

Case study: ESBL E. coli recreational water exposure to treated wastewater effluent

Team:

- Ashley Heida, ASU
- Julia Gambino, Seaford High School, NY
- Kaylee Sanderson, Seaford High School, NY
- Mary E. Schoen, Soller Environmental
- Michael A. Jahne, USEPA
- Jay Garland, USEPA
- Lucia Ramirez, ASU
- Allison J. Lopatkin, Barnard College/ Columbia U.
- Kerry Hamilton, ASU

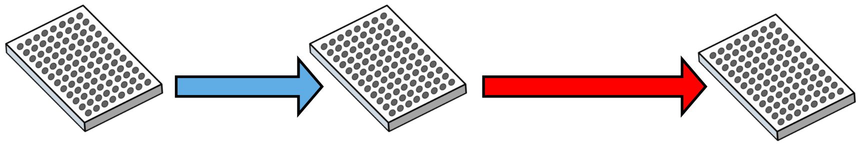


Metabolism and antibiotic resistance

1. Model → experiment

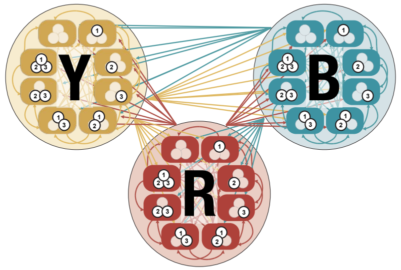
- Predictive models inform experimental design to identify metabolic mutants
- Metabolic mutations highly prevalent in pathogens
- Mutations independently confer resistance

Metabolic-dependent selection

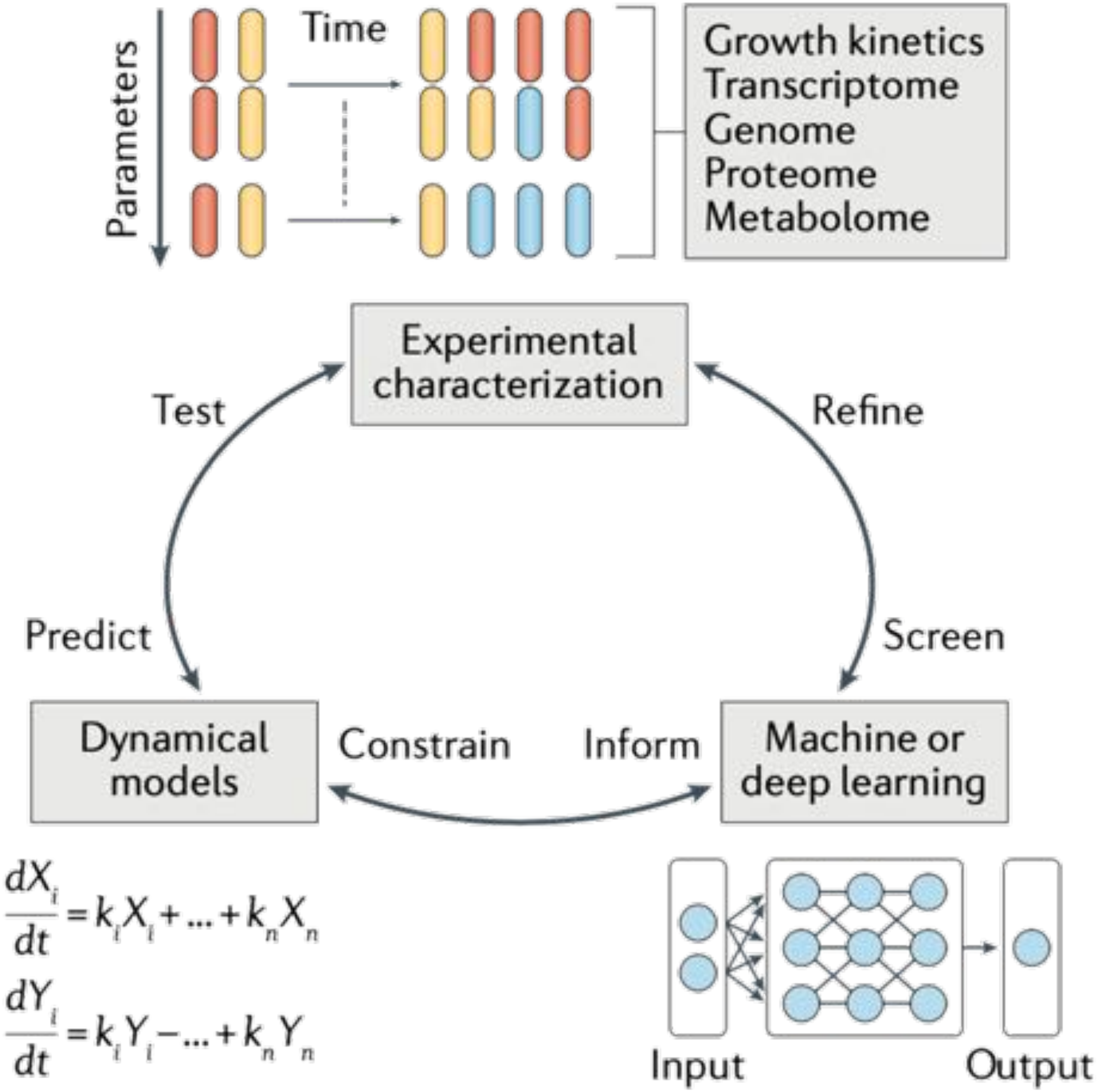


2. Experiment → model

- Conjugation efficiency and growth dynamics depend on bacterial metabolism
- Modeling accurately predicts plasmid persistence
- Interfering in conjugation dynamics can predictably modulate plasmid outcome



Paradigm of predictive microbiology



Lopatkin AJ & Collins J, Nature Reviews Microbiology (2020)

Thank you!

Check out the Lopatkin lab at:
www.lopatkinlab.com

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Jenifer Moralez
Alana Palomino
Hannah Prensky
Karolina Szenkiel

Collaborators:

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Kerry Hamilton (ASU)
Anne-Catrin Uhlemann (CUMC)
Amy Pruden (Virginia tech)
Rob Smith (NSU)

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Jon Stokes, PhD
Melissa Takahashi, PhD
Sarah Bening
Ashlee Earl, PhD
Abigail Mason, PhD

HGT work:

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Rob Smith, PhD
Jaydeep Srimani, PhD
Shuqiang Huang, PhD
Hannah Meredith,PhD
Tatyana Sysoeva, PhD