



*Cape Cod Morning*, Edward Hopper, 1950

## Finding hopes in a hopeless time

How Predictive Modeling and Data Analytics shifts our perspectives about antimicrobial discovery

J. David Zhang, Ph.D.

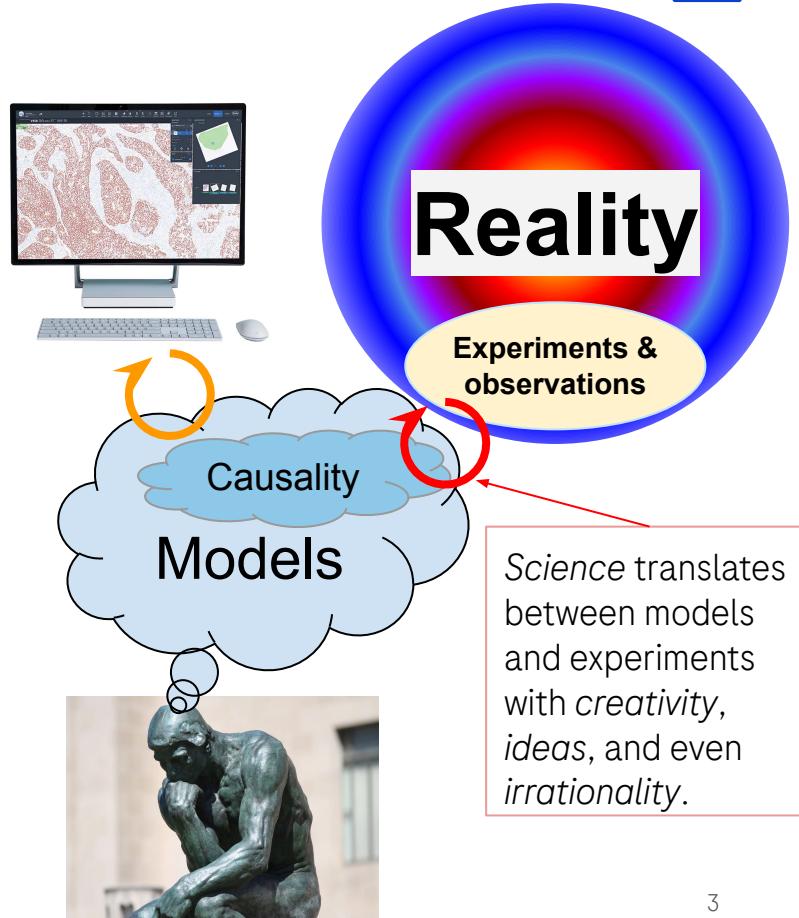
Pharmaceutical Sciences, Pharma Research and Early Development  
Roche Innovation Center Basel  
F. Hoffmann-La Roche Ltd

# Outline

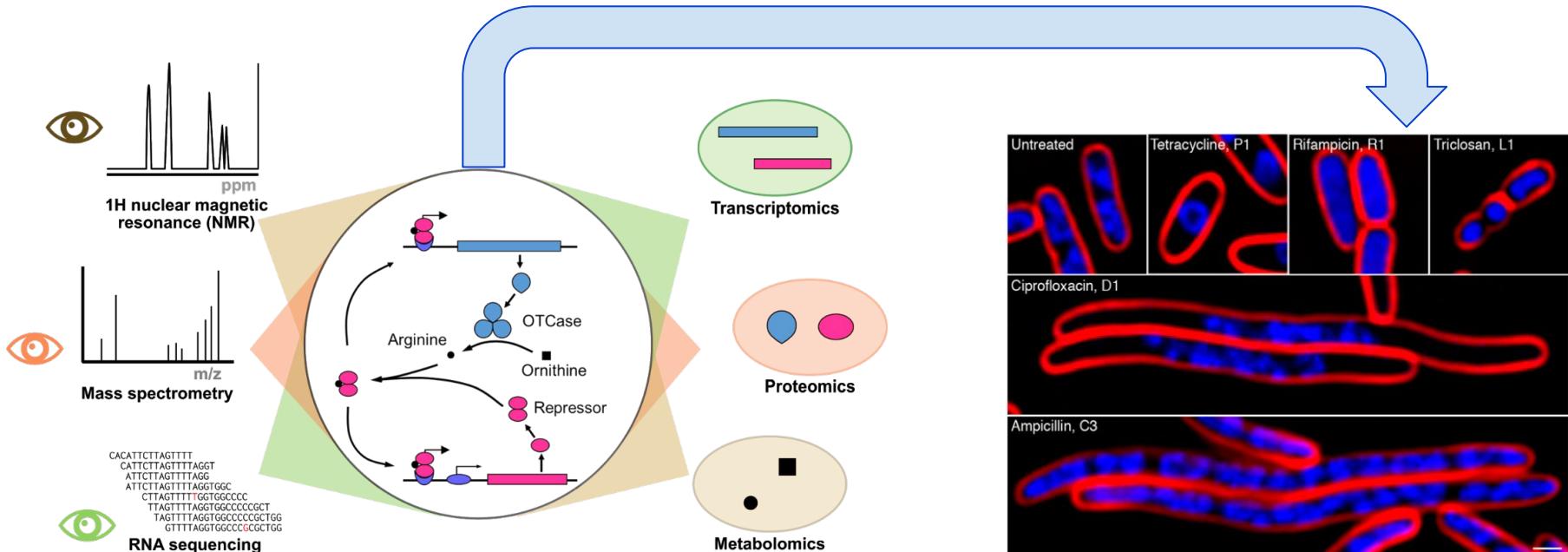
- Predictive Modeling and Data Analytics integrates knowledge, experiments, and machine intelligence to establish causality.
- Three case studies of PMDA in antimicrobial drug discovery:
  1. Imaging and machine learning for MoA;
  2. Molecular phenotyping for safety assessment;
  3. Single-cell sequencing for cellular heterogeneity;
- Challenges and prospects

# *Five hallmarks of predictive modeling in drug discovery*

1. *Human intelligence*
2. *Machine intelligence*
3. *Experiments & observations*
4. *Models, including causality*
5. *Iterations*

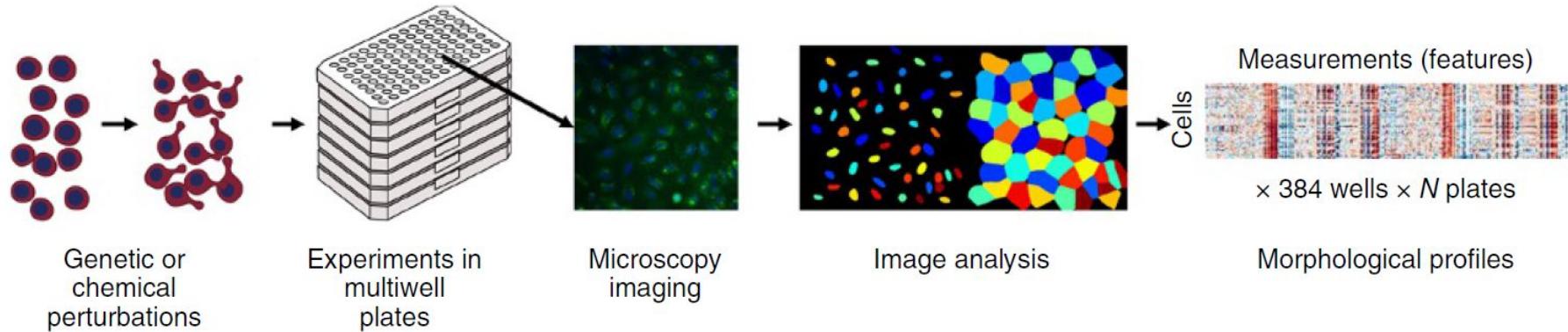
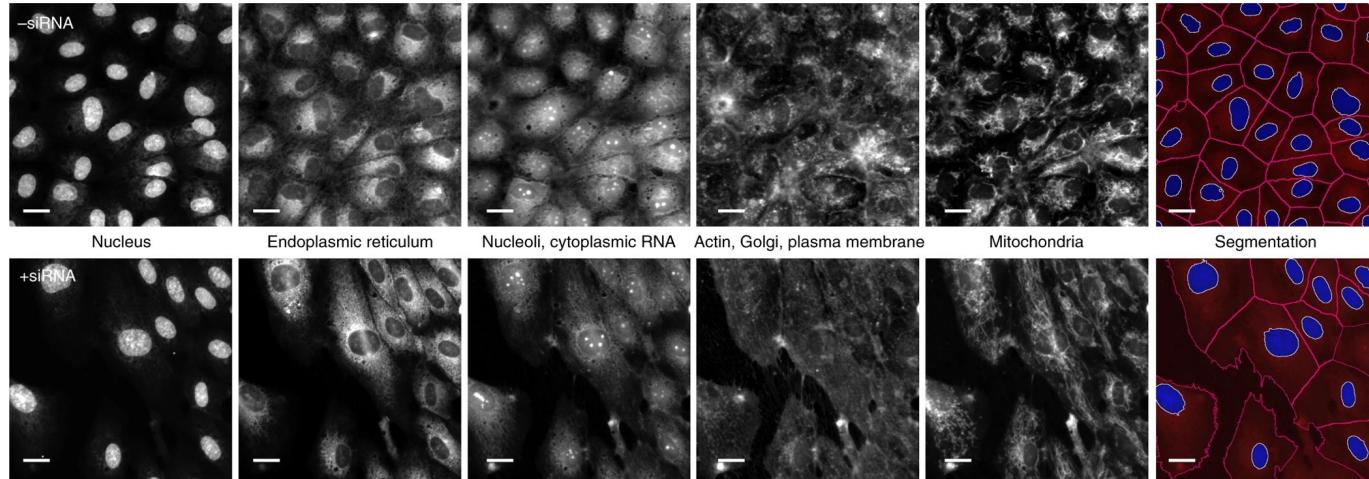


# Omics and morphology offer rich biological information



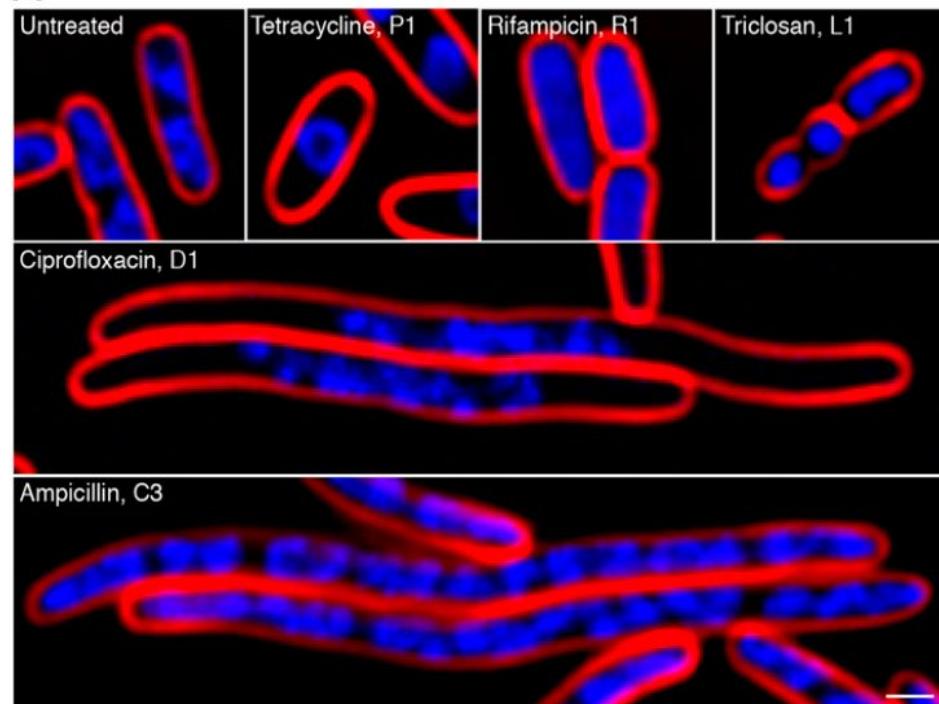
Cell identity and state represented as high-dimensional data

# Imaging-based screening and machine learning empower phenotypic drug discovery

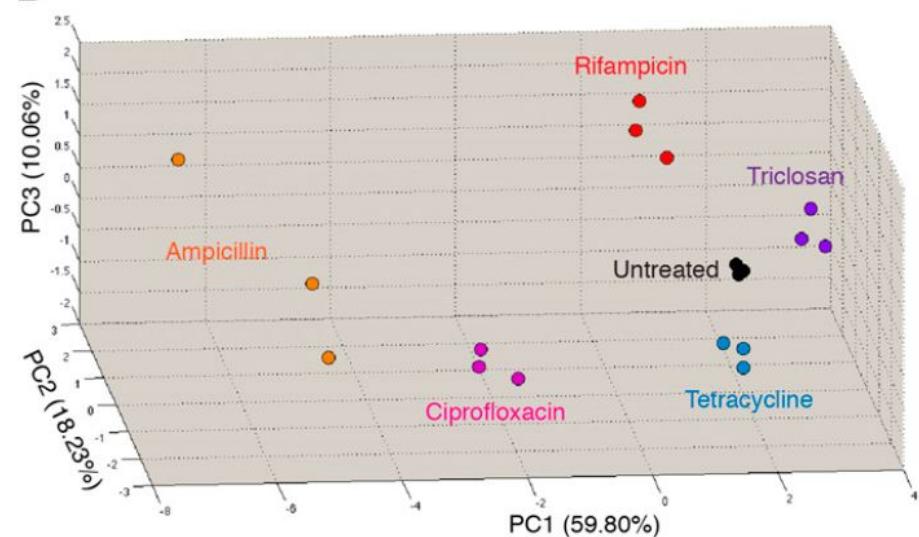


# Bacterial cytological profiling identifies cellular pathways targeted by antibacterial molecules

A



B



P: Protein translation inhibitors

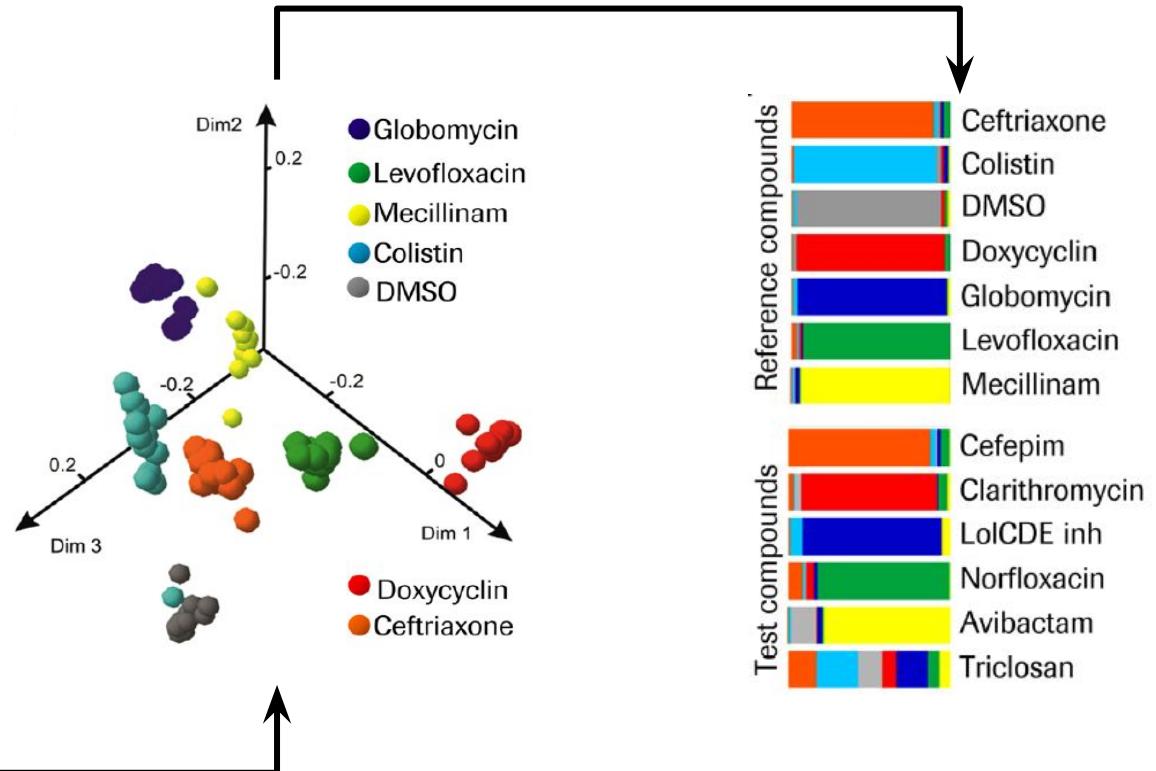
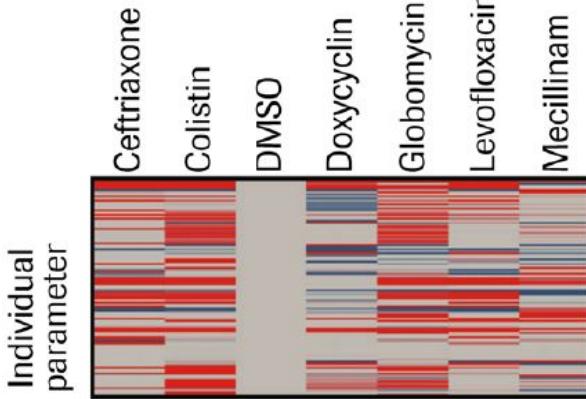
R: RNA transcription inhibitors

D: DNA replication inhibitors

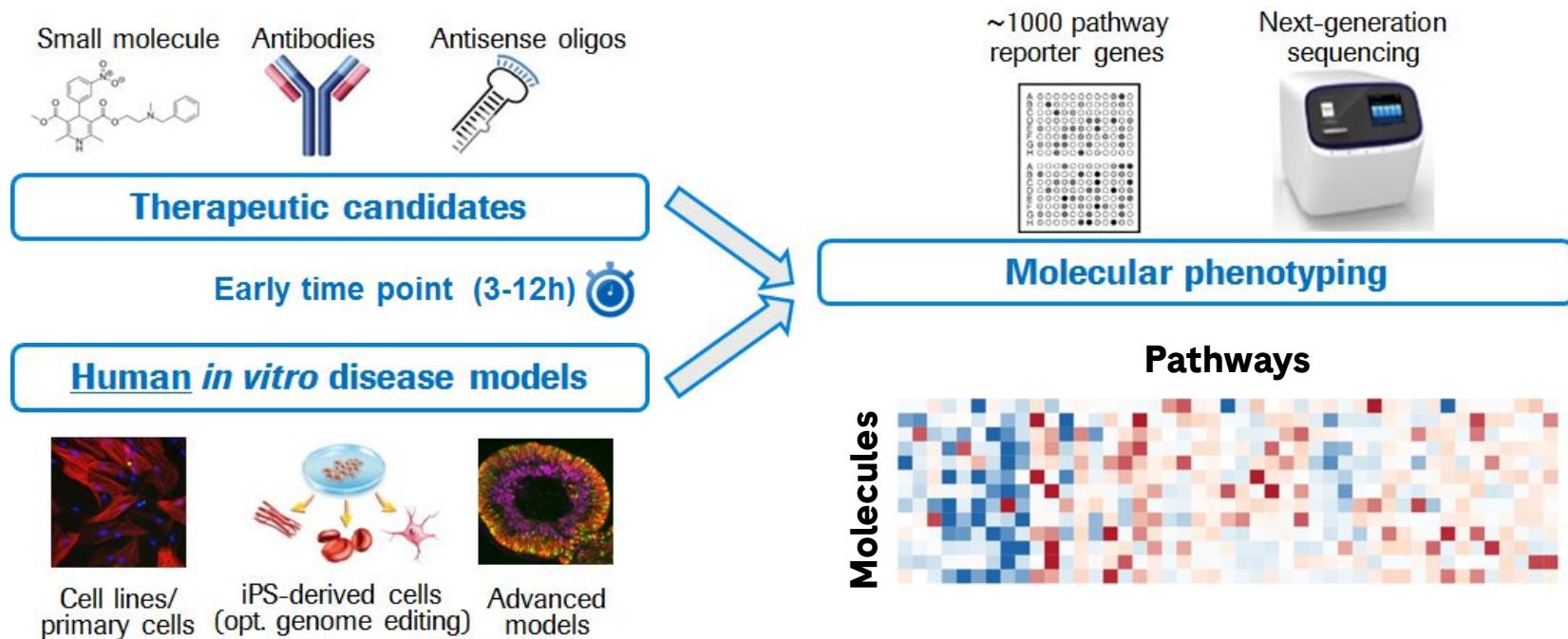
L: Lipid biosynthesis inhibitors

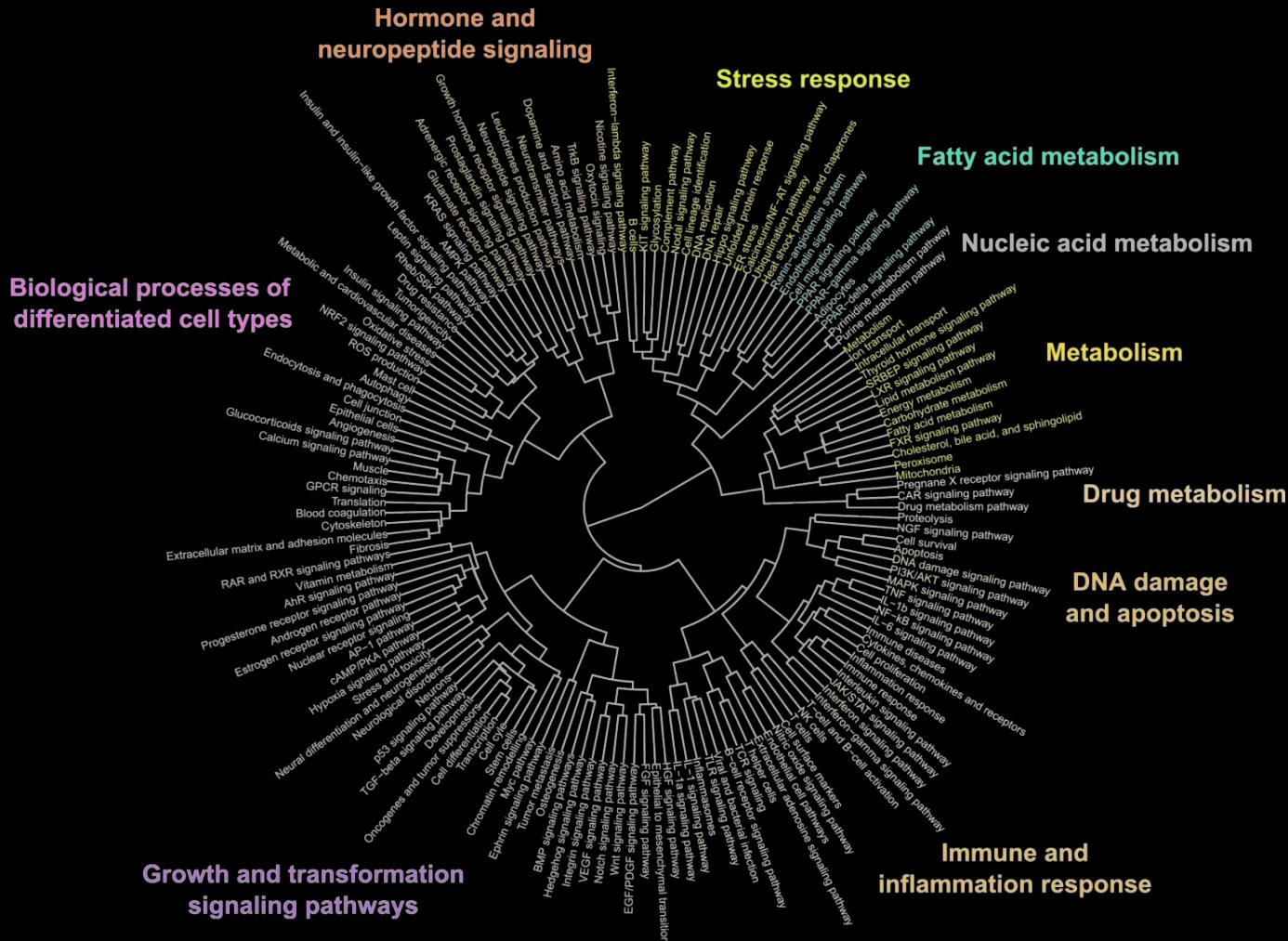
C: Cell-wall synthesis inhibitors (peptidoglycan)

# Morphology classifies compounds by MoA



# Molecular phenotyping reveals modulation of human pathway activities by compounds



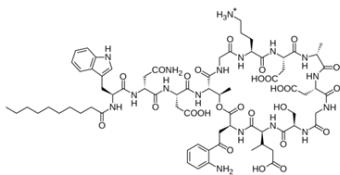


# Three antibiotics profiled in three cell systems with molecular phenotyping for safety assessment

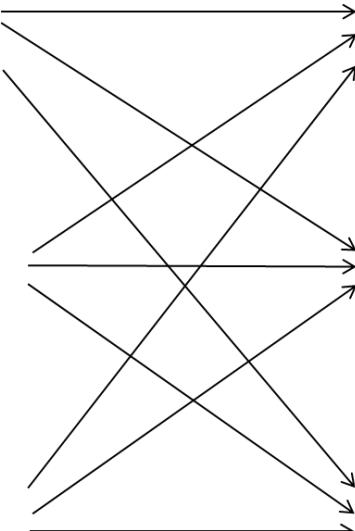
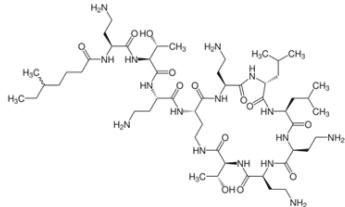
**R0xyz**



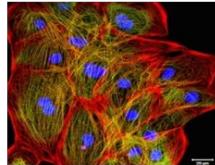
**Daptomycin**



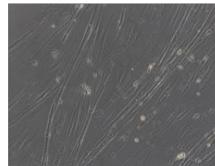
**Colistin**



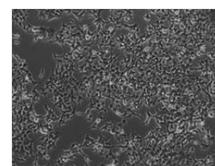
iPS-derived human cardiomyocytes



Primary human skeletal muscle cells



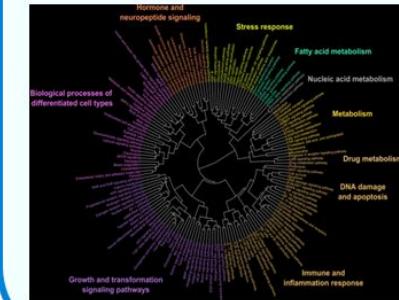
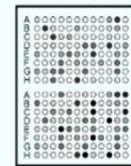
Human embryonic kidney cells



**6h treatment at  
sub-cytotoxic concentration**

**Molecular  
phenotyping**

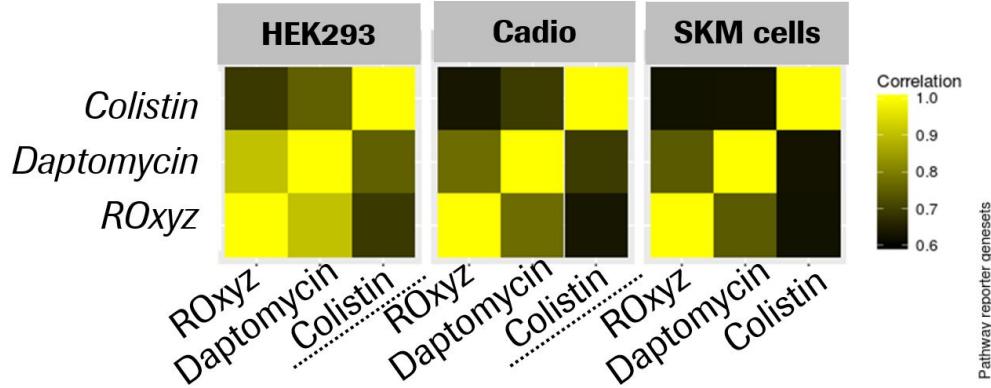
Pathway Reporter Genes



# Roche compound is more similar with daptomycin than colistin, irrespective of cell type

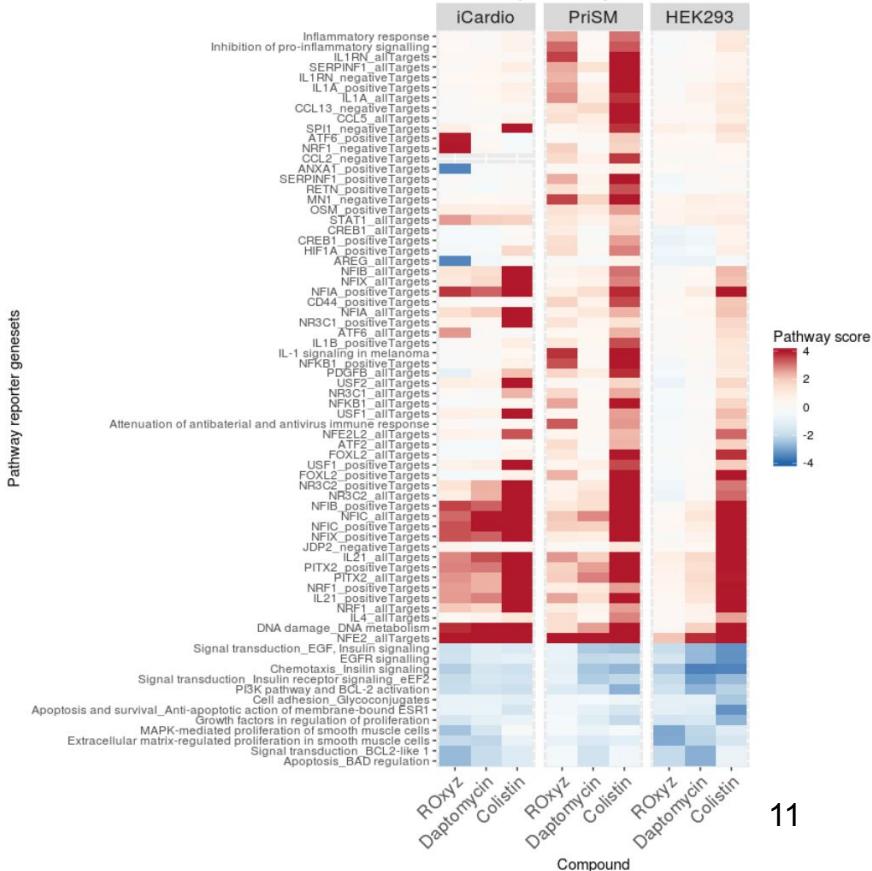


## Similarity of differential expression profiles

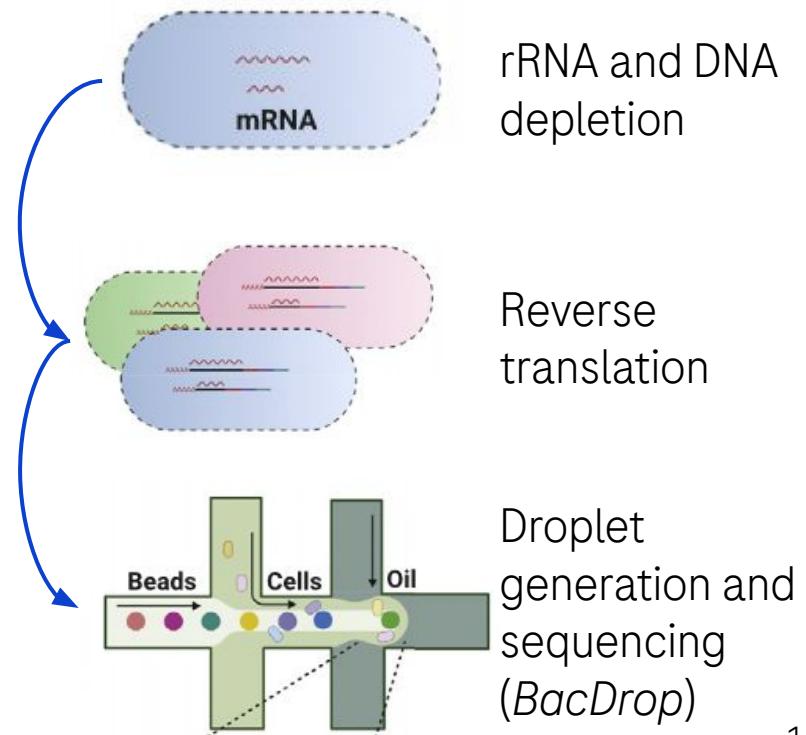
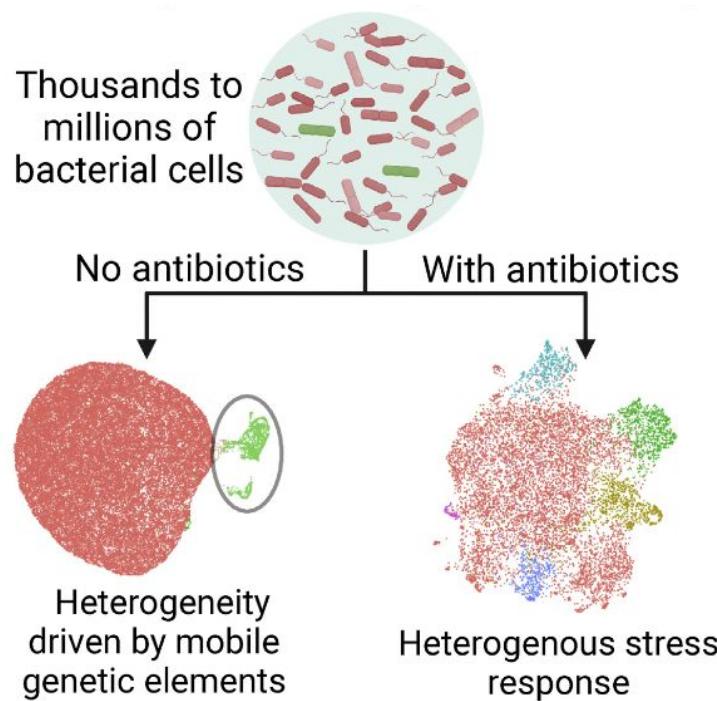


Roche compound shows molecular phenotypes more similar with daptomycin than with colistin, consistent with *in vivo* findings.

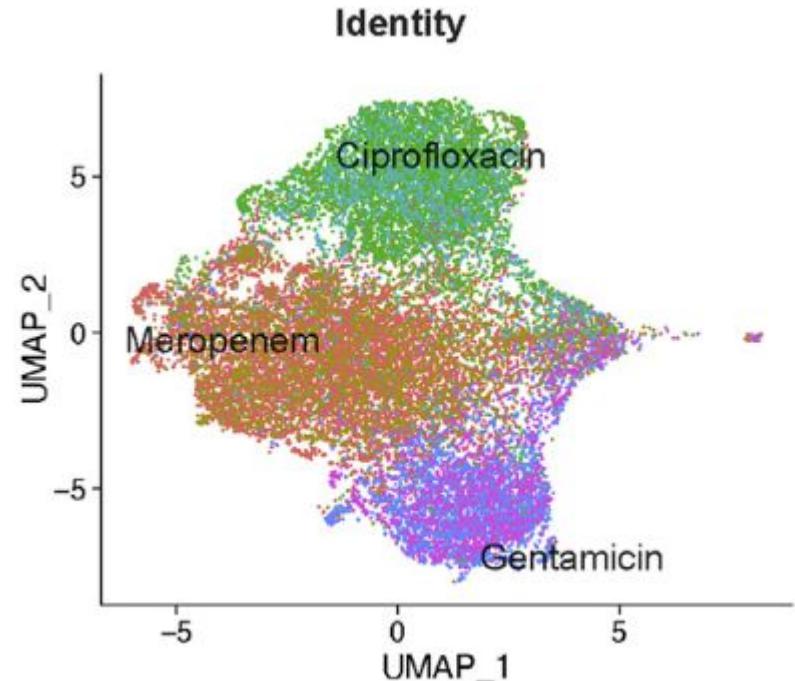
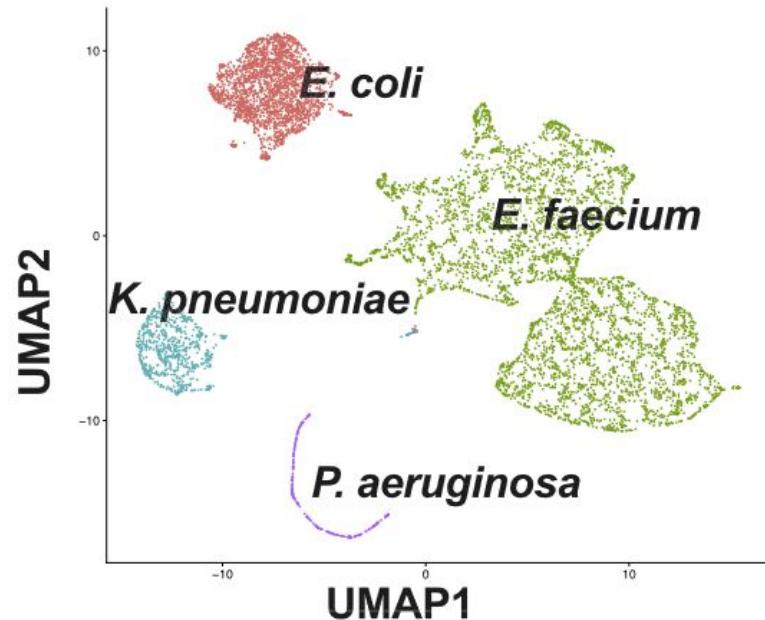
## Pathway regulation



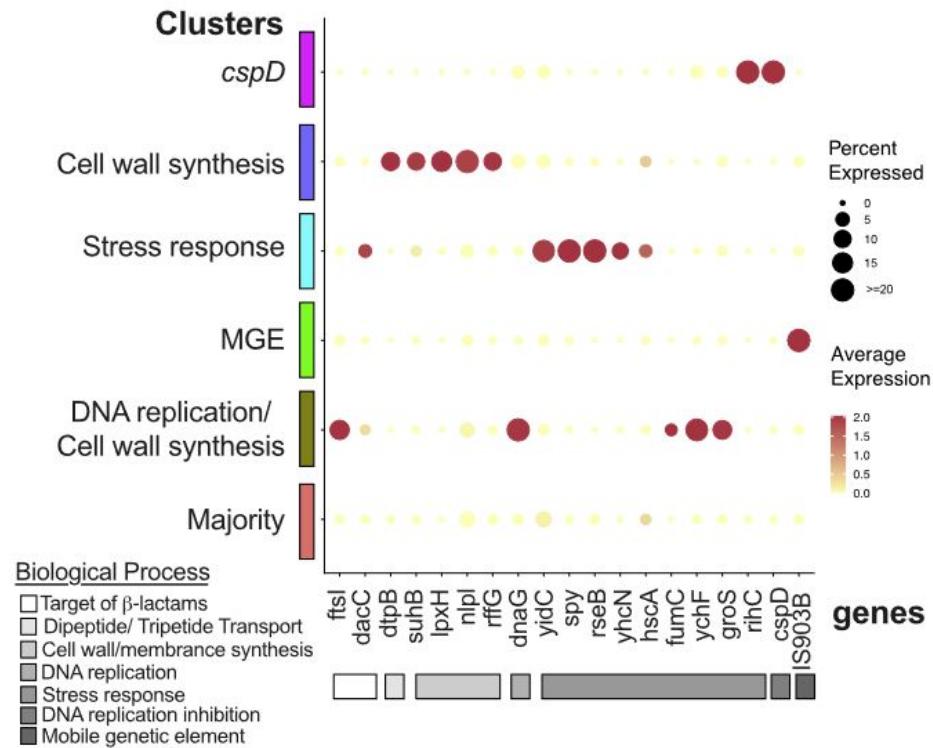
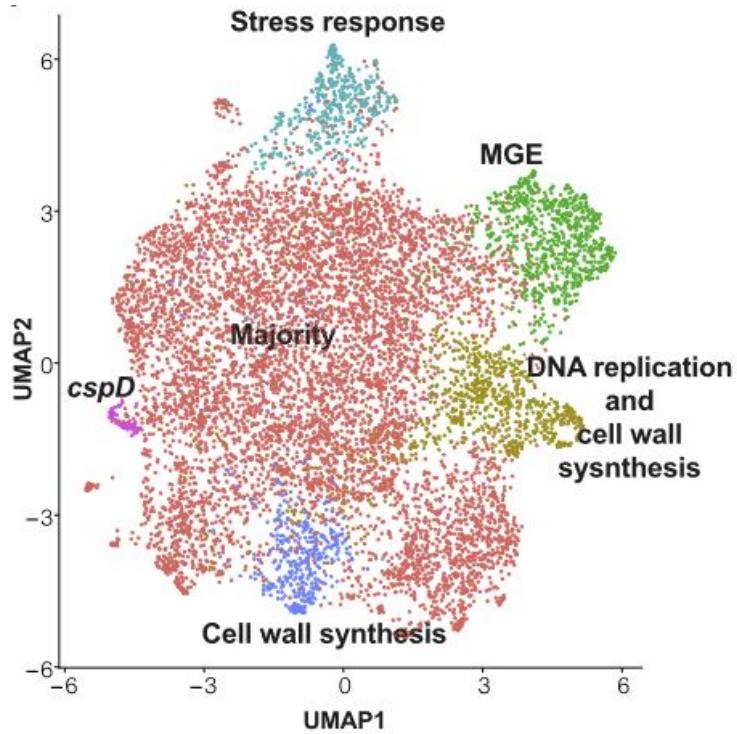
# Single-cell RNA-seq reveals cellular heterogeneity of response to antimicrobial drug treatment



# BacDrop recovers bacterial species and reveals effects of antibiotics treatment



# **Meropenem treatment induced heterogeneous responses**



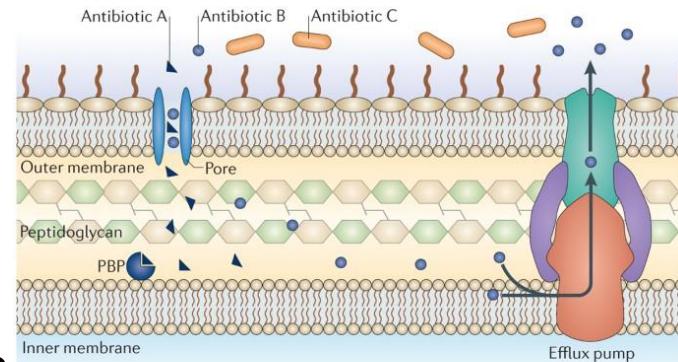
# Challenges and prospects

- **Fighting Gram-negative bugs**
  - New chemistry to penetrate Gram-negative cell walls;
  - Learning from failures is as important as from successes.
- **(Molecular) phenotypic drug discovery**
  - Dissecting pharmacology from toxicology;
  - Preclinical models with high predictivity for clinical outcome;
- **Acting against resistance from day 0**
  - Omics-enhanced resistance detection and understanding;
  - Precompetitive knowledge sharing is essential.

**Doing now what patients need next**

# What's X?

- X is protected by magical walls.
- Few secret passages connect inside of X with outside.
- X constantly changes its internals.
- Relatively little is known how to destroy X.
- In fact, X has never been completely extinct.

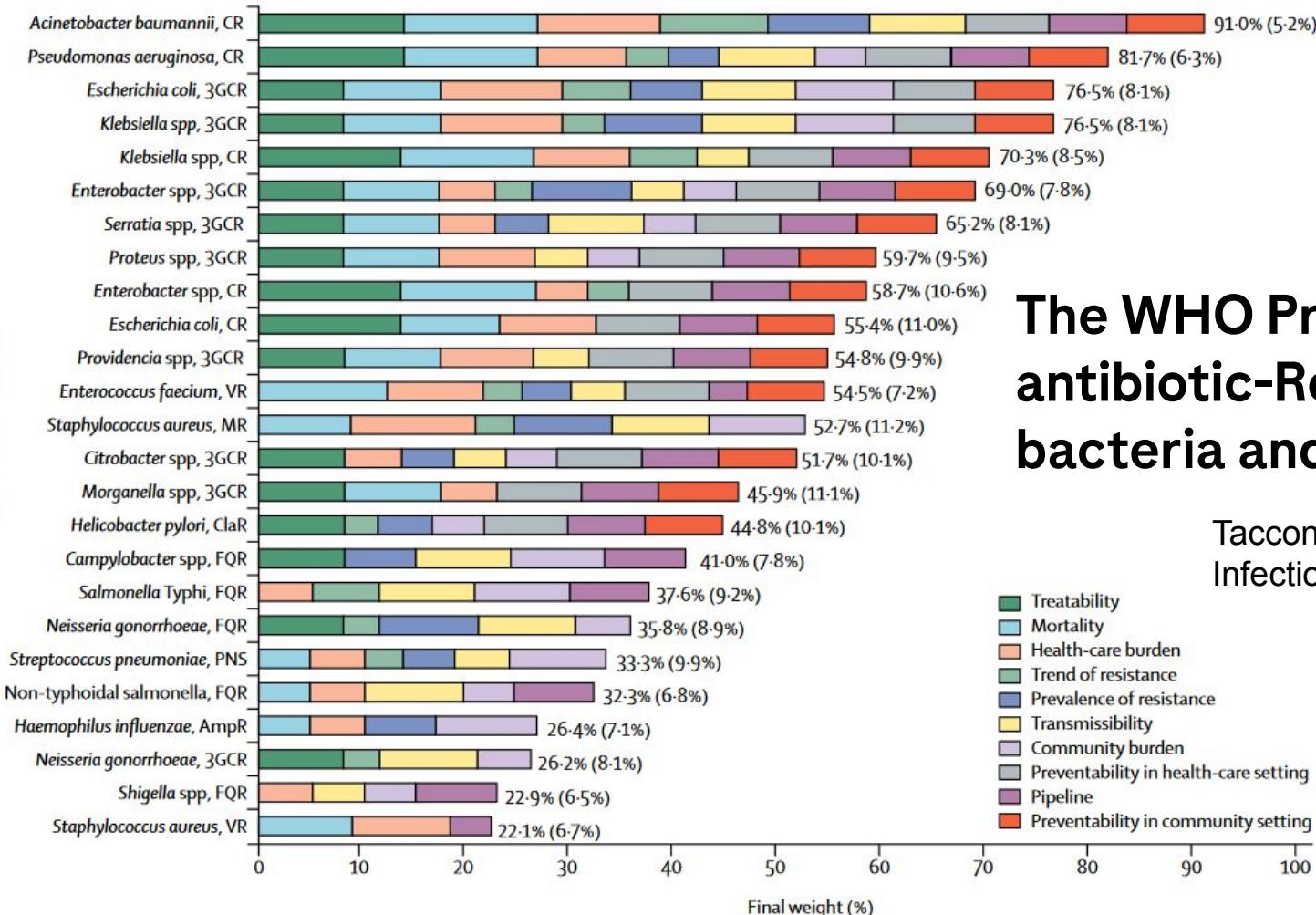


**Hogwarts? Multiresistant bacteria?**

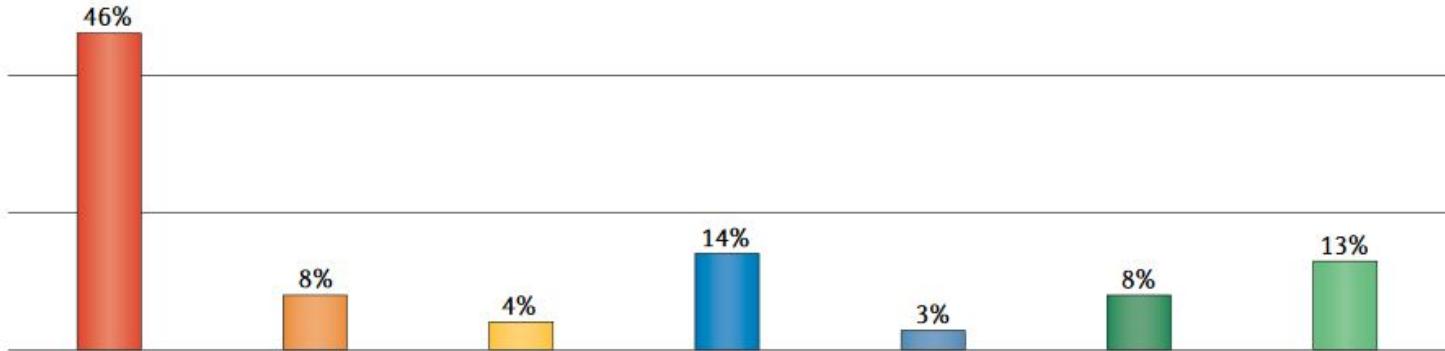
# The WHO Priority list of antibiotic-Resistant bacteria and tuberculosis

Tacconelli *et al.*, The Lancet Infectious Diseases, 2018

Antibiotic-resistant bacteria



**407 preclinical antibiotic projects from  
314 institutions (81% small and medium-sized enterprises)**



#### Direct-acting small molecules

- ~70% new and ~20% old targets
- ~50% targeting Gram-negative bacteria

#### Potentiators

- $\beta$ -Lactamase or efflux pump inhibitors
- Expanding spectrum
- Enhancing or restoring activity
- Protectors

#### Repurposed drugs

- FDA-approved drugs

#### Antibodies and vaccines

- Against select pathogens

#### Immuno-modulators

- Support pathogen elimination

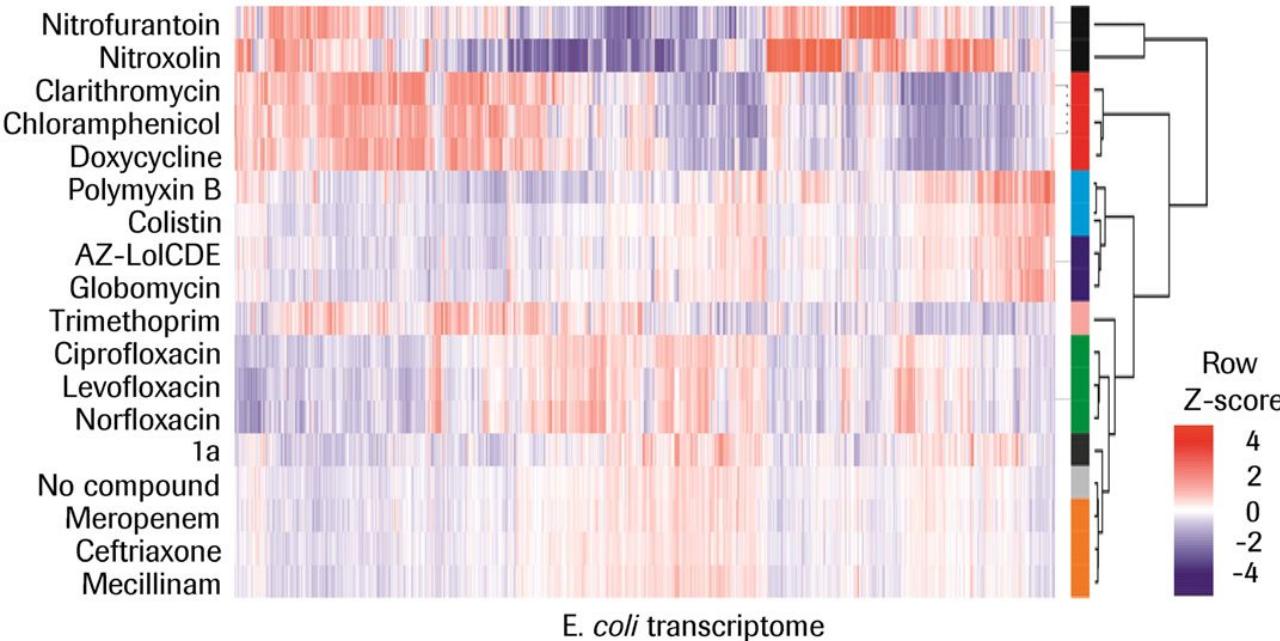
#### Antivirulence approaches

- Adjunctive
- Targeting different virulence factors and strategies
- Against select pathogens

#### Phages and microbiota

- Phages against select pathogens
- Endolysins
- Modulators of microbiota (mostly gut)

# Morphology and gene expression offer complementary information

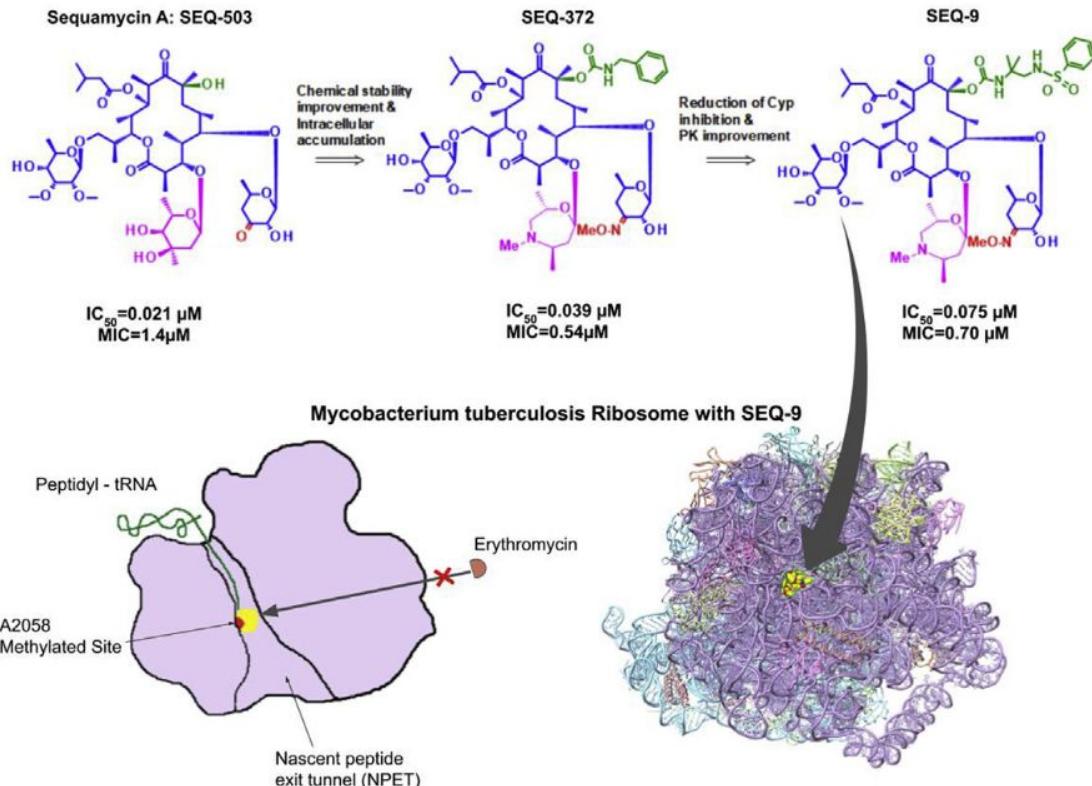


Pathway and network analysis

Alternative assays

Pathway-  
Phenotype associations

# CryoEM reveals molecular interactions of antimicrobials



- Structure-based optimization of SEQ-9, a sequanamycin derivative.
- Sequanamycins overcome Mtb macrolide resistance.
- SEQ-9 adjusts its binding mode to the resistant Mtb ribosome.
- SEQ-9 kills Mtb in vitro and is efficacious in mouse models of TB.

# Prospects



- New experimental approaches, empowered by computational methods, shift our perspective of antimicrobial drug discovery.
- Make the new wet-lab and *in silico* approaches available to researchers is indispensable to overcome the challenges of AMR.

# Copyright, sources, and references

1. *Cape Cod Morning*, Edward Hopper, 1950: SAAM, <https://americanart.si.edu/artwork/cape-cod-morning-10760>
2. Theuretzbacher, Ursula, Kevin Outterson, Aleks Engel, and Anders Karlén. "The Global Preclinical Antibacterial Pipeline." *Nature Reviews Microbiology* 18, no. 5 (May 2020): 275-85. <https://doi.org/10.1038/s41579-019-0288-0>.
3. Tacconelli, Evelina, Elena Carrara, Alessia Savoldi, Stephan Harbarth, Marc Mendelson, Dominique L Monnet, Céline Pulcini, et al. "Discovery, Research, and Development of New Antibiotics: The WHO Priority List of Antibiotic-Resistant Bacteria and Tuberculosis." *The Lancet Infectious Diseases* 18, no. 3 (March 1, 2018): 318-27. [https://doi.org/10.1016/S1473-3099\(17\)30753-3](https://doi.org/10.1016/S1473-3099(17)30753-3).
4. Michoel, Tom, and Jitao David Zhang. "Causal Inference in Drug Discovery and Development." arXiv, September 29, 2022. <https://doi.org/10.48550/arXiv.2209.14664>.
5. Zhang, Jitao David, Lisa Sach-Peltason, Christian Kramer, Ken Wang, and Martin Ebeling. "Multiscale Modelling of Drug Mechanism and Safety." *Drug Discovery Today* 25, no. 3 (March 1, 2020): 519-34. <https://doi.org/10.1016/j.drudis.2019.12.009>.
6. Nonejuie, Poochit, Michael Burkart, Kit Pogliano, and Joe Pogliano. "Bacterial Cytological Profiling Rapidly Identifies the Cellular Pathways Targeted by Antibacterial Molecules." *Proceedings of the National Academy of Sciences* 110, no. 40 (October 1, 2013): 16169-74. <https://doi.org/10.1073/pnas.1311066110>.
7. Drawnel, Faye Marie, Jitao David Zhang, Erich Kung, Natsuyo Aoyama, Fethallah Benmansour, Andrea Araujo Del Rosario, Sannah Jensen Zoffmann, et al. "Molecular Phenotyping Combines Molecular Information, Biological Relevance, and Patient Data to Improve Productivity of Early Drug Discovery." *Cell Chemical Biology* 18, no. 24(5) (April 20, 2017): 624-34. <https://doi.org/10.1016/j.chembiol.2017.03.016>.
8. Nonejuie, Poochit, Michael Burkart, Kit Pogliano, and Joe Pogliano. "Bacterial Cytological Profiling Rapidly Identifies the Cellular Pathways Targeted by Antibacterial Molecules." *Proceedings of the National Academy of Sciences* 110, no. 40 (October 1, 2013): 16169-74. <https://doi.org/10.1073/pnas.1311066110>.
9. Zoffmann, Sannah, Maarten Vercruyse, Fethallah Benmansour, Andreas Maunz, Luise Wolf, Rita Blum Marti, Tobias Heckel, et al. "Machine Learning-Powered Antibiotics Phenotypic Drug Discovery." *Scientific Reports* 9, no. 1 (March 21, 2019): 1-14. <https://doi.org/10.1038/s41598-019-39387-9>.
10. Ma, Peijun, Haley M. Amemiya, Lorrie L. He, Shivam J. Gandhi, Robert Nicol, Roby P. Bhattacharyya, Christopher S. Smillie, and Deborah T. Hung. "Bacterial Droplet-Based Single-Cell RNA-Seq Reveals Antibiotic-Associated Heterogeneous Cellular States." *Cell* 186, no. 4 (February 16, 2023): 877-891.e14. <https://doi.org/10.1016/j.cell.2023.01.002>.
11. Photo of Hogwarts: [https://commons.wikimedia.org/wiki/File:Hogwarts\\_at\\_Wizarding\\_World\\_of\\_Harry\\_Potter.jpg](https://commons.wikimedia.org/wiki/File:Hogwarts_at_Wizarding_World_of_Harry_Potter.jpg). Creative Commons.
12. Photo of Hogwarts Express: <https://www.rawpixel.com/image/6111756/photo-image-public-domain-free-harry-potter>. Public Domain.
13. Blair, Jessica M. A., Mark A. Webber, Alison J. Baylay, David O. Ogbolu, and Laura J. V. Piddock. "Molecular Mechanisms of Antibiotic Resistance." *Nature Reviews Microbiology* 13, no. 1 (January 2015): 42-51. <https://doi.org/10.1038/nrmicro3380>.
14. Zhang, Jidong, Christine Lair, Christine Roubert, Kwame Amaning, María Belén Barrio, Yannick Benedetti, Zhicheng Cui, et al. "Discovery of Natural-Product-Derived Sequanamycins as Potent Oral Anti-Tuberculosis Agents." *Cell* 186, no. 5 (March 2, 2023): 1013-1025.e24. <https://doi.org/10.1016/j.cell.2023.01.043>.