Cape Cod Morning, Edward Hopper, 1950

Finding hope in a hopeless time

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

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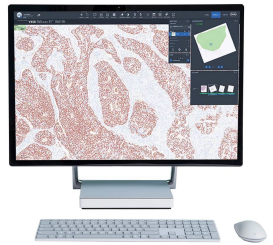
Outline

- 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

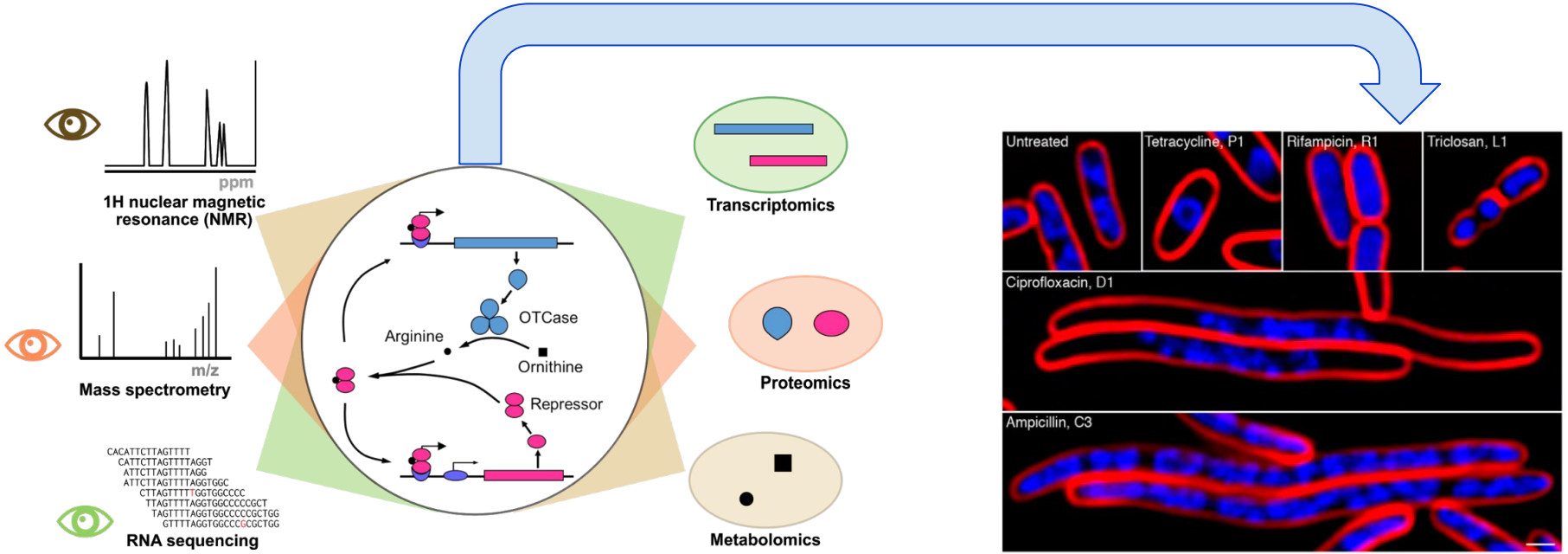
Five hallmarks of predictive modeling in drug discovery

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



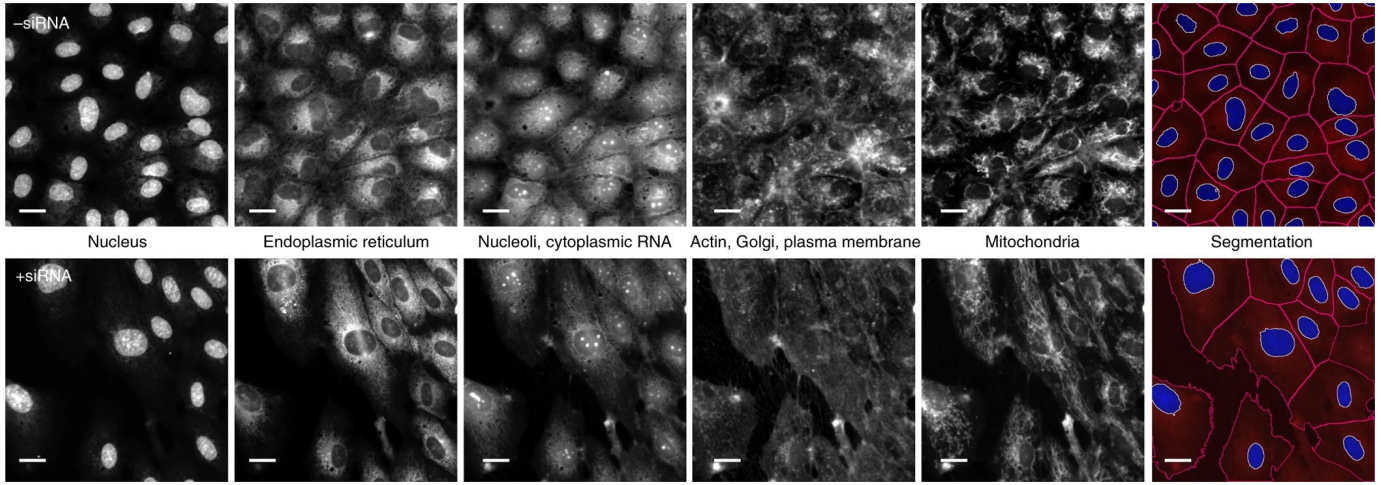
Science translates between models and experiments with *creativity*, *ideas*, and even *irrationality*.

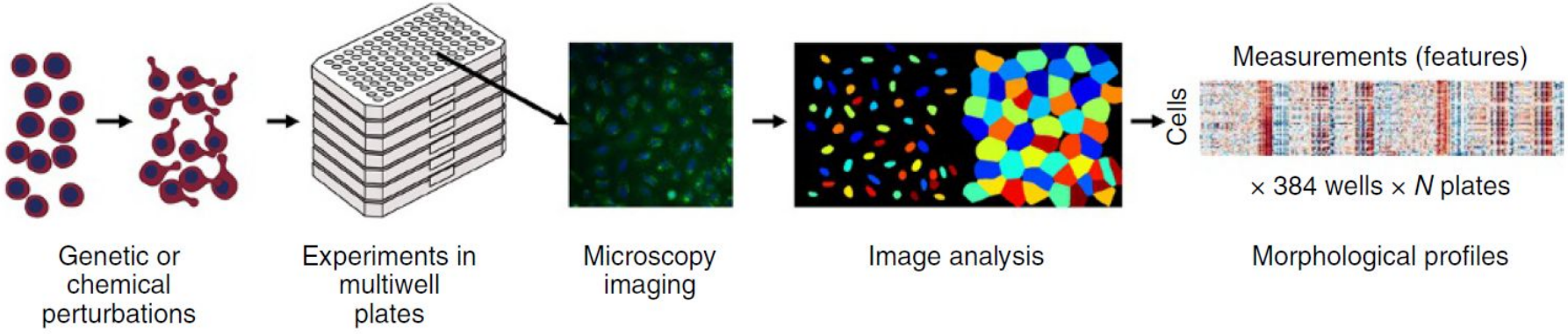
Omics and morphology offer rich biological information



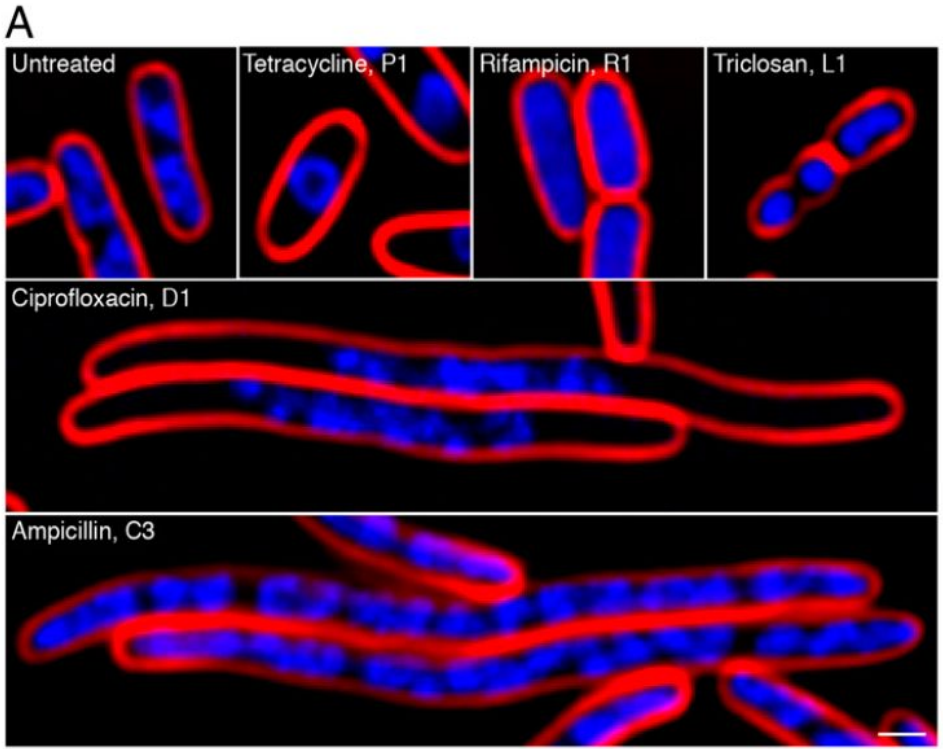
Cell identity and state represented as high-dimensional data

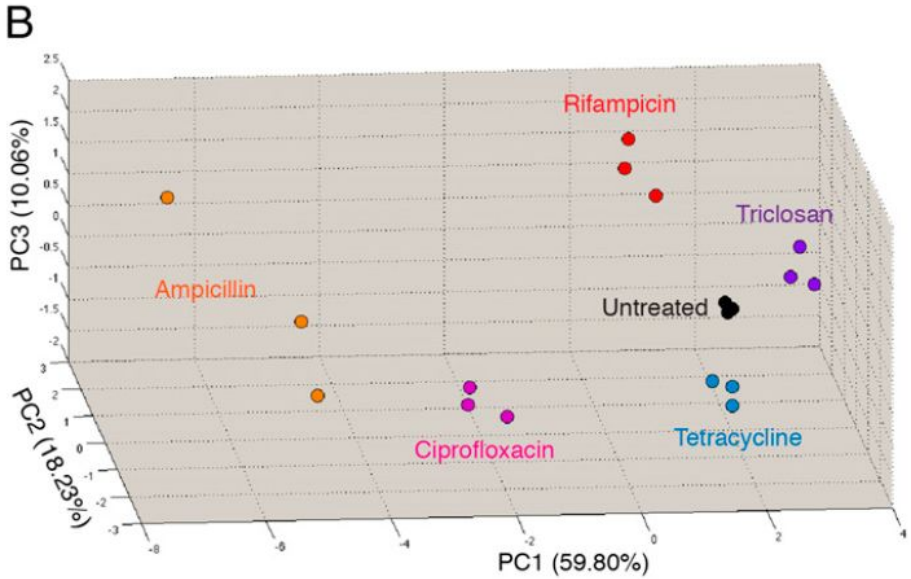
Imaging-based screening and machine learning empower phenotypic drug discovery





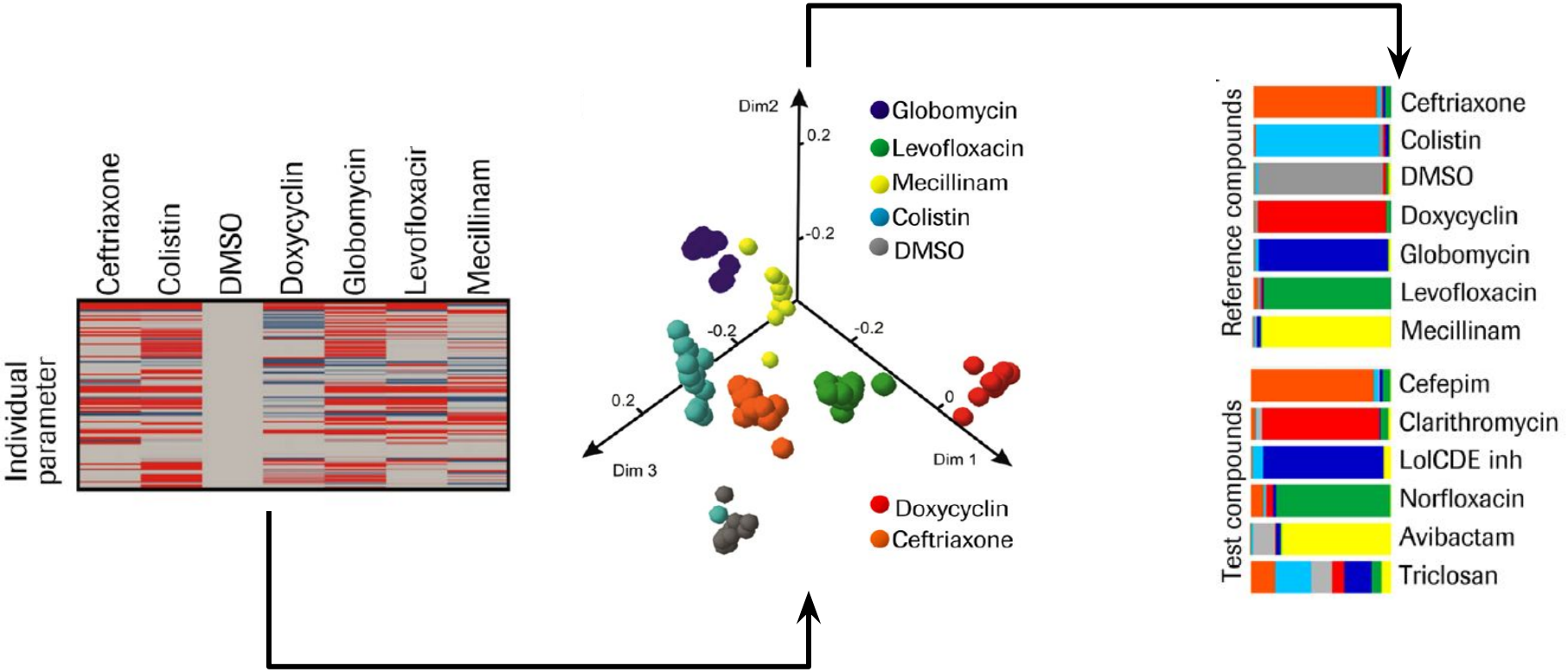
Bacterial cytological profiling identifies cellular pathways targeted by antibacterial molecules



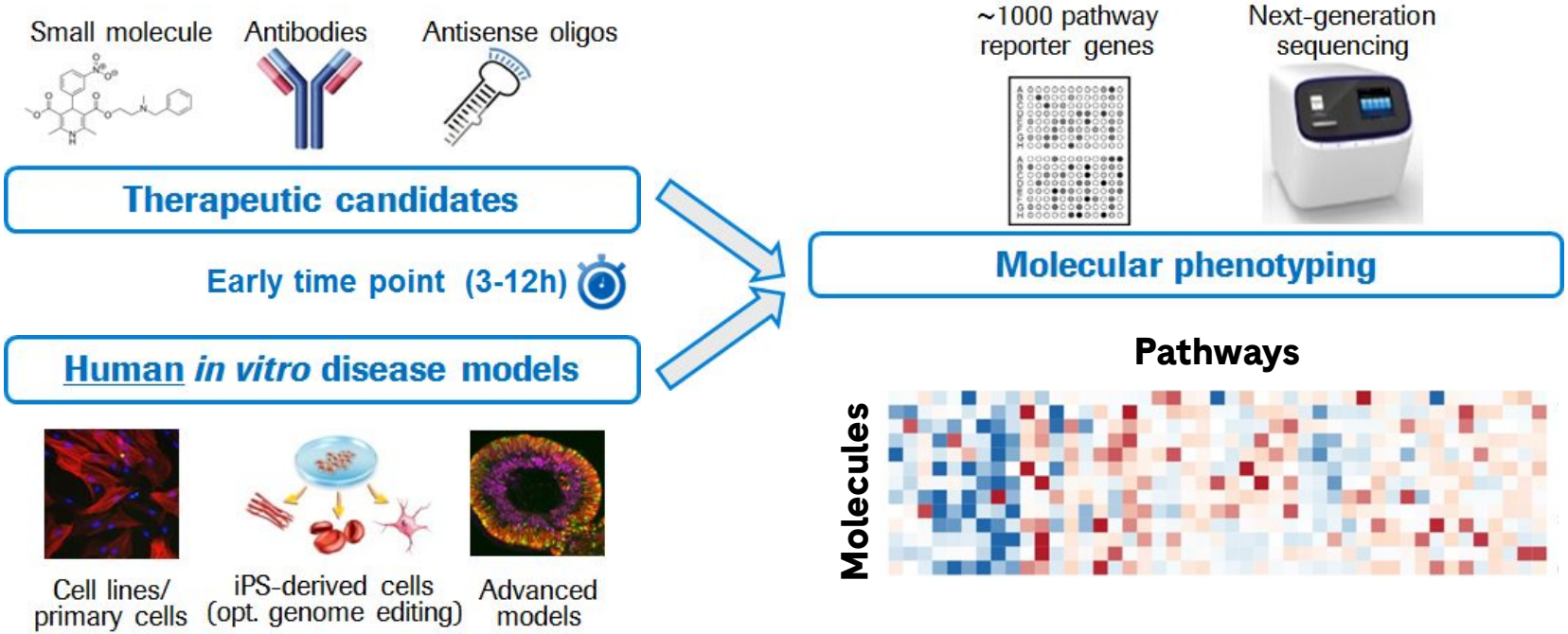


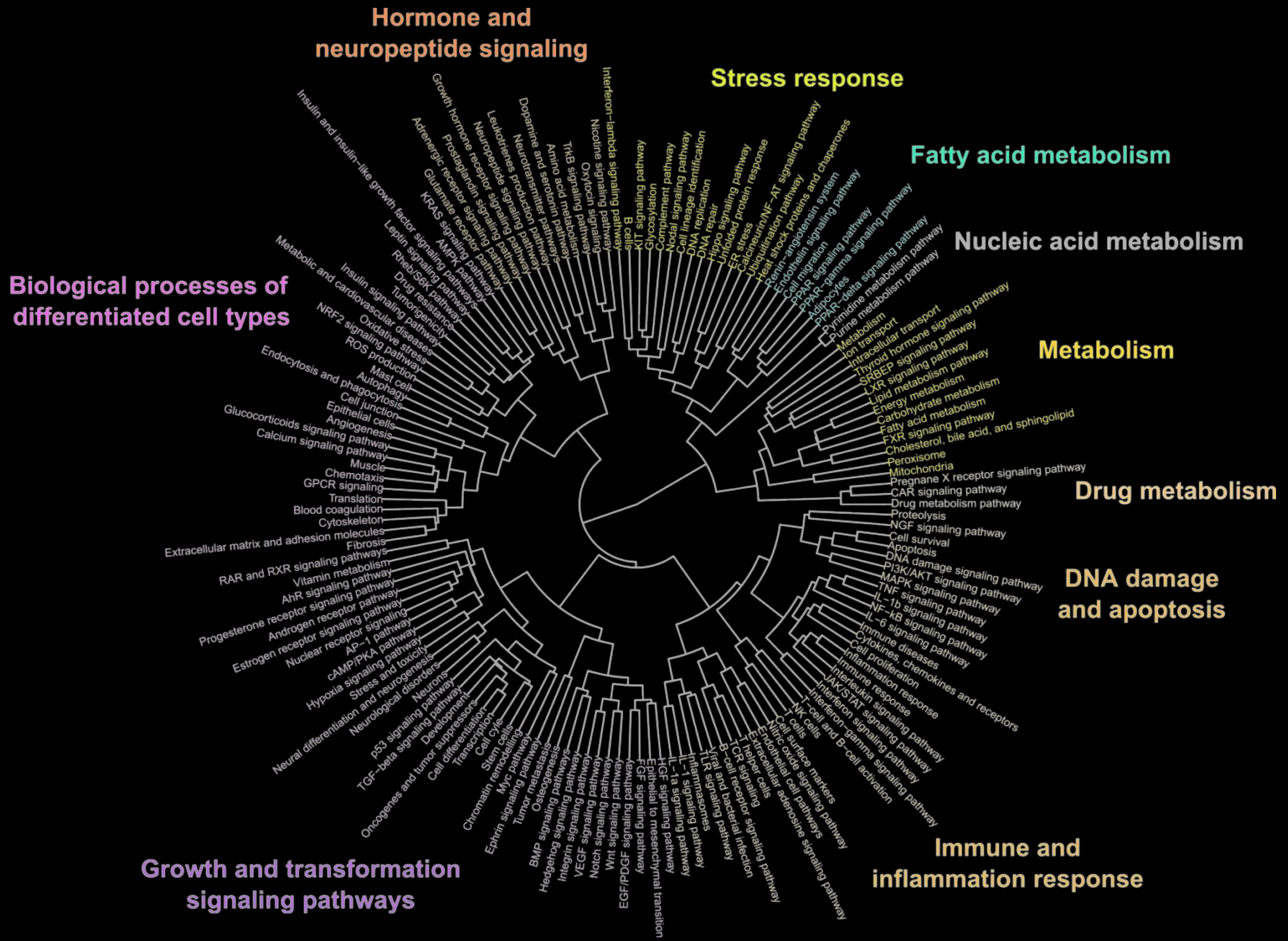
P: Protein translation inhibitors
R: RNA transcription inhibitors
D: DNA replication inhibitors
L: Lipid biosynthesis inhibitors
C: Cell-wall synthesis inhibitors (peptidoglycan)

Morphology classifies compounds by MoA



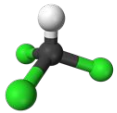
Molecular phenotyping reveals modulation of human pathway activities by compounds



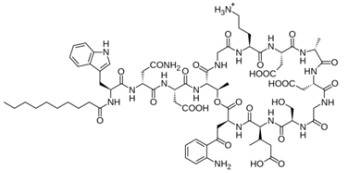

Hormone and neuropeptide signaling
Stress response
Fatty acid metabolism
Nucleic acid metabolism
Metabolism
Drug metabolism
DNA damage and apoptosis
Immune and inflammation response
Growth and transformation signaling pathways
Biological processes of differentiated cell types
Interferon−lambda signaling pathway
B cells
KIT signaling pathway
Glycosylation
Complement pathway
Nodal signaling pathway
Cell lineage identification
DNA replication
DNA repair
Hippo signaling pathway
Unfolded protein response
ER stress
Calcineurin/NF−AT signaling pathway
Ubiquitination pathway
Heat shock proteins and chaperones
Renin−angiotensin system
Endothelin signaling pathway
Cell migration
PPAR signaling pathway
PPAR−gamma signaling pathway
Adipocytes
PPAR−delta signaling pathway
Pyrimidine metabolism pathway
Purine metabolism pathway
Metabolism
Ion transport
Intracellular transport
Thyroid hormone signaling pathway
SRBEP signaling pathway
LXR signaling pathway
Lipid metabolism pathway
Energy metabolism
Carbohydrate metabolism
Fatty acid metabolism
FXR signaling pathway
Cholesterol, bile acid, and sphingolipid
Peroxisome
Mitochondria
Pregnane X receptor signaling pathway
CAR signaling pathway
Drug metabolism pathway
Proteolysis
NGF signaling pathway
Cell survival
Apoptosis
DNA damage signaling pathway
PI3K/AKT signaling pathway
MAPK signaling pathway
TNF signaling pathway
IL−1b signaling pathway
NF−kB signaling pathway
IL−6 signaling pathway
Immune diseases
Cytokines, chemokines and receptors
Cell proliferation
Inflammation response
Immune response
Interleukin signaling pathway
JAK/STAT signaling pathway
Interferon signaling pathway
Interferon−gamma signaling pathway
T−cell and B−cell activation
NK cells
T cells
Cell surface markers
Nitric oxide signaling pathway
Extracellular adenosine signaling pathway
Endothelial cell pathways
T helper cells
TCR signaling
B−cell receptor signaling pathway
Viral and bacterial infection
TLR signaling pathway
Inflammasomes
IL−1a signaling pathway
IL−1 signaling pathway
HGF signaling pathway
Epithelial to mesenchymal transition
FGF signaling pathway
EGF/PDGF signaling pathway
Wnt signaling pathway
Notch signaling pathway
VEGF signaling pathway
Integrin signaling pathway
Hedgehog signaling pathway
BMP signaling pathway
Osteogenesis
Tumor metastasis
Ephrin signaling pathway
Myc pathway
Chromatin remodelling
Stem cells
Cell cycle
Transcription
Cell differentiation
Oncogenes and tumor suppressors
Development
TGF−beta signaling pathway
p53 signaling pathway
Neurons
Neurological disorders
Neural differentiation and neurogenesis
Stress and toxicity
Hypoxia signaling pathway
cAMP/PKA pathway
AP−1 pathway
Nuclear receptor signaling
Estrogen receptor signaling pathway
Androgen receptor pathway
Progesterone receptor signaling pathway
AhR signaling pathway
Vitamin metabolism
RAR and RXR signaling pathways
Fibrosis
Extracellular matrix and adhesion molecules
Cytoskeleton
Blood coagulation
Translation
GPCR signaling
Chemotaxis
Muscle
Calcium signaling pathway
Glucocorticoids signaling pathway
Angiogenesis
Epithelial cells
Cell junction
Endocytosis and phagocytosis
Autophagy
Mast cell
ROS production
NRF2 signaling pathway
Oxidative stress
Metabolic and cardiovascular diseases
Insulin signaling pathway
Tumorigenicity
Drug resistance
Rheb/S6K pathway
Leptin signaling pathway
Insulin and insulin−like growth factor signaling pathway
KRAS signaling pathway
AMPK signaling pathway
Glutamate receptor signaling pathway
Adrenergic receptor signaling pathway
Prostaglandin signaling pathway
Growth hormone receptor signaling pathway
Neuropeptide signaling pathway
Leukotrienes production pathway
Neurotransmitter pathway
Dopamine and serotonin pathway
Amino acid metabolism
TrkB signaling pathway
Oxytocin signaling
Nicotine signaling pathway

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

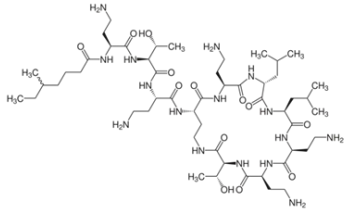
ROxyz



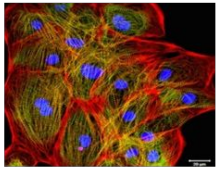
Daptomycin



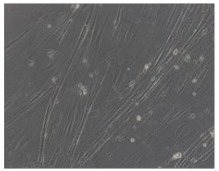
Colistin



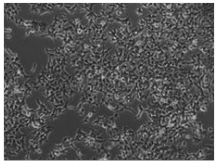
iPS-derived human cardiomyocytes



Primary human skeletal muscle cells

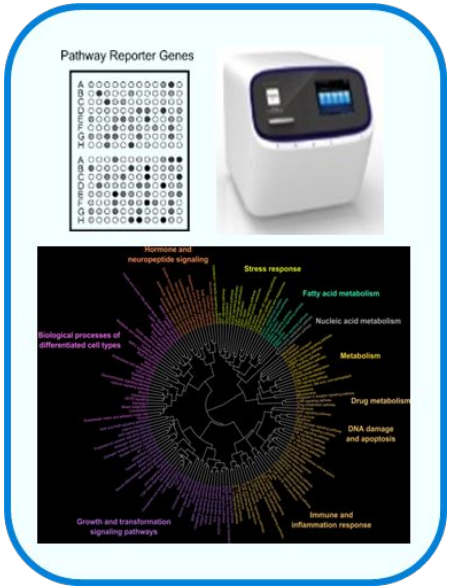


Human embryonic kidney cells



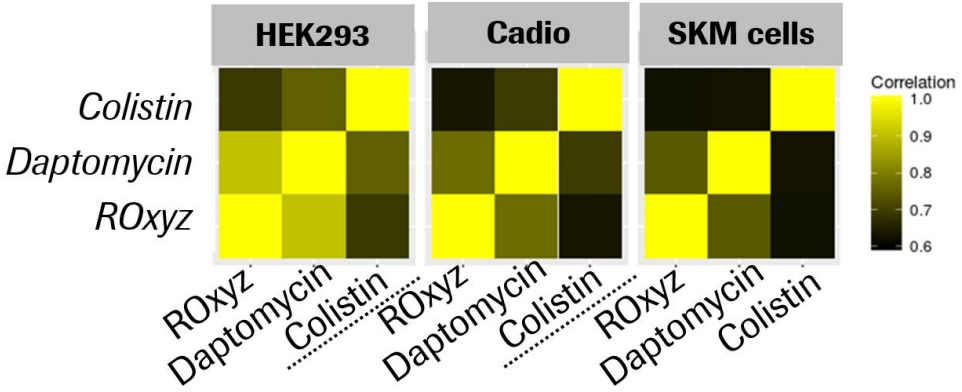
6h treatment at
sub-cytotoxic concentration

Molecular phenotyping

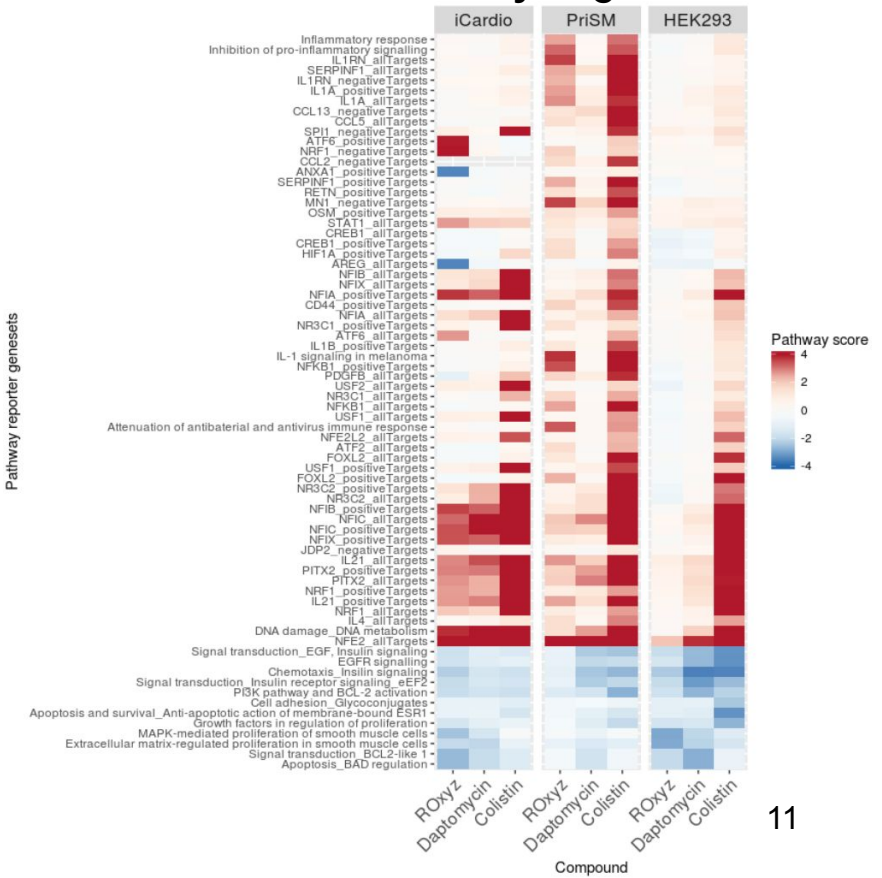


Roche compound is more similar to daptomycin than colistin, irrespective of cell type

Similarity of differential expression profiles

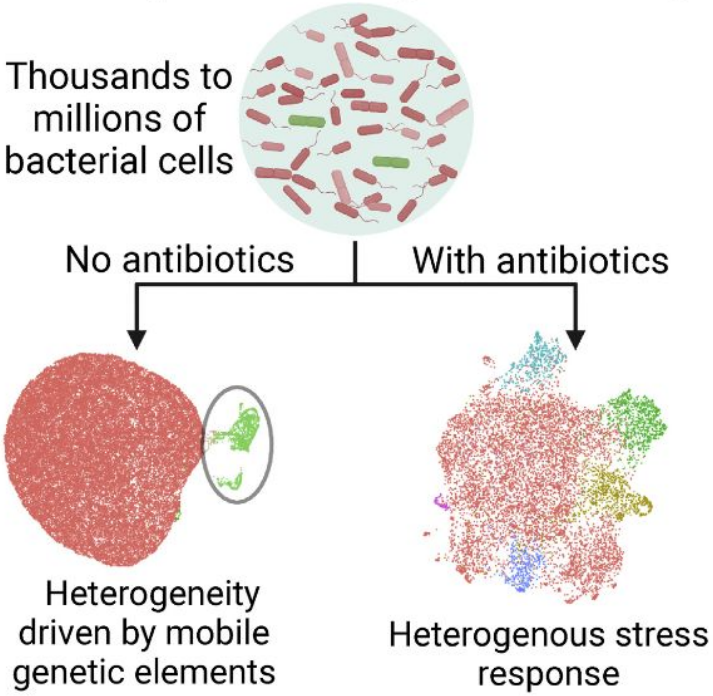


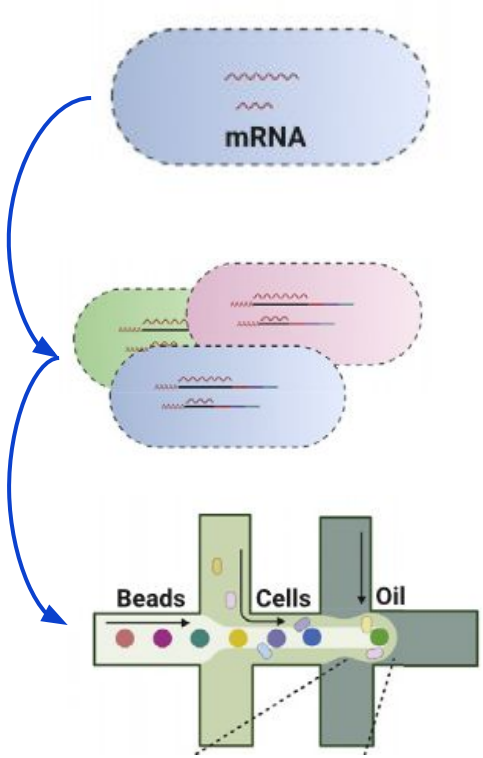
Pathway regulation



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

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



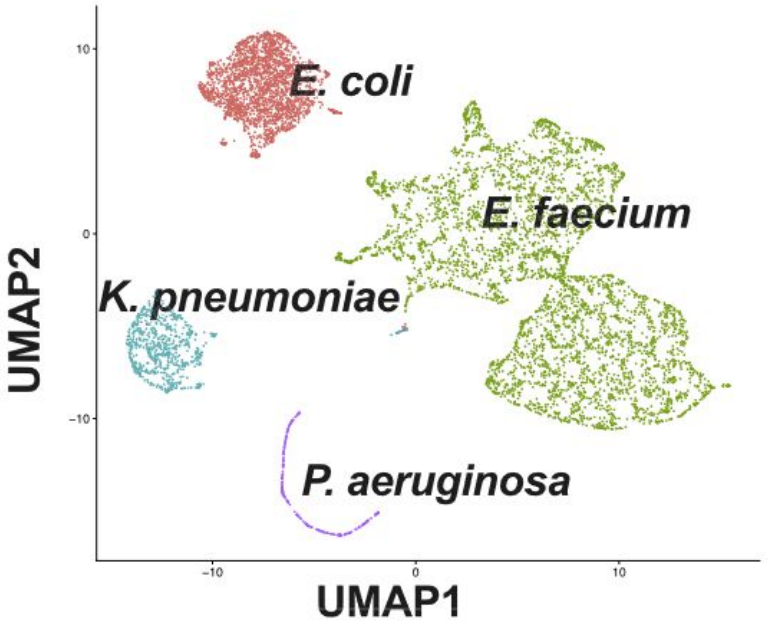


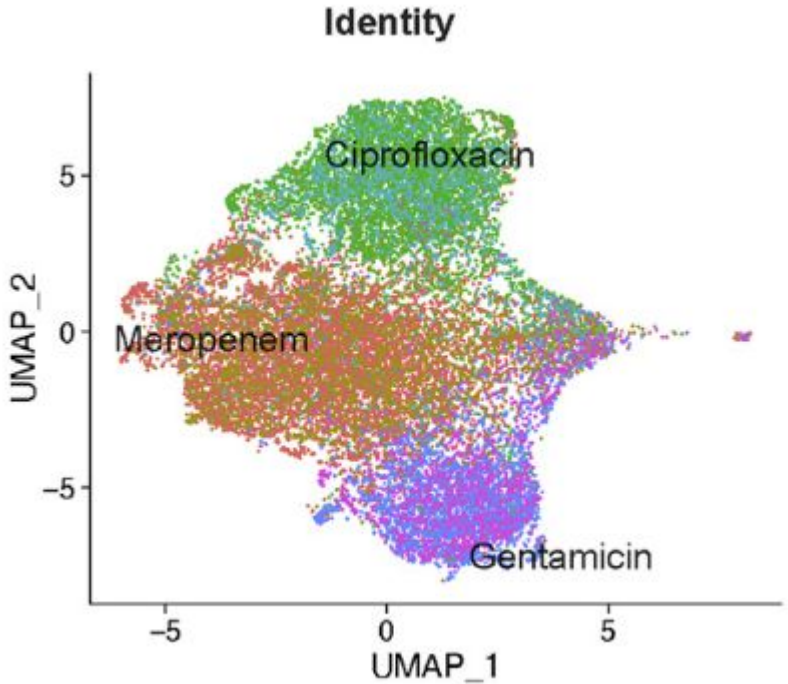
rRNA and DNA depletion

Reverse translation

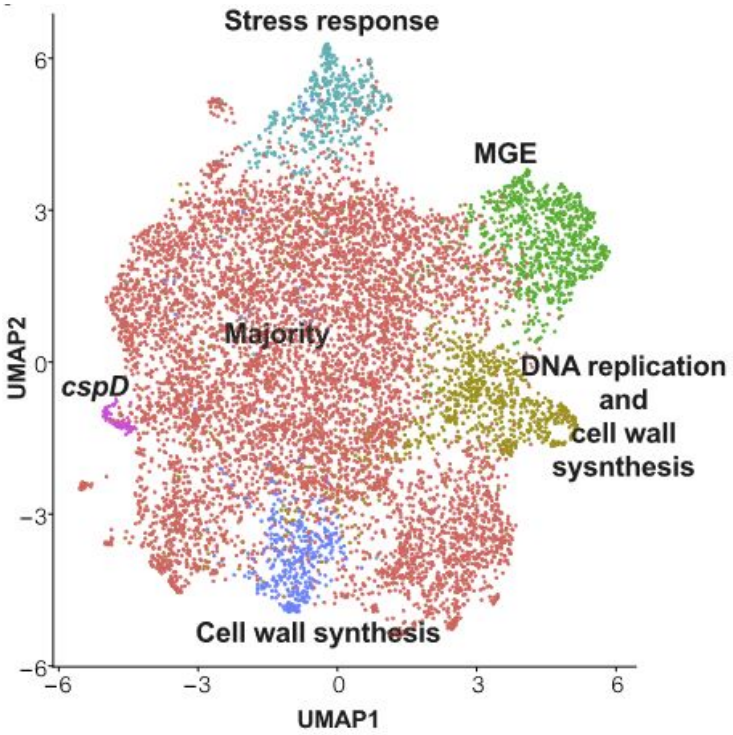
Droplet generation and sequencing (*BacDrop*)

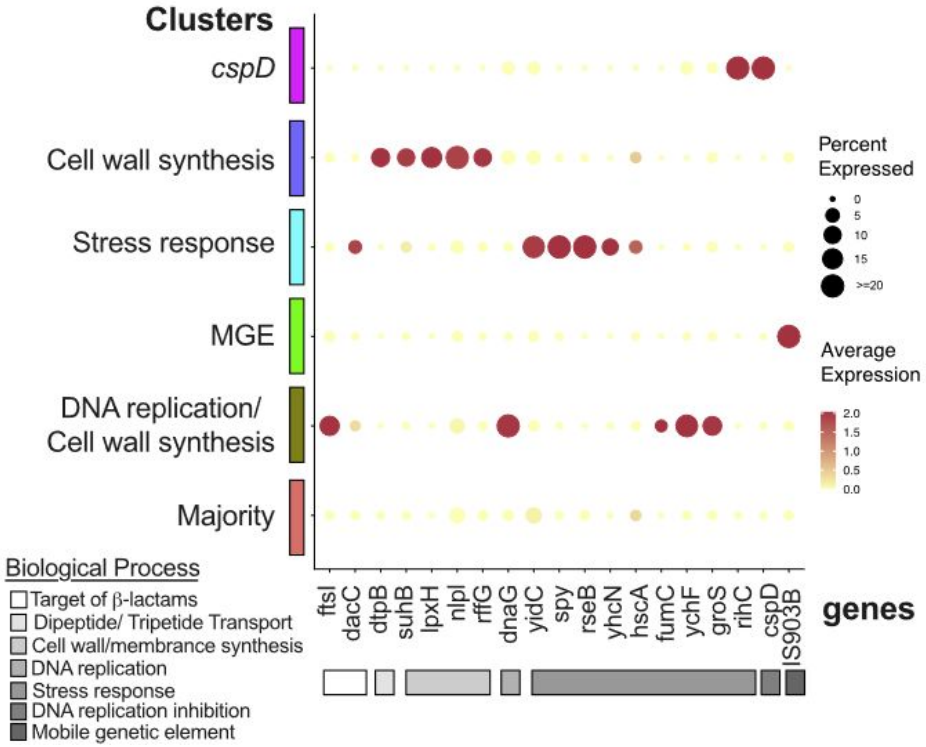
BacDrop recovers bacterial species and reveals effects of antibiotics treatment





Meropenem treatment induced heterogeneous responses





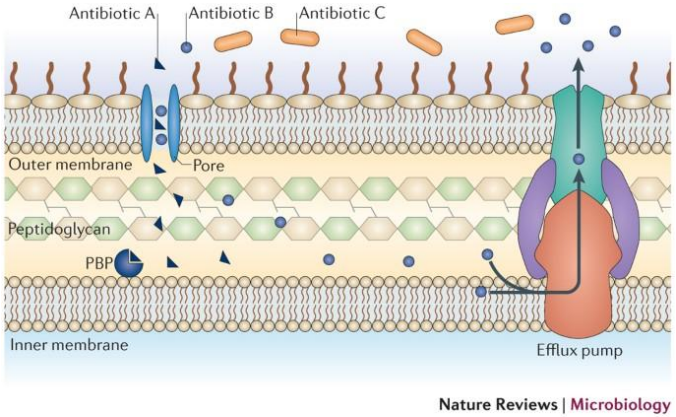
Challenges and prospects

- **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.

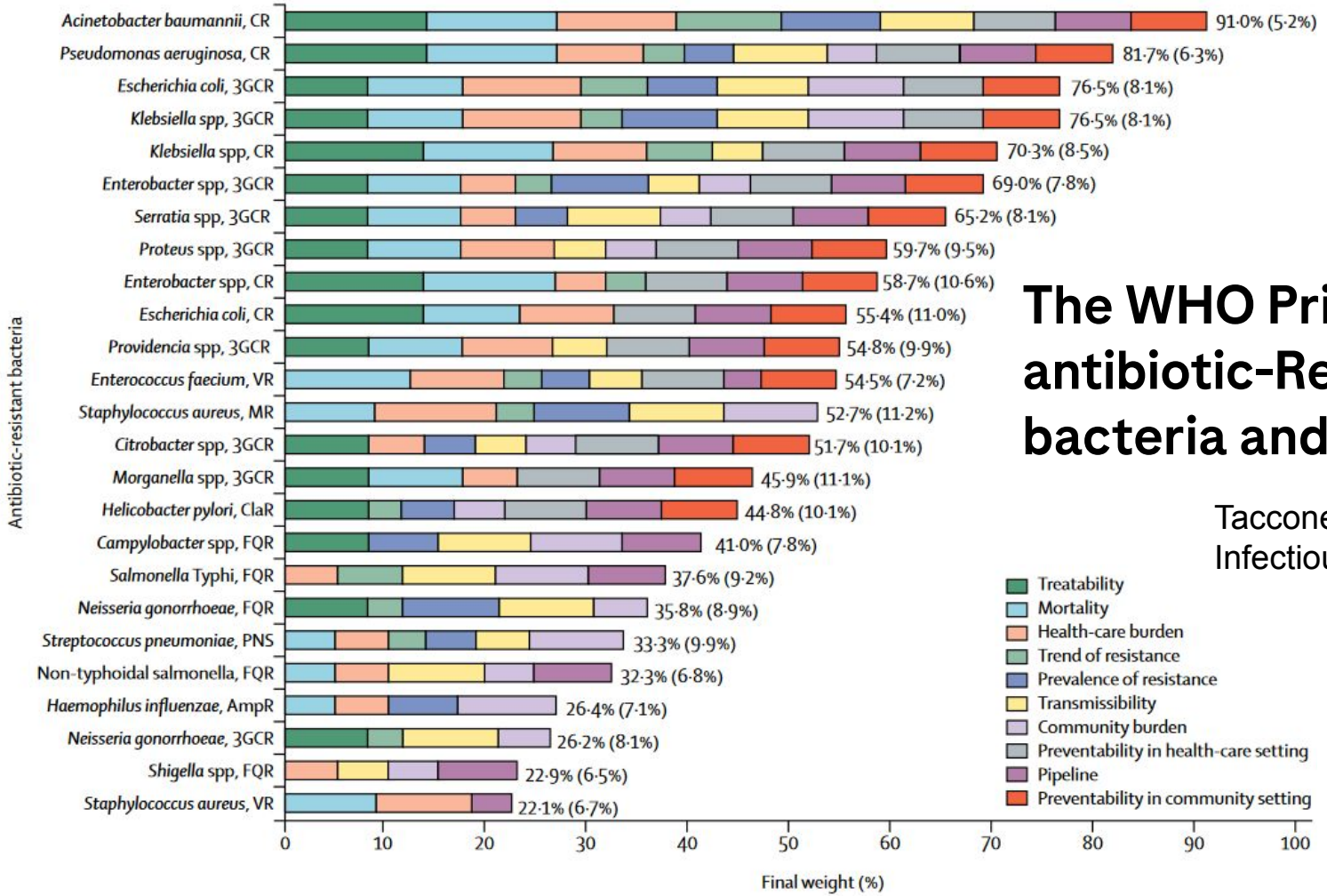
Doing now what patients need next

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.



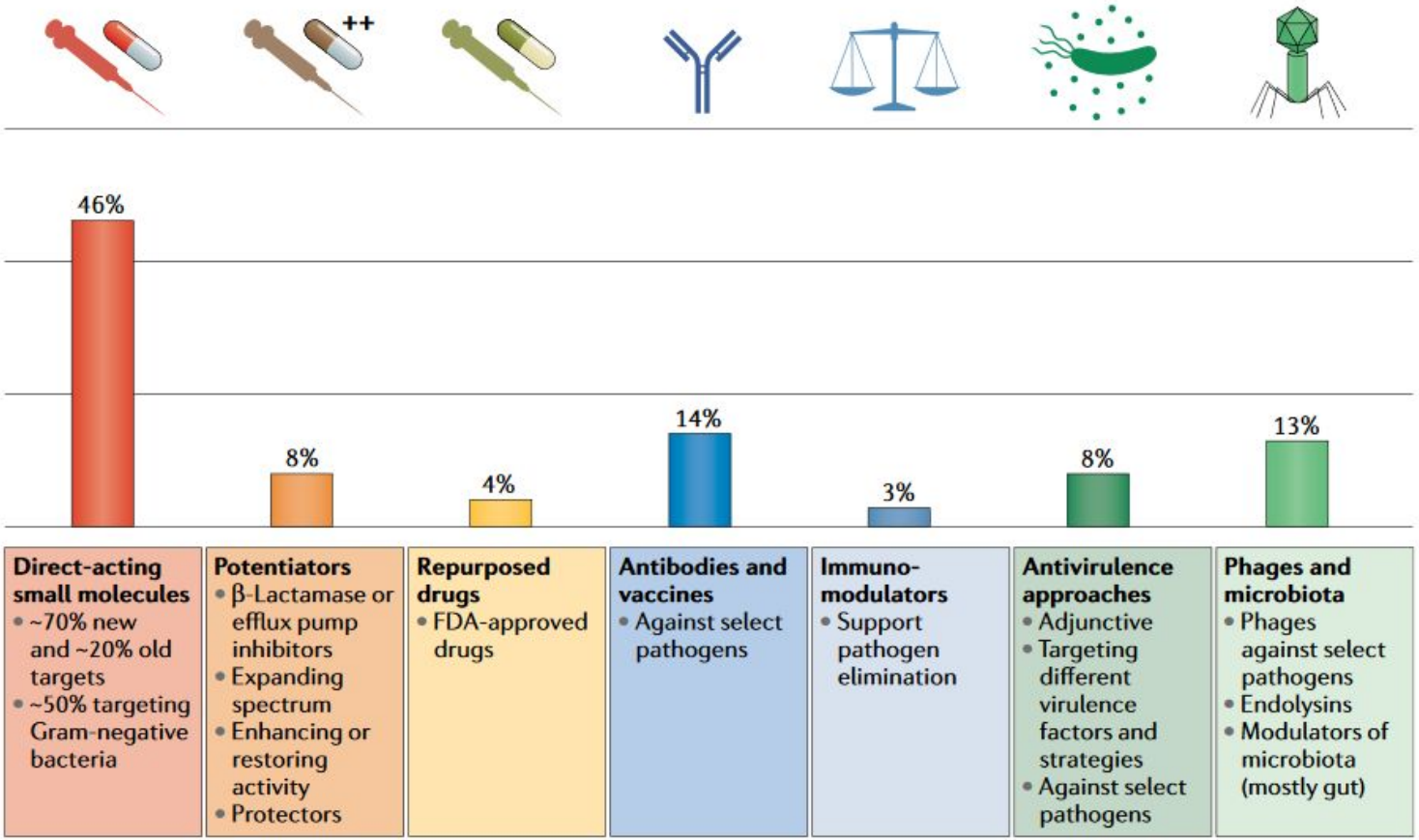
Hogwarts? Multiresistant bacteria?



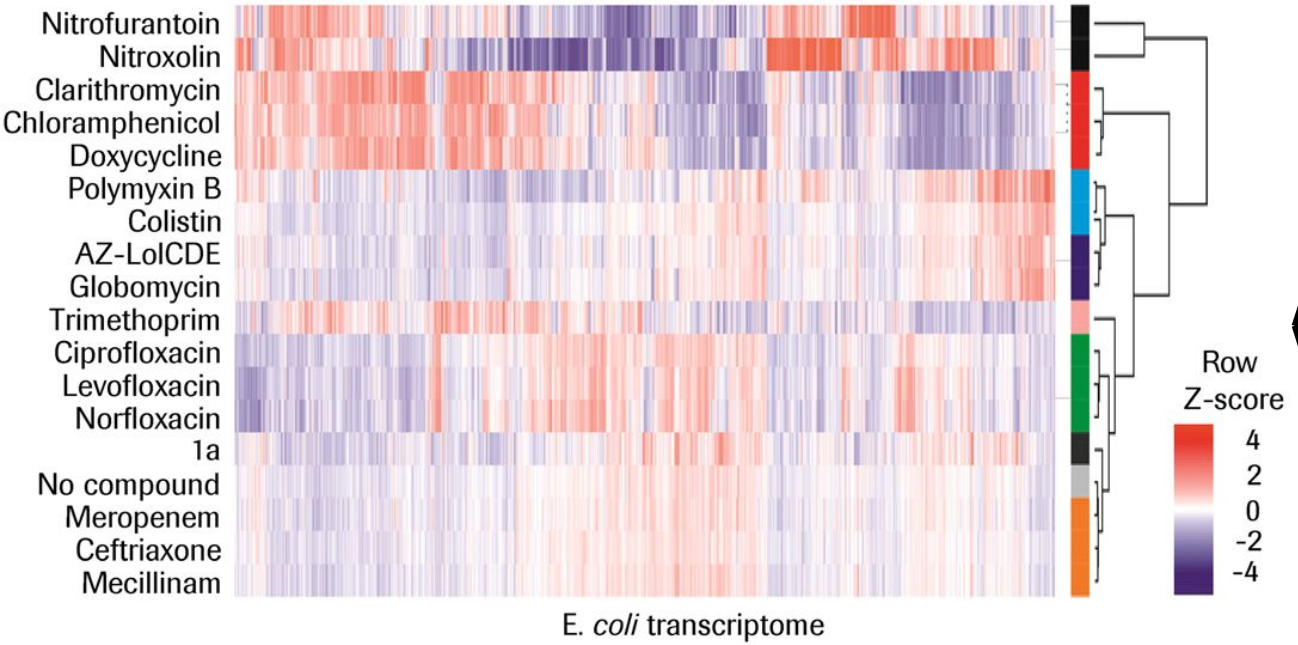
The WHO Priority list of antibiotic-Resistant bacteria and tuberculosis

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

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



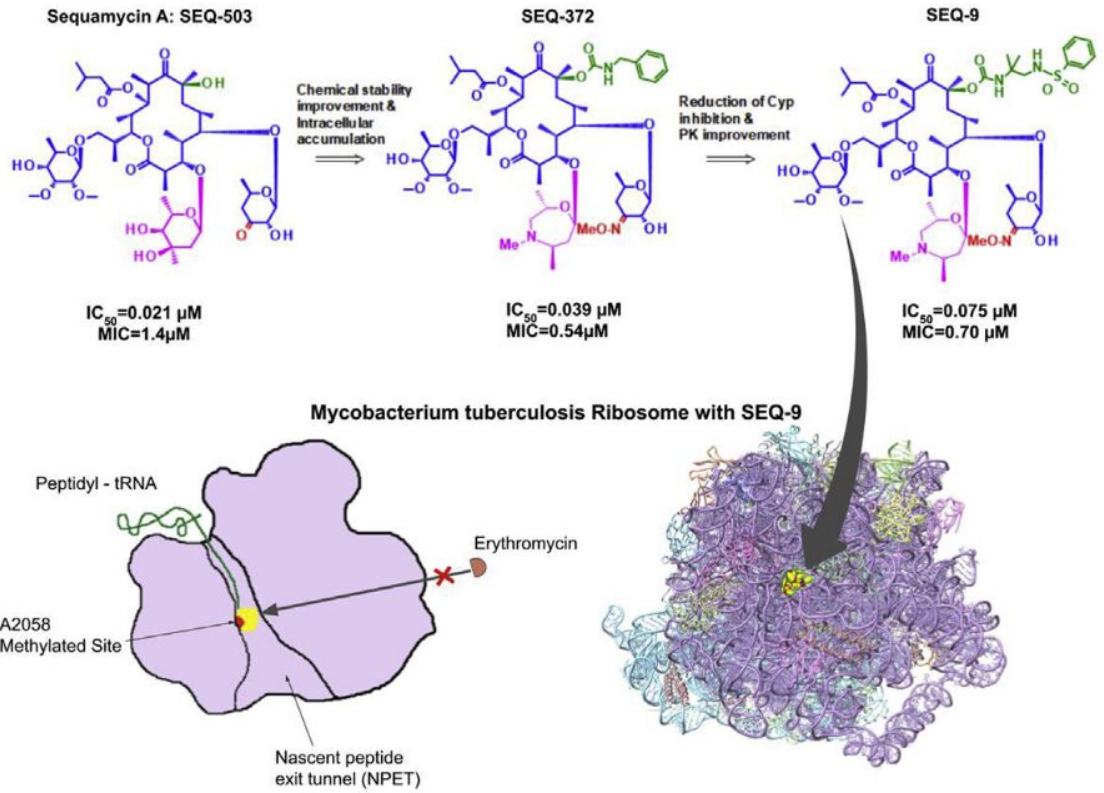
Morphology and gene expression offer complementary information



E. *coli* transcriptome

- 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.
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 Küng, 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 Vercruysse, 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, 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.