Design of combination therapies for tuberculosis

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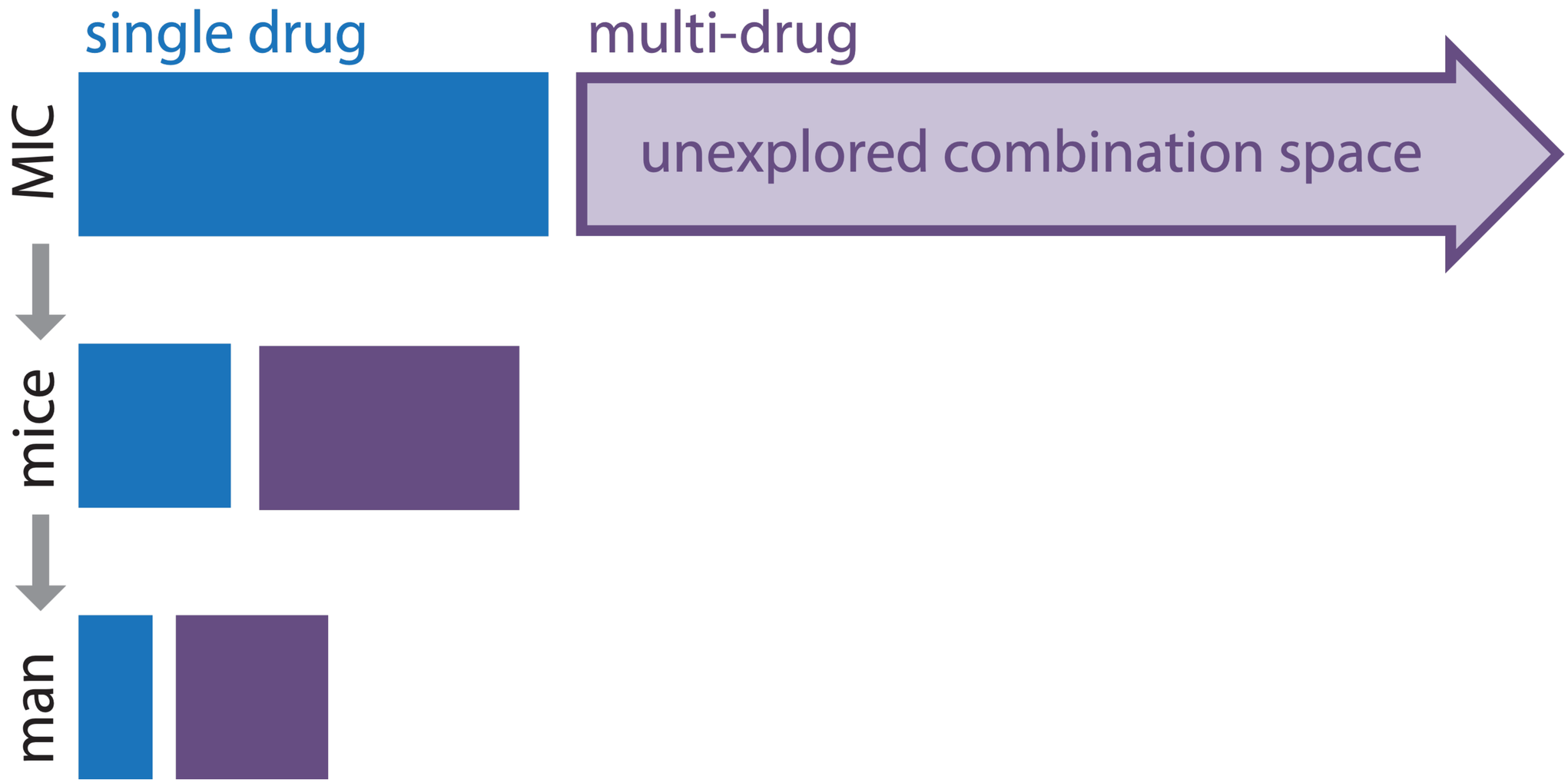
Tufts Stuart B. Levy Center for Integrated Management of Antimicrobial Resistance

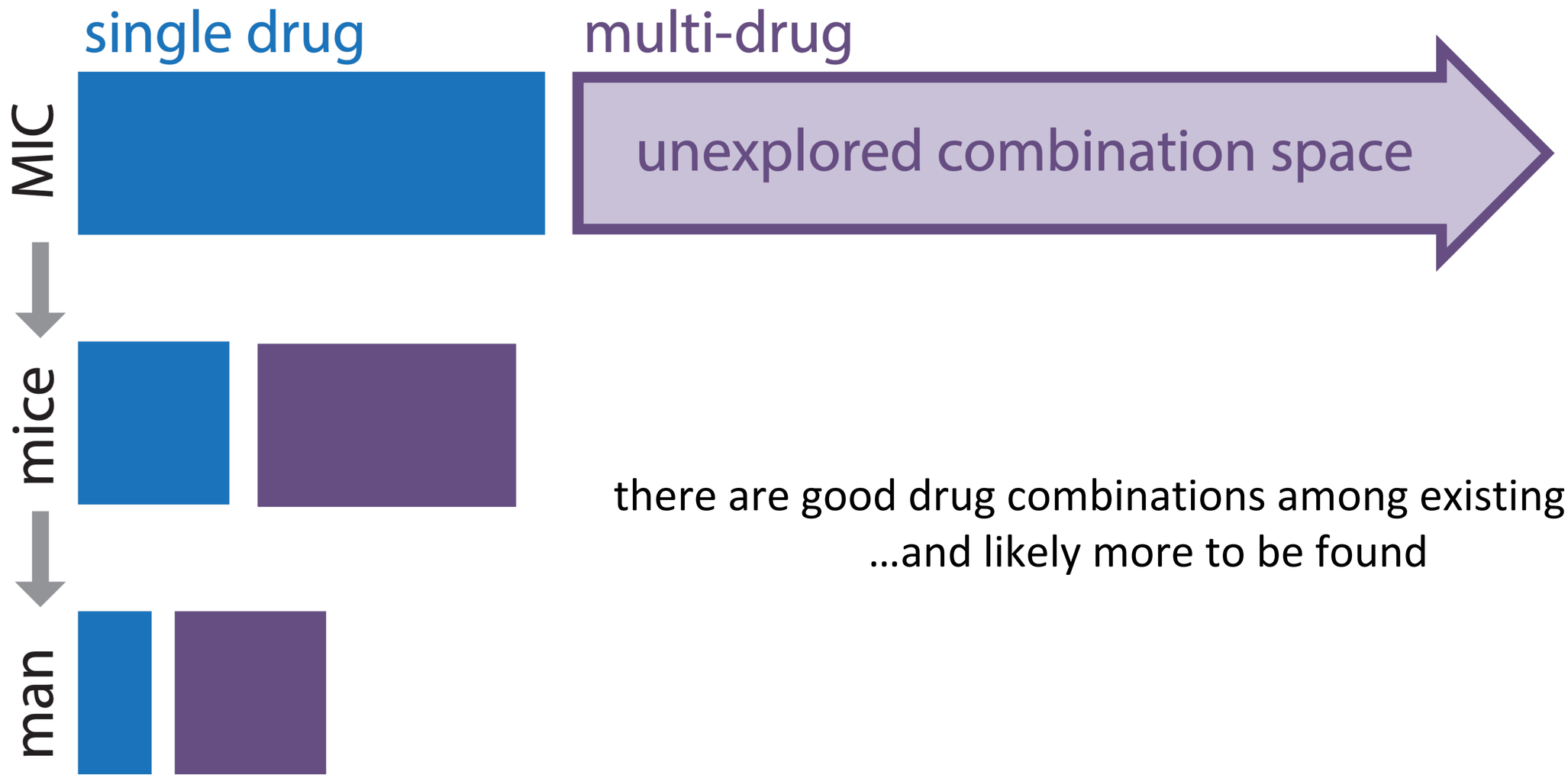
TB must be treated with multidrug therapies because of heterogeneity

- innate (bacterial)
- lesion

How can we prioritize drug combinations?

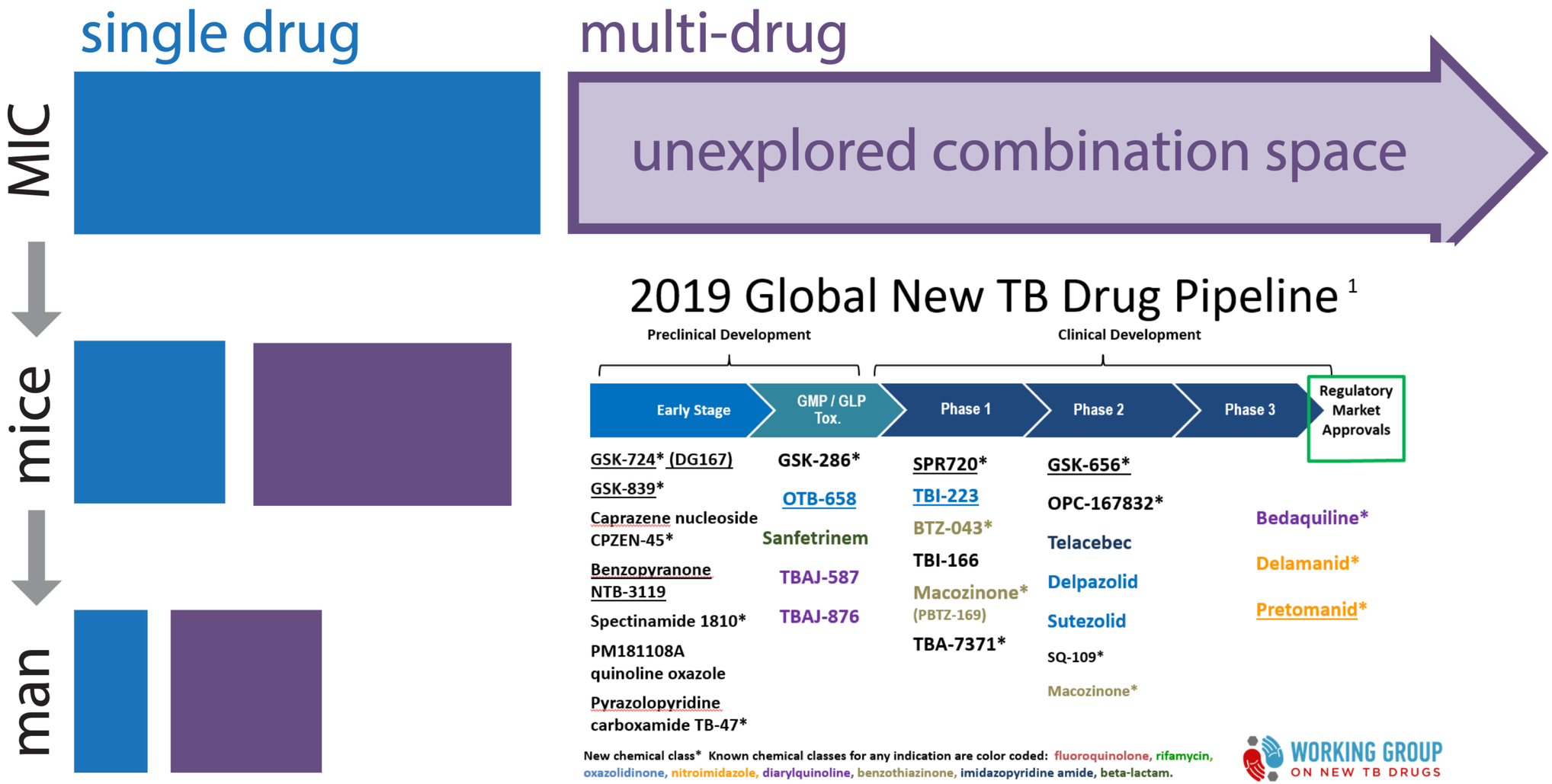


The vast combination space is sparsely considered in drug regimen design



there are good drug combinations among existing drugs
…and likely more to be found

The idea: prioritize combinations for animal studies based on practical systematic *in vitro* measurement



2019 Global New TB Drug Pipeline [1]

Preclinical Development | Clinical Development

Early Stage	GMP / GLP Tox.	Phase 1	Phase 2	Phase 3	Regulatory Market Approvals
GSK-724* (DG167)	GSK-286*	SPR720*	GSK-656*		
GSK-839*	OTB-658	TBI-223	OPC-167832*	Bedaquiline*	
Caprazene nucleoside CPZEN-45*	Sanfetrinem	BTZ-043*	Telacebec	Delamanid*	
Benzopyranone NTB-3119	TBAJ-587	TBI-166	Delpazolid	Pretomanid*	
Spectinamide 1810*	TBAJ-876	Macozinone* (PBTZ-169)	Sutezolid		
PM181108A quinoline oxazole		TBA-7371*	SQ-109*		
Pyrazolopyridine carboxamide TB-47*			Macozinone*		

New chemical class* Known chemical classes for any indication are color coded: fluoroquinolone, rifamycin, oxazolidinone, nitroimidazole, diarylquinoline, benzothiazinone, imidazopyridine amide, beta-lactam.

[1] New Molecular Entities not yet approved, being developed for TB or only conditionally approved for TB. Showing most advanced stage reported for each. Details for projects listed can be found at http://www.newtbdrugs.org/pipeline/clinical Underline = new to Phase since March 2019

Ongoing projects without a lead compound series identified: http://www.newtbdrugs.org/pipeline/discovery

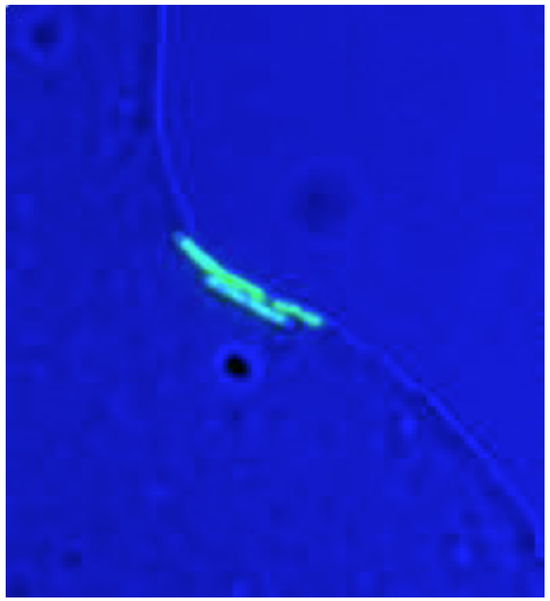
WORKING GROUP ON NEW TB DRUGS

www.newtbdrugs.org

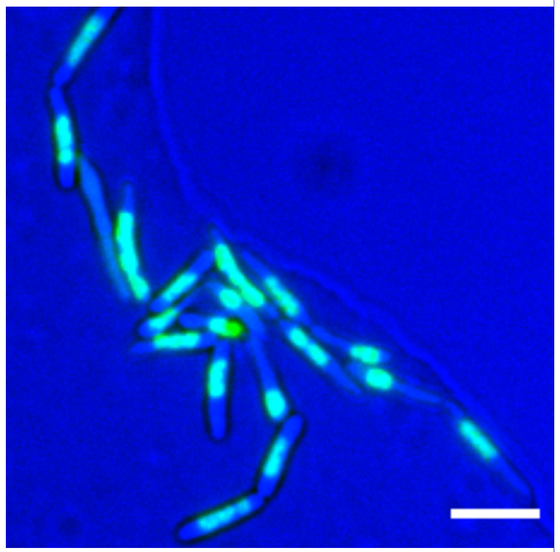
Updated: October 2019

Different drugs induce different morphological changes

rifampicin:

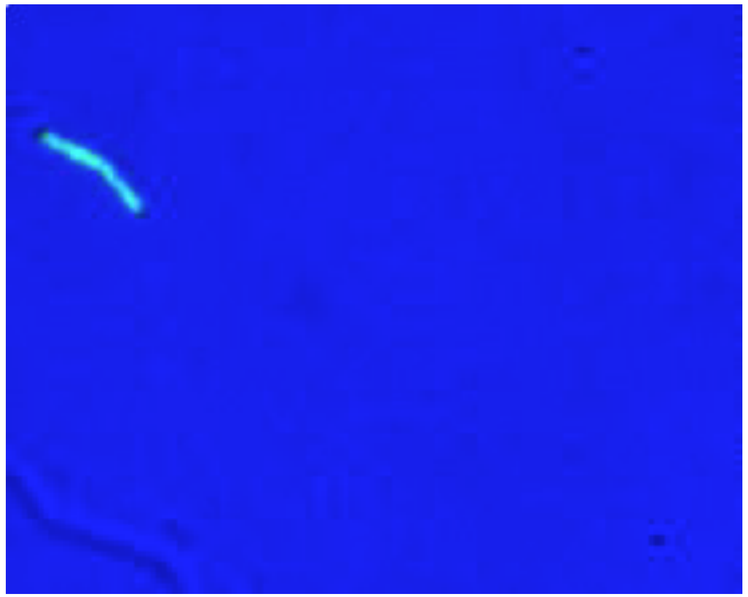


snapshot:

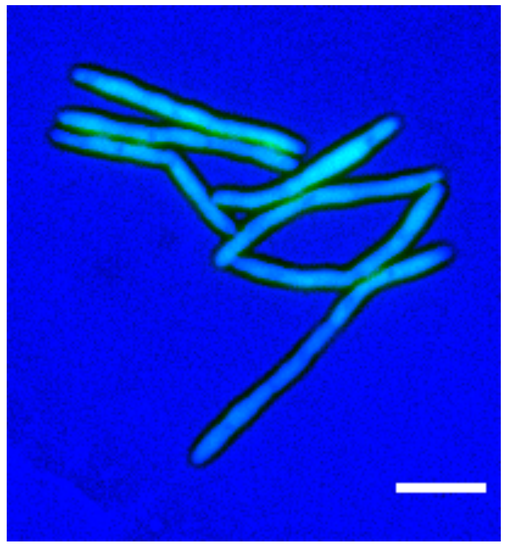


M. smegmatis RpoB-GFP

moxifloxacin:



snapshot:



Can we rapidly capture these morphological changes to classify drugs with similar pathways of action?

PNAS

Bacterial cytological profiling rapidly identifies the cellular pathways targeted by antibacterial molecules

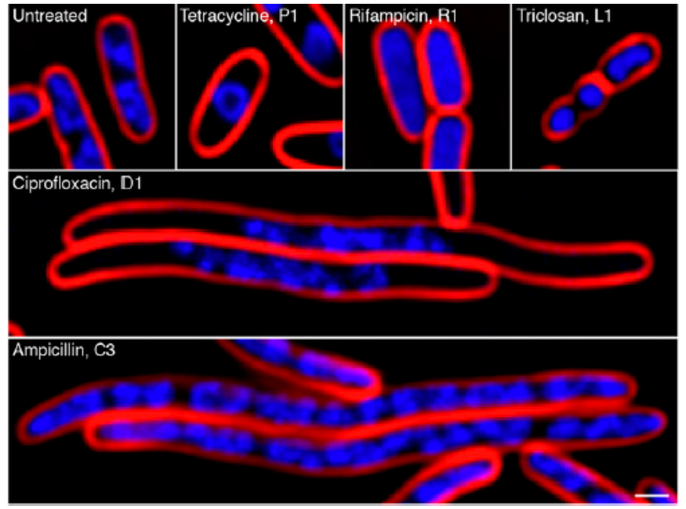
Poochit Nonejuie[a], Michael Burkart[b], Kit Pogliano[a], and Joe Pogliano[a,1]

[a]Division of Biological Sciences, University of California, San Diego, CA 92093; and [b]Department of Chemistry and Biochemistry, University of California, San Diego, CA 92093

Edited by Christopher T. Walsh, Harvard Medical School, Boston, MA, and approved August 22, 2013 (received for review June 10, 2013)

Identifying the mechanism of action for antibacterial compounds is essential for understanding how bacteria interact with one an-

MMS assays suffer from low resolution, low accuracy, and relatively low throughput.



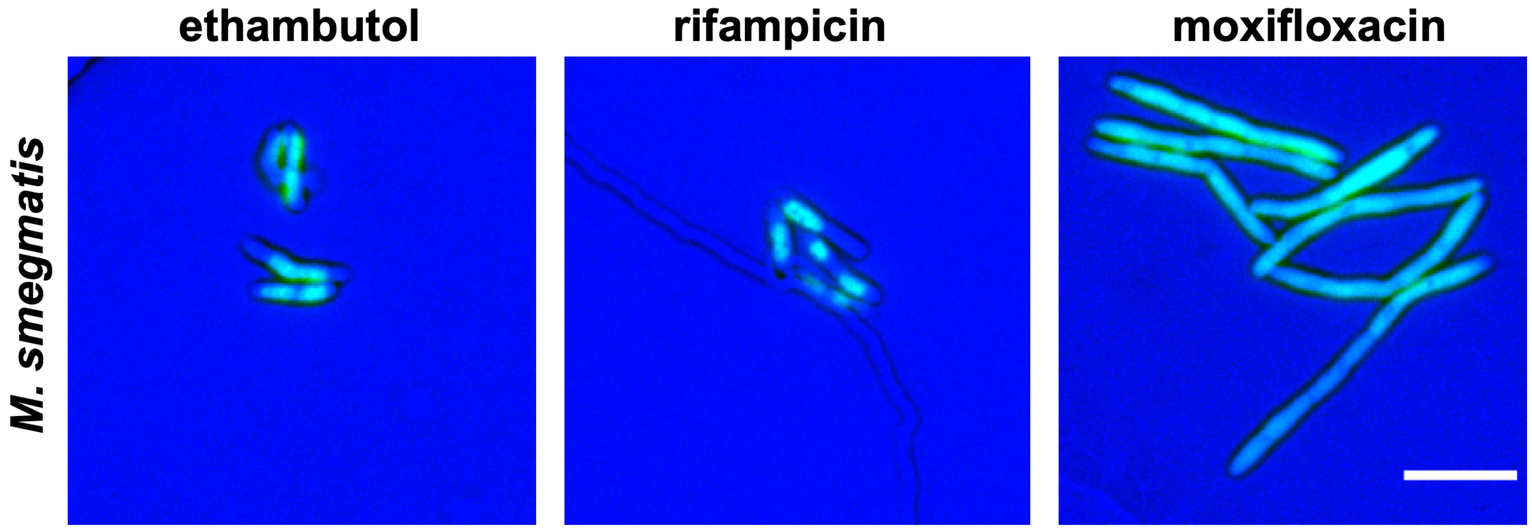
Trever Smith, PhD

Krista Pullen

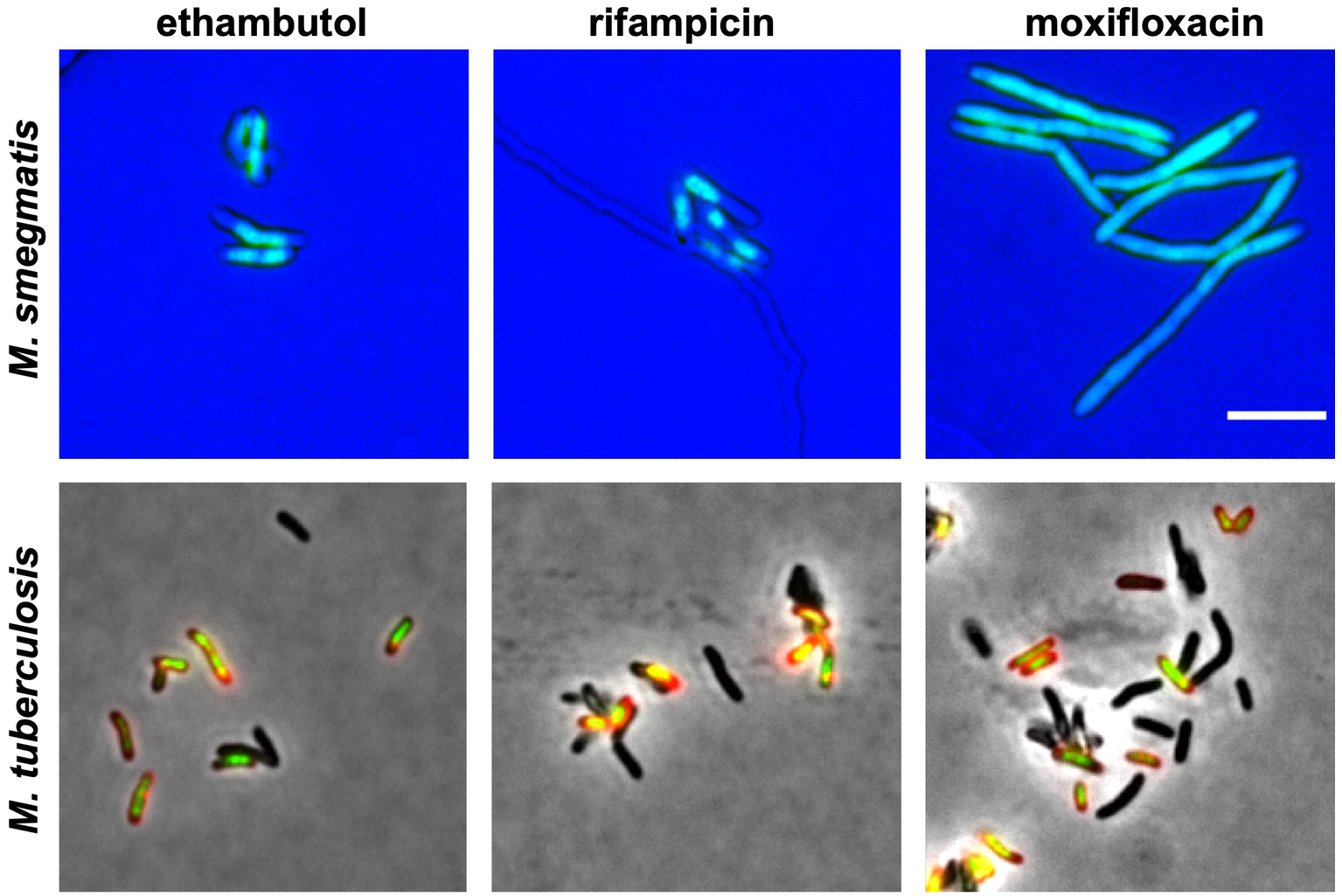
Michaela Olson

Morgan McNellis

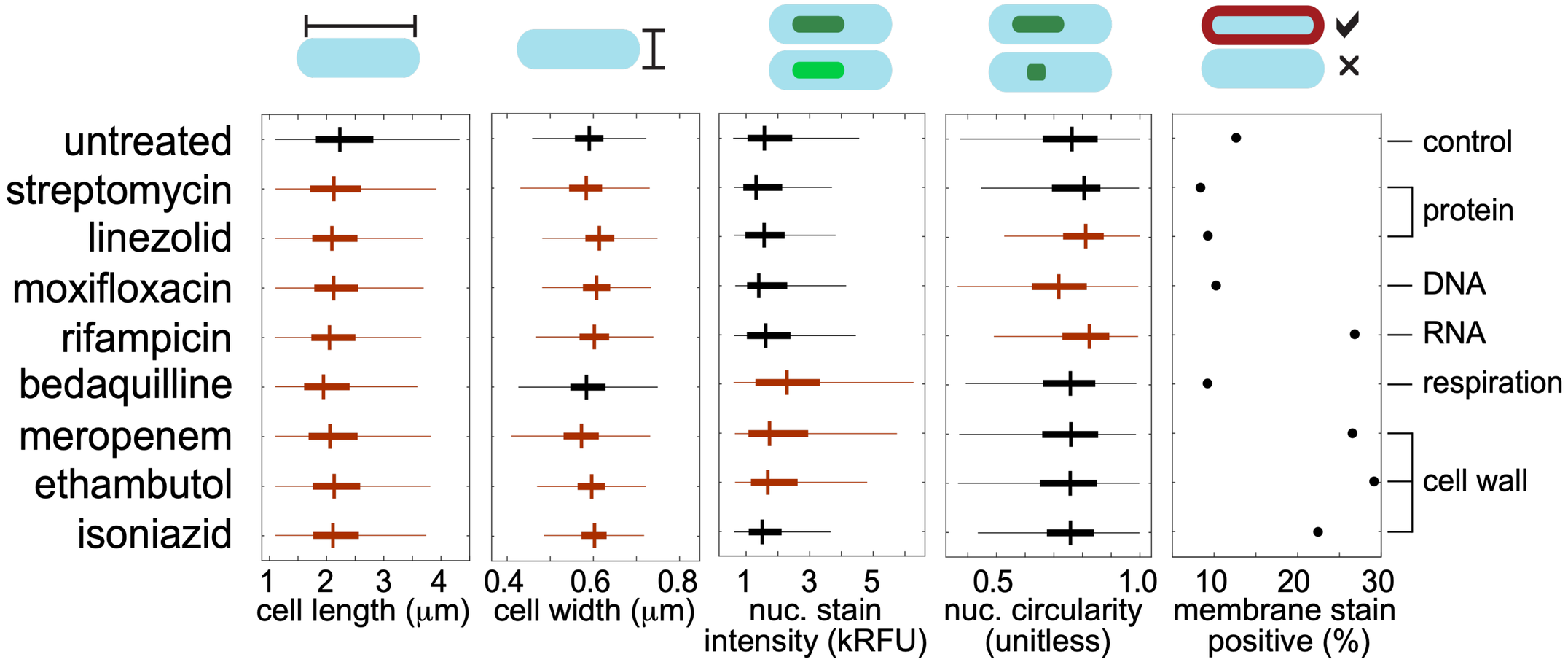
Can we rapidly capture these morphological changes to classify drugs with similar pathways of action?



Morphological features in drug-treated Mtb are subtle and extremely variable

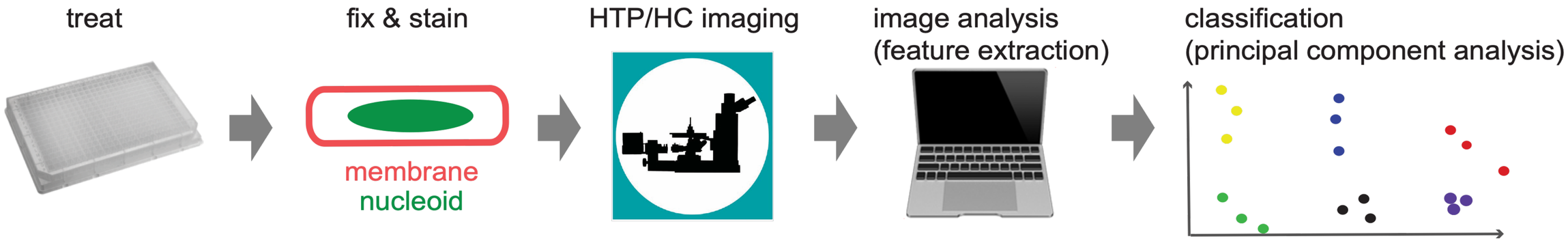


Morphological differences in Mtb are measurable using high-throughput fixed-cell imaging



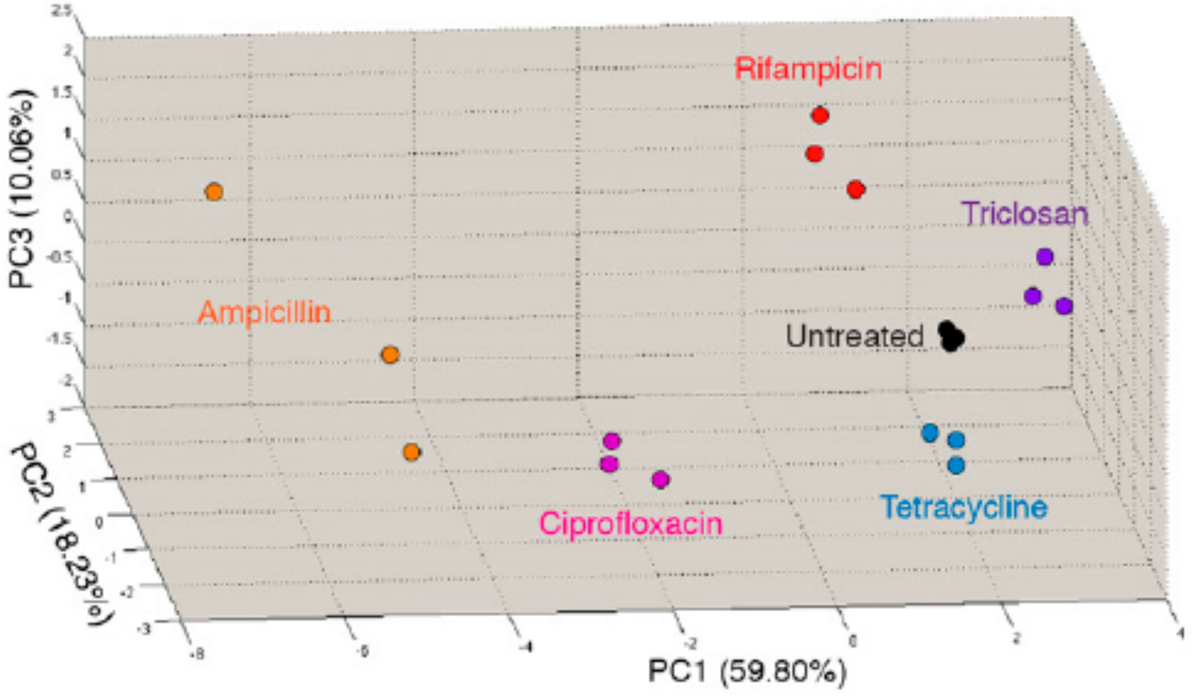
Smith et. al, PNAS, 2020

Bacterial cytological profiling pipeline



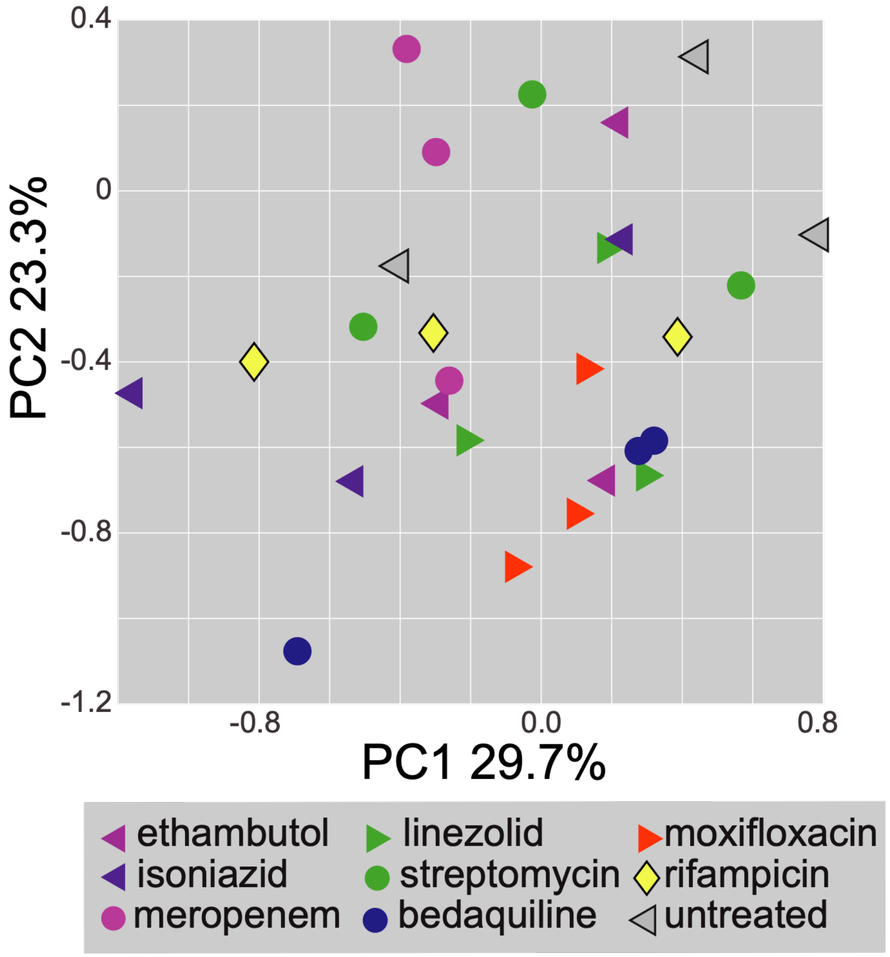
Mtb samples cannot be classified by drug mechanism of action using the standard pipeline

E. coli (Nonejuie et al)



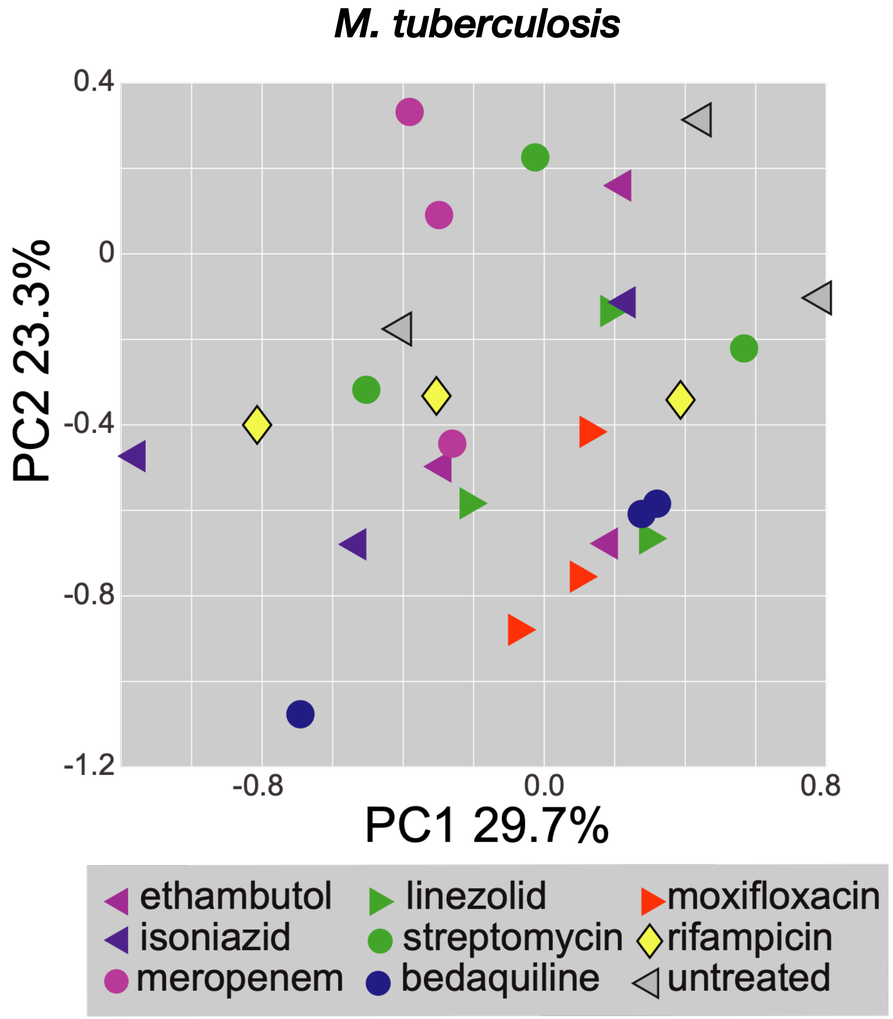
Nonejuie et al. PNAS 2013

M. tuberculosis

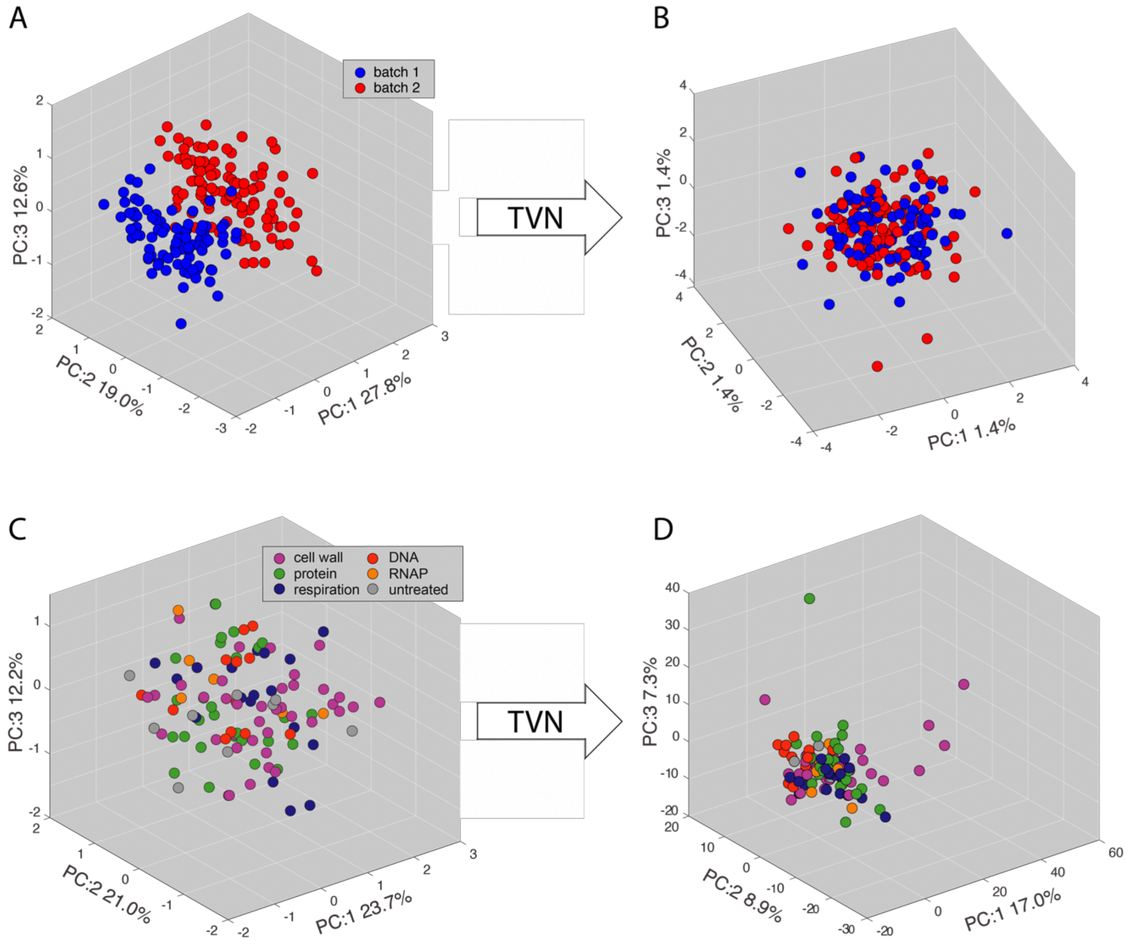


Mtb samples cannot be classified by drug mechanism of action using the standard pipeline

- **Sample-sample heterogeneity (batch)**
- **Cell-to-cell heterogeneity**
- **Subtle features**
- **Nonlinear clustering**



Accounting for batch-to-batch and cell-to-cell heterogeneity with Typical Variation Normalization



TVN: D. Michael Ando, Cory McLean, Marc Berndl
https://www.biorxiv.org/content/10.1101/161422v1.full

Accounting for batch-to-batch and cell-to-cell heterogeneity improves clustering

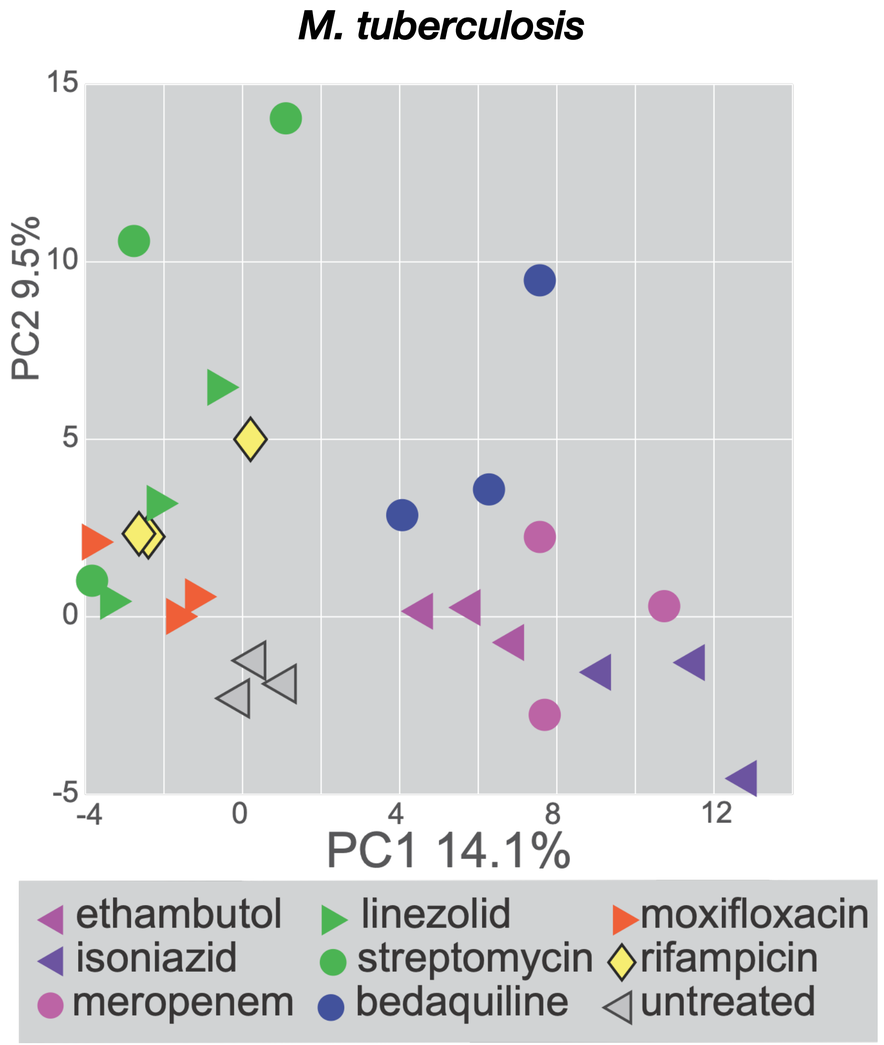
- **Sample-sample heterogeneity (batch)**
- **Cell-to-cell heterogeneity**

- **Subtle features**

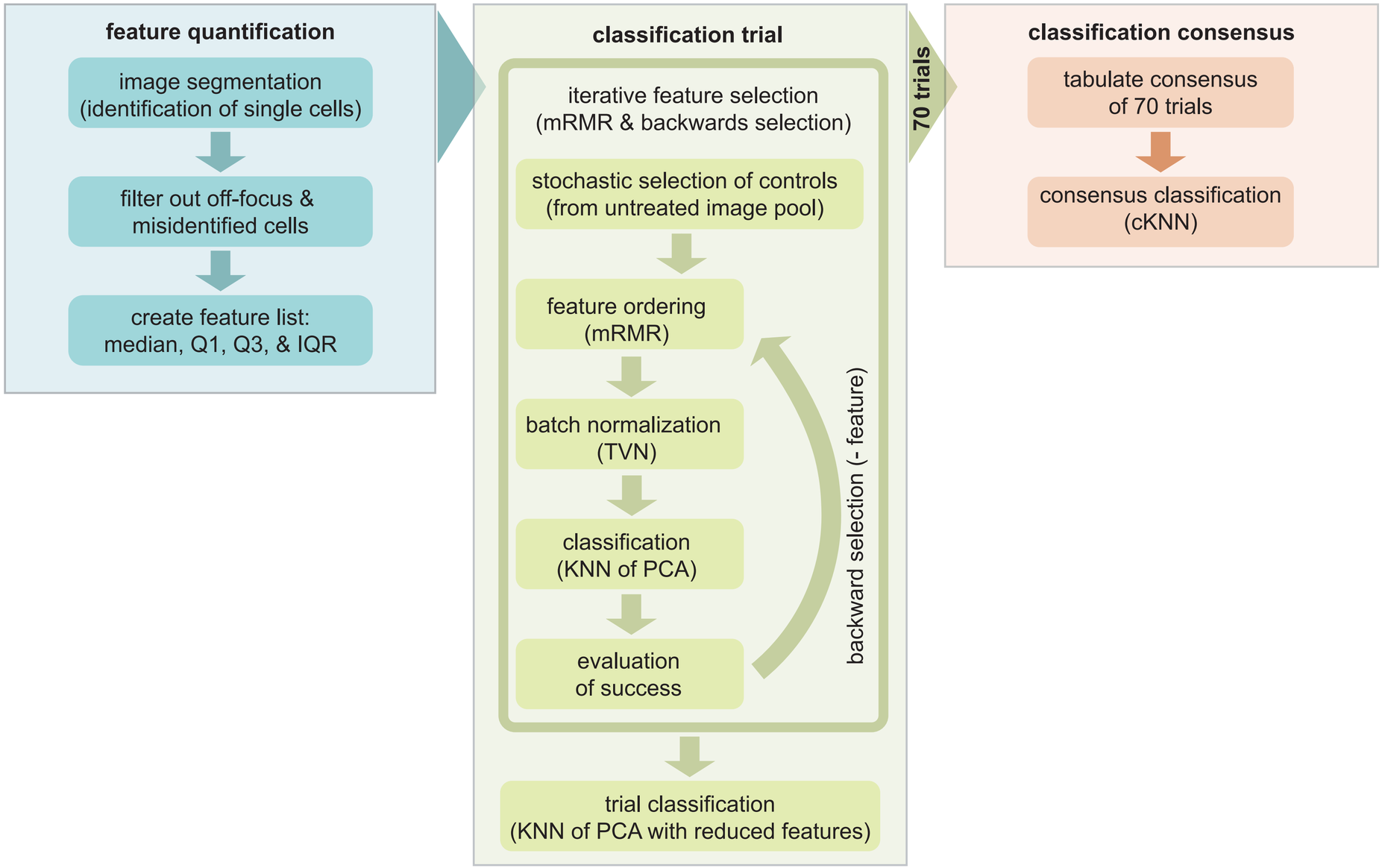
- **Nonlinear clustering**

~100 features:

- nucleoid shape
- cell shape
- stain solidity
- stain intensity
- heterogeneity
- number of stained regions
- texture of staining



MorphEUS: Morphological Evaluation and Understanding of Stress



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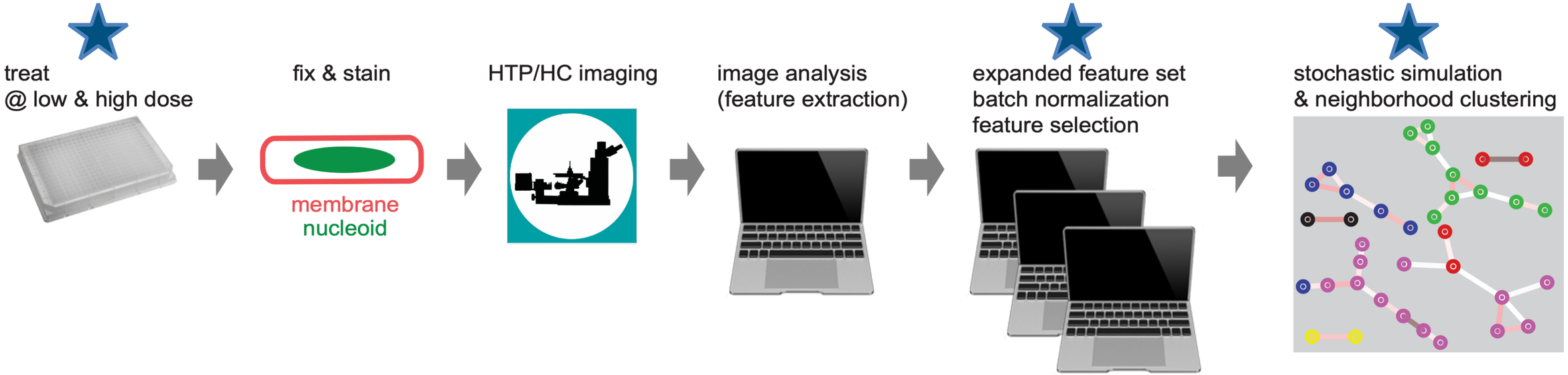
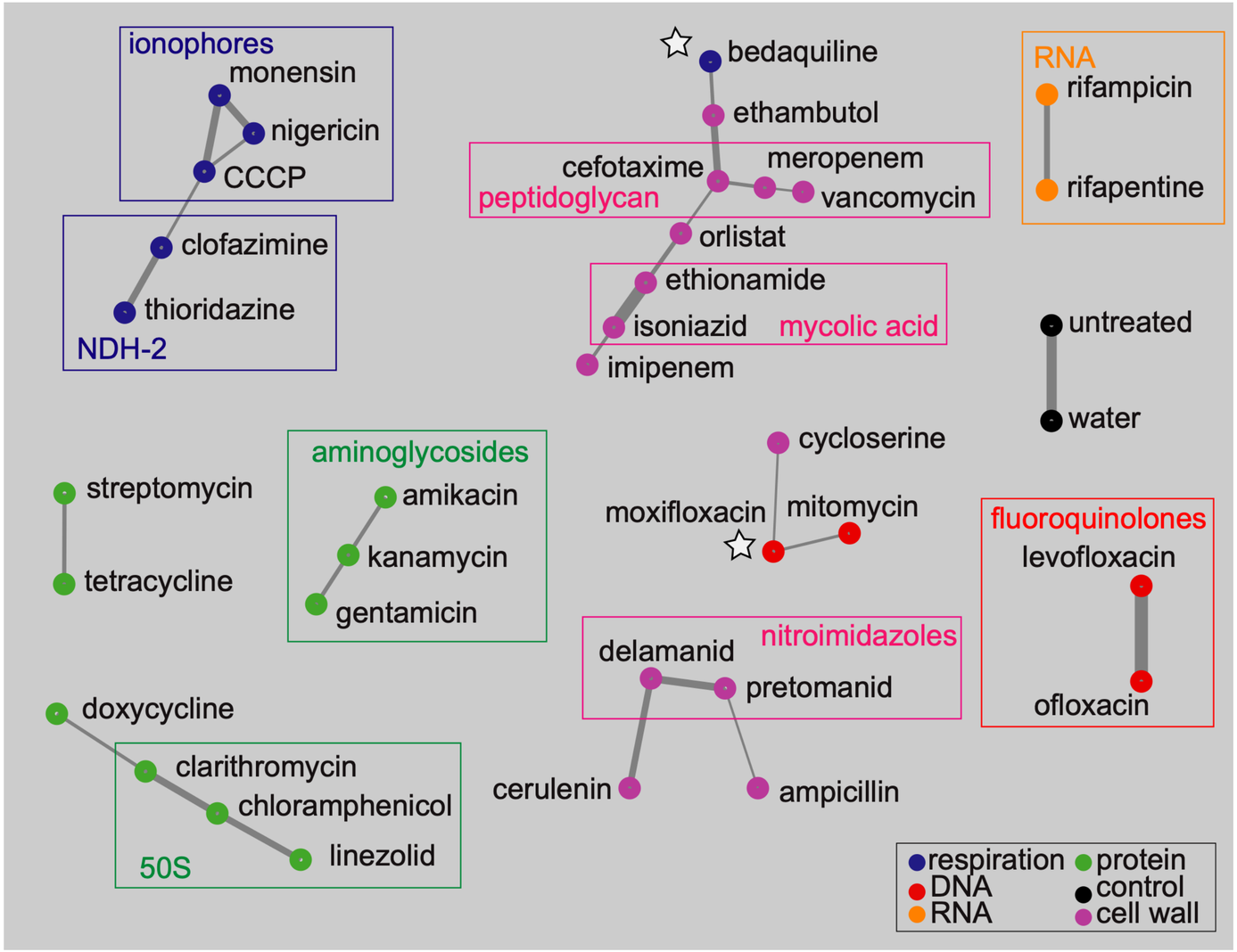
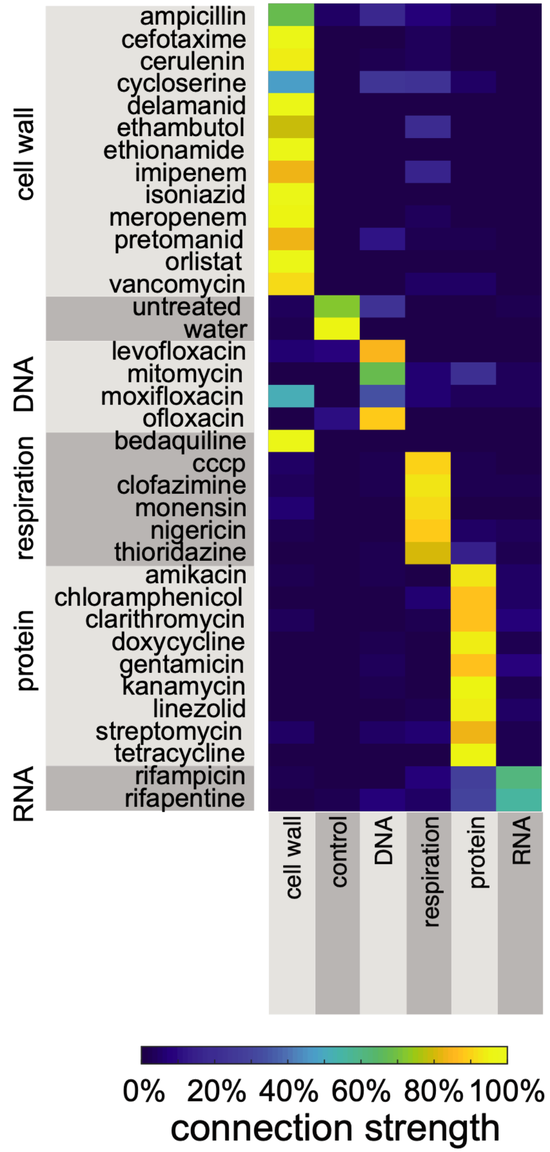


- Sample-sample heterogeneity (batch)
- Cell-to-cell heterogeneity
- Subtle features

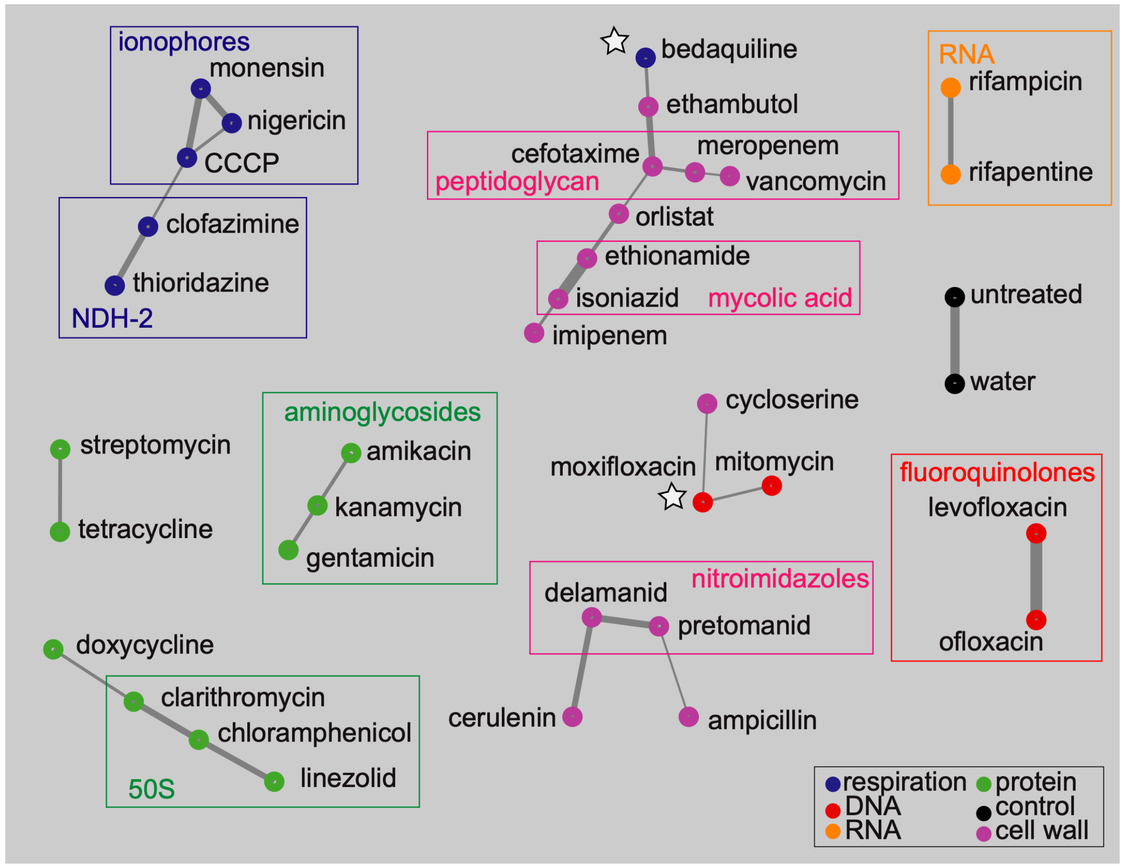
Antibiotics induce morphological changes that are similar within broad cellular targets

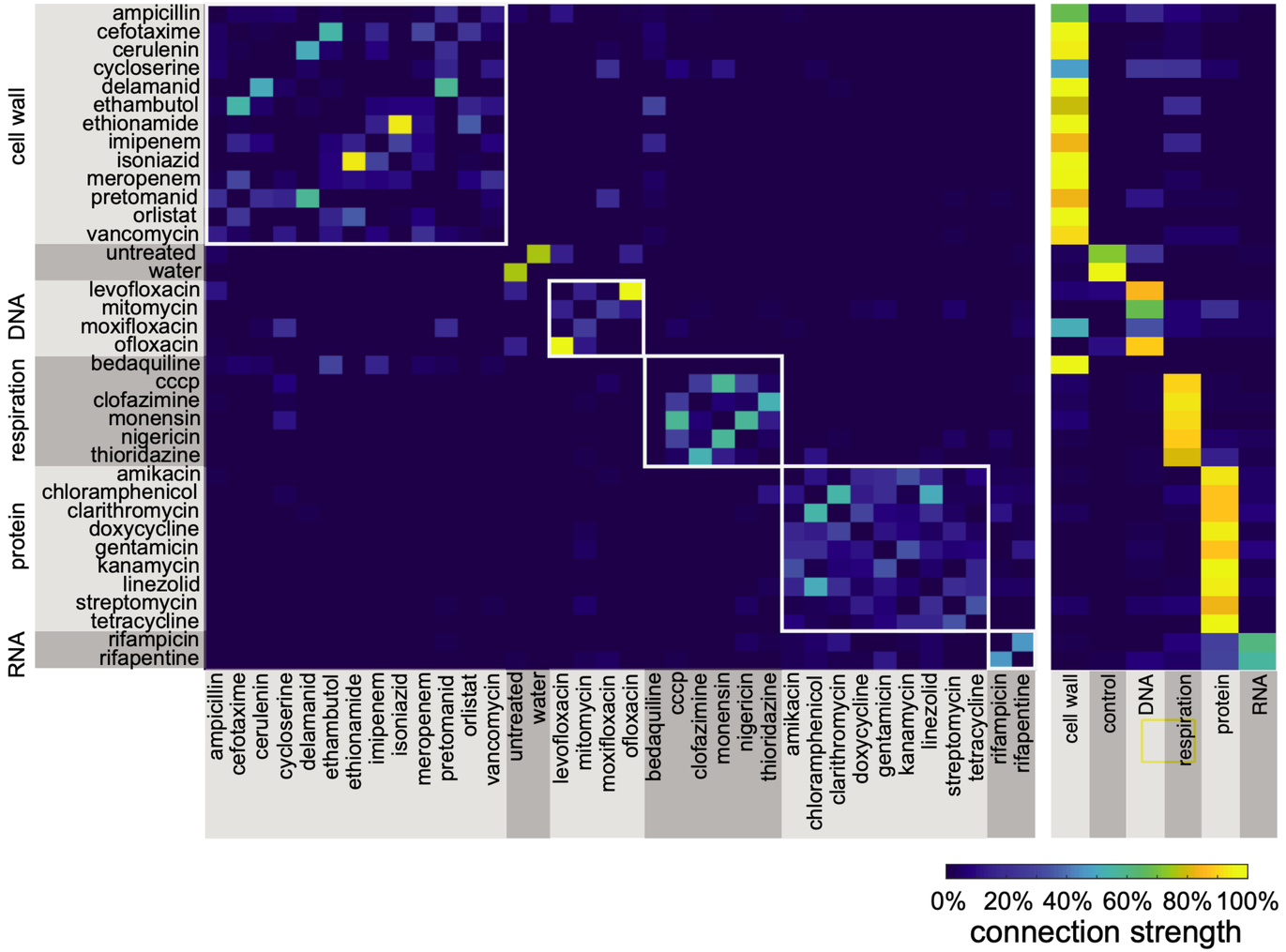


accuracy 94% (cross validation 76%)

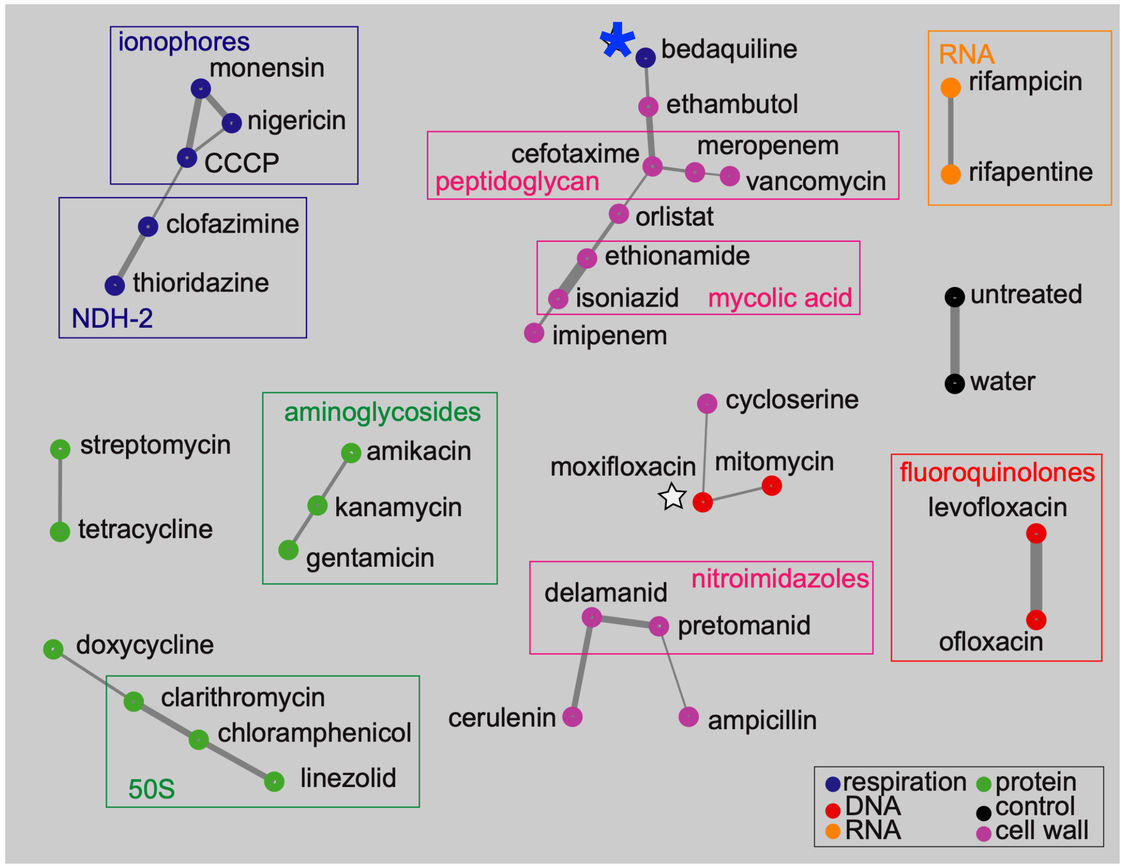


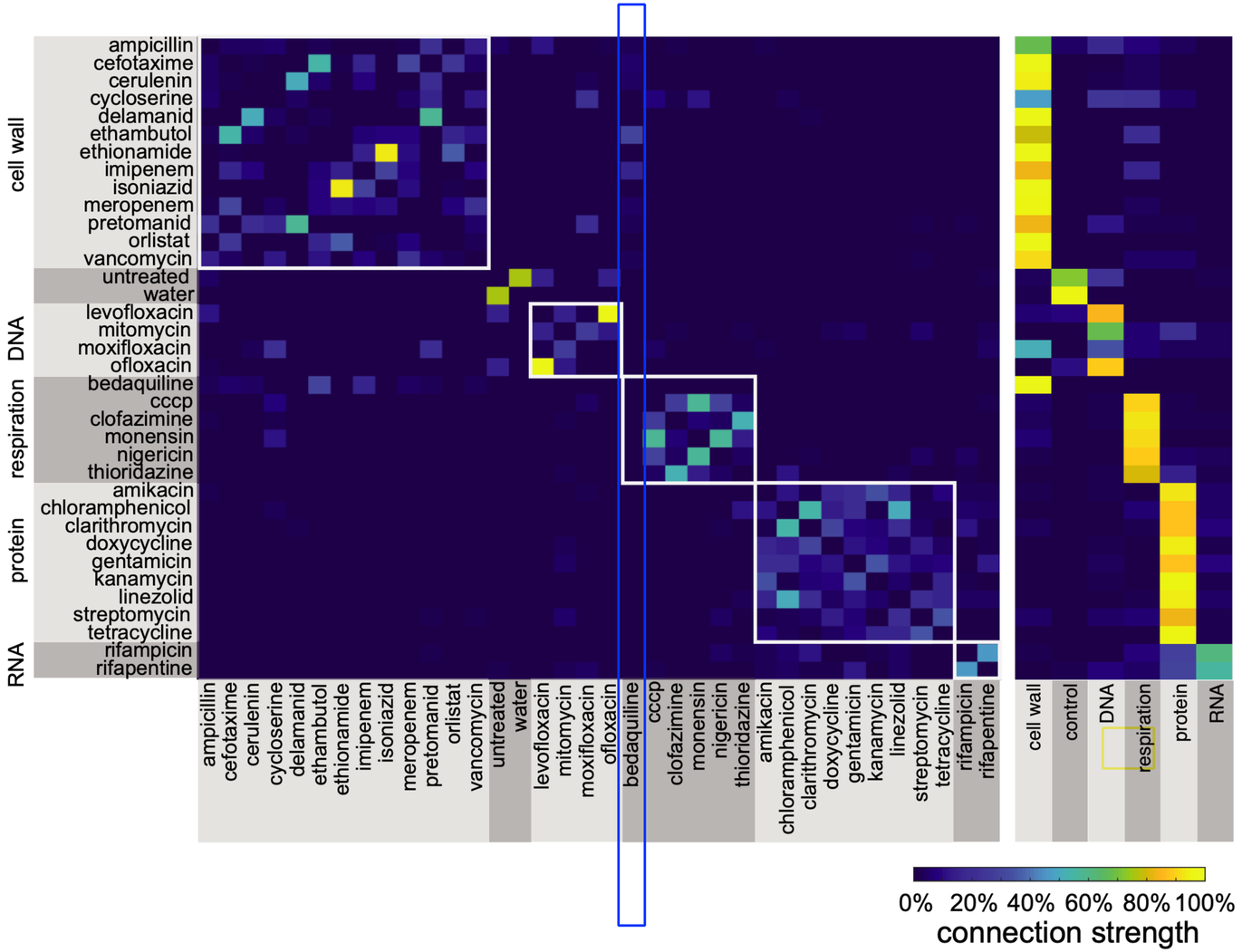
Antibiotics with similar target pathways are well-grouped





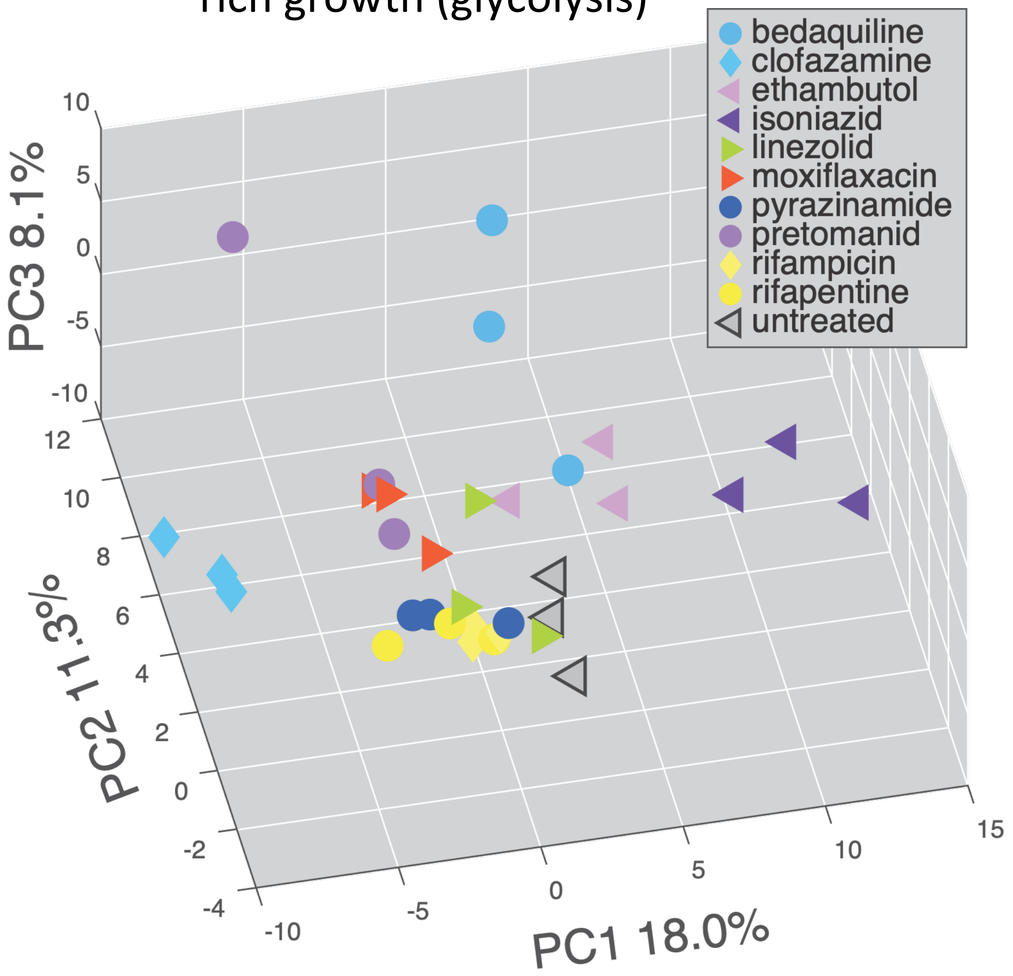
Bedaquiline paradox: energy crisis





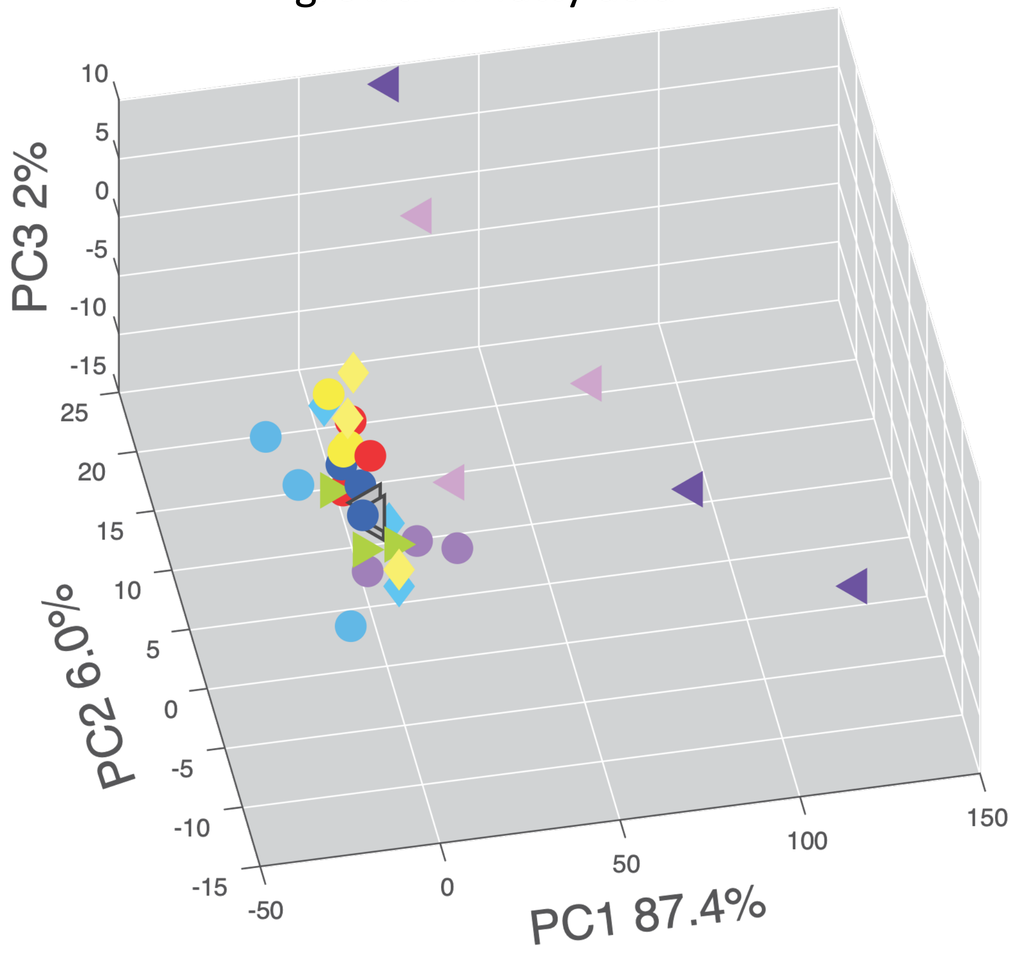
Bedaquiline paradox: energy crisis

rich growth (glycolysis)



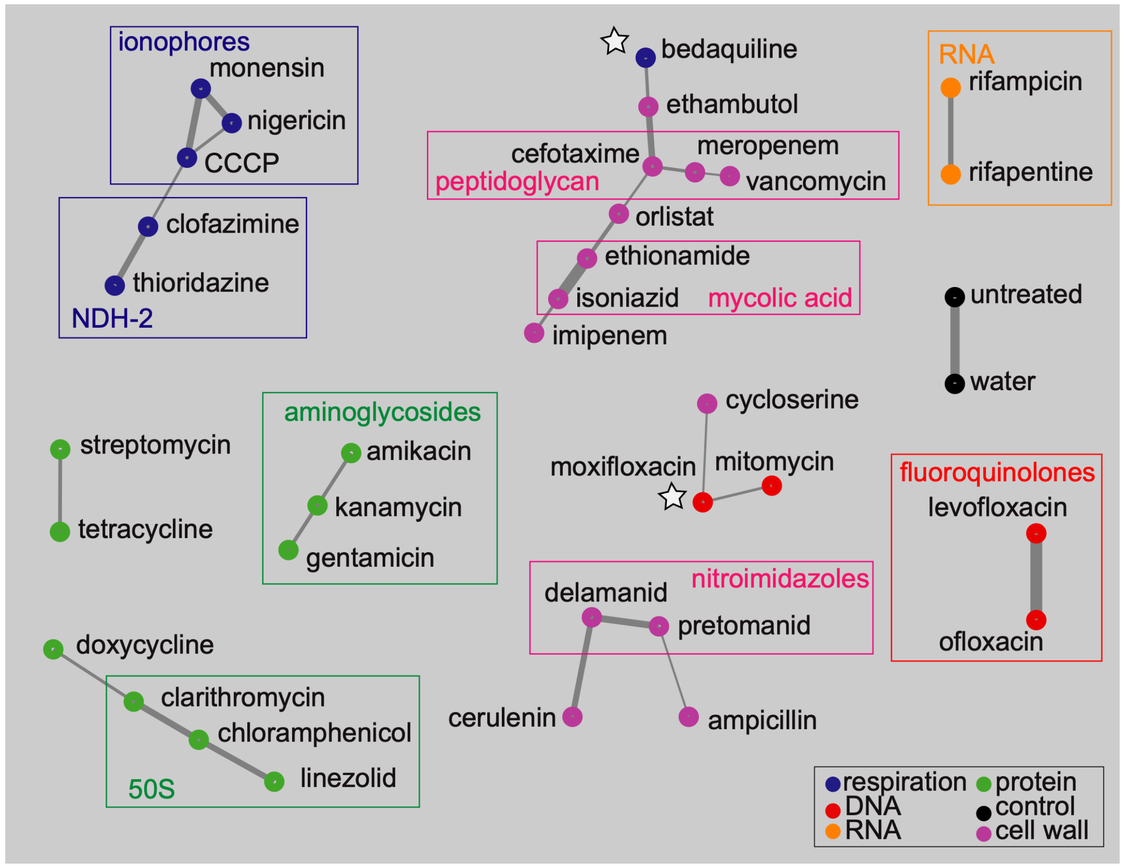
bedaquiline similar to ethambutol & isoniazid

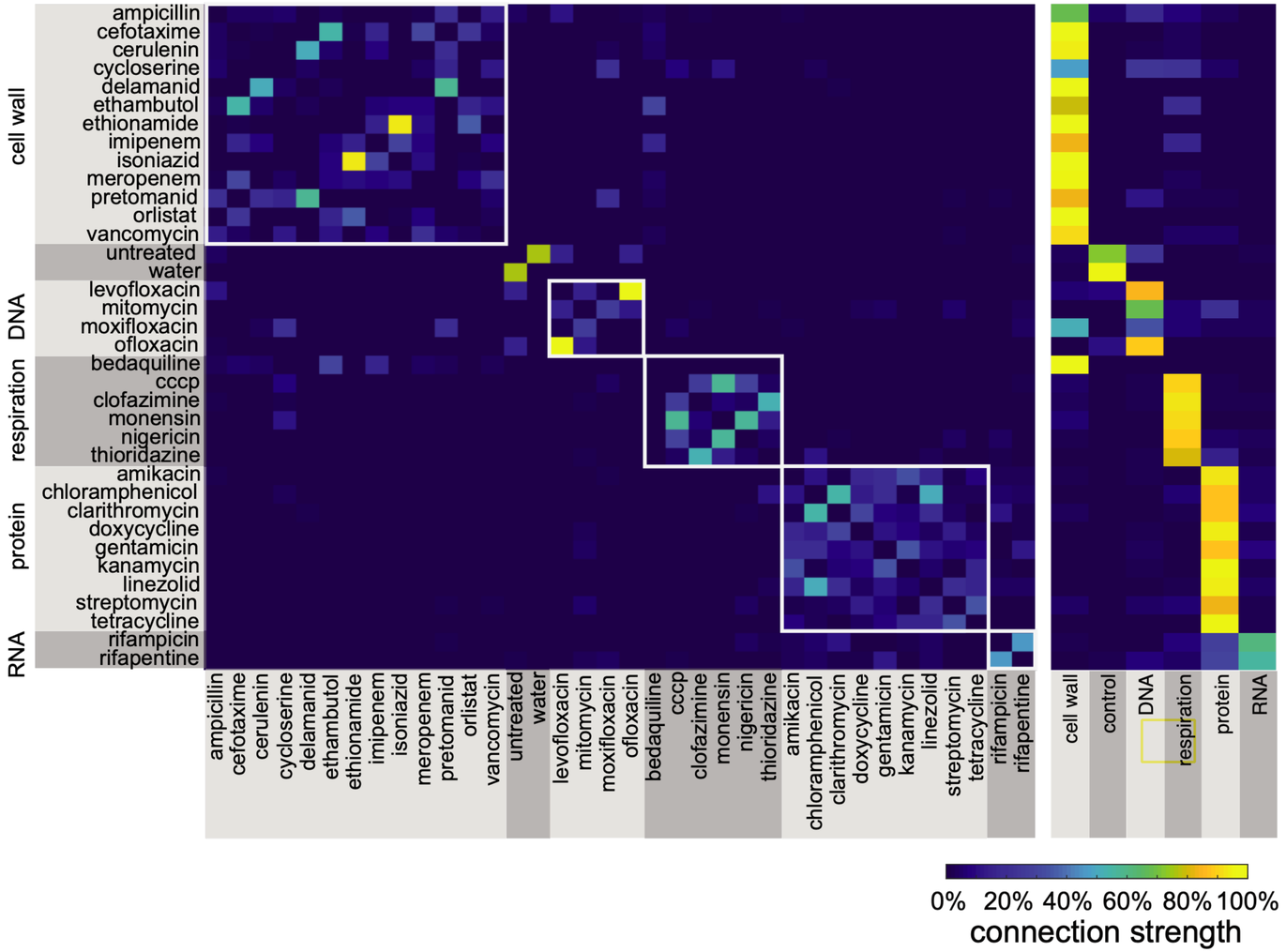
growth in fatty acid



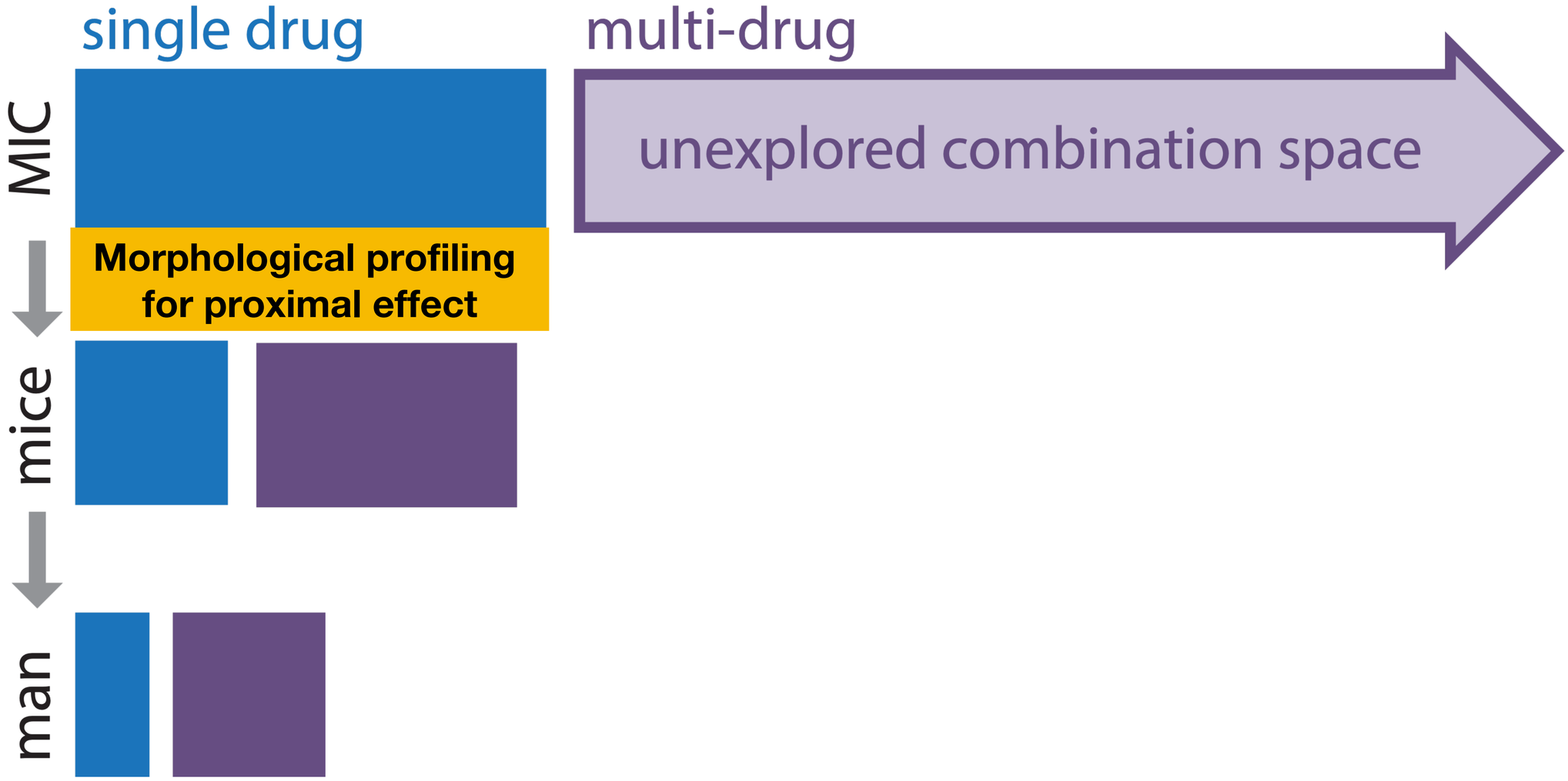
bedaquiline NOT similar to ethambutol & isoniazid

Morphological profiling captures the signatures for the proximal cause of cellular damage

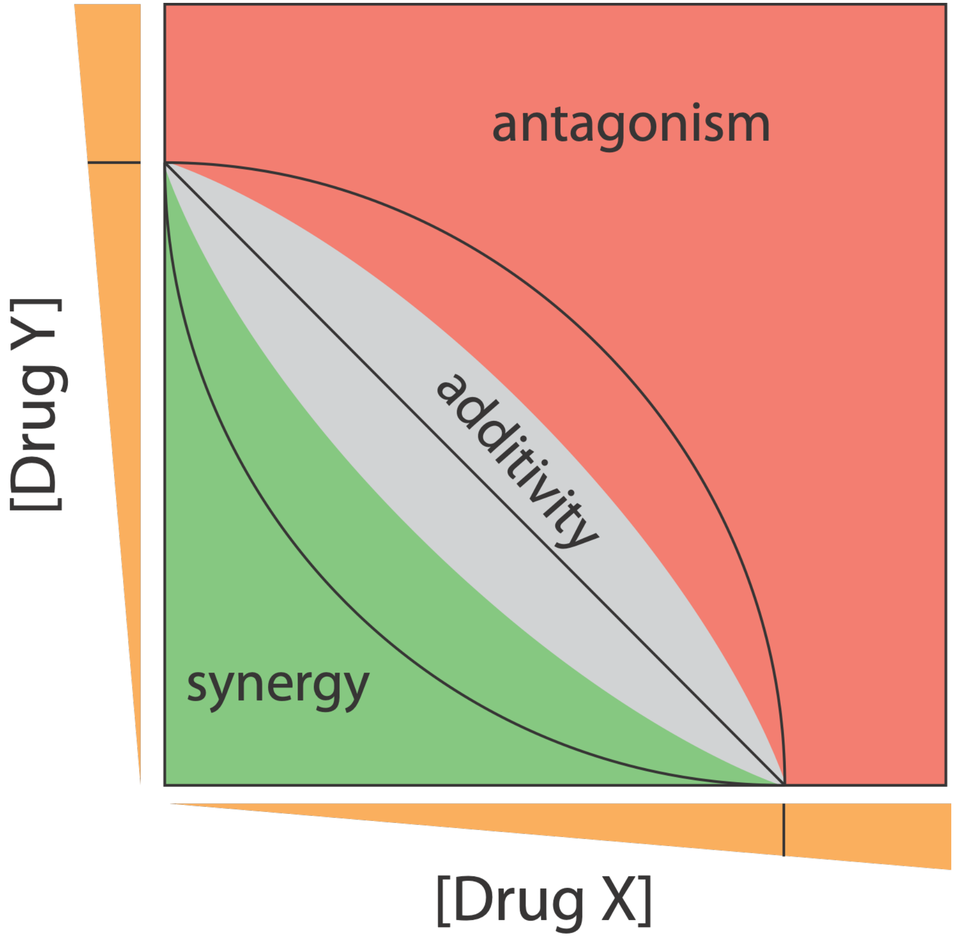


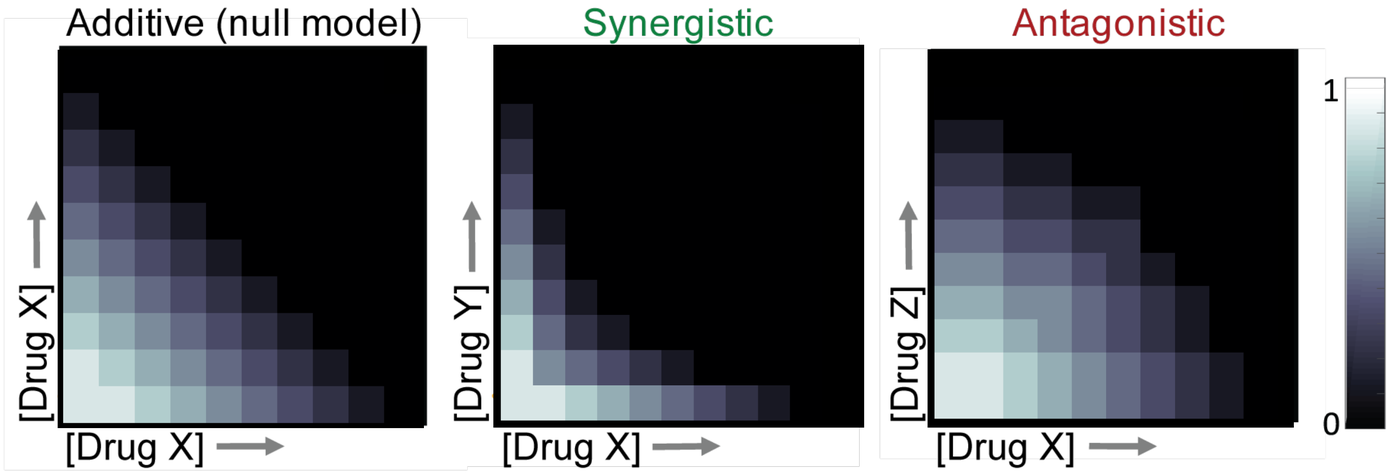


Design of multi-drug therapies

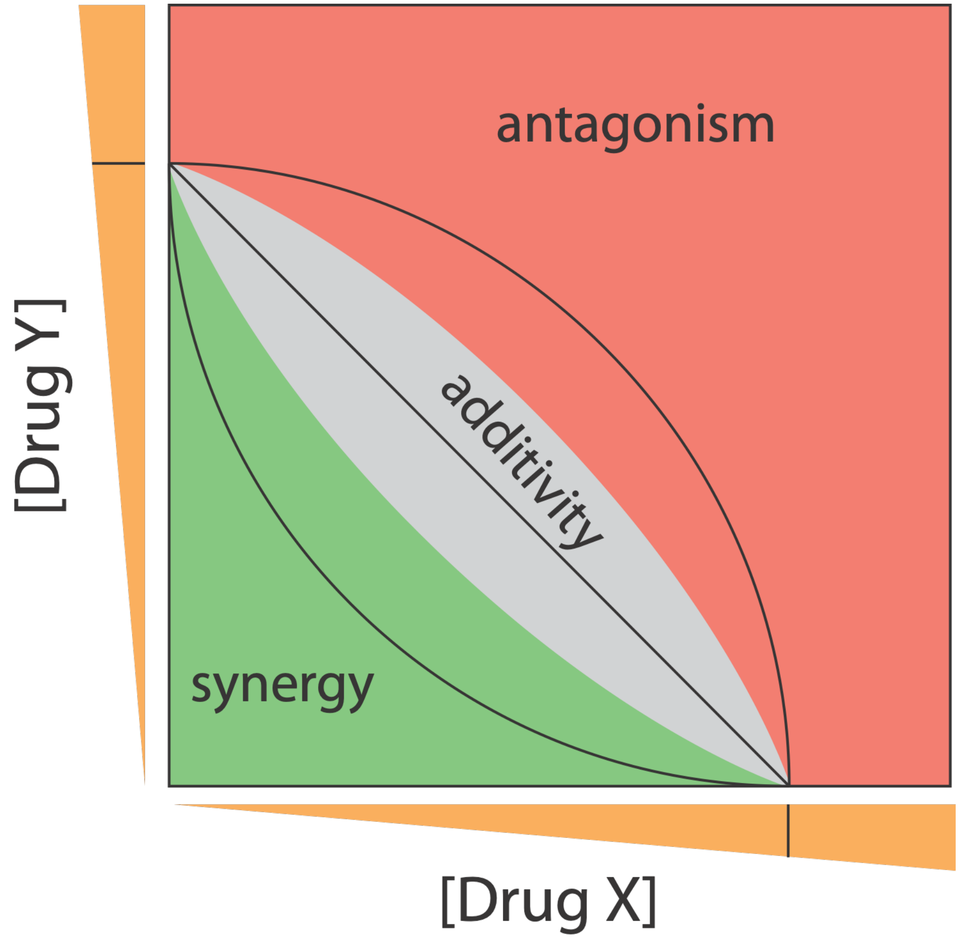


The contour of the checkerboard is a measurement of drug synergy and antagonism





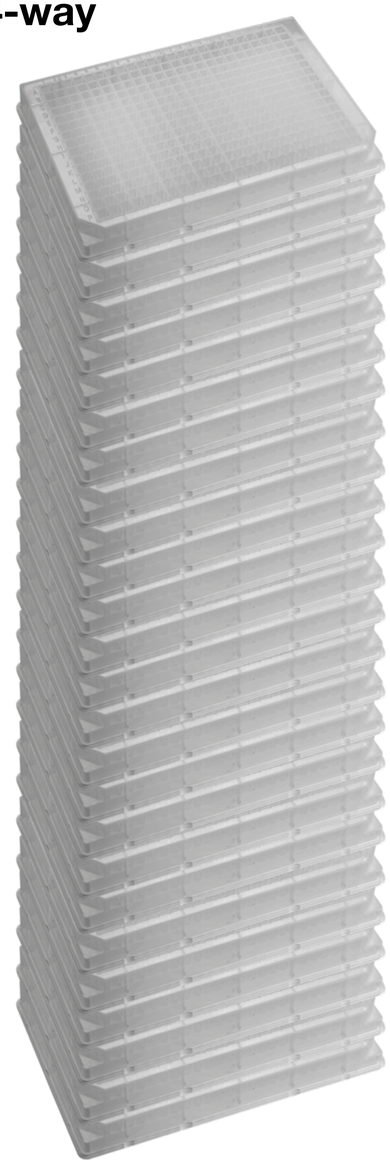
Checkerboard assays are prohibitively inefficient for systematic study



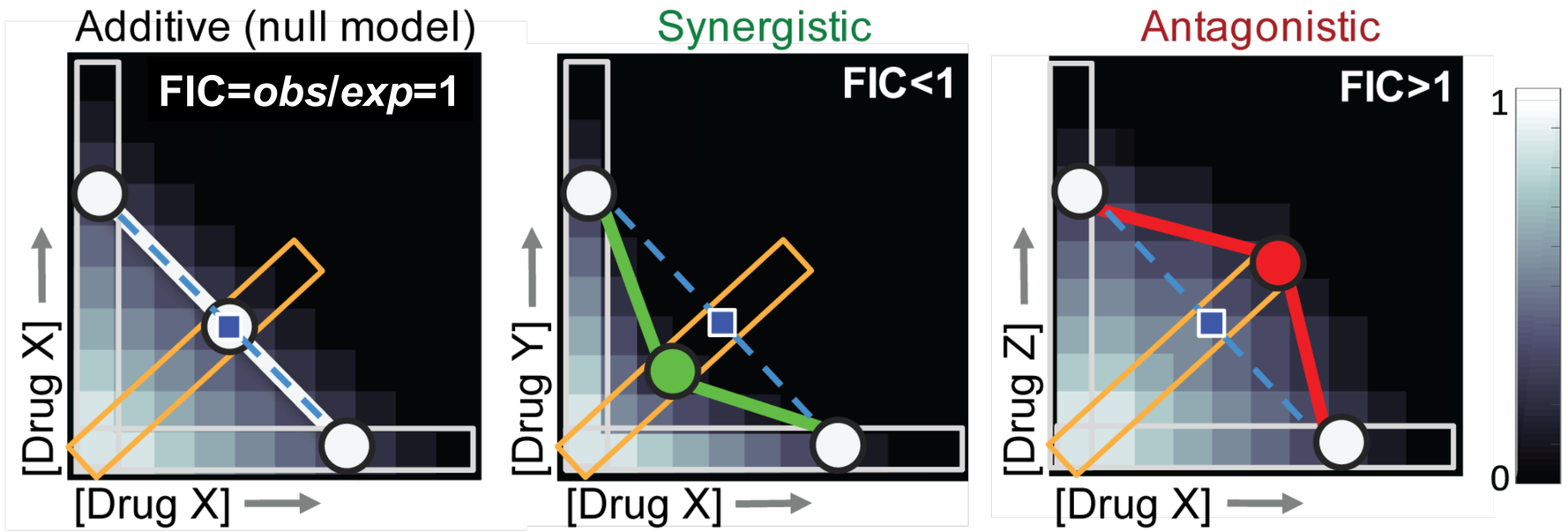
2-way

3-way

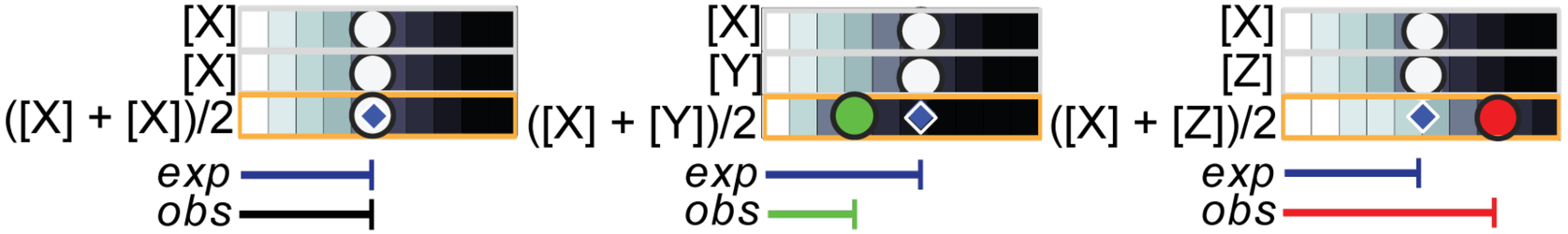
4-way



DiaMOND:
Diagonal measurement of n-way drug interactions

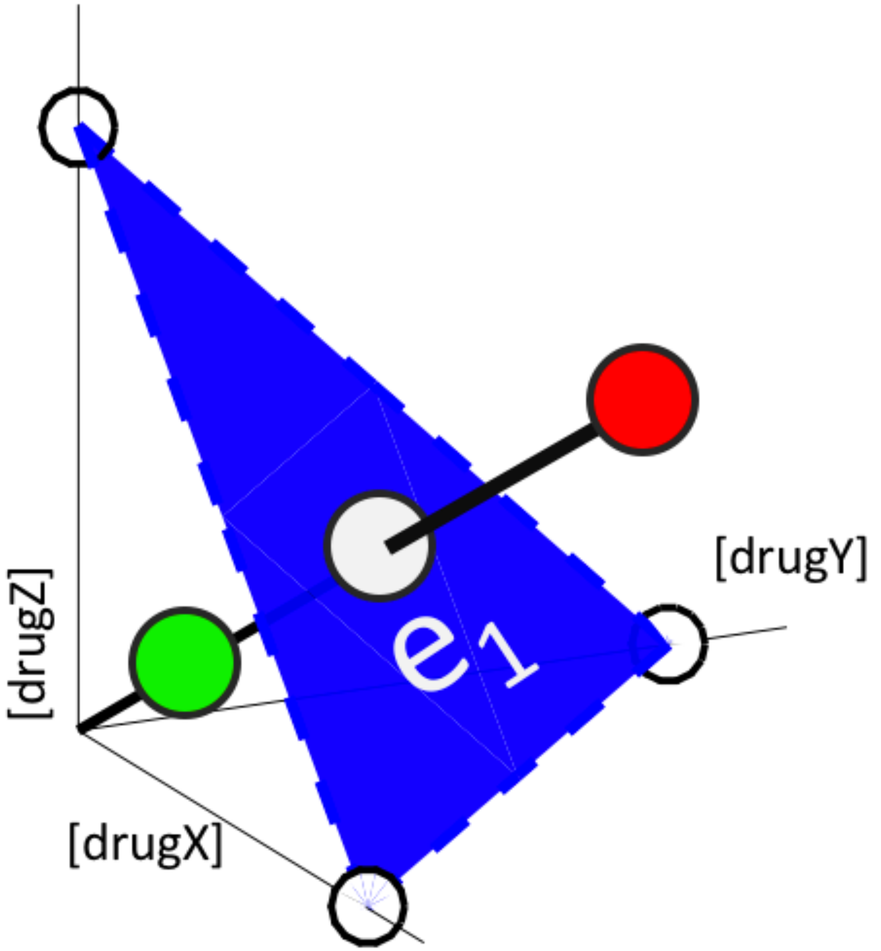


DiaMOND's single-drug and combination dose responses:



Cokol *et al.*, *Science Advances* (2017)

DiaMOND can be extended to high-order interactions



Cokol *et al.*, *Science Advances* (2017)
Katzir et al, *PLoS Computational Biology* (2019)

The idea: prioritize combinations for animal studies based on practical systematic *in vitro* measurement

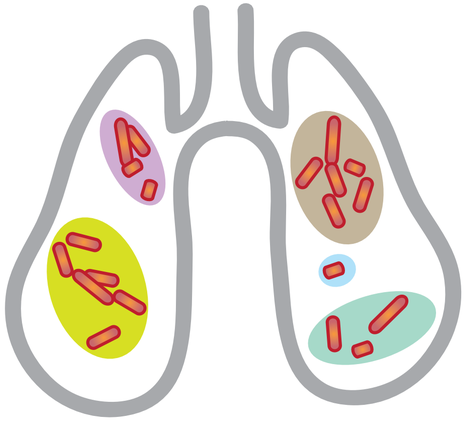
drug combinations

10 drugs

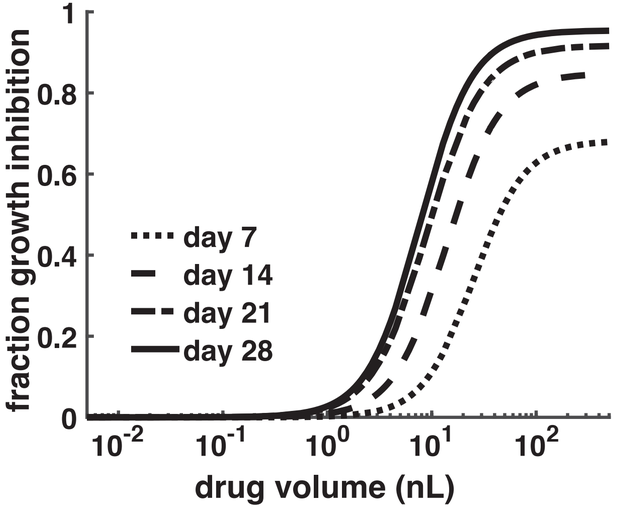
10 single
45 pairwise
120 three-way

X

stress conditions



dynamic dose responses



(1) vast space was not practical to systematically explore
(2) lacked standardized methods to map *in vitro* to animal studies

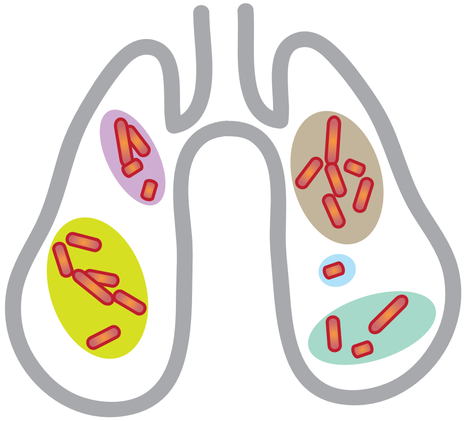
A compendium of drug combination potency and interaction metrics using DiaMOND

drug combinations

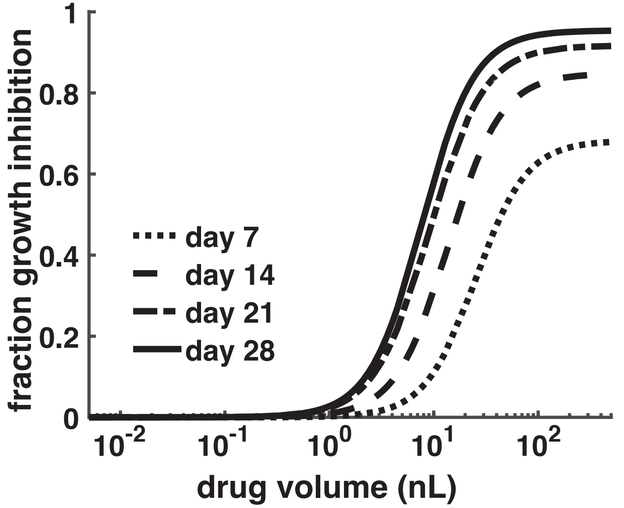
10 drugs ⇄ 10 single, 45 pairwise, 120 three-way

X

stress conditions



dynamic dose responses




Jonah Larkins-Ford


Nhi Van


Yonatan Degefu

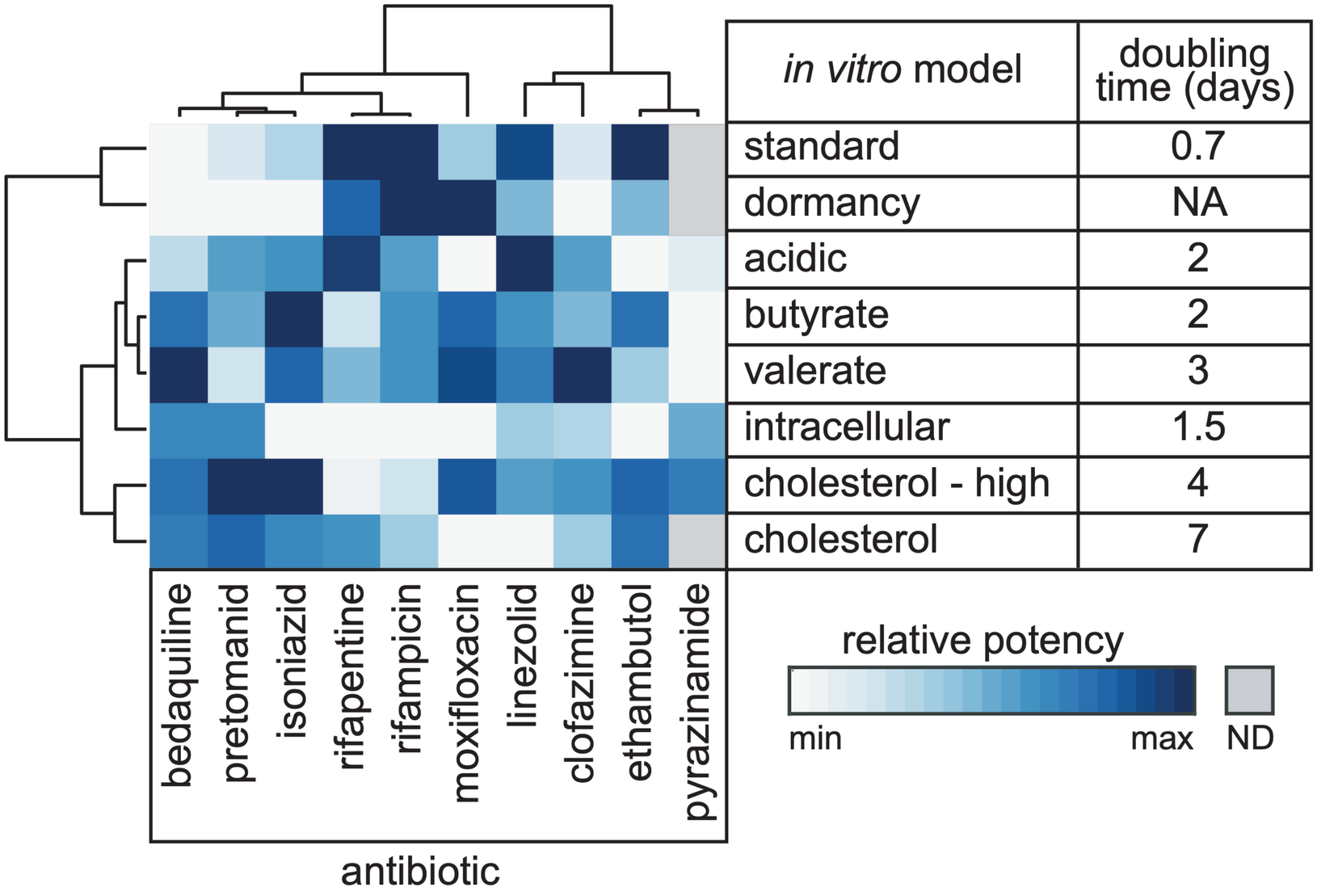

Talia Greenstein


Michaela Olson

175 combinations
>50,000 dose response curves

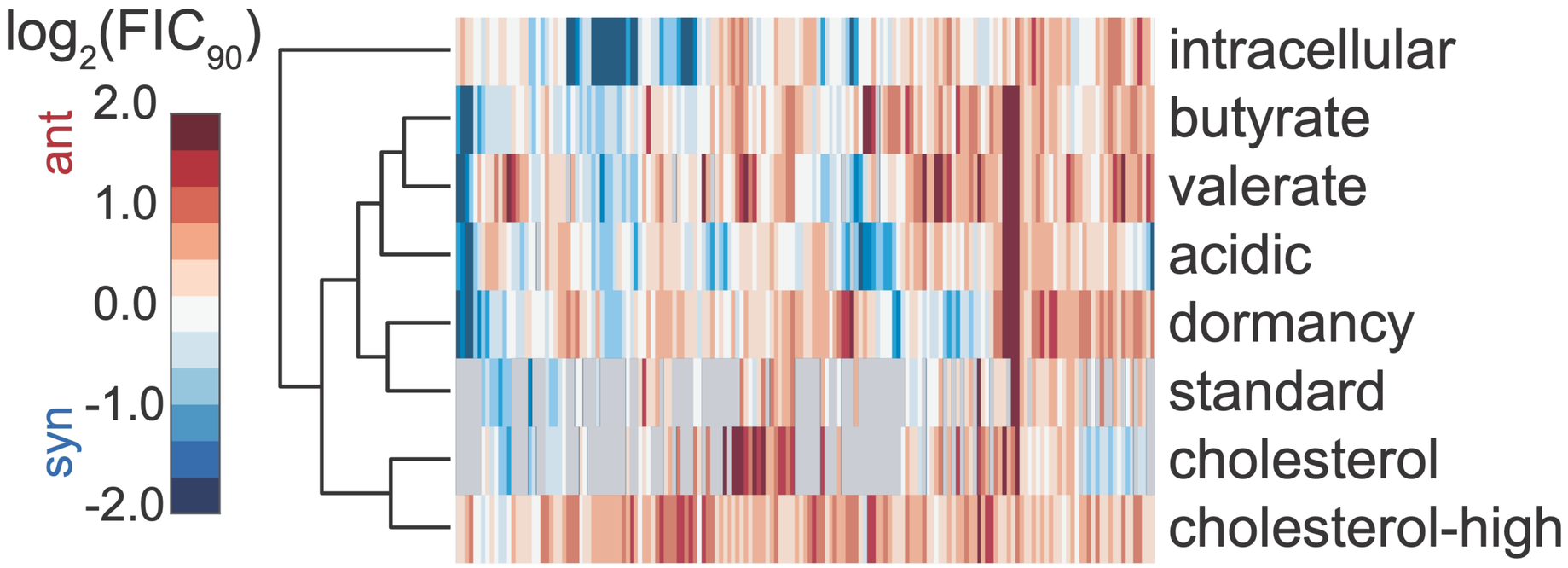
Larkins-Ford et al., *bioRxiv*, 2021

Drug potencies are highly dependent on the growth environment and pathway of action

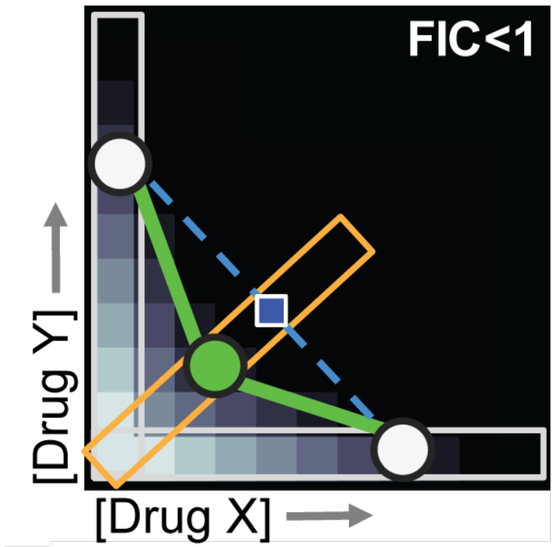


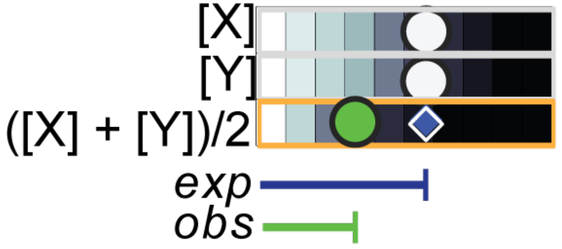
in vitro model	doubling time (days)
standard	0.7
dormancy	NA
acidic	2
butyrate	2
valerate	3
intracellular	1.5
cholesterol - high	4
cholesterol	7

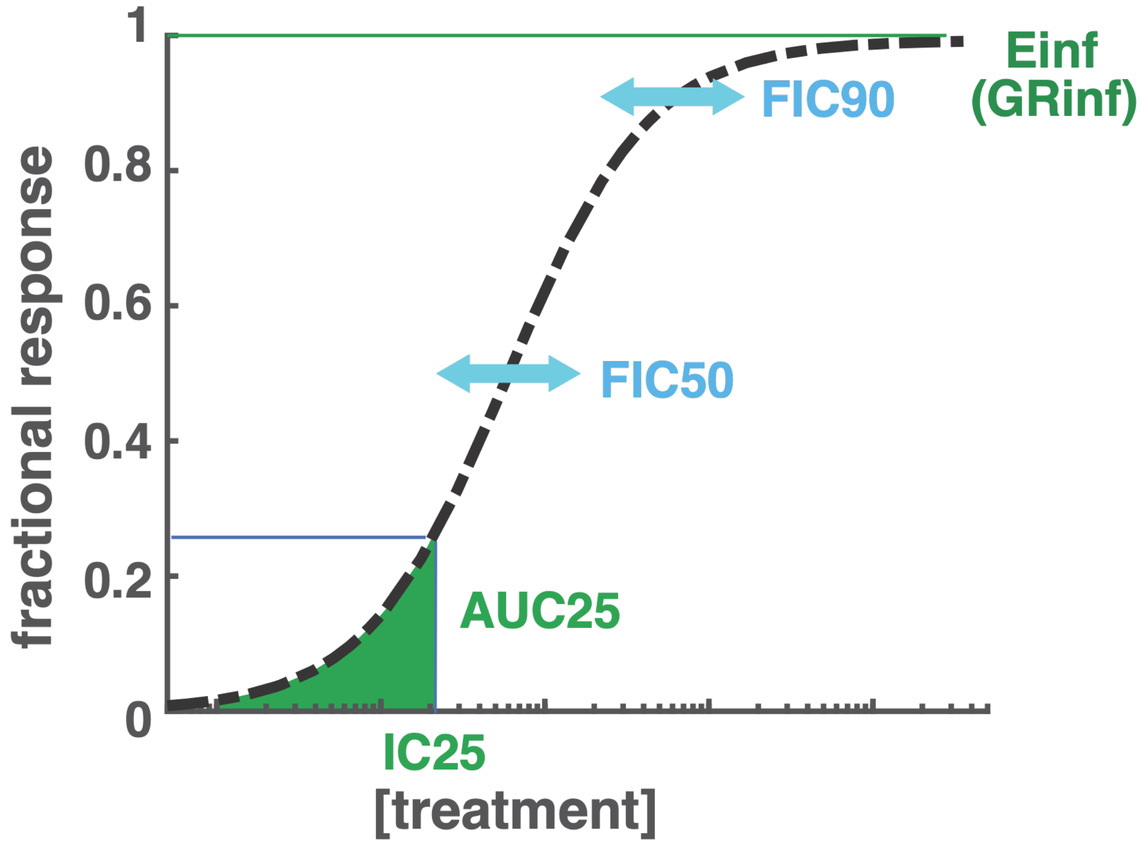
Drug interactions are dependent on the growth environment



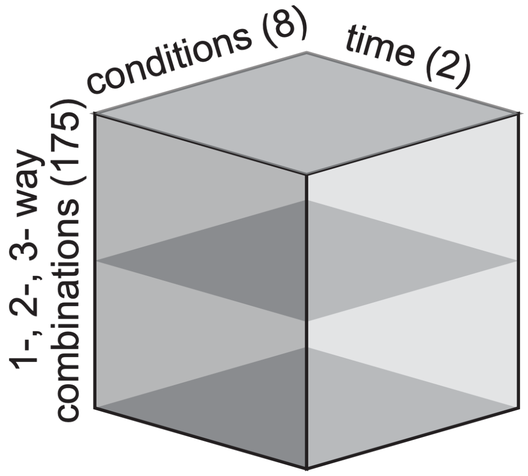
Metrics of drug interactions and potencies from DiaMOND combination dose response curves

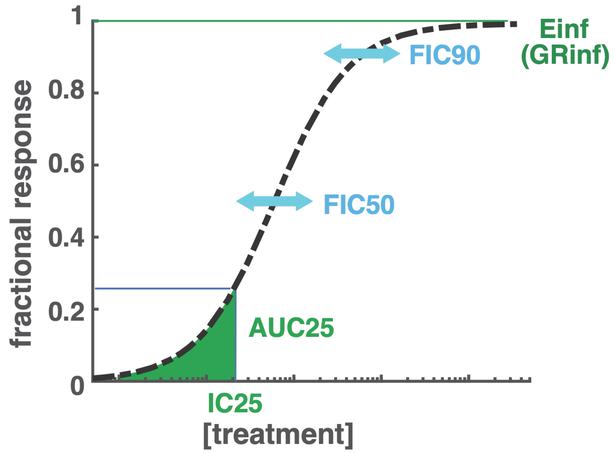


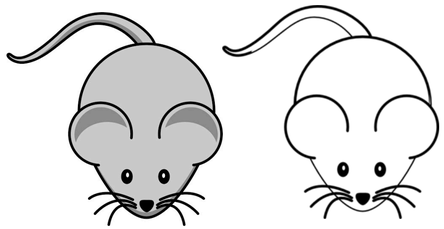




Can combinations of *in vitro* metrics predict *in vivo* outcomes?





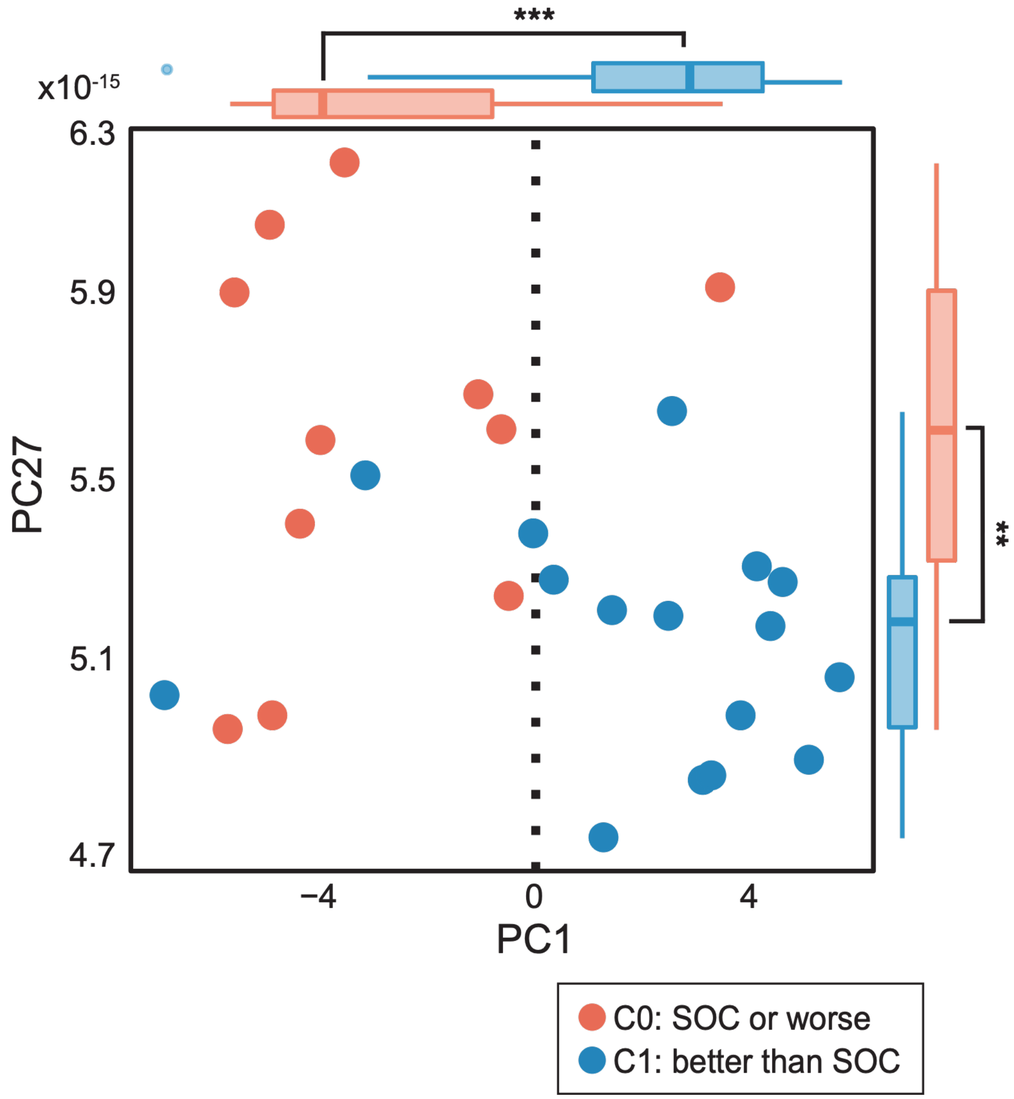


Outcomes in BALB/c relapsing mouse model

- C0: SOC or worse
- C1: better than SOC

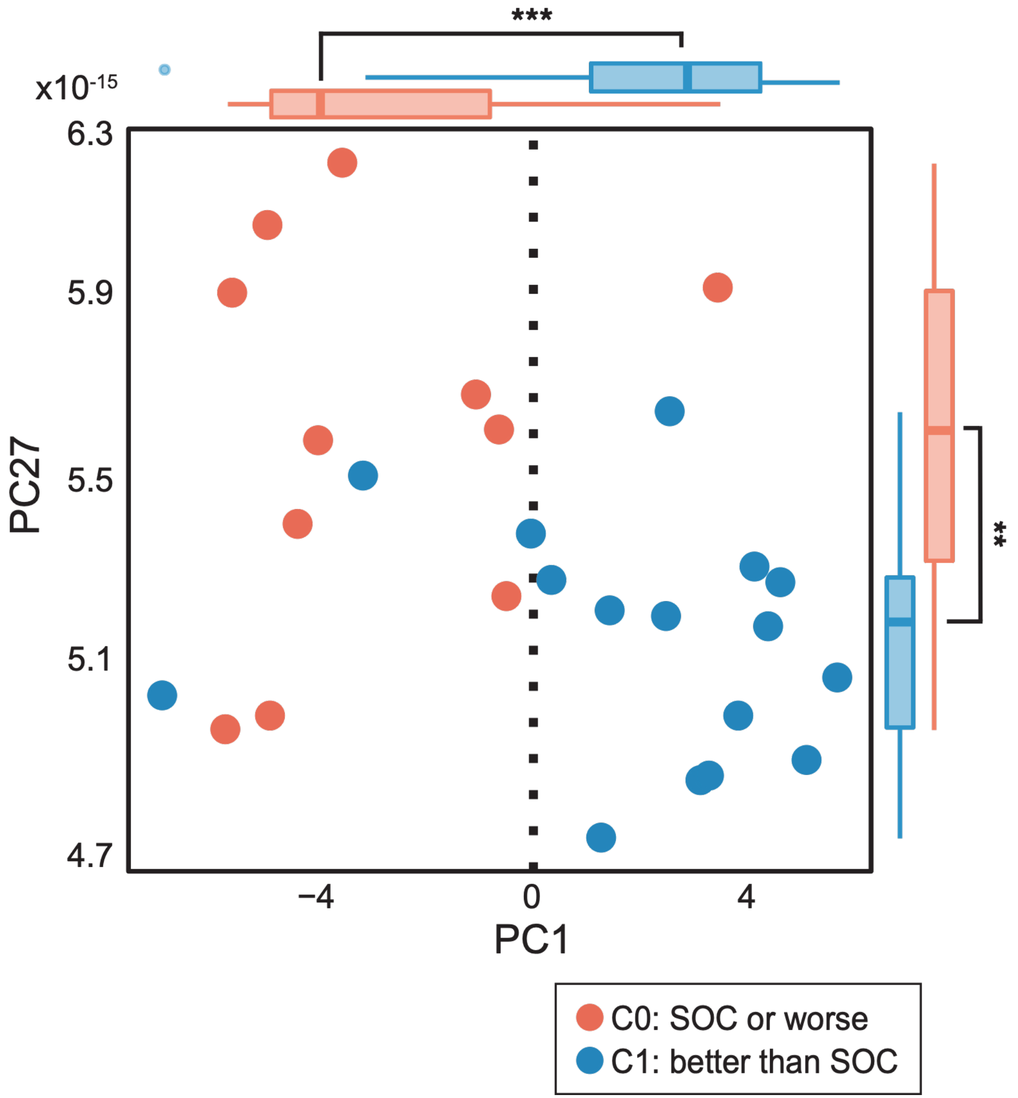
- Trained on 27 combinations
- Tested on 19 combinations

Unsupervised, *in vitro* DiaMOND data distinguishes outcomes in the BALB/c relapsing model



Potency metrics in lipid-rich and acidic environments drive classification of relapse outcome

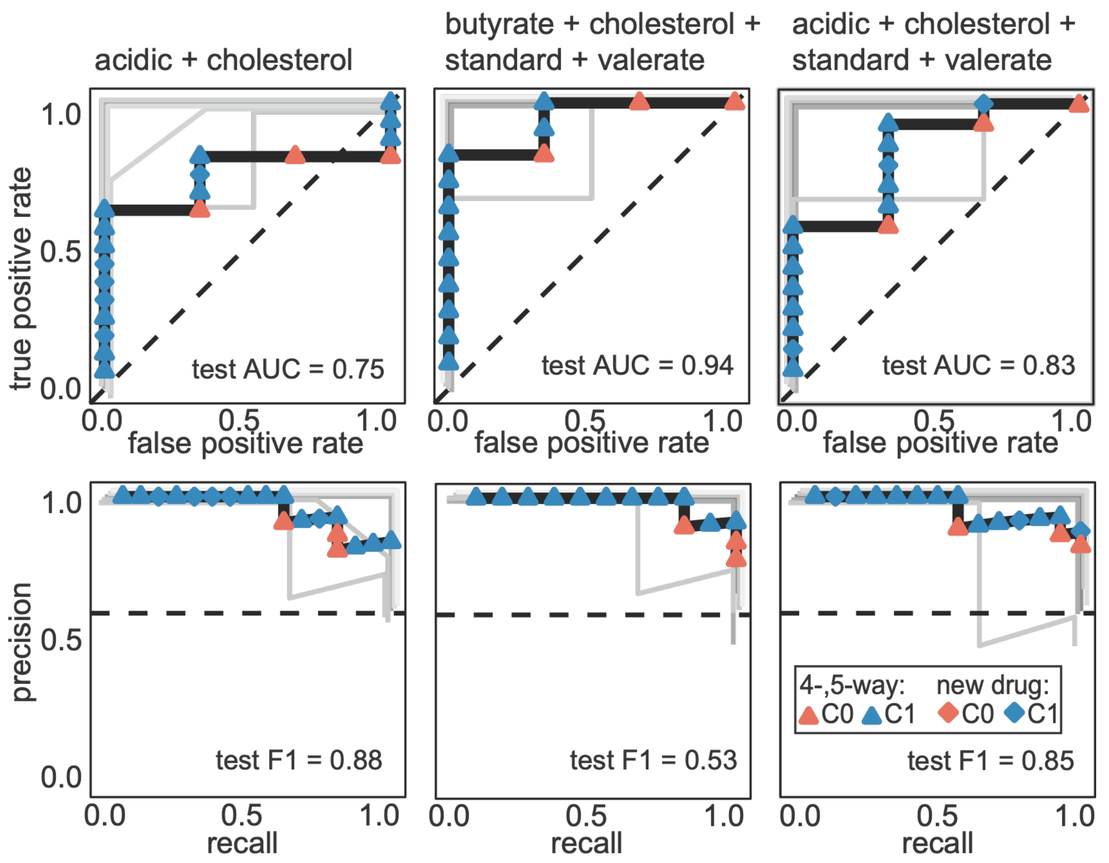
model	metric
standard	GR_{inf}
standard	E_{inf}
acidic	$GR_{inf}(T)$
cholesterol	$E_{inf}(C)$
acidic	$GR_{inf}(C)$
butyrate	$E_{inf}(C)$
valerate	$E_{inf}(C)$
valerate	$AUC_{25}(T)$
valerate	$GR_{inf}(T)$
cholesterol-high	$FIC_{50}(T)$
dormancy	$E_{inf}(C)$
cholesterol	$FIC_{50}(T)$
cholesterol	$E_{inf}(T)$
valerate	$AUC_{25}(T)$
cholesterol-high	$E_{inf}(C)$
cholesterol	$E_{inf}(C)$
standard	AUC_{25}
valerate	$GR_{inf}(C)$
cholesterol	$GR_{inf}(C)$
acidic	$AUC_{25}(T)$



A suite of simple *in vitro* models can be used for predictive drug response measurement

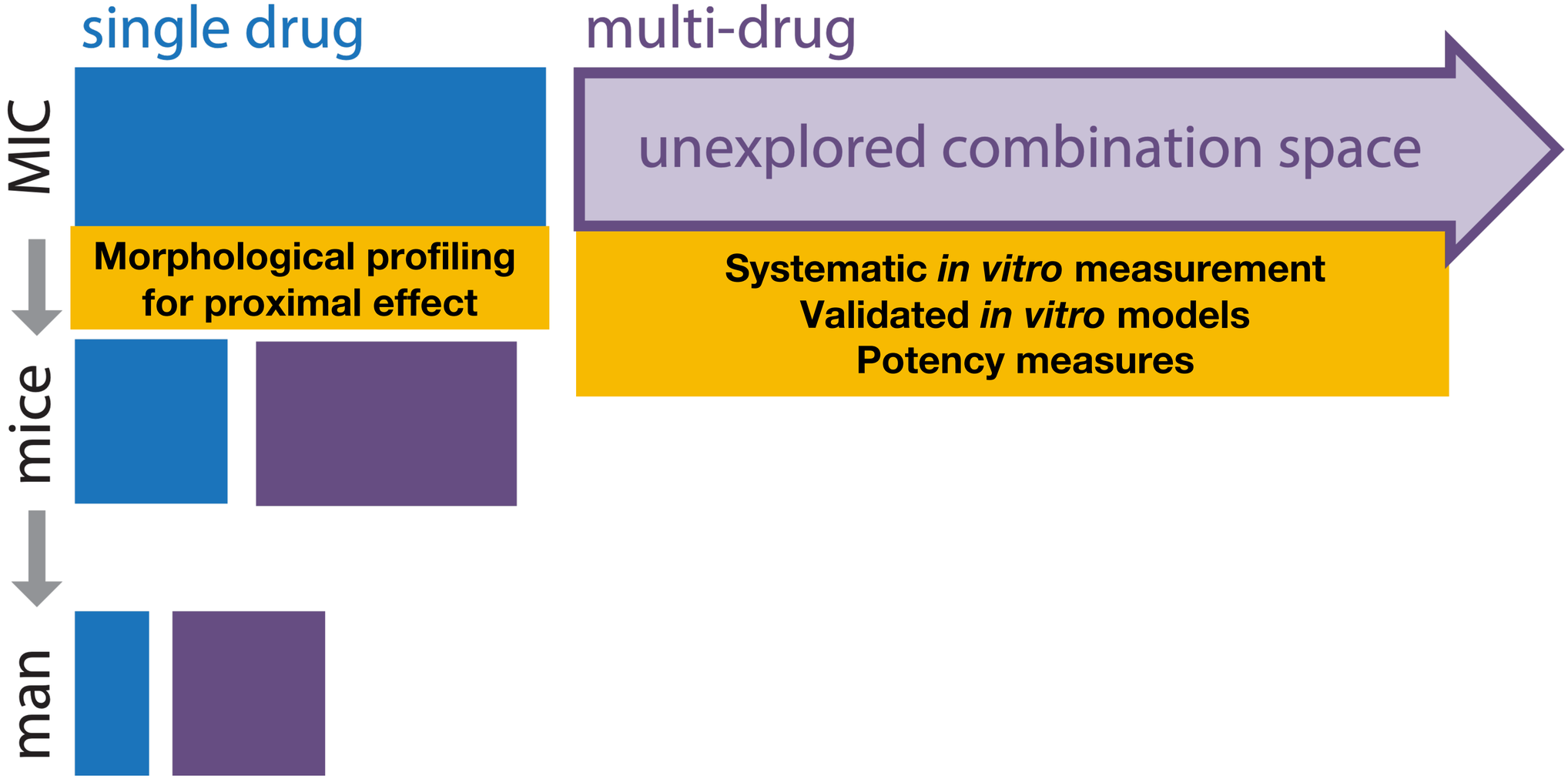
model	metric
standard	GR_{inf}
standard	E_{inf}
acidic	$GR_{inf}(T)$
cholesterol	$E_{inf}(C)$
acidic	$GR_{inf}(C)$
butyrate	$E_{inf}(C)$
valerate	$E_{inf}(C)$
valerate	$AUC_{25}(T)$
valerate	$GR_{inf}(T)$
cholesterol-high	$FIC_{50}(T)$
dormancy	$E_{inf}(C)$
cholesterol	$FIC_{50}(T)$
cholesterol	$E_{inf}(T)$
valerate	$AUC_{25}(T)$
cholesterol-high	$E_{inf}(C)$
cholesterol	$E_{inf}(C)$
standard	AUC_{25}
valerate	$GR_{inf}(C)$
cholesterol	$GR_{inf}(C)$
acidic	$AUC_{25}(T)$

Top classifiers using subsets of simple growth conditions:



Relapsing mouse model

Design of multi-drug therapies



Many thanks to…

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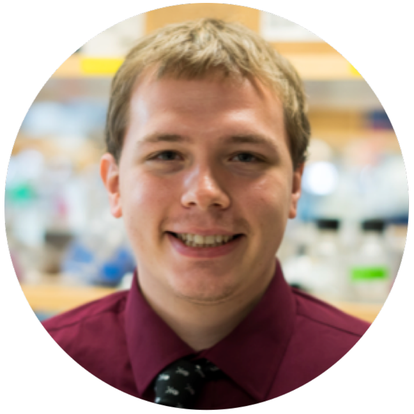
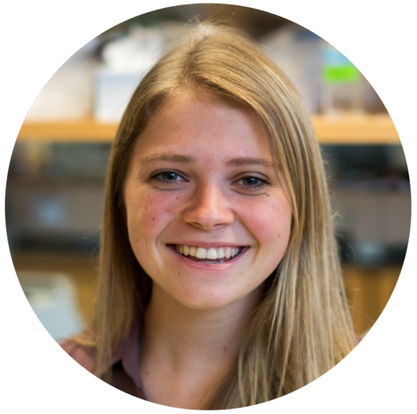


Trever Smith, PhD



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