Probing metabolic variation in Mycobacterium Tuberculosis using qHTS; a systems approach to uncovering novel targets

Clifton E. Barry, 3rd
Tuberculosis Research Section, NIAID

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CDD Community Meeting

TBRS

1/3 of the worlds population is infected with TB.

- TB is responsible for 25% of adult deaths in the developing world.
- 8-12 million new infections with *M. tuberculosis* per year, 2-3 million people die from TB per year.
- TB and AIDS exhibit a deadly synergy
- There has been a surge of multi-drug resistant (MDR) TB and eXtensively-drug resistant (XDR) TB that threatens even the developed world

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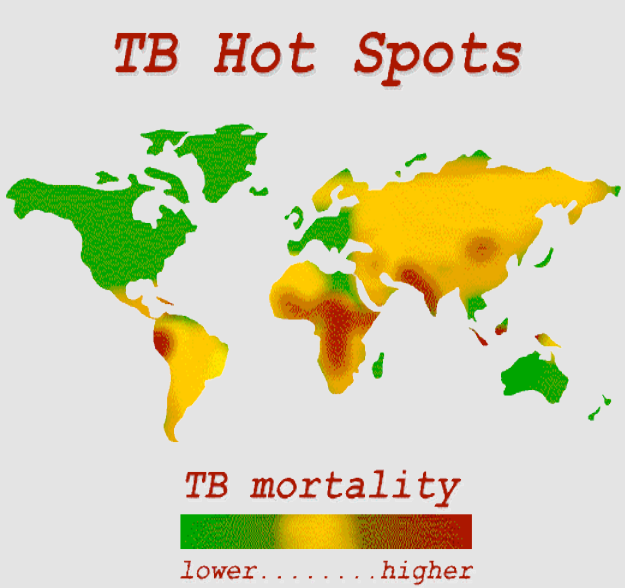
TB Chemotherapy is highly effective, greater than 95% of patients who complete their therapy are cured under ideal circumstances. But in the real world circumstances are far from ideal and delivering 6 to 8 months of therapy is a logistical nightmare



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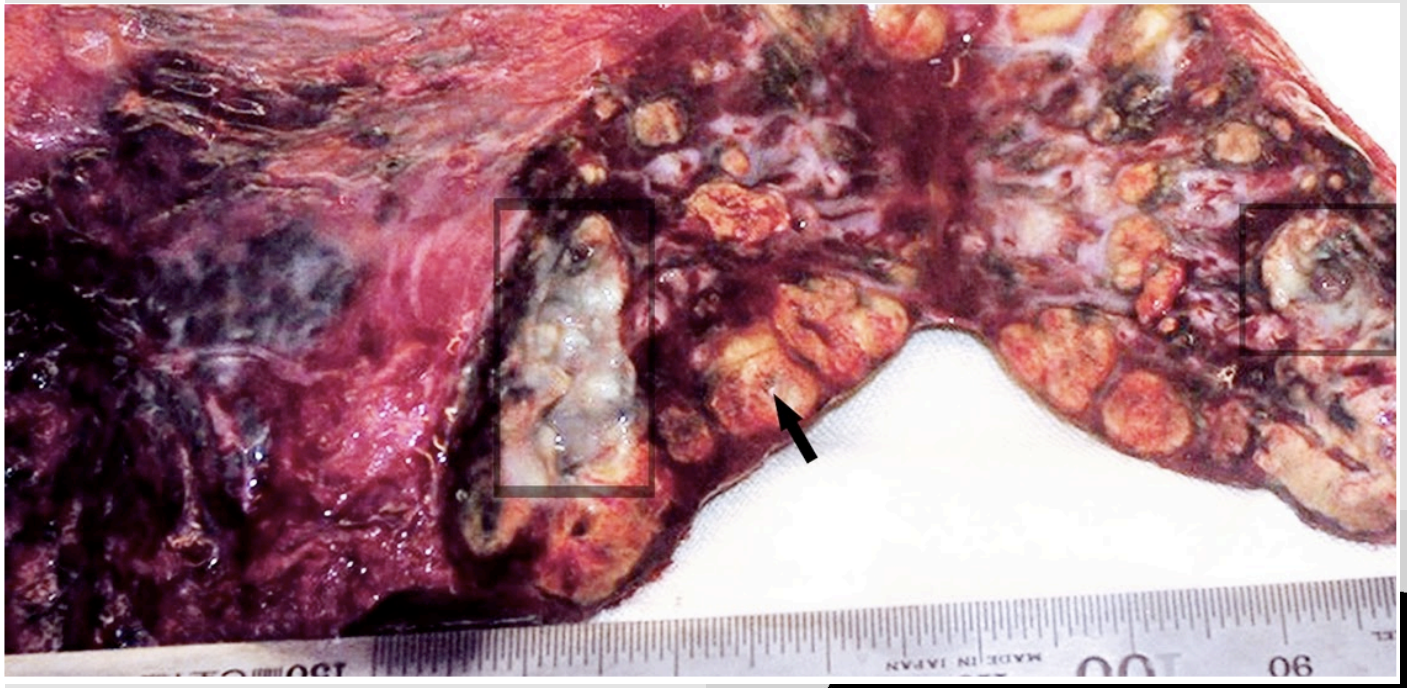
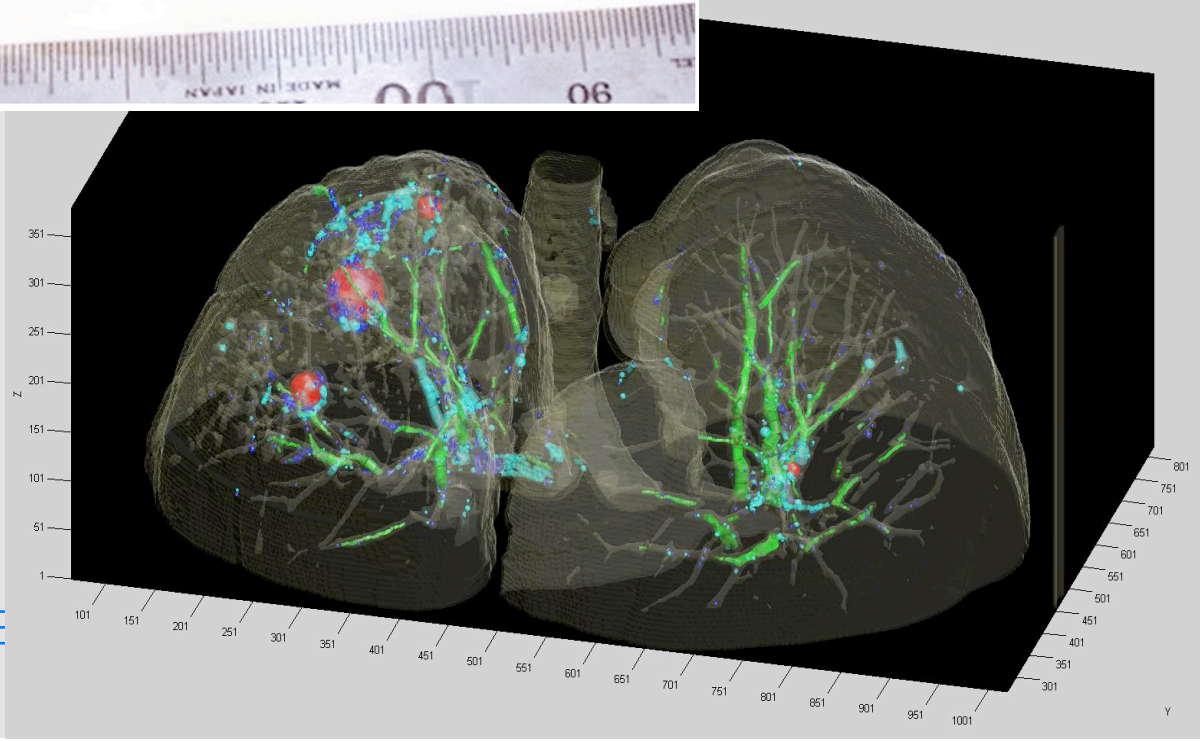
The key to shortening therapy – understanding the system

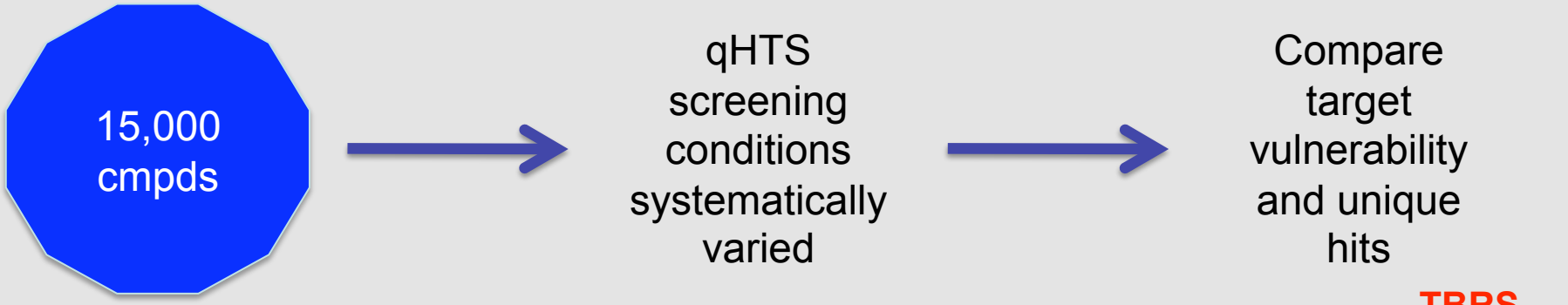


Upper lobectomy of a
19 year old male with MDR-TB



Towards a more predictive *in vitro* screen

- We don't know the precise environmental conditions
- We don't know the replication status of the organism
- Target vulnerability is thought to vary as a function of both of these
- Can we explore the limits of target vulnerability and identify new targets by smarter screening?



7H9/Tween-80/Glucose/Glycerol

↓

7H9/Tyloxapol/Glucose/Glycerol

↓

7H9/Tyloxapol/Glucose

↓

7H9/Tyloxapol/Glycerol

↓

7H9/Tyloxapol/Acetate

↓

7H9/Tyloxapol/Butyrate

↓

7H9/Tyloxapol/Cholesterol

↓

7H9/Tyloxapol/Isovalerate

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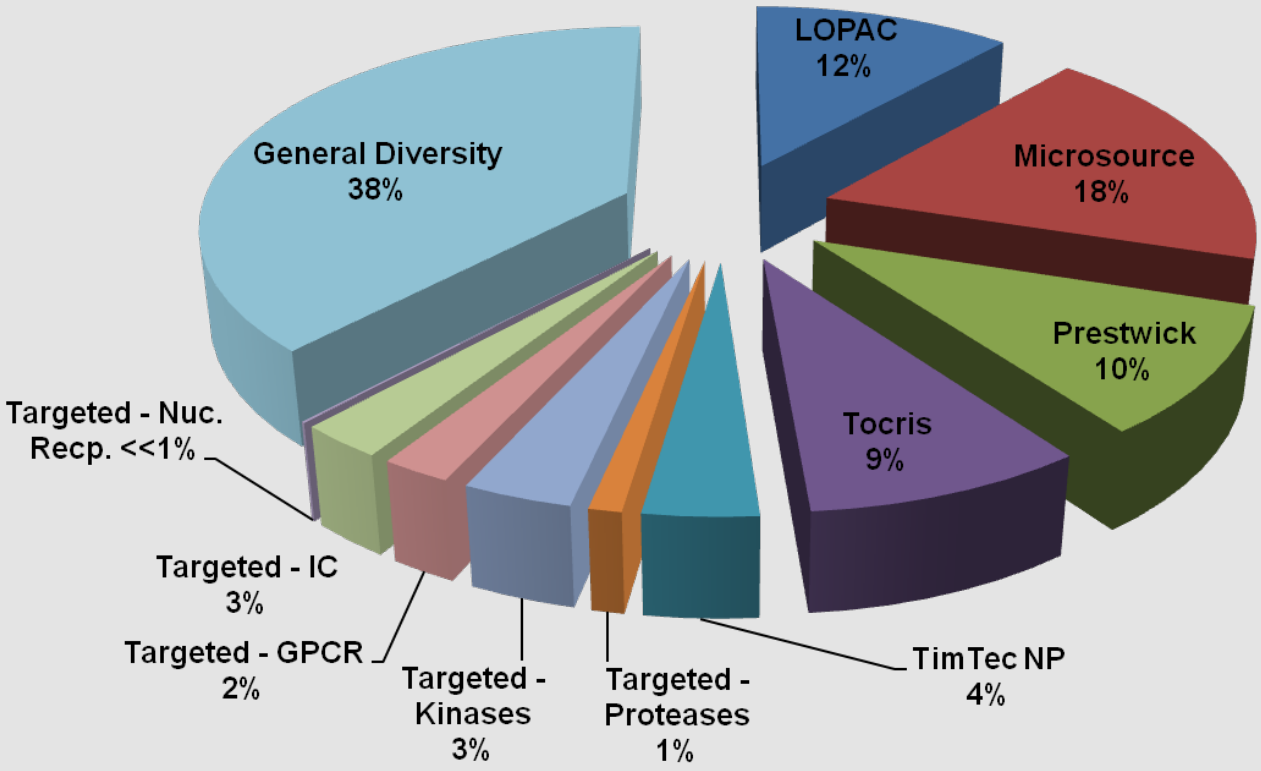
Screening library: Compound Classification of 15k Chemical Library



Composition:

•Targeted Libraries:
- Chemotypes focused on proteases, kinases, GPCRs, ion channels, nuclear receptors

•Diversity Collection:
- 'Drug like' compounds with MW < 500, LogP < 5

- Small collection of natural product derived compounds

•Known actives collection:
-Sigma's LOPAC^{1280}
-Microsource collection
-Prestwick library
-Tocris

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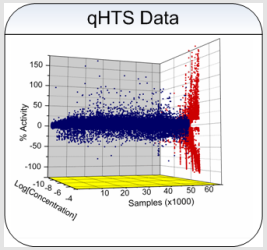
qHTS Data Analysis Process

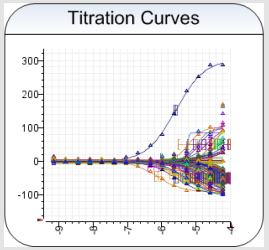
Normalization of qHTS Data

1



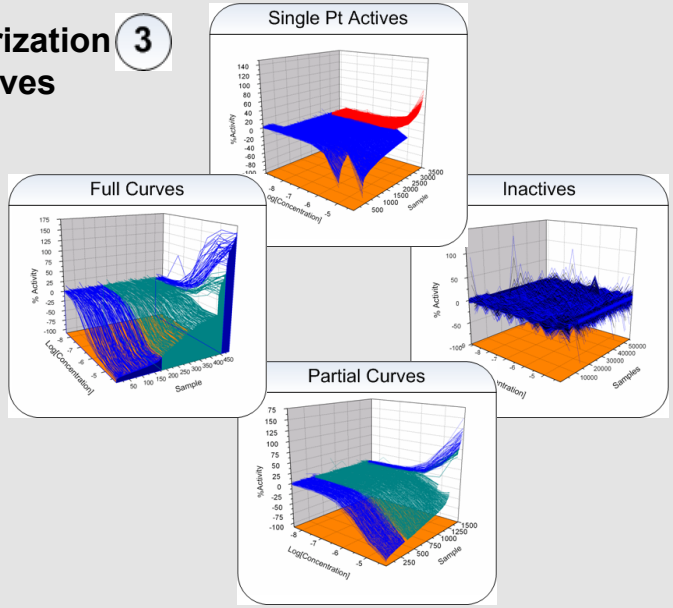
Fitting of Titration Response Curves

2



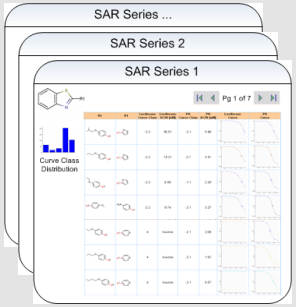
Characterization of Curves

3



Elimination of Artifacts Generation of SAR, Profiles

4



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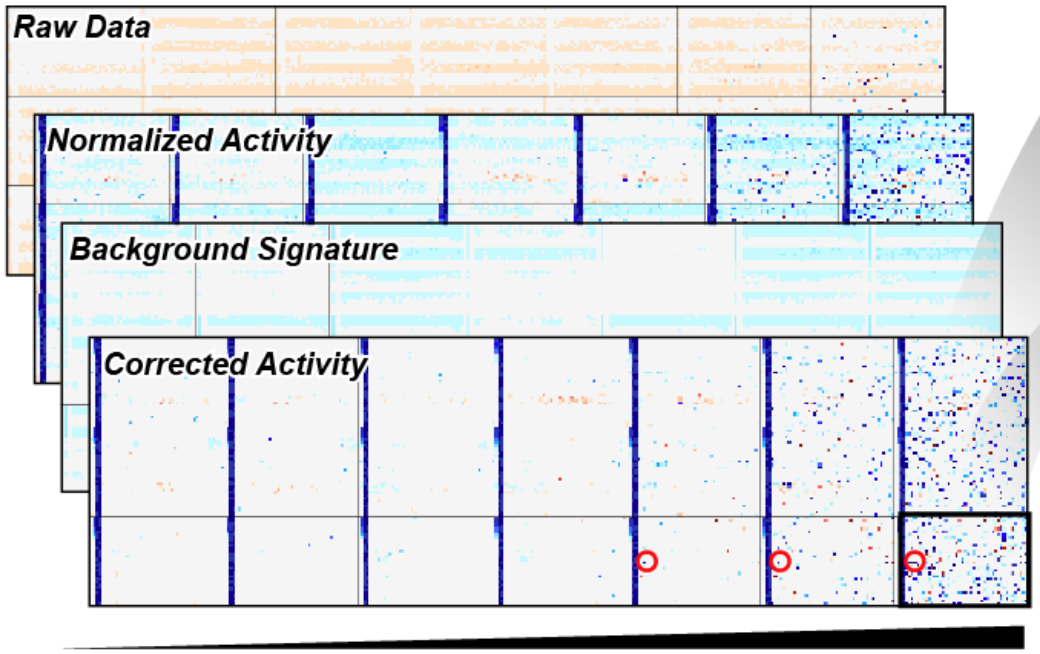
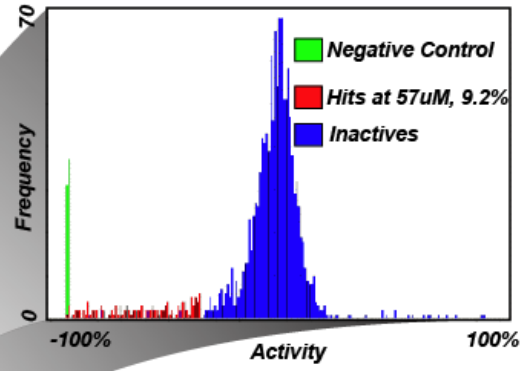
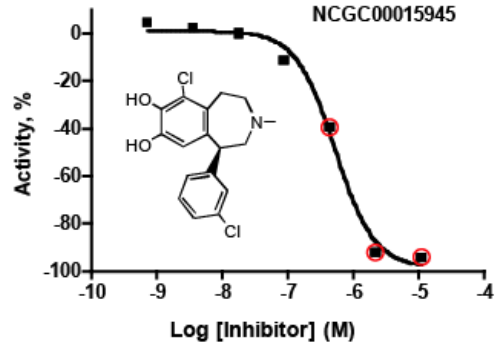
qHTS Data Analysis Process

Typical Processing of Raw Data:



Typical Single Pt. Results:



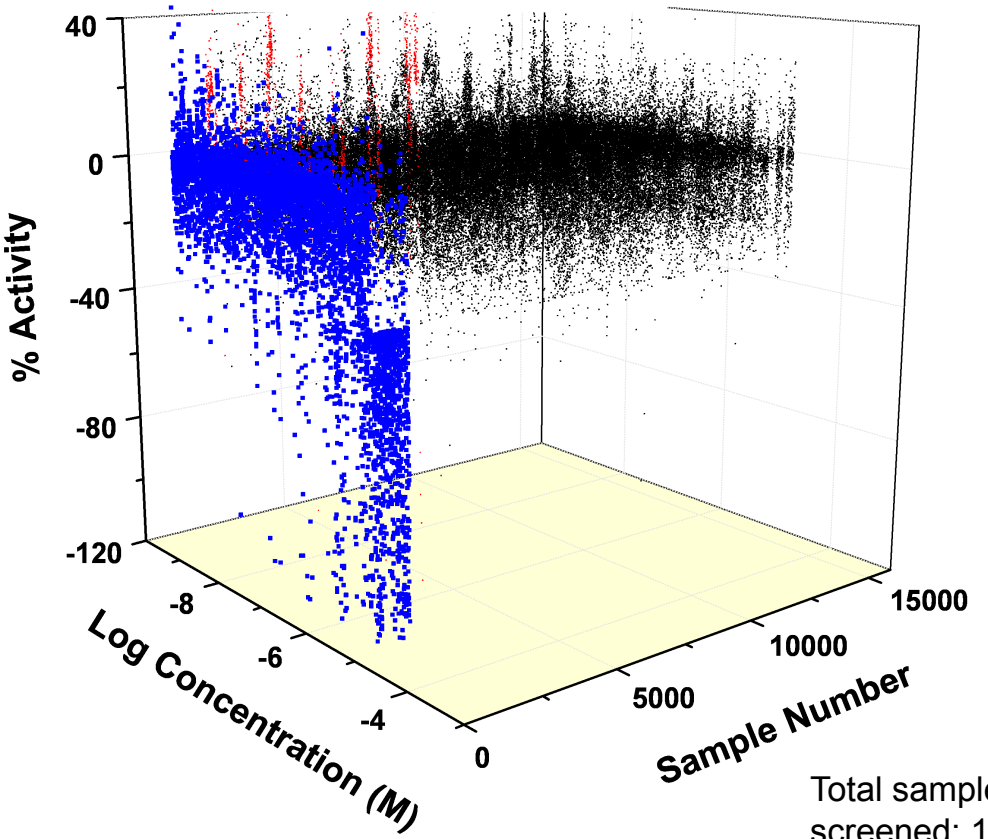


RS

Typical qHTS Results

MTB-GFP Activity

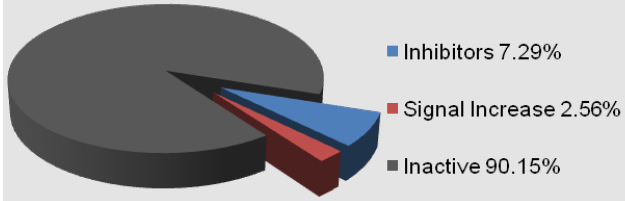
A) Normalized activity of entire screen



Sample concentration range:
1.73nM to 27.03uM

Total samples
screened: 15,141

B) Activity distribution



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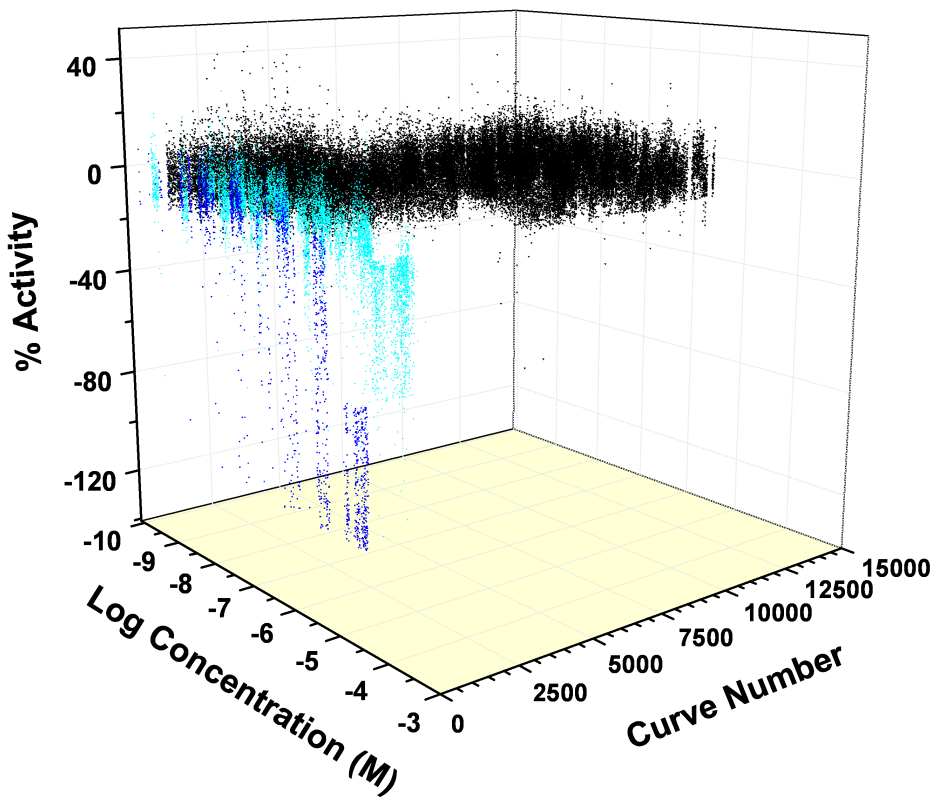
Butyrate qHTS Activity

MTB "Butyrate" Activity Scatter Plot



408 samples identified as top actives (dark blue) and 1,759 identified as inconclusive (light blue). The remaining samples were inactive (black).

Concentration response curves that gave full or partial curves and had maximum response greater than six sigma activity (~60% inhibition) were declared as top actives.

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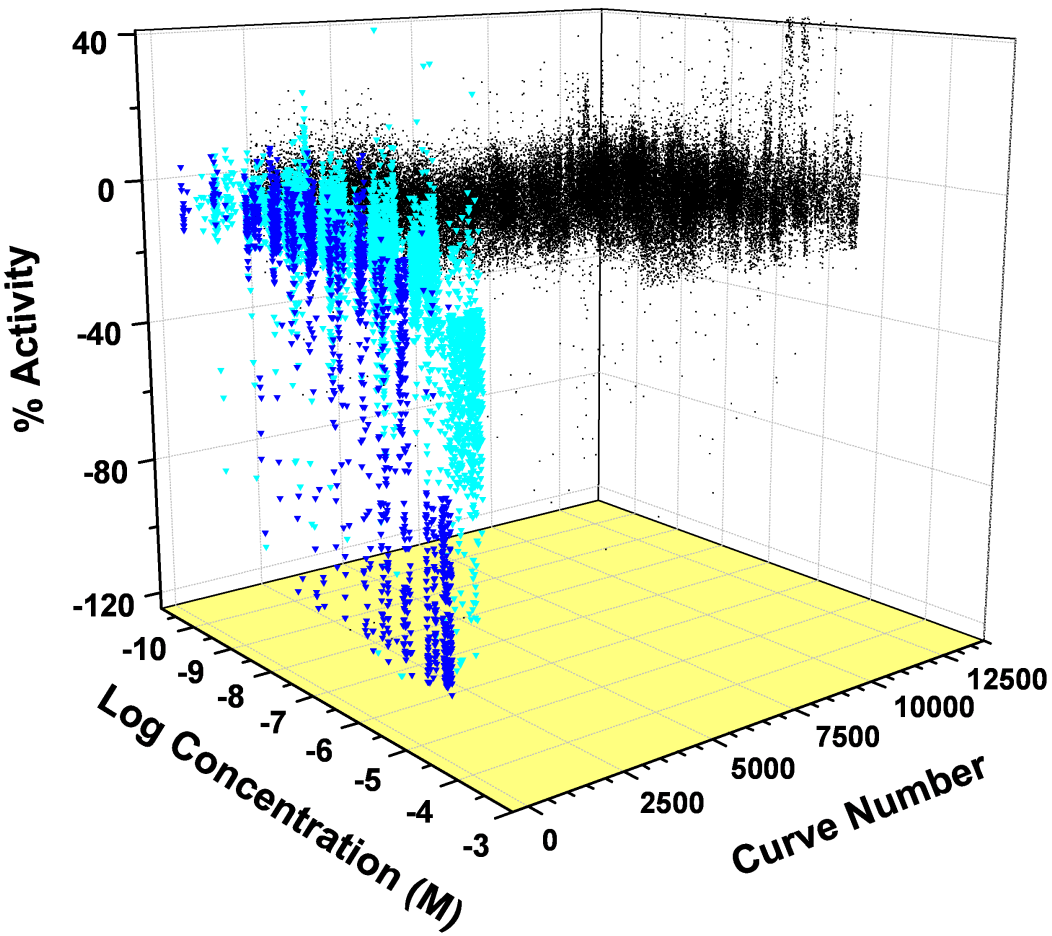
Isovalerate qHTS Activity

MTB “Isovalerate” Activity Scatter Plot



2.2% identified as top actives (dark blue) and 1,030 or 7.9% identified as inconclusive (light blue).

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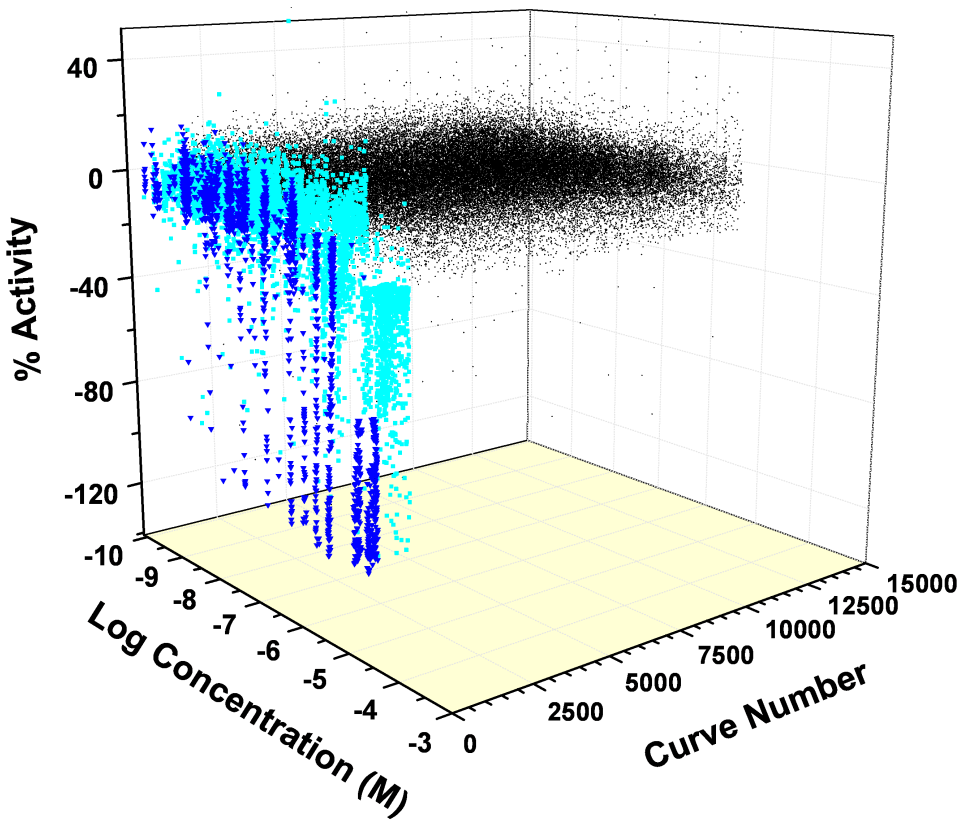
Cholesterol qHTS Activity

MTB “Cholesterol” Activity Scatter Plot



337 samples or 2.5% identified as top actives (dark blue) and 999 or 7.5% identified as inconclusive (light blue). The remaining 90% samples were inactive (black).

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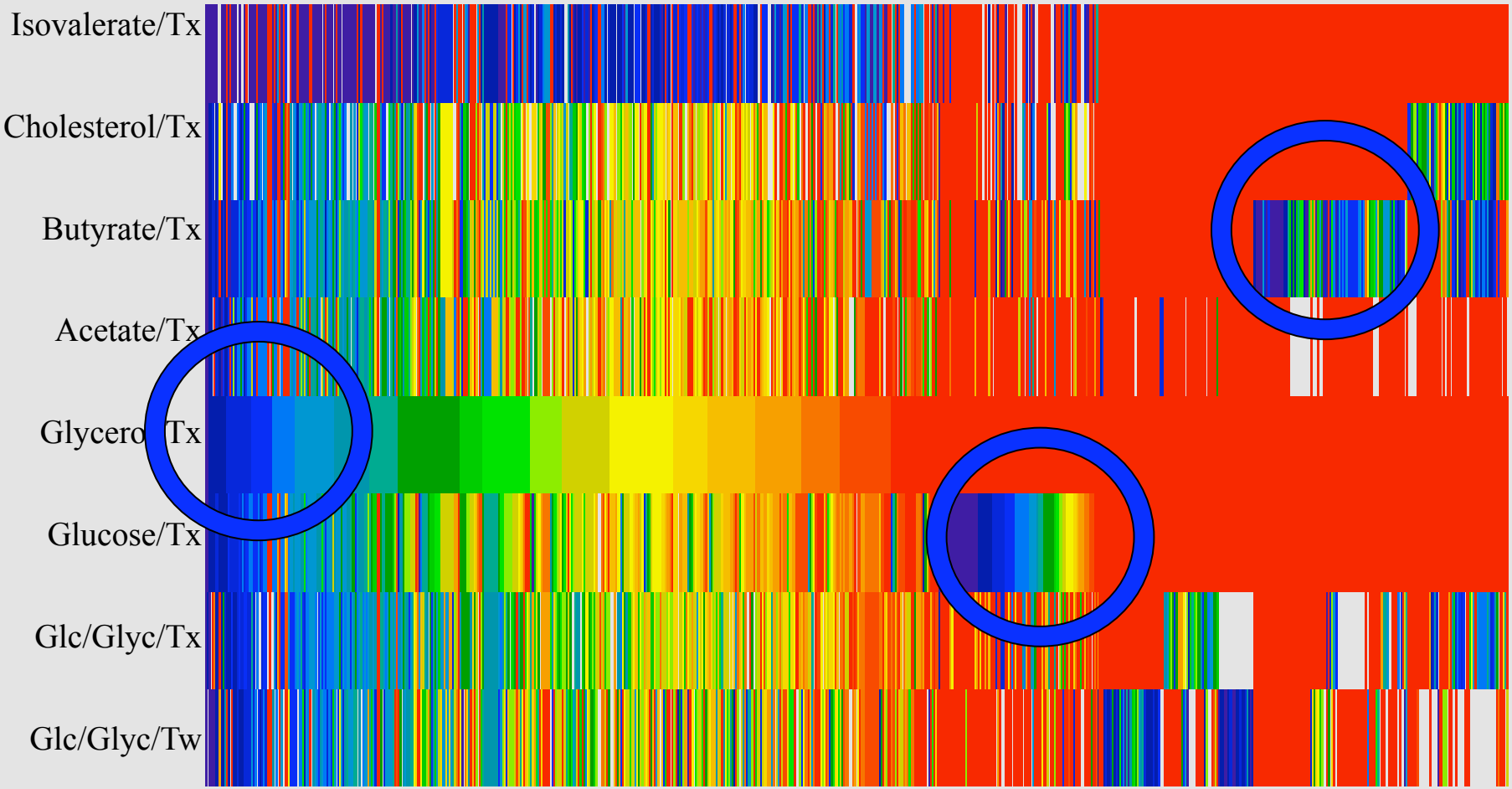
Comparative analysis of screens: pan-actives vs condition-selectives



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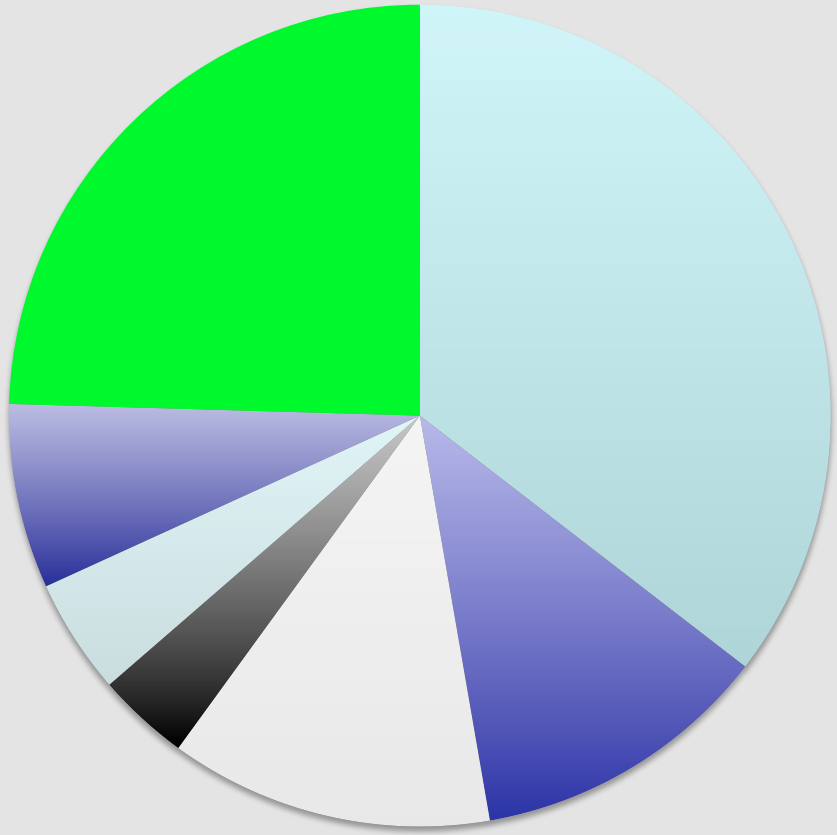
Analysis of the top 112 compounds (<10μM potency in all assays)



- Known AntiTB Activity
- Known MOA
- DNA Damaging
- Assay Interference
- Reactive/Prodrugs
- Ionophore/Channel Blocking/Detergent
- Interesting Molecules

"Known AntiTB" Includes: rifamycins, fluoroquinolones, tetracyclines, INH like, prothionamide, aminoglycosides, macrolides, beta-lactams
"Known MOA" Includes: redox cyclers, protein synthesis inhibitors, vancomycin, nucleobase analogs

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761 Compounds “Any Hitters”

↓ -Fluorescent, - Nuisance, -PubChem

254 Compounds

↓

24 Clusters

46 Singletons

↓ Pwdr confirmed, Limited Analoging

11 Clusters

6 Singletons

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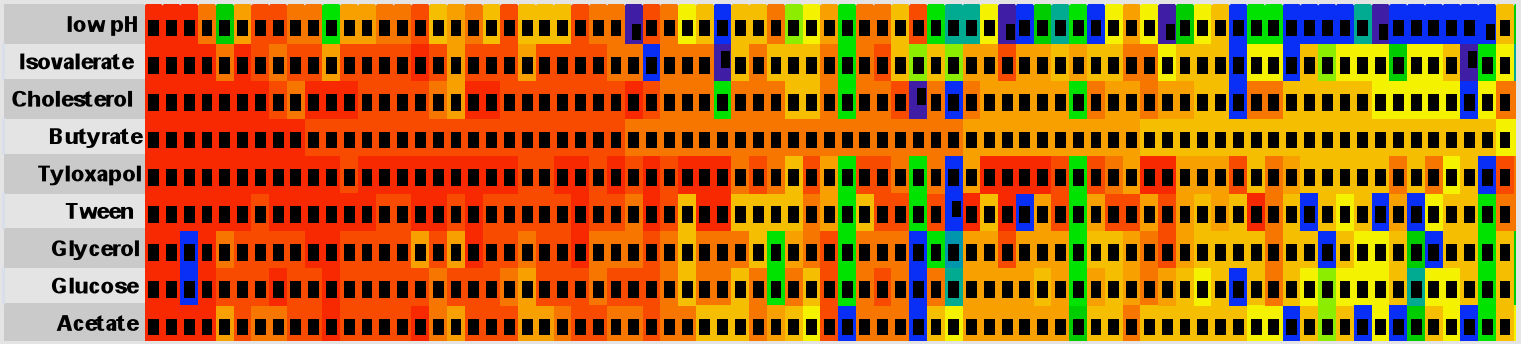
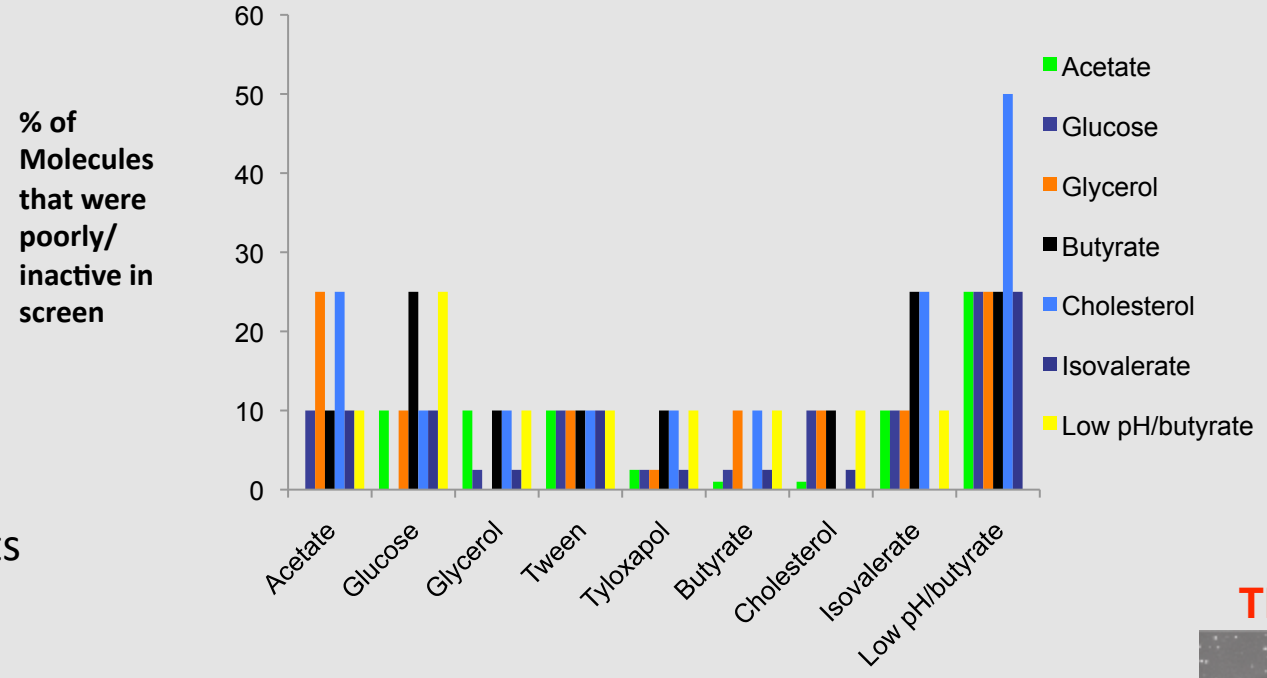
What are the minimum screening conditions to avoid missing hits?





Pairwise comparisons show that you can observe >90% of the hits with three screening conditions

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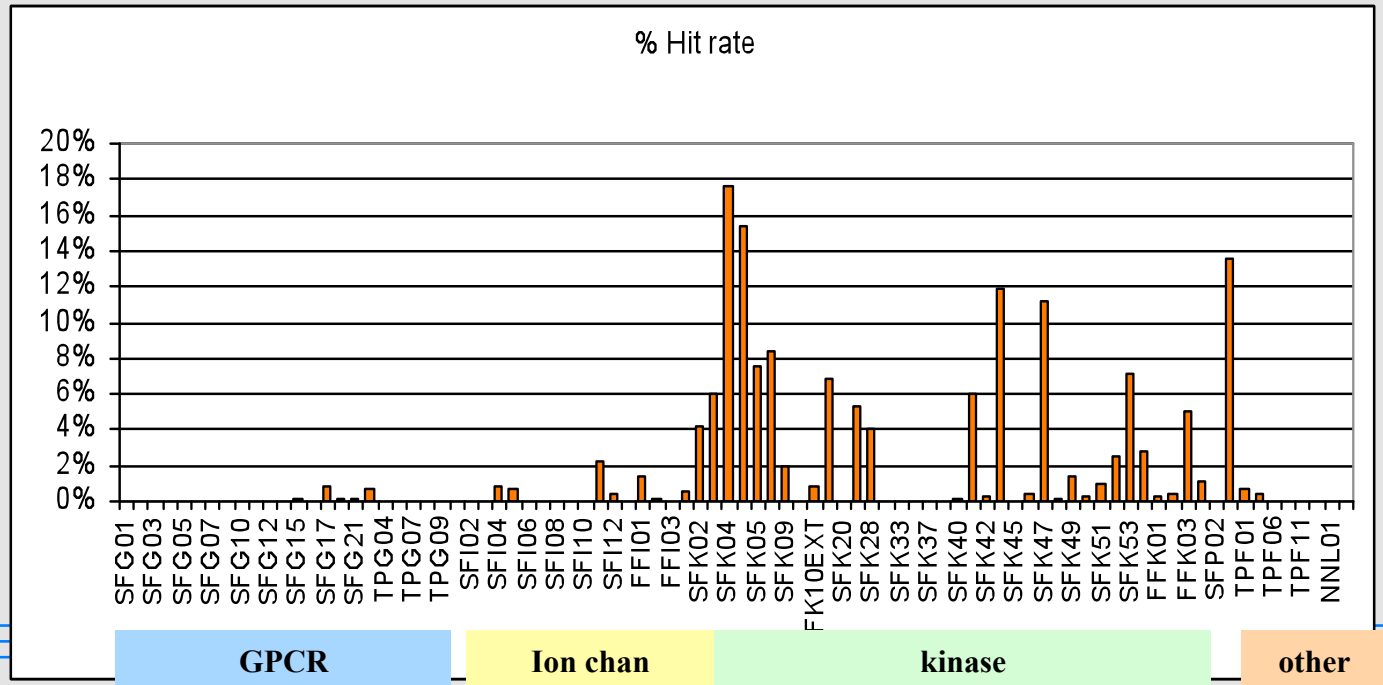
BioFocus Screen

- 35,108 compounds supplied to NIAID for screening from across 86 different BioFocus' SoftFocus® libraries
- Collaborative program with a group of South African investigators who will be contributing chemistry



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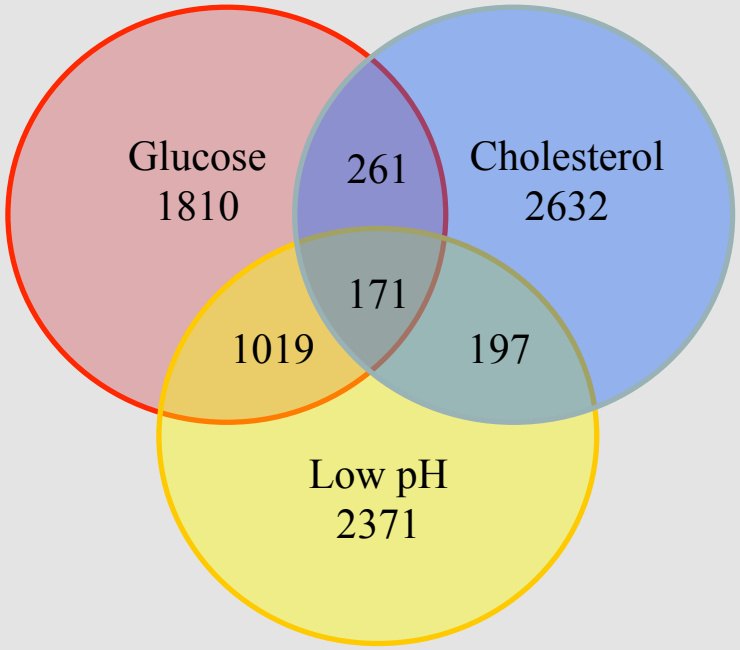
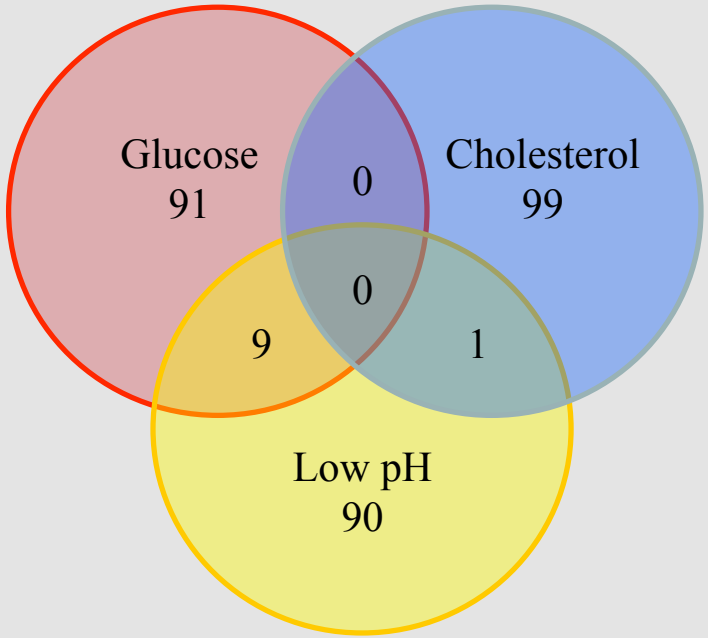
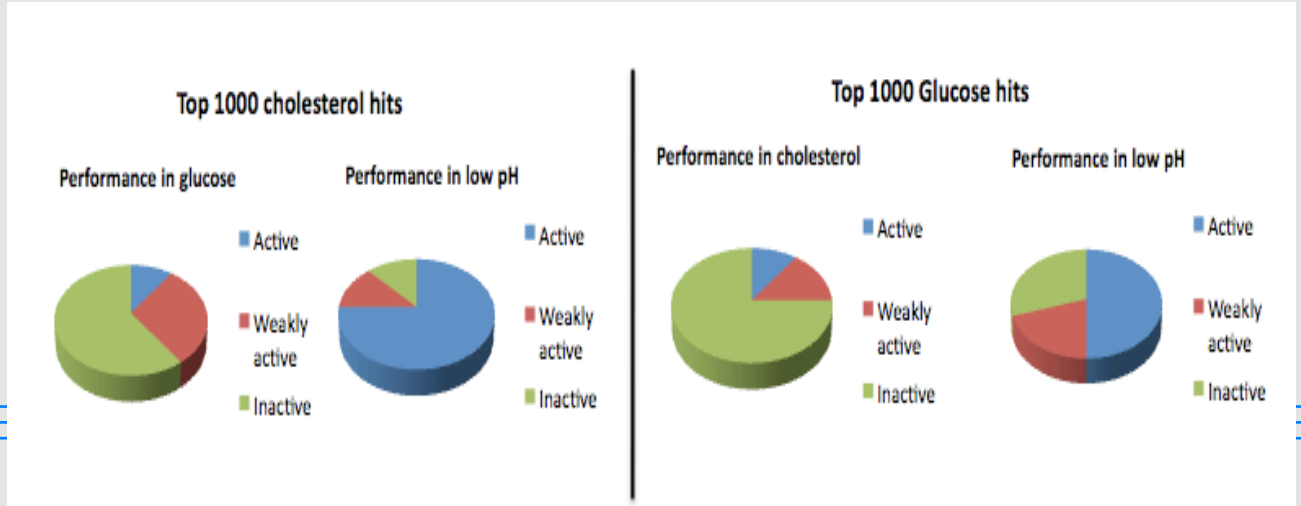
BioFocus Screen Comparisons

Top 3000 hits



Top 100 hits





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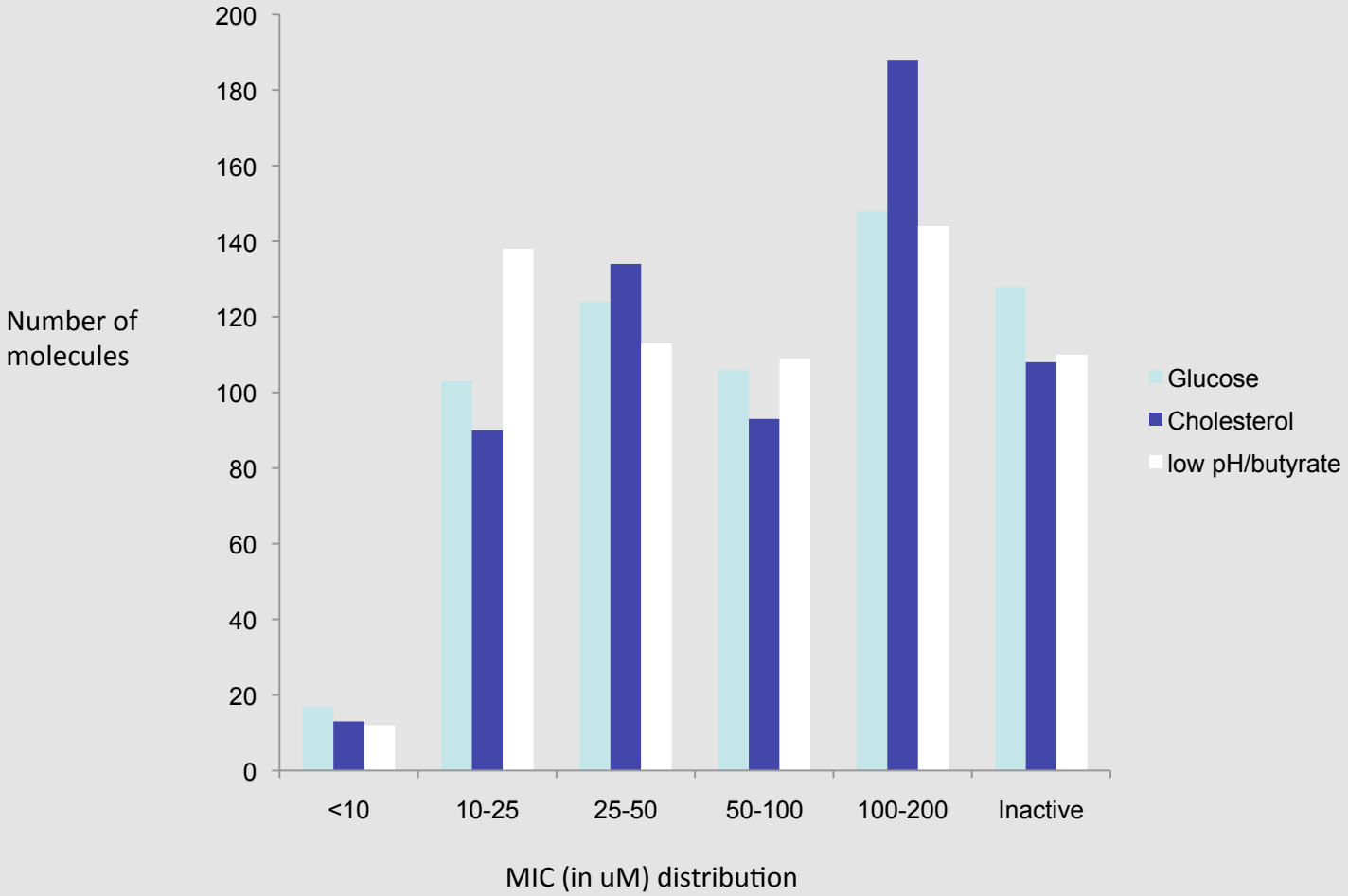
BioFocus reconfirmation statistics



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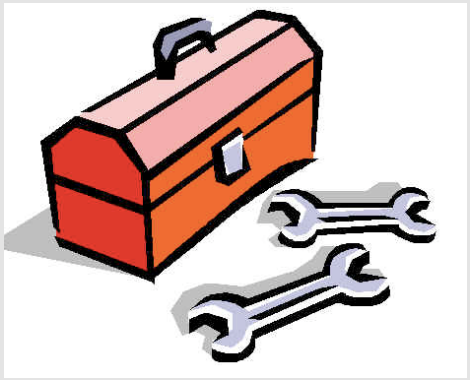
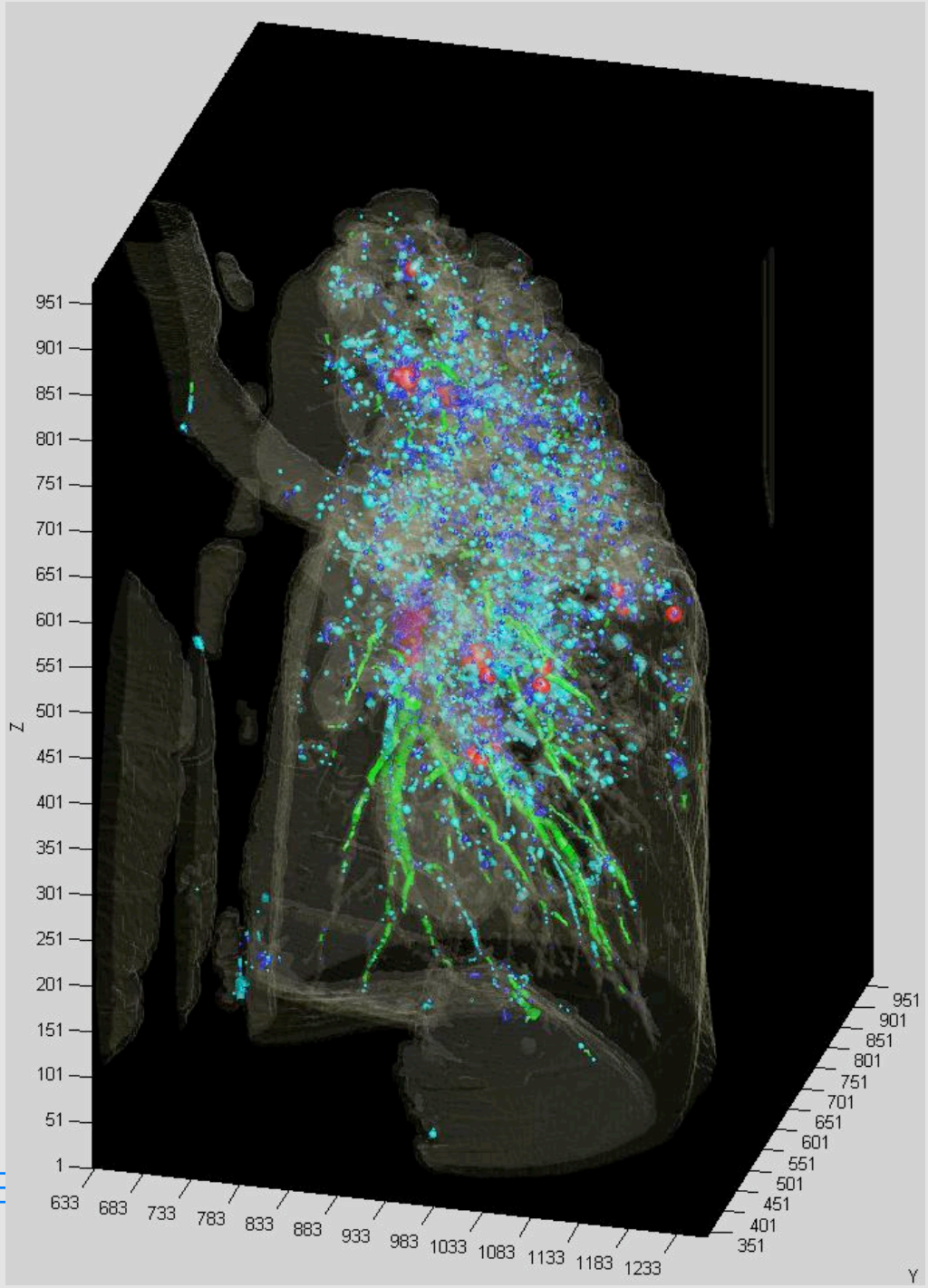
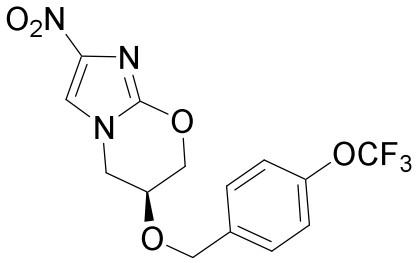


Condition-selective hits will lead to a better understanding of the parameters correlating *in vivo* efficacy and *in vitro* screening parameters. But how do we test them early?



Building a pipeline from hood to bedside



Lead Compound

qHTS
Systems biology
Whole cell screens

Marmoset POC

Quantitative Imaging Tools
Biomarkers
Pilot trial design

Phase II POC

Quantitative Imaging Tools
Biomarkers
MDR focused

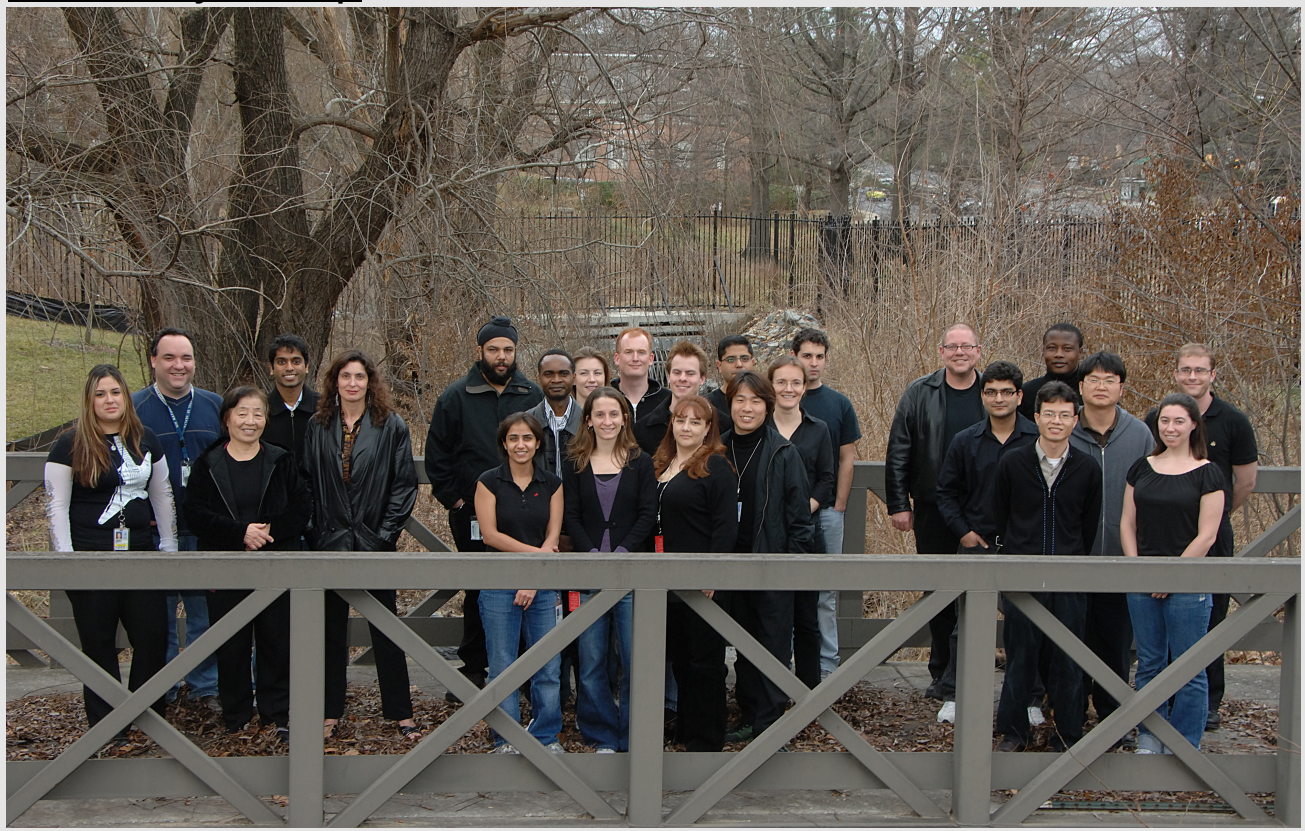
Take Homes:

- TB is a chronic heterogeneous disease with discrete lesion types
- Varied screening conditions give very different results
- Without any clear idea what lesions are rate-limiting we need to be expansive in screening conditions
- Always have an end game POC in mind at the start

"Order and simplification are the first steps toward the mastery of a subject — the actual enemy is the unknown."

-Thomas Mann, "The Magic Mountain"

The Barry Group



TBRS:
Sabrina Garcia
Matthew Carroll
Yong Ae Shin
Vignesh Rajan
Laura Via
*Ramandeep Singh
Kriti Arora
Chola Shamputa
Katie Smith
Kristin Burns
Richard Ledwidge
Nick McBride
Jacquie Gonzalez
Kapil Tahlan
Il-Dong Choi
*Helena Boshoff
Mark Carroll
Amit Nayyar
*Young Hwan Ha
Abayomi Orisadipe
Maggie Collins
David Kastrinsky
Lisa Goldfeder
Pradeep Kumar
*Liang Zhang
Sunhee Kang
*Michael Goodwin

NIH Chemical Genomics Center:
Chris Austin
Ajit Jadhav
Adam Yasgar
Carleen Klumpp
Anton Simeonov
Sam Michael

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