

A Systematic Approach to Early Drug Discovery

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Amphora

Acknowledgements

Target Production

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Screen Development

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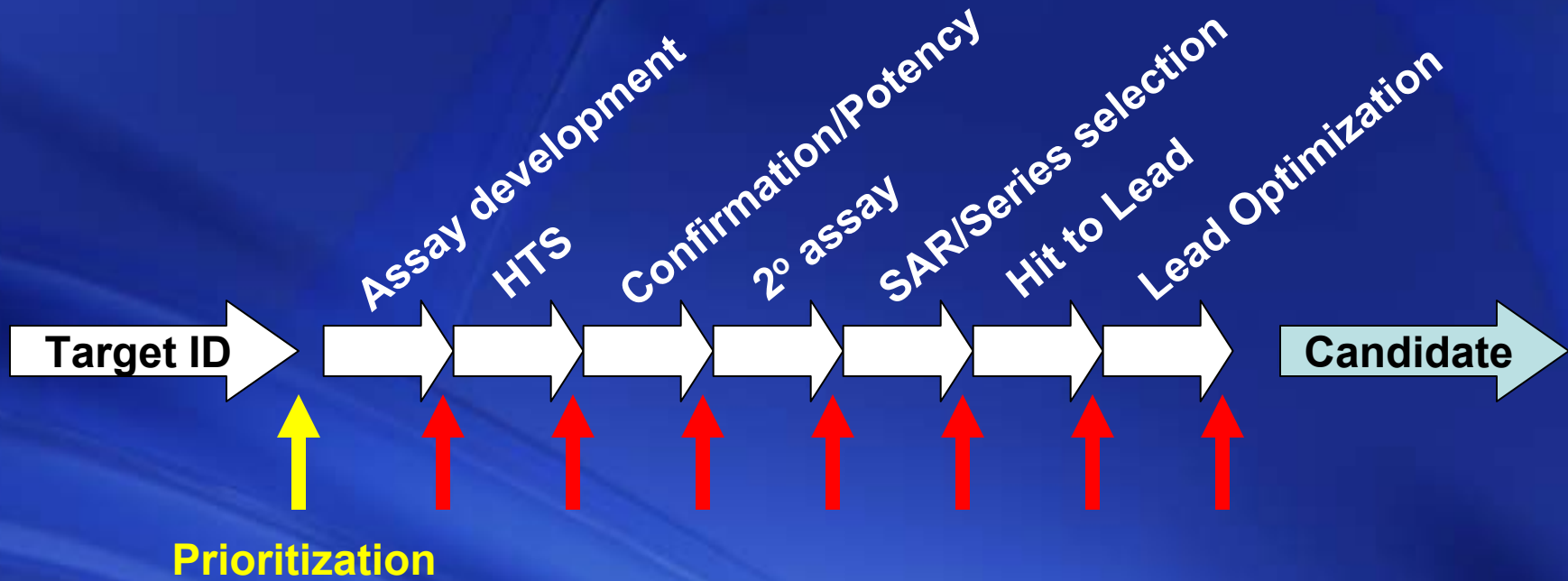
Project Management

Karen McKenzie

Founders

Bill Janzen
Nick Hodge

The Early Discovery Pipeline



↑ Go/No Go decisions

Our approach

Requirements to Improve Productivity

Data Quality and Consistency

Precision and Accuracy

Breadth and Depth

Without sacrificing speed, cost or biological relevance...

Solutions

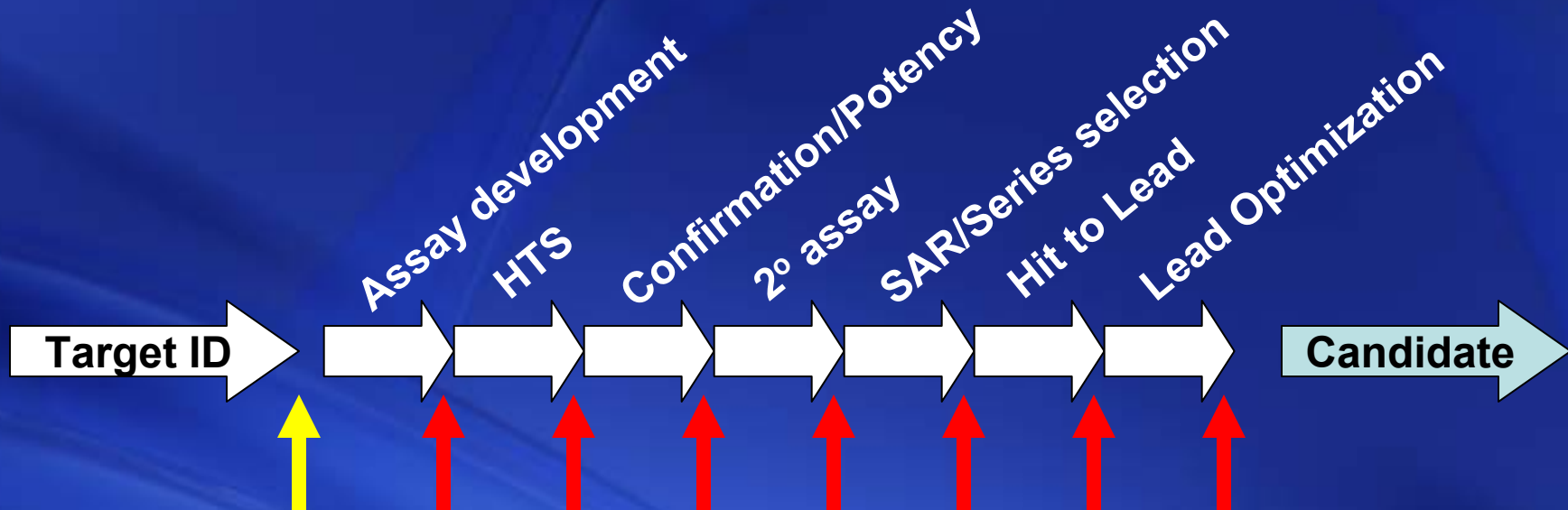
Consistent technology

Focus on **quality** at all stages of the process

Establish a comprehensive database with all data collected and comparable

Industrialization and parallel processing

The Early Discovery Pipeline



Prioritization

Consistent technology
Quality at all stages of the process
Comprehensive database with comparable data

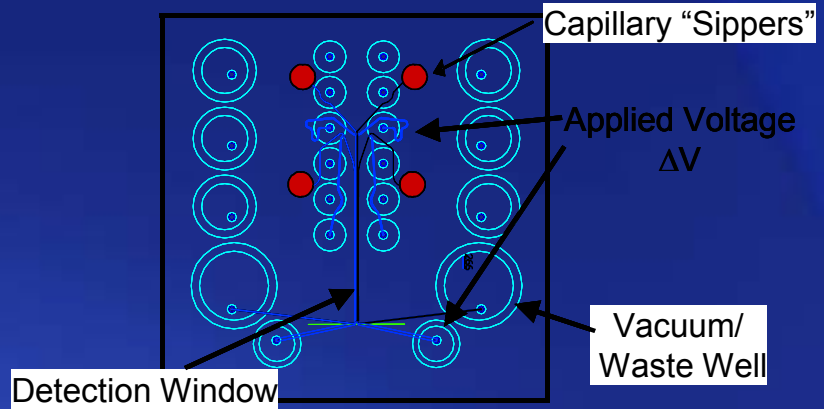
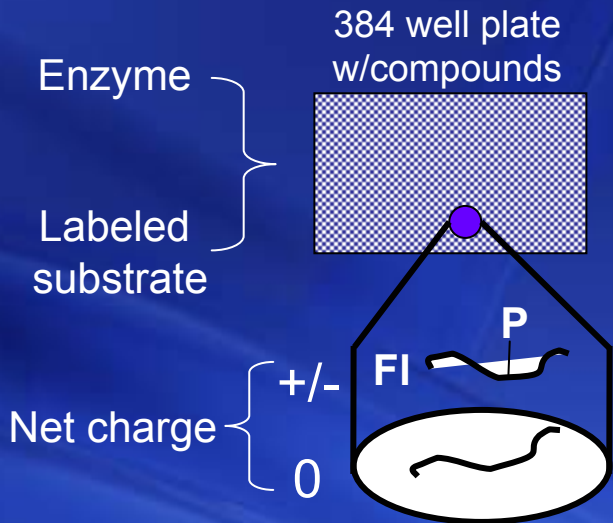
↑ Go/No Go decisions

Microfluidic Technology for multiple target families

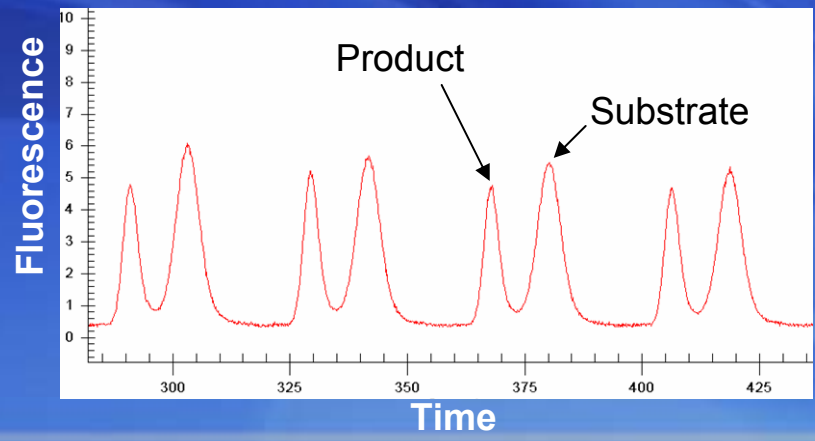
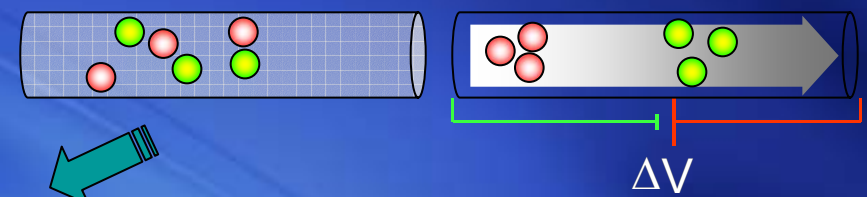
Step 1: Enzyme reaction



Step 2: On-chip separation of substrate and product



● Substrate } Use charge difference to separate
● Product



Step 3: Intervention-free detection

Assay Development

Substrate Identification

- >120 peptide library*
- ~9-15 mers*

Biochemical Parameters

- Specific activity*
- Substrate Km*
- Inhibition Profile*
- Stability*

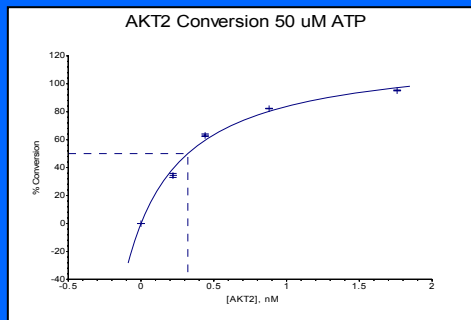
SOPs

- Common buffer*
- Binned [substrate]*
- Two determined incubation periods (3 or 17 hr)*
- Constant [peptide]*

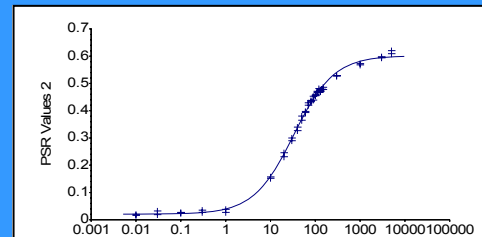
- Highly standardized procedure
- Development on many enzymes completed in parallel
- Process can be applied to several peptide-based target classes (kinases, phosphatases, proteases, epigenetic targets, etc.)

Uniform Biochemical Parameters for Data Comparability

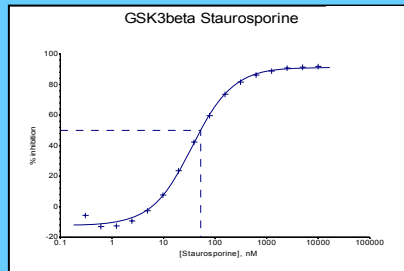
Phosphorylation Rate



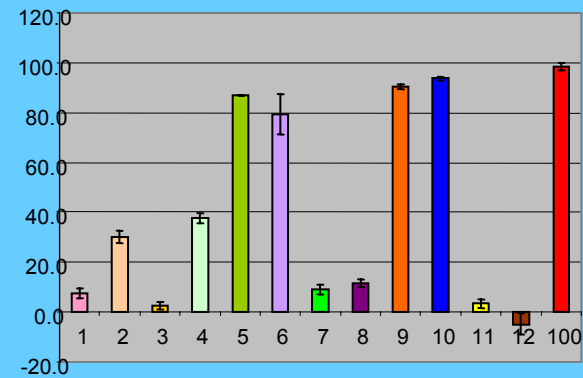
ATP/Peptide Km



Control Inhibition

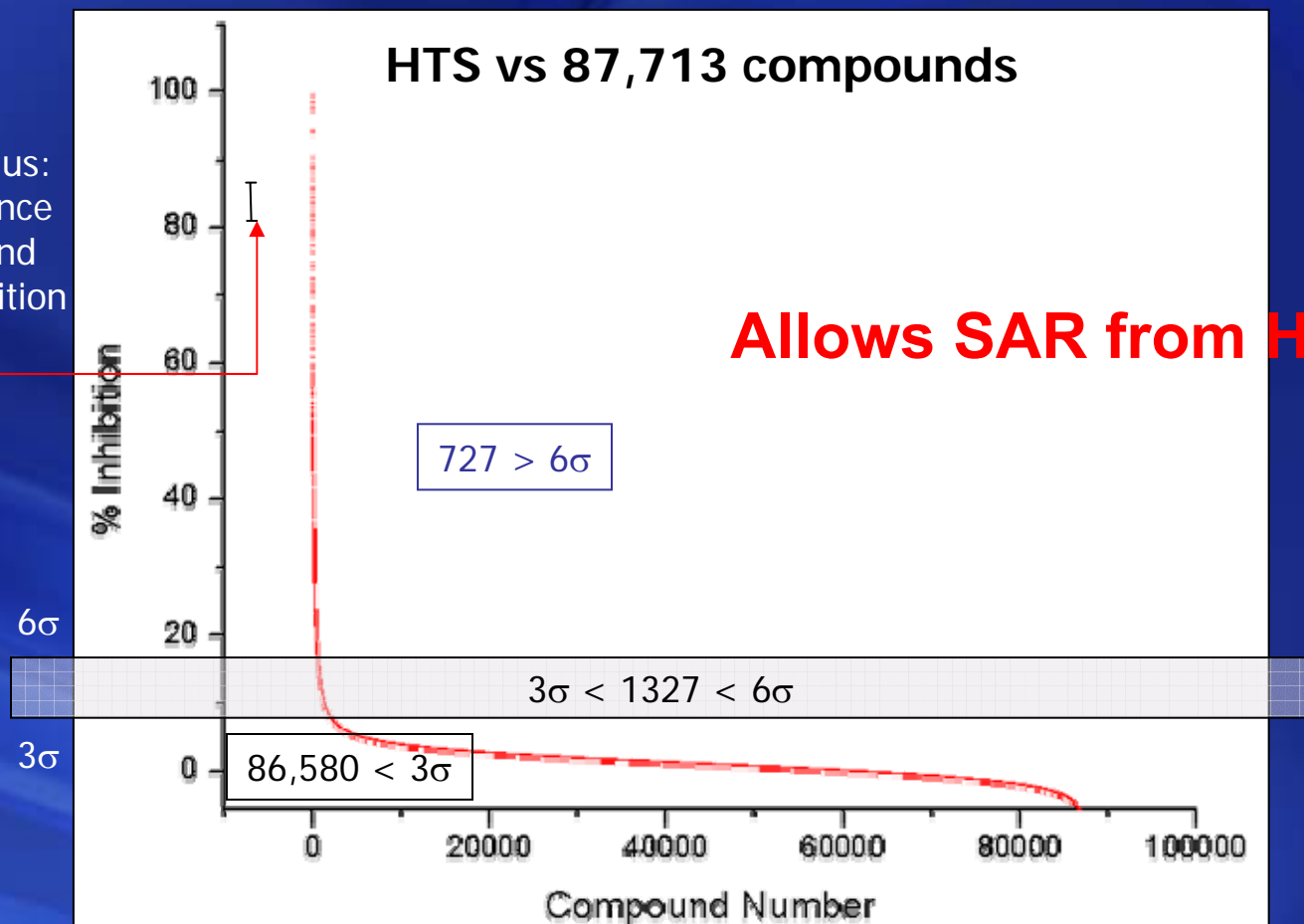


Inhibition Profile



Quality Screening

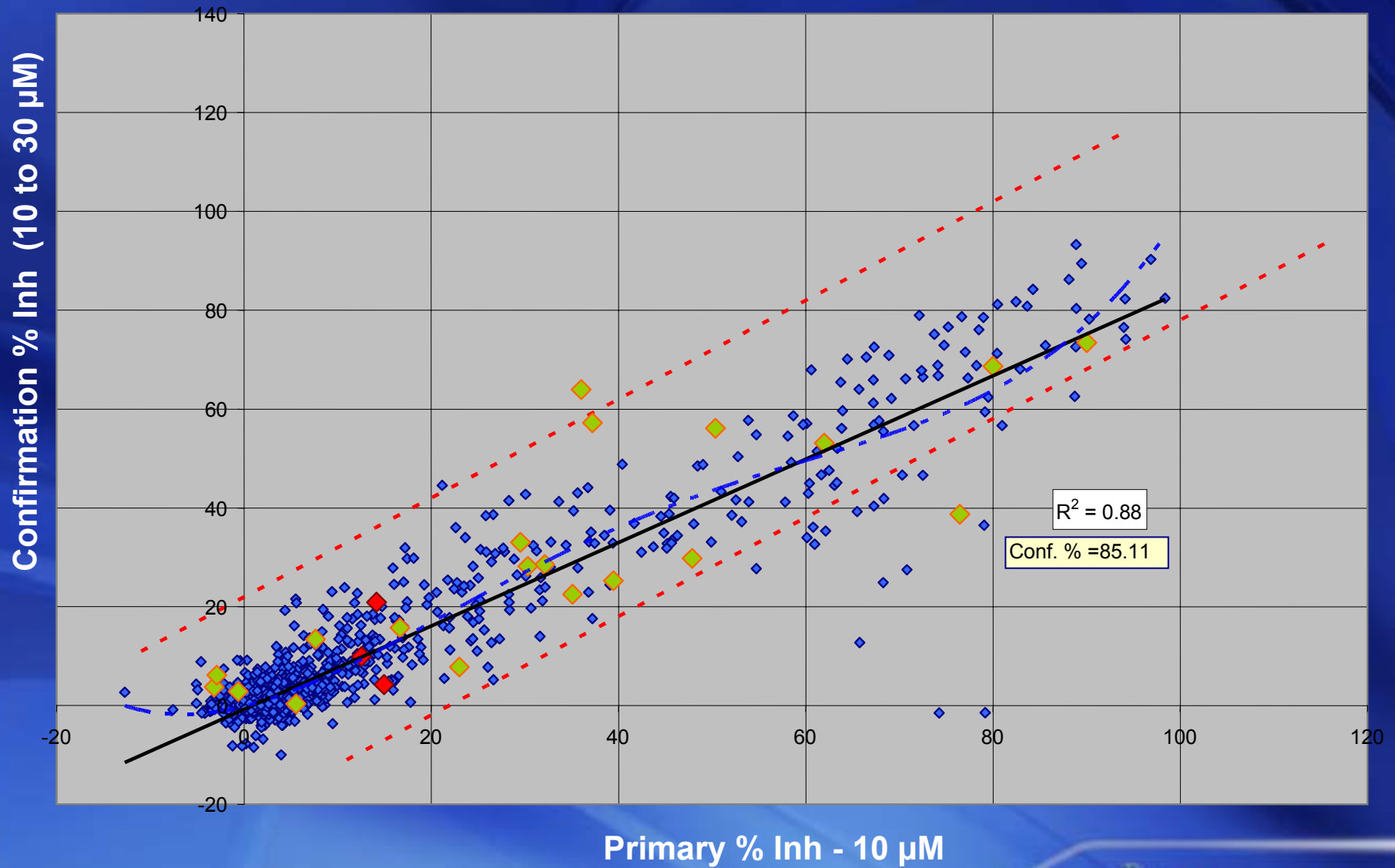
Precision radius:
95% Confidence
Interval around
percent inhibition



Allows SAR from HTS data

As the precision radius decreases, the power to distinguish between compounds exhibiting similar inhibition increases.

Primary Screen & Confirmation



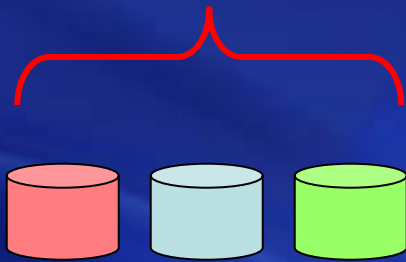
Library Purity - Critical for Generating High Quality Data

- Produces credible negative data, a differentiating feature of the Amphora database
 - Identity of sample known
 - All samples purified and characterized
 - Concentration at time of screening known

Library Quality

Characteristics of successful drugs look like?

Compound sources



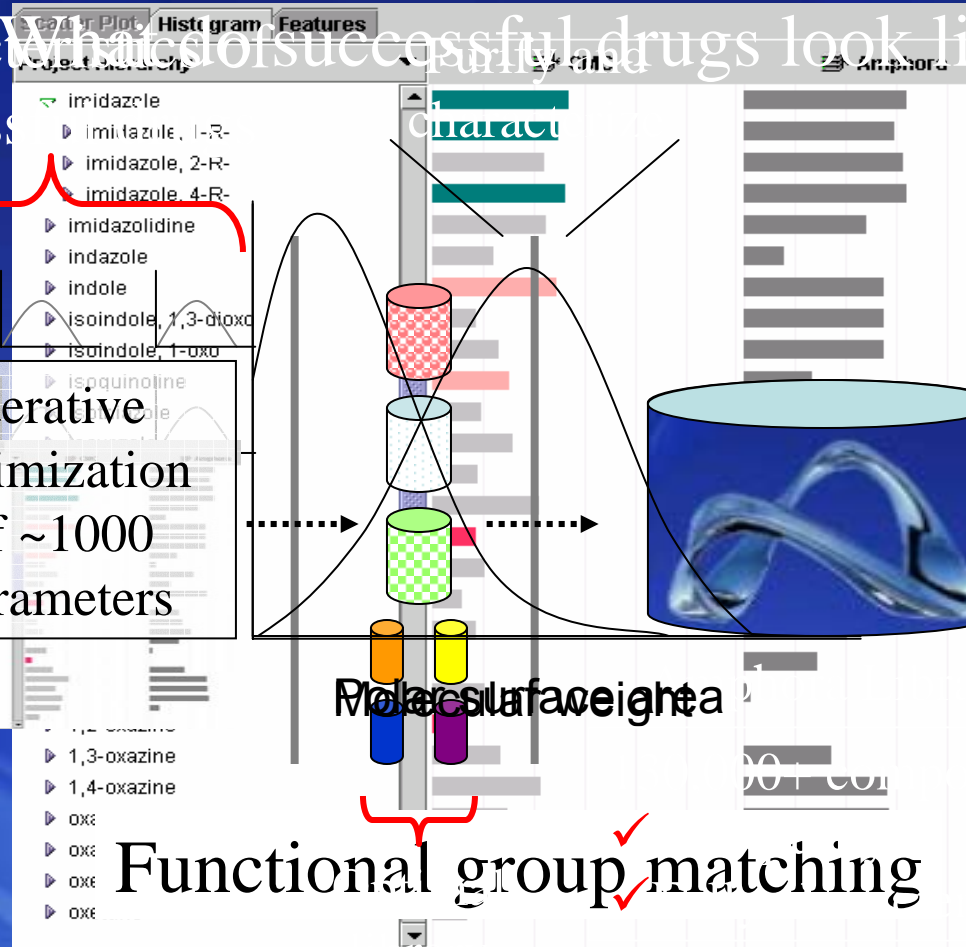
Commercial libraries



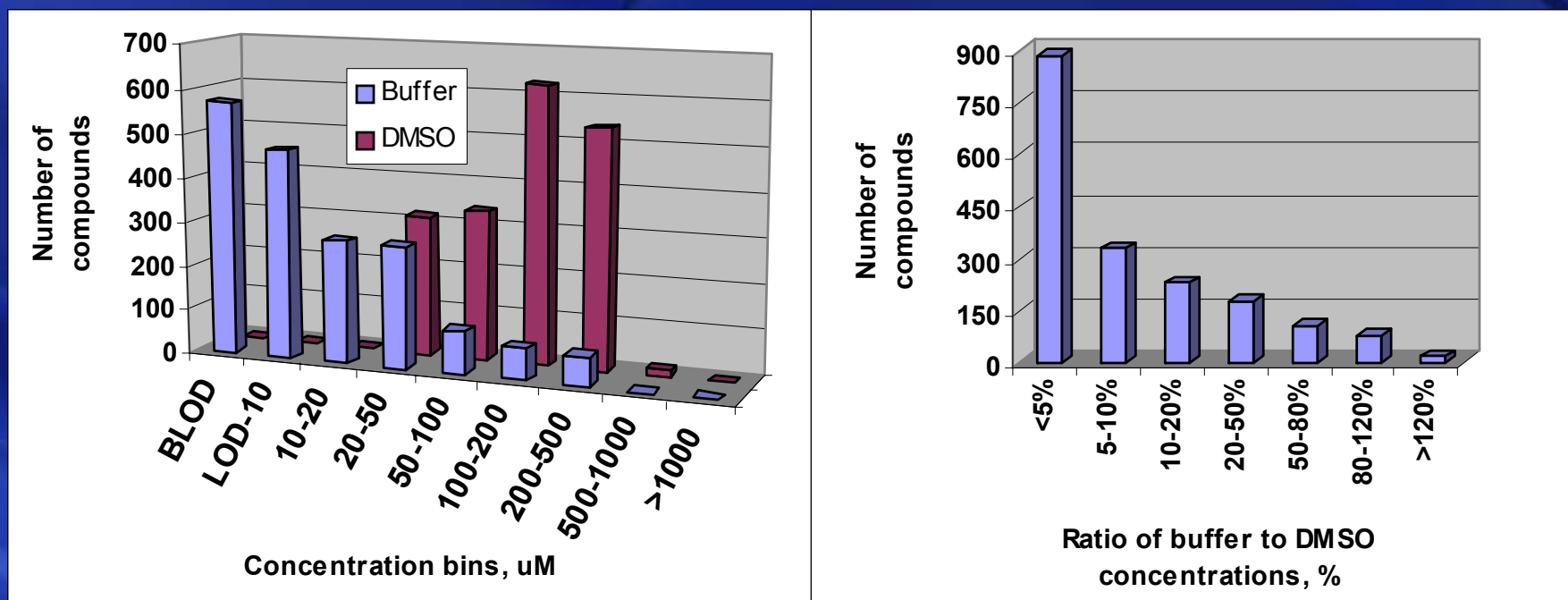
In-house libraries

Filter problematic functional groups

Iterative optimization of ~1000 parameters



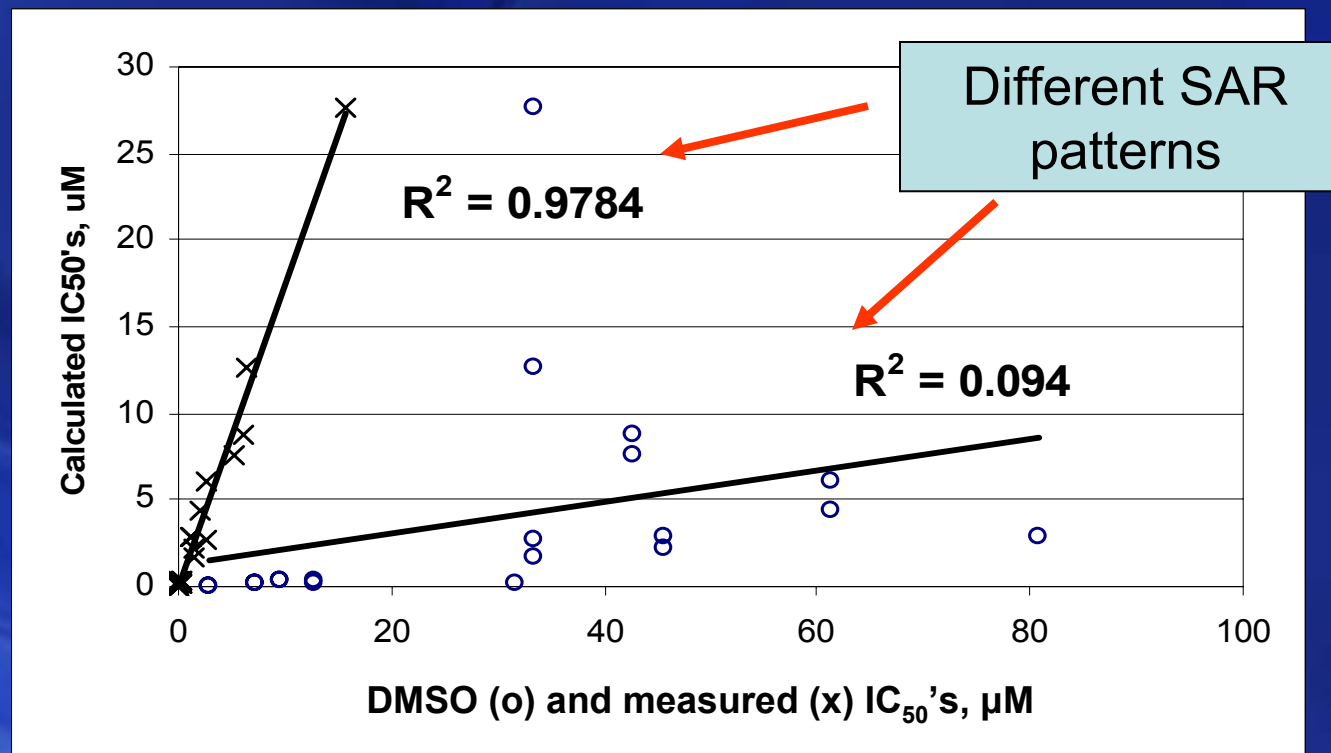
Aqueous Quantitation - A Key to Data Quality



Analysis for a random sample of 2000 compounds from the Amphora library

(Popa-Burke et al., *Anal. Chem.* 2004, 76, 7278 – 7287)

Allows Determination of *Actual* IC₅₀ Values



$$\text{Inh} = \frac{[\text{conc}]}{[\text{conc}] + \text{IC}_{50}}$$

Irwin H. Siegel, Enzyme Kinetics, John Wiley & Sons, 1975

Actives Analysis

Identify all “actives” above the 6σ threshold

1. Remove any known inhibitors
2. Sort by selectivity across all targets
3. Remove promiscuous inhibitors

↓
Cluster by structure

↓
Identify series of interest

- ↓
1. Evaluate SAR including negative SAR
 2. Determine IP position
 3. Evaluate diversity points
 4. Identify syntheses

↓
Initiate chemistry



Amphora

Data Analysis Example:
P38 α Actives Analysis

p38α HTS: Screen#1 Statistics Output

Assay Id: 1-P38-ALPHA-550-PRI-2	Type: PRIMARY
Assay Name: Primary P38-ALPHA 17 hr ATP 100.000000 µM PEP-55 1.000000 µM	Method: FS266 - Off-Chip Mobility Shift
Assay Status: Approved	Purpose: The one true protocol.
Time Period: 4th Quarter 2004	
First Entered: Friday, October 15, 2004	
Last Edited: Monday, February 07, 2005	
Target: P38-ALPHA	Target Family: MAPK
Target Name: MAP Kinase p38 alpha	Sub-Family: p38
Target Class: Kinase	Progress: In Lead Optimization
Target Type: serine/threonine protein kinase	Study of: Inhibition
Target Group: CMGC	
	Protocol Id: R-ENZYM-OFF-CHIP-PRIMARY-P01A
	Protocol Name: The one
	Validation: NOT VALIDATED
	Study Id: P38-ALPHA
	Study Name: MAP Kinase p38 alpha

Total # of samples: 129187

Incubation Time: 17 hr
Substrates: ATP 100.000 µM
PEP-55 1.000 µM

Cut-off Value > 16.5%

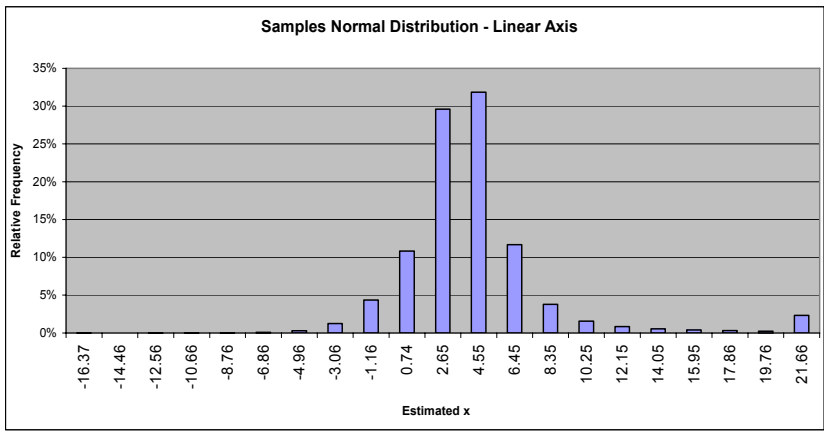
Total # of Points: 80,542	Average: 3.65		
# Points within 3 σ: 69,786	Standard Deviation: 7.00		
# Diversity Smpl n > 1: 36,345	Average: 2.65		
	3σ Stdev: 2.38		
	Repeatability (within): 45.0%		
	Distance: 6.44		
# Boolean Samples: 74	65+ Cutoff Value: 16.90		
# Samples with Quant: 509	65- Cutoff Value: -11.61		
# Points / Sample: 2.0			

Min: -60.8	66-: -11.6	36-: -4.2	Avg: 2.6	36+: 9.5	66+: 16.9	Max: 102.6
12	250					
0.03%	0.62%					
	Median: -5.14					
	Average: -5.65				Average: 26.70	
	Pooled Stdev: 2.51				Pooled Stdev: 2.29	
	Singles Rate: 18.7%				Singles Rate: 1.4%	
	Noise Rate: 17.4%				Noise Rate: 9.7%	
	Boolean Rate: 15.8%				Boolean Rate: 1.7%	
	P(-6σ)Cpop2-: 5.7%				P(+6σ)Cpop2+: 18.7%	

Precision Radius: 5.5%

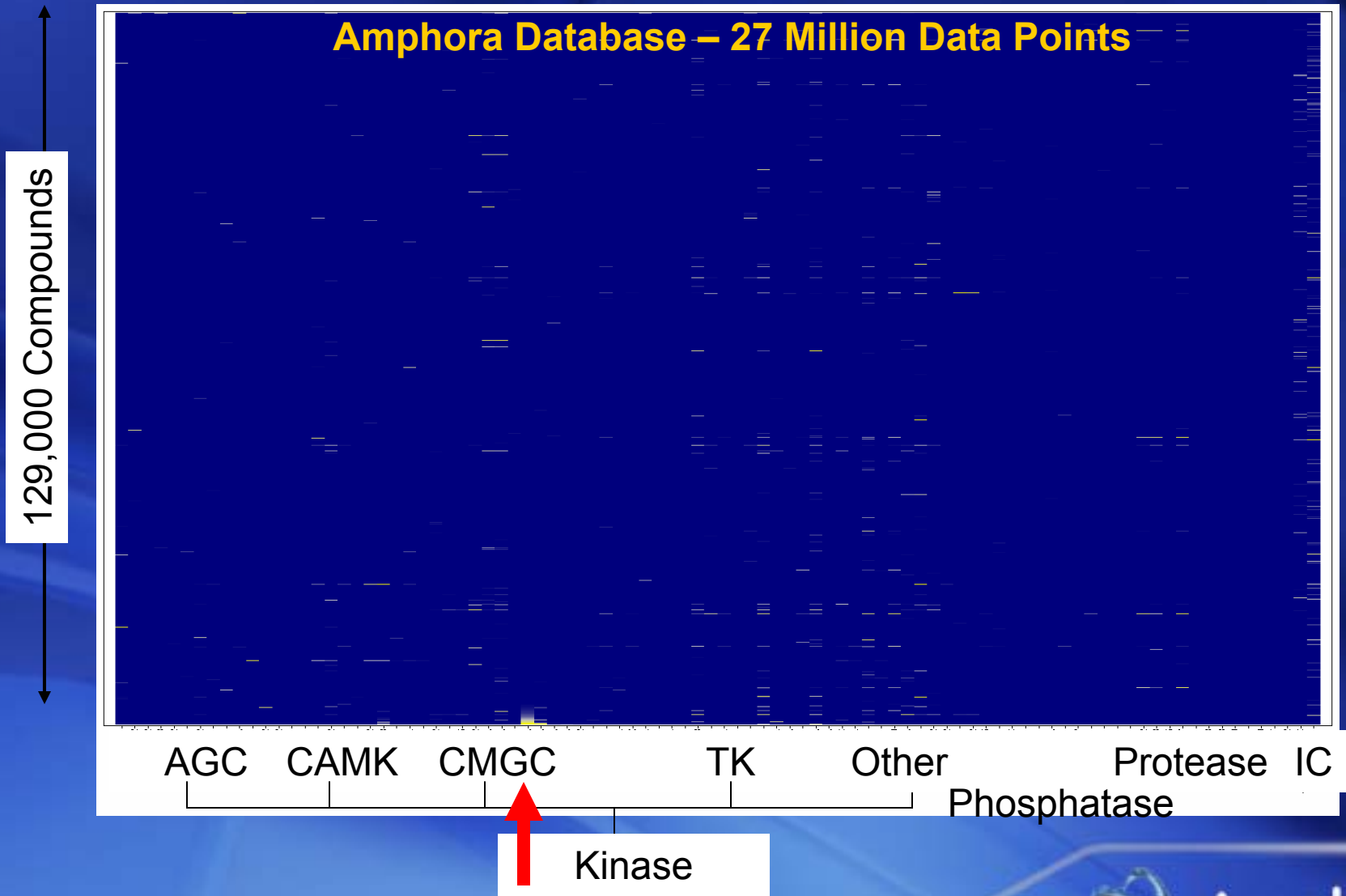
Number of Actives: 1790

Boolean Samples Excluded!

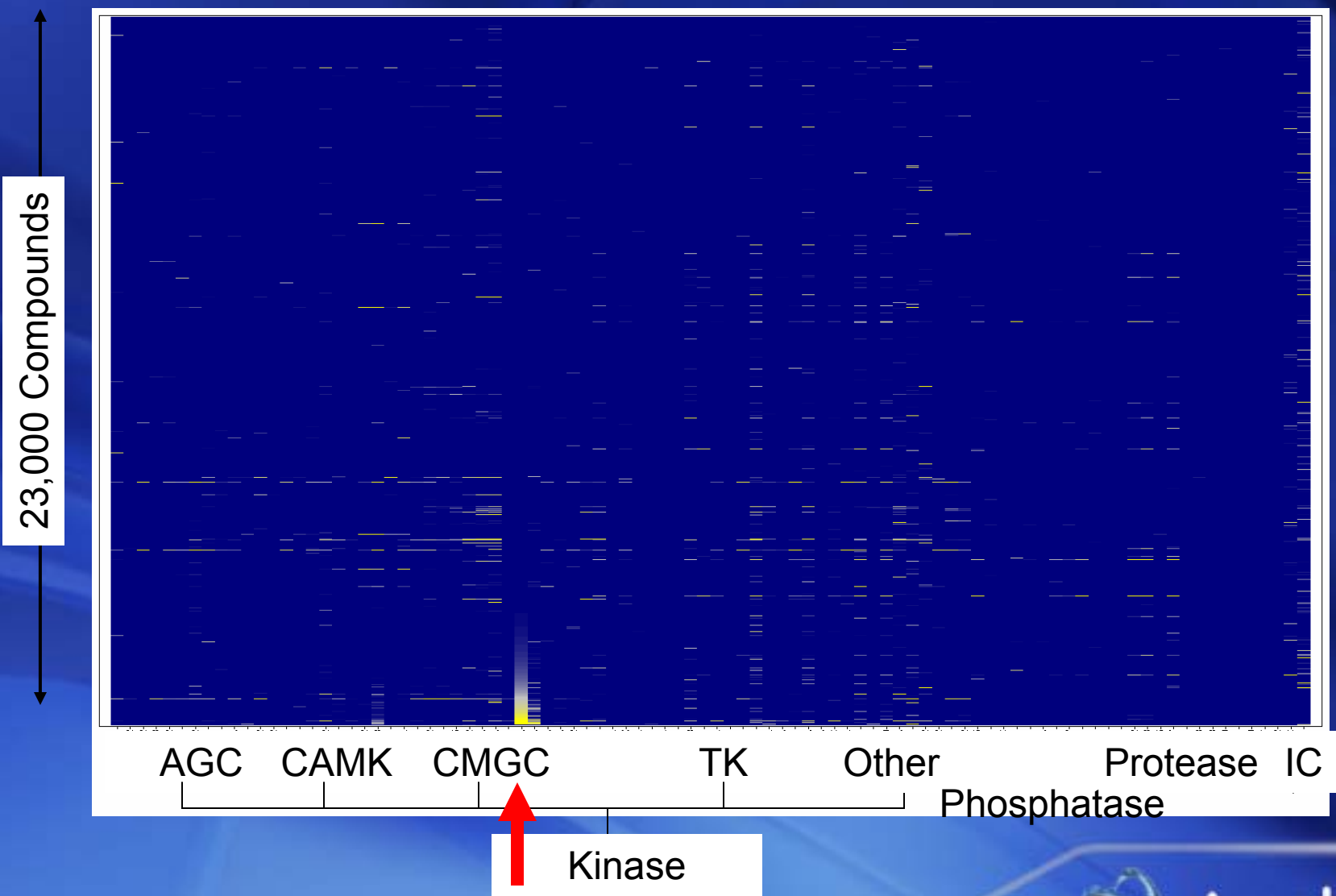


Percentile	Estimated x	Frequency	Relative Frequency
0.0000%	-16.37	7	0.02%
0.0000%	-14.46	4	0.01%
0.0000%	-12.56	6	0.01%
0.0000%	-10.66	9	0.02%
0.0001%	-8.76	6	0.01%
0.0032%	-6.86	32	0.08%
0.0687%	-4.96	119	0.29%
0.8198%	-3.06	499	1.23%
5.4799%	-1.16	1,765	4.35%
21.1855%	0.74	4,392	10.83%
50.0000%	2.65	11,994	29.58%
78.8145%	4.55	12,908	31.84%
94.5201%	6.45	4,737	11.68%
99.1802%	8.35	1,536	3.79%
99.9313%	10.25	638	1.57%
99.9968%	12.15	342	0.84%
99.9999%	14.05	221	0.55%
100.0000%	15.95	156	0.38%
100.0000%	17.86	135	0.33%
100.0000%	19.76	91	0.22%
100.0000%	21.66	945	2.33%

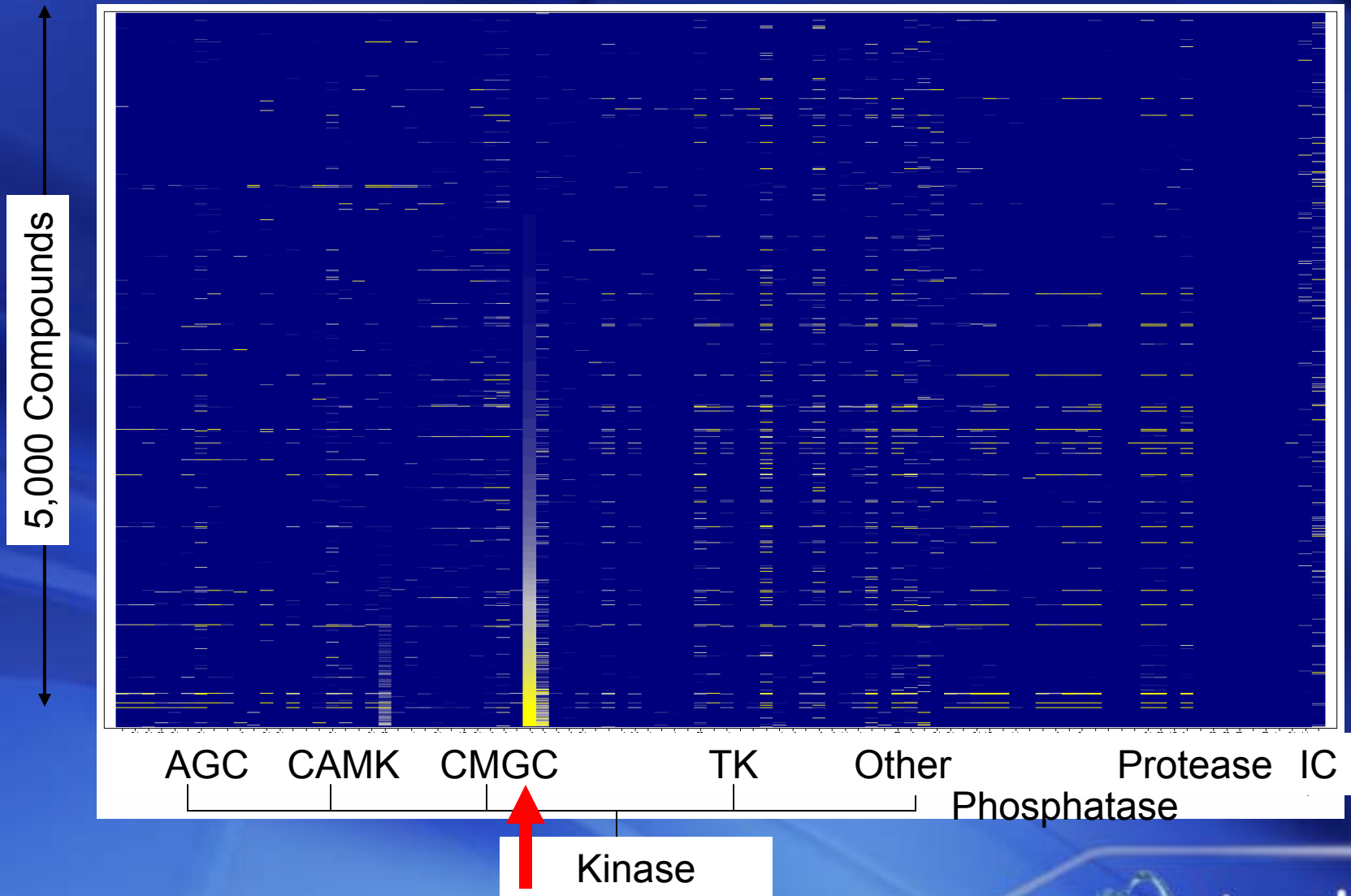
Series Selection p38 α



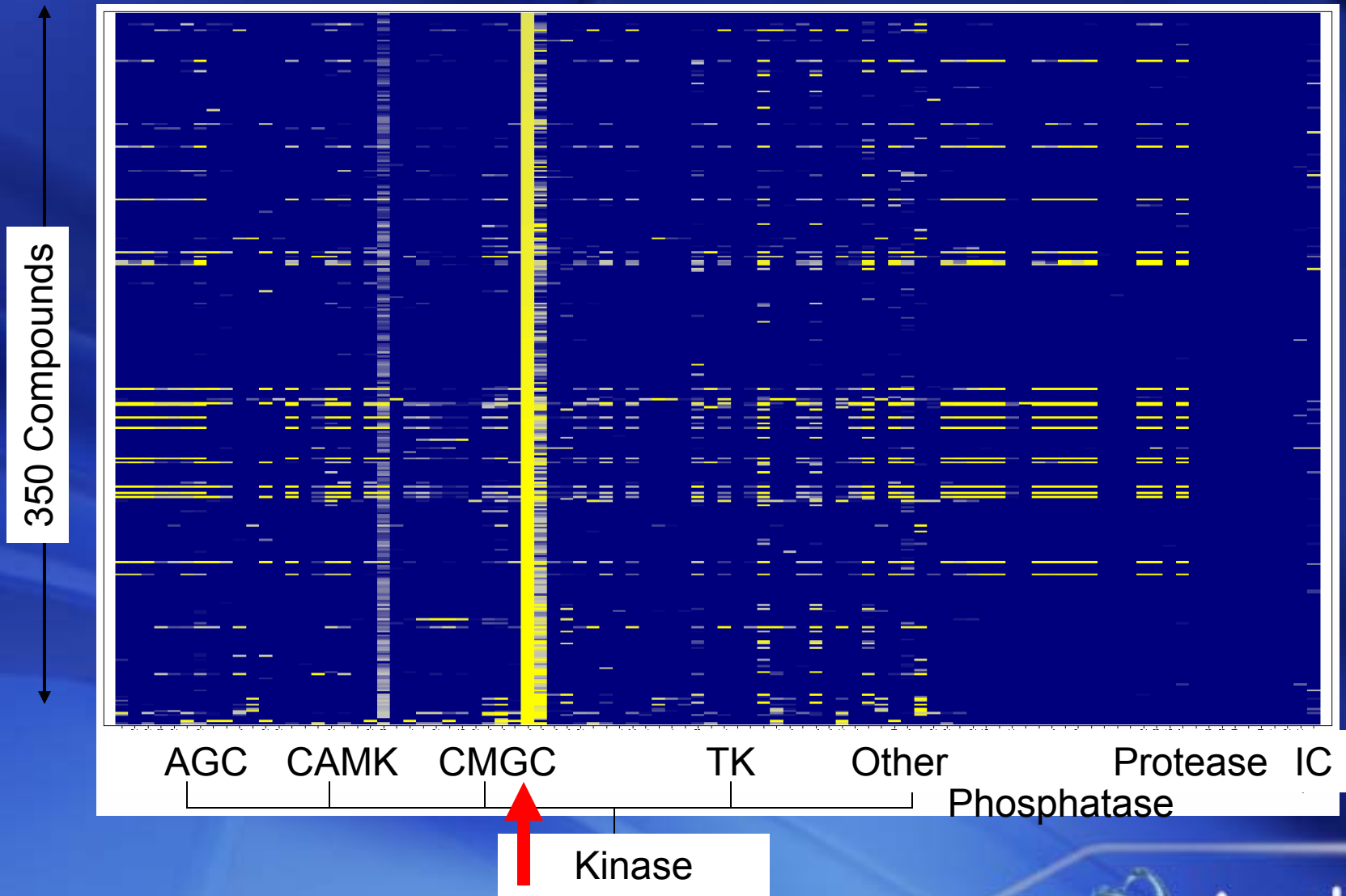
Series Selection p38 α



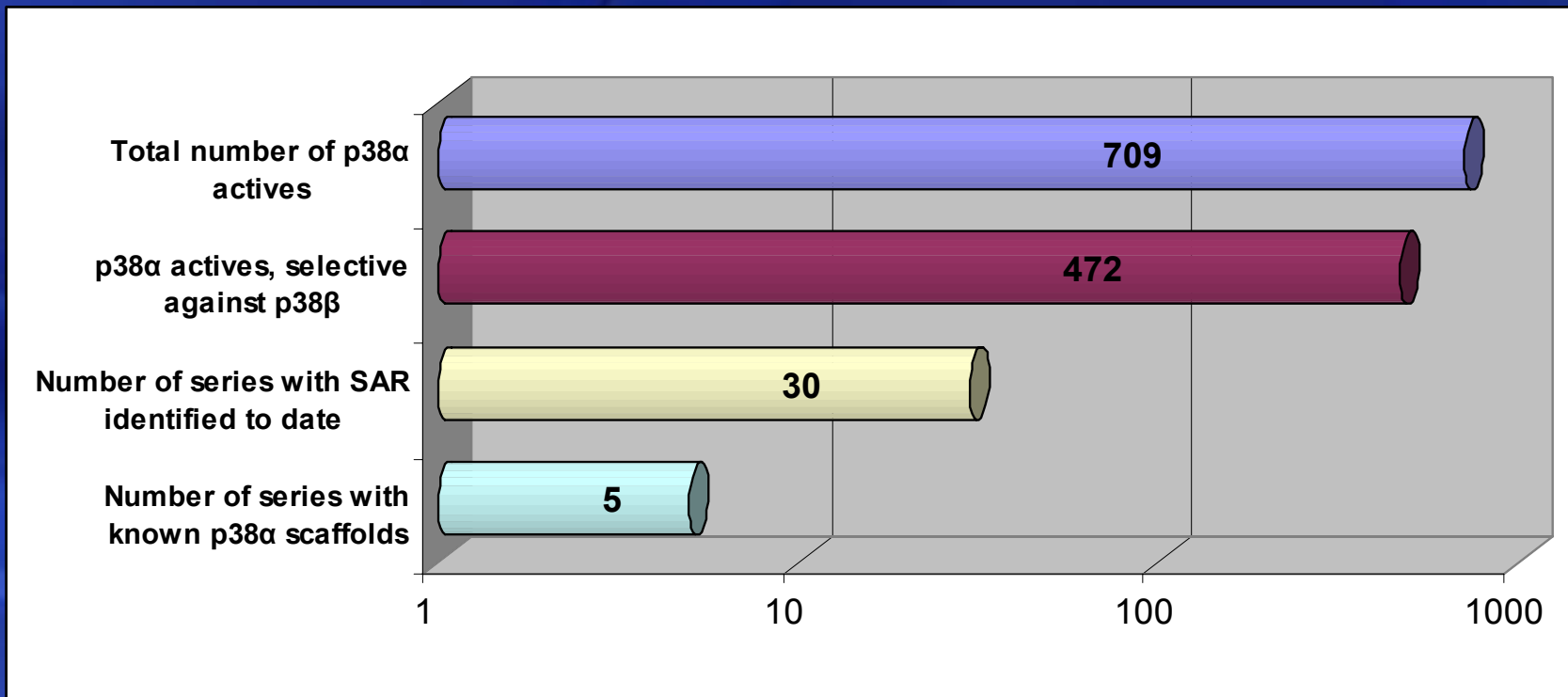
Series Selection p38 α



Series Selection p38 α



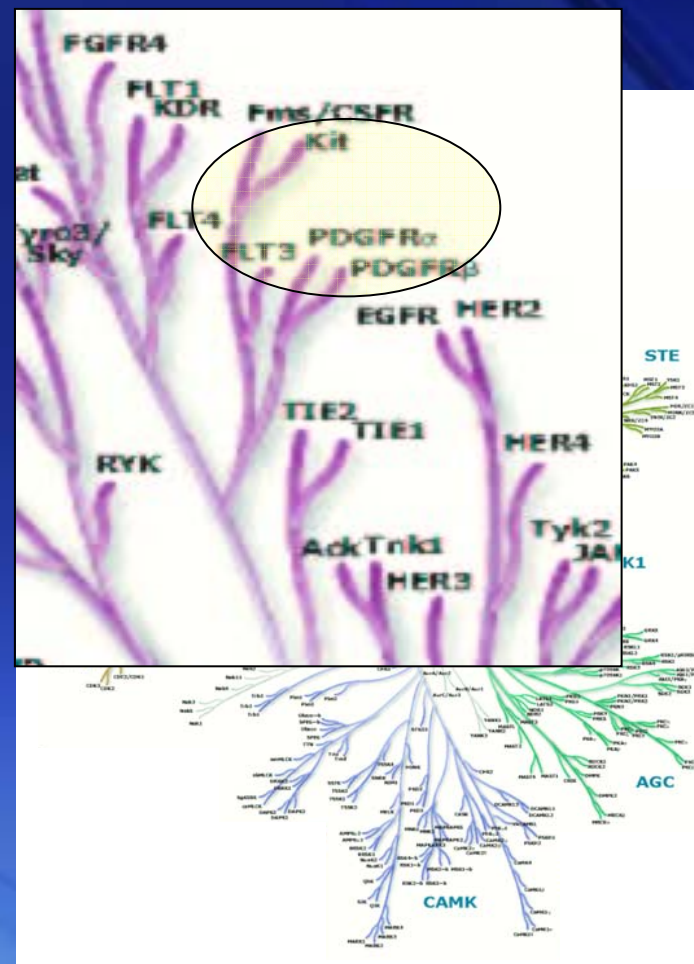
p38 α HTS: Actives Analysis Summary



Data Analysis Example:
Targeted Tyrosine Kinase Program
Actives Analysis

Starting Point for a Selective Multi-Kinase Series

1. Group FLT-3, c-KIT, and PDGFR as primary target
 - Sequence similarity at the active site
 - c-KIT, FLT-3, PDGFRs commonly mis-regulated in cancers
2. Mining and evaluation of multiple scaffolds from the existing profiling database
3. Use SAR from 1^o data and start new chemistry

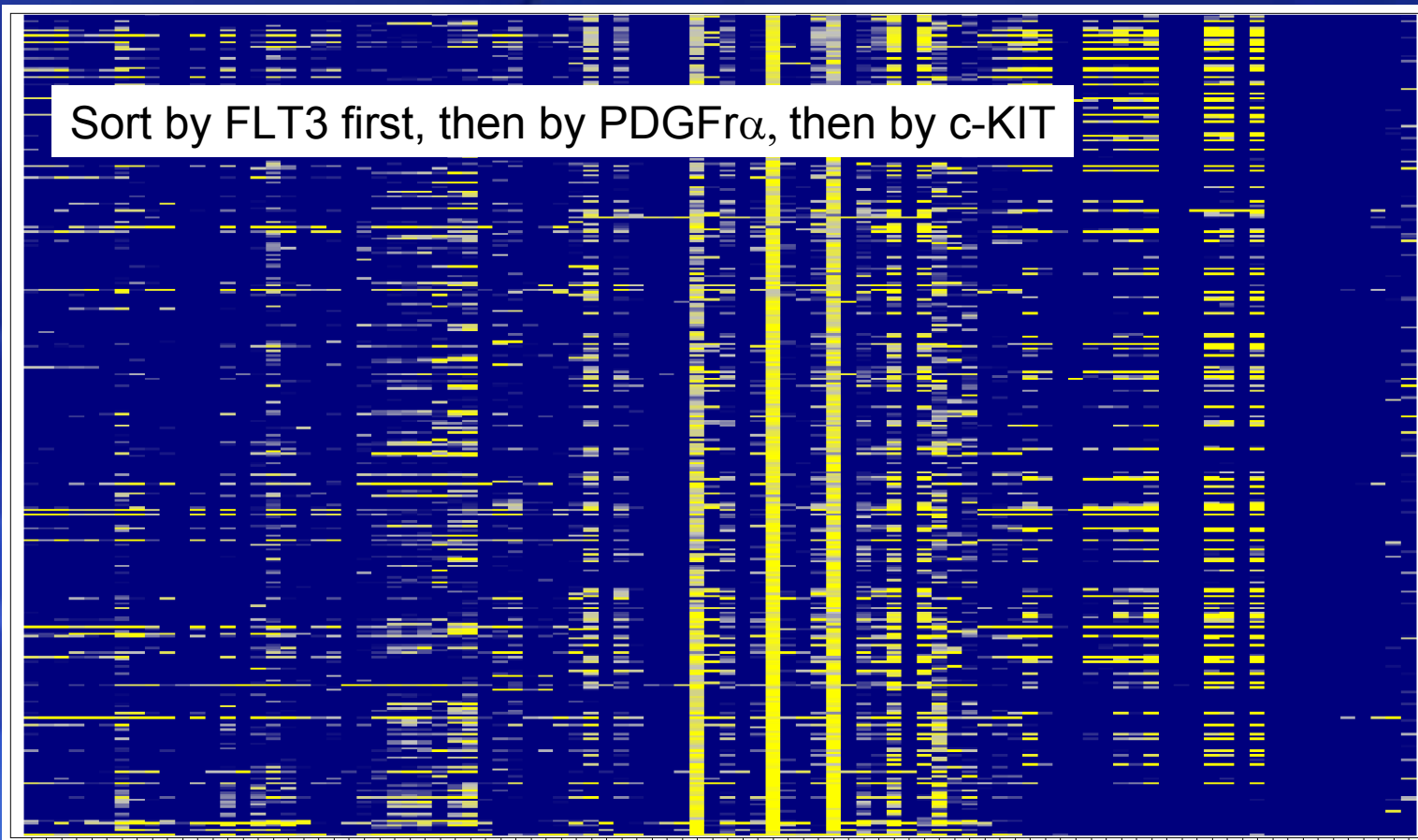


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Database Mining for TTK Inhibitors

350 compounds

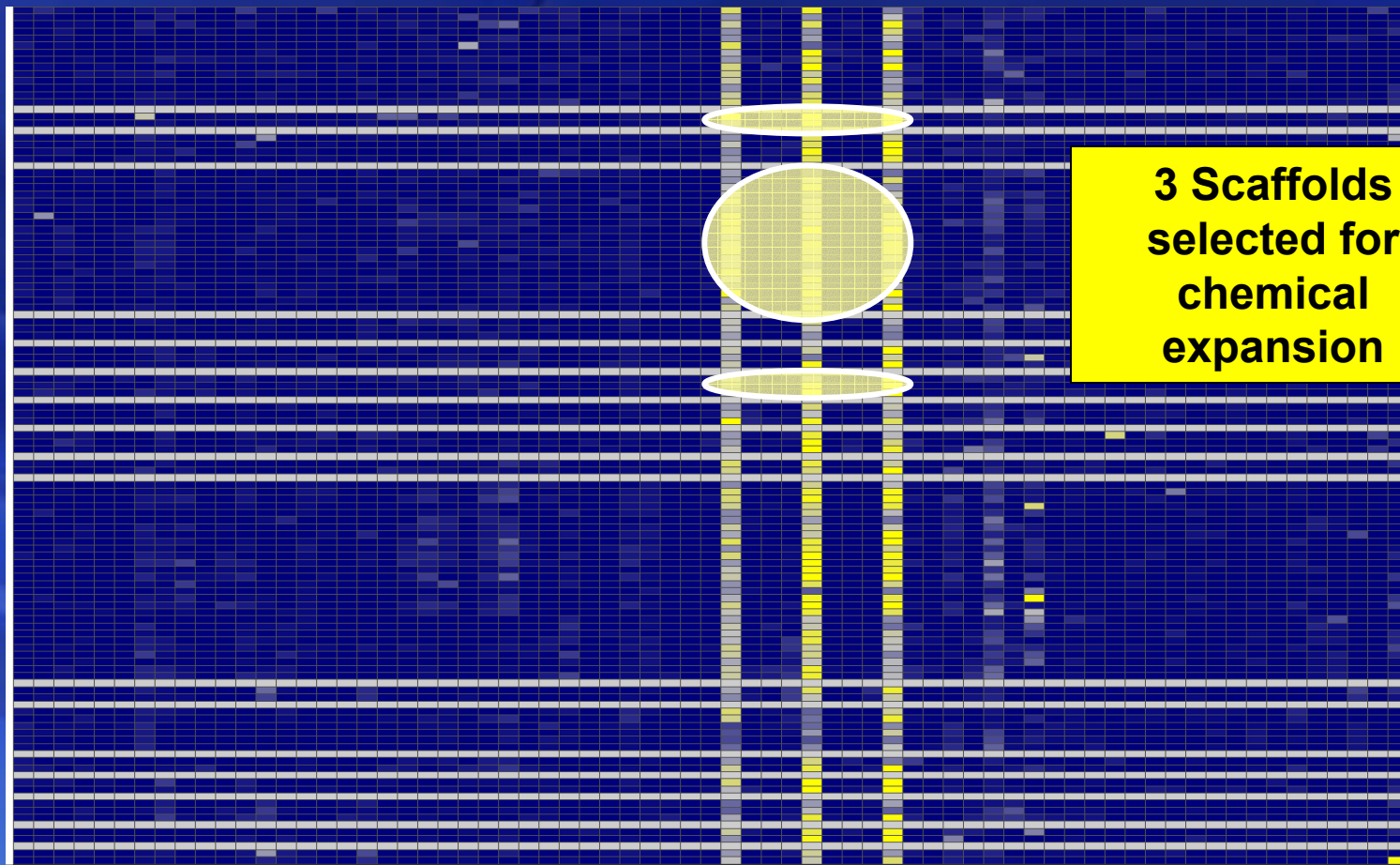
Sort by FLT3 first, then by PDGFR α , then by c-KIT



Active Weakly active Inactive No data

Series Selection TTK Inhibitors

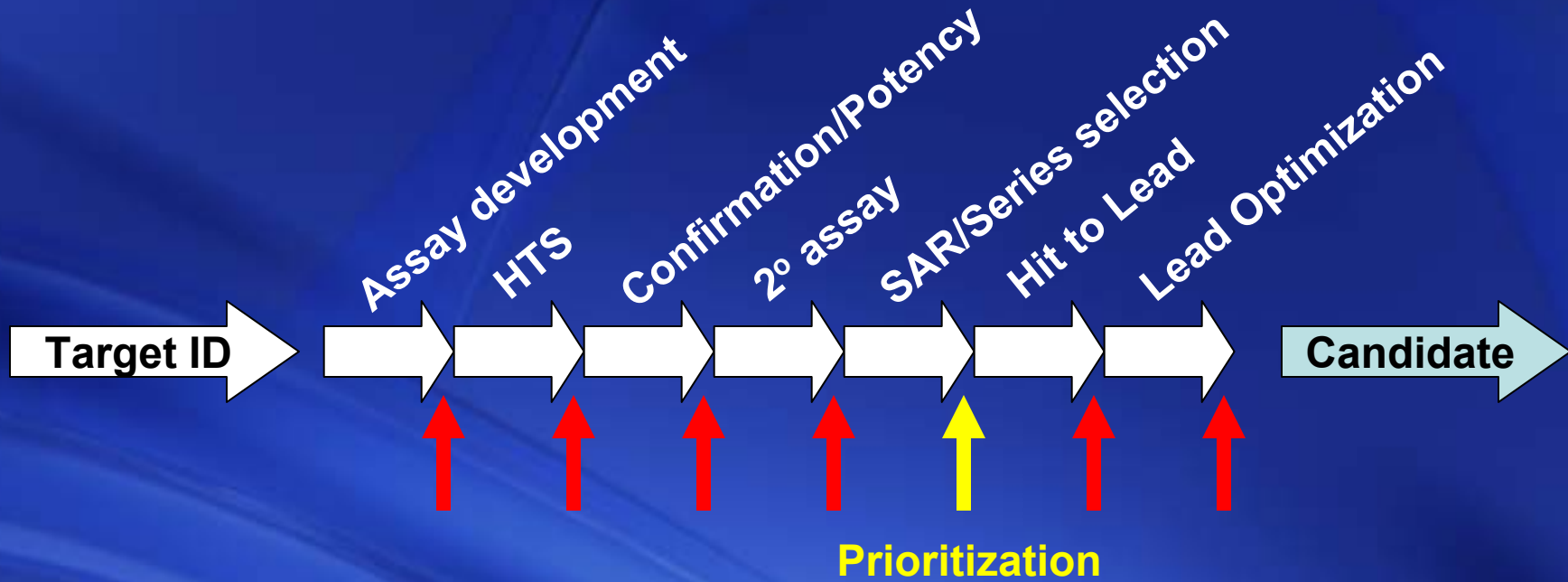
18 distinct, selective chemotypes identified



**3 Scaffolds
selected for
chemical
expansion**

Active Weakly active Inactive No data

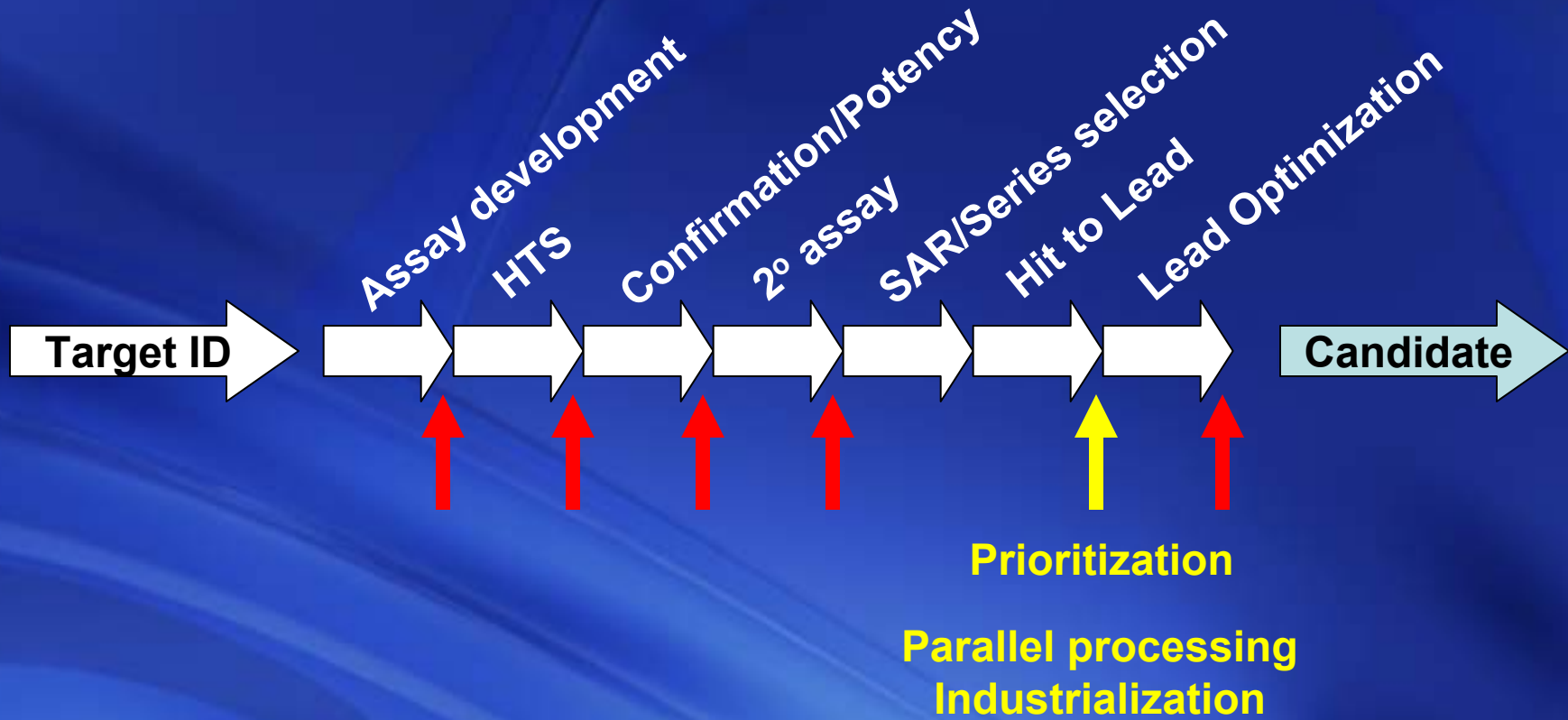
The Early Discovery Pipeline



Consistent technology
Quality at all stages of the process
Comprehensive database with comparable data

↑ **Go/No Go decisions**

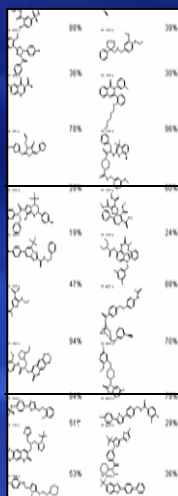
The Early Discovery Pipeline



 **Go/No Go decisions**

Industrialization of Lead Identification Process

New compound synthesis SAR from 1^o screen



Multiple scaffolds enter queue

Monthly profiling

- Kinases (66)
- Phosphatases (6)
- Proteases (24)
- ADME Surrogates (3)

Establish Inhibition Mechanism

- K_i evaluation

Kinetic Filters

- Reversibility
- Time dependence
- Tight binding
- Aggregation

Quantitation



- Nitrogen detection in aqueous buffer

Full characterization in 30 days

- Immediate selectivity
- Take advantage of automation, liquid handling, automatic loading & meta-analysis
- Evaluate SAR with K_i & correct mechanism
- Recognize divergence
- Eliminate misleading data
- Understand solubility & true K_i

Industrialization of Lead Identification Process

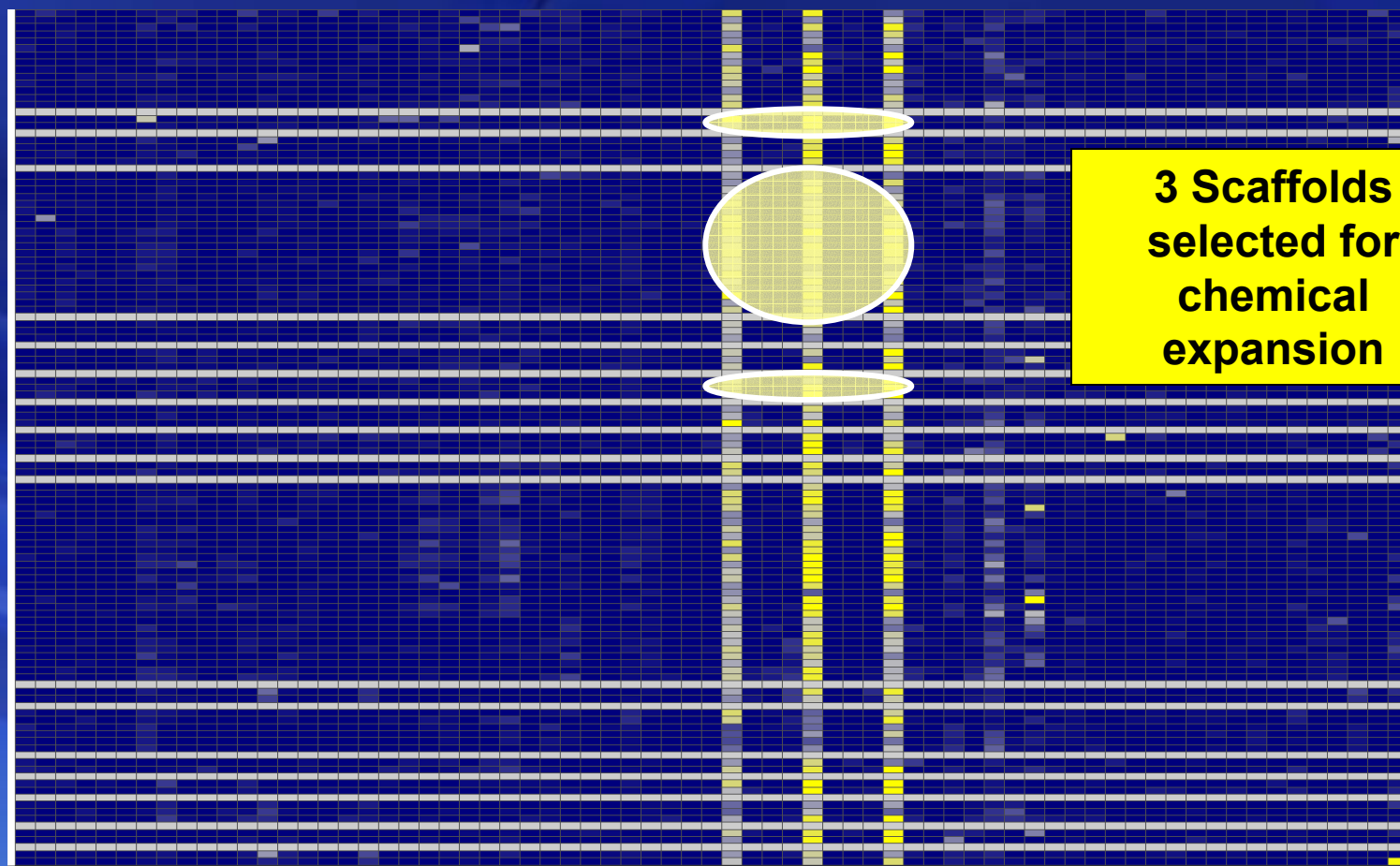
	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	
Program 1		Actives Analysis	Design	Synthesis / Purification/LM	ELF-Panel-ADME	Cell Pharm	Analyze data - Team review	Design	Synthesis / Purification/LM	ELF-Panel-ADME	Cell Pharm	Analyze data - Team review				
Program 2			Actives Analysis	Design	Synthesis / Purification/LM	ELF-Panel-ADME	Cell Pharm	Analyze data - Team review	Design	Synthesis / Purification/LM	ELF-Panel-ADME	Cell Pharm	Analyze data - Team review			
Program 3				Actives Analysis	Design	Synthesis / Purification/LM	ELF-Panel-ADME	Cell Pharm	Analyze data - Team review	Design	Synthesis / Purification/LM	ELF-Panel-ADME	Cell Pharm	Analyze data - Team review		
Program 4										Actives Analysis	Design	Synthesis / Purification/LM	ELF-Panel-ADME	Cell Pharm	Analyze data - Team review	

 Go/NoGo Decision for 3 Programs

Series Selection TTK Inhibitors

18 distinct, selective chemotypes identified



**3 Scaffolds
selected for
chemical
expansion**

Active Weakly active Inactive No data

Outcome After First Round



Potency – Ki	160 nM (X) 4 nM (Y) 2 nM (Z)	33% inh (X) 30 nM (Y) 22 nM (Z)
Enzyme selectivity	Good selectivity over all primary screen enzymes	
Mechanism	ATP Competitive	
Cell proliferation	Low nM	High nM
ADME surrogate	Good metabolic stability (rat liver microsomes)	

*Data is from one cycle of chemistry optimization

Conclusion

- Parallel processing and focus on quality at all stages from target to candidate increases efficiency, reduces timelines, lowers cost and gives an improved probability of success.

Thank you for your attention!