Causal Discovery from Mass Cytometry Data

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FORTH-ICS

ΠΑΝΕΠΙΣΤΗΜΙΟ ΚΡΗΤΗΣ

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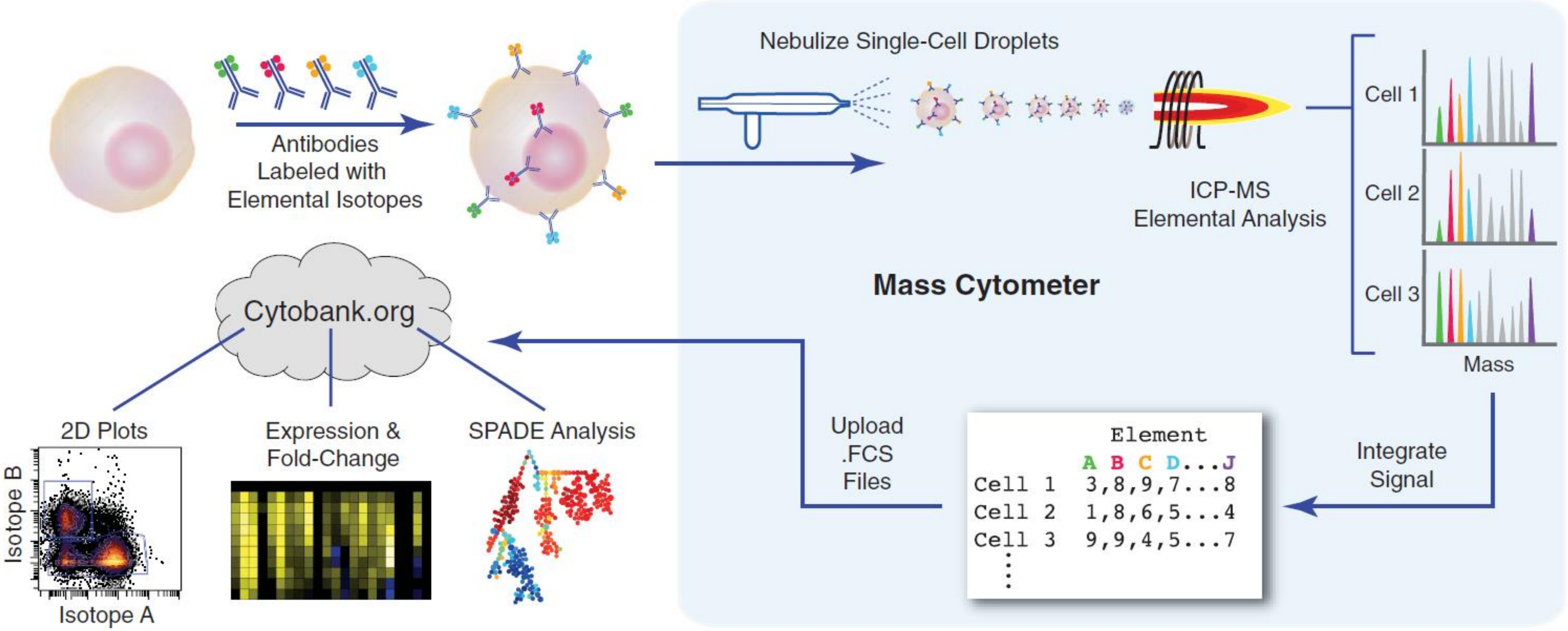
The Measuring Technology

Mass Cytometry

- **Single cells** measurements
- **Sample sizes in the millions**, minimal cost
- Public data available
- Up to ~30 proteins measured at a time
- Applications
  1. Cell counting
  2. Cell sorting (gating)
  3. Identifying signaling responses
  4. Drug screening
  5. De novo, personalized pathway / causal discovery (?)

Mass Cytometry



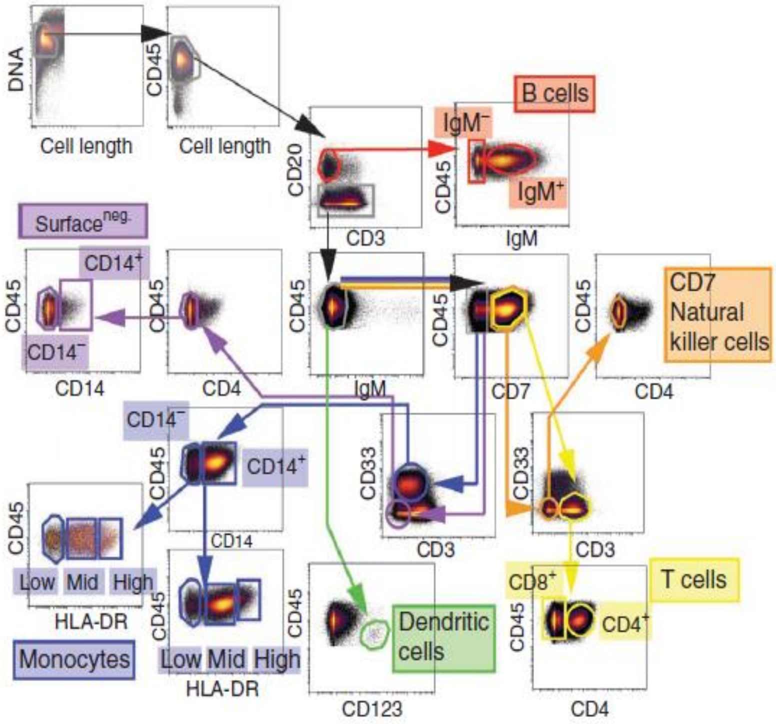
	Element
	A B C D...J
Cell 1	3,8,9,7...8
Cell 2	1,8,6,5...4
Cell 3	9,9,4,5...7
⋮	

[Image by Bendall et al., Science 2011]

Cell Sorting (Gating)

- **Immune system cells** can be distinguished based on specific surface markers.

- Process resembles a decision tree

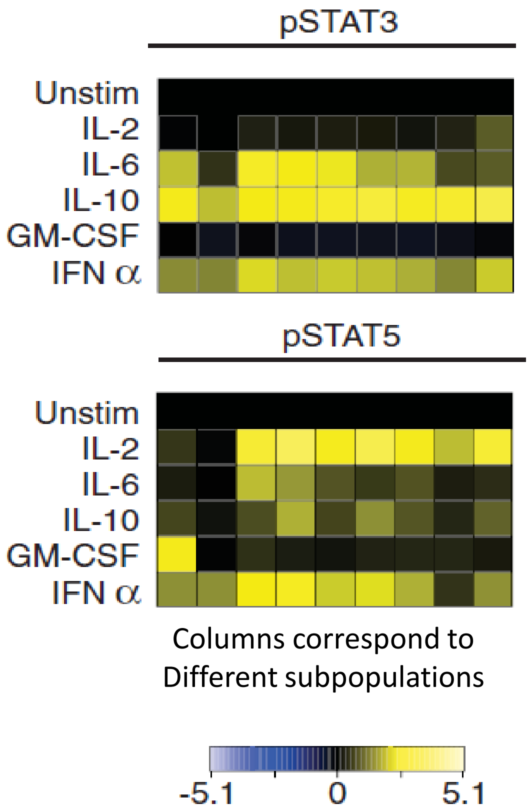


[Image by Bodenmiller et al., Nat. Biotech. 2012]

Identifying Signaling Responses

- Immune responses are triggered by specific **activators**
- Signaling responses are sub-population specific.
- Mass cytometry for identifying signaling effects:

1. Functional proteins (non-surface) are also marked (e.g., pSTAT3 and pSTAT5)
2. Activators are applied to stimulate a response to disease
3. Cells are sorted by sub-population
4. Changes in protein abundance/phosphorylation in each subpopulation are quantified



Columns correspond to Different subpopulations

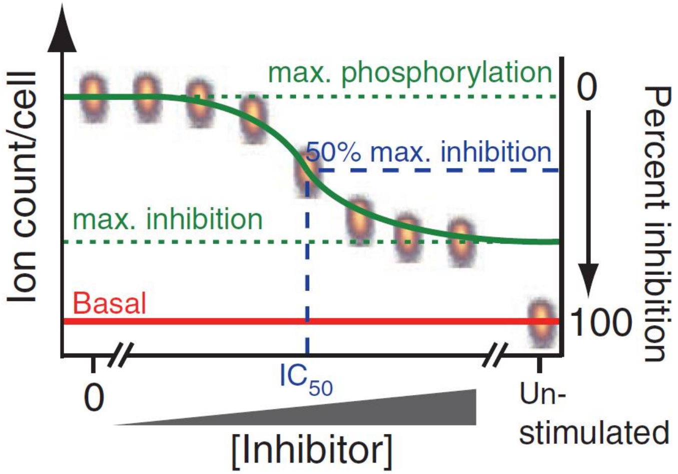
Difference in log2 mean intensity of the stimulated condition compared with the unstimulated control

[Image by Bendall et al., Science 2011]

Drug Screening

- Unwanted signaling responses should be suppressed for disease treatment
- Mass cytometry for drug screening

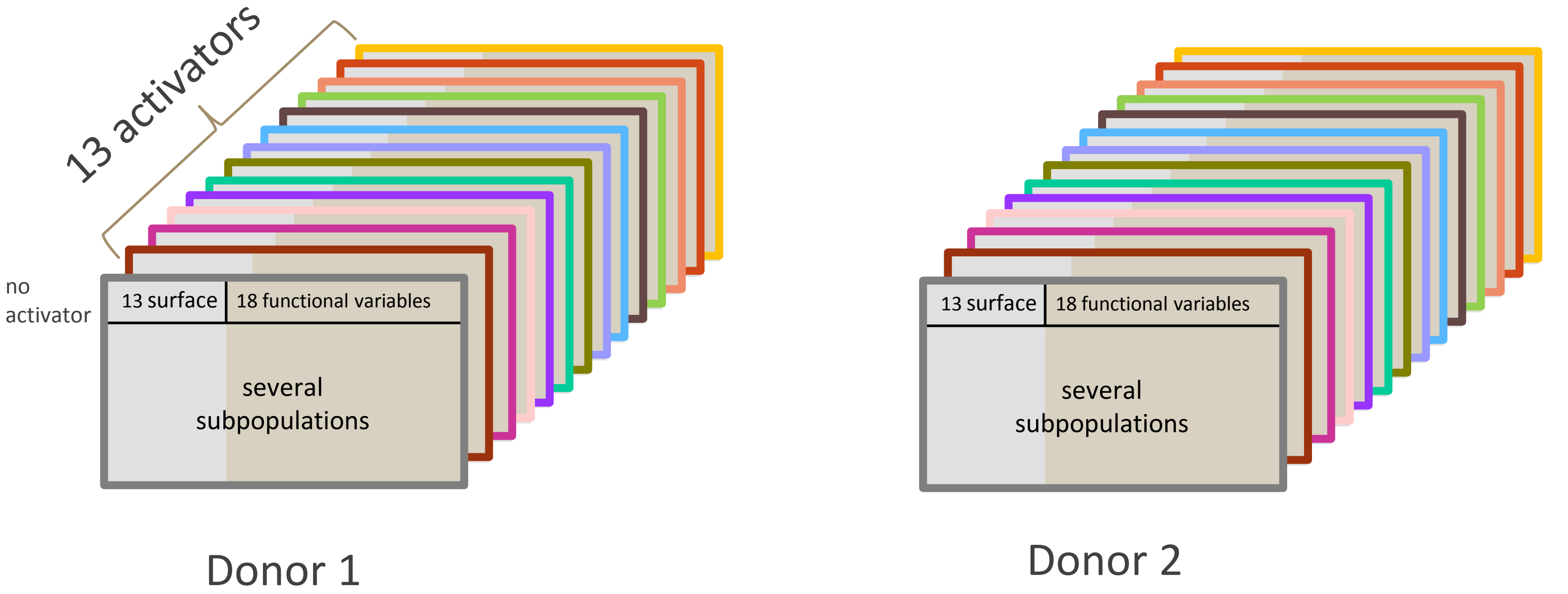
1. **After stimulation**, cells are treated with potential drugs (**inhibitors**)
2. Cells are sorted by sub-population
3. Dose-response curves are identified
   - Per activator
   - Per sub-population
   - Per inhibitor



[Image by Bodenmiller et al., Nat. Biotech. 2012]

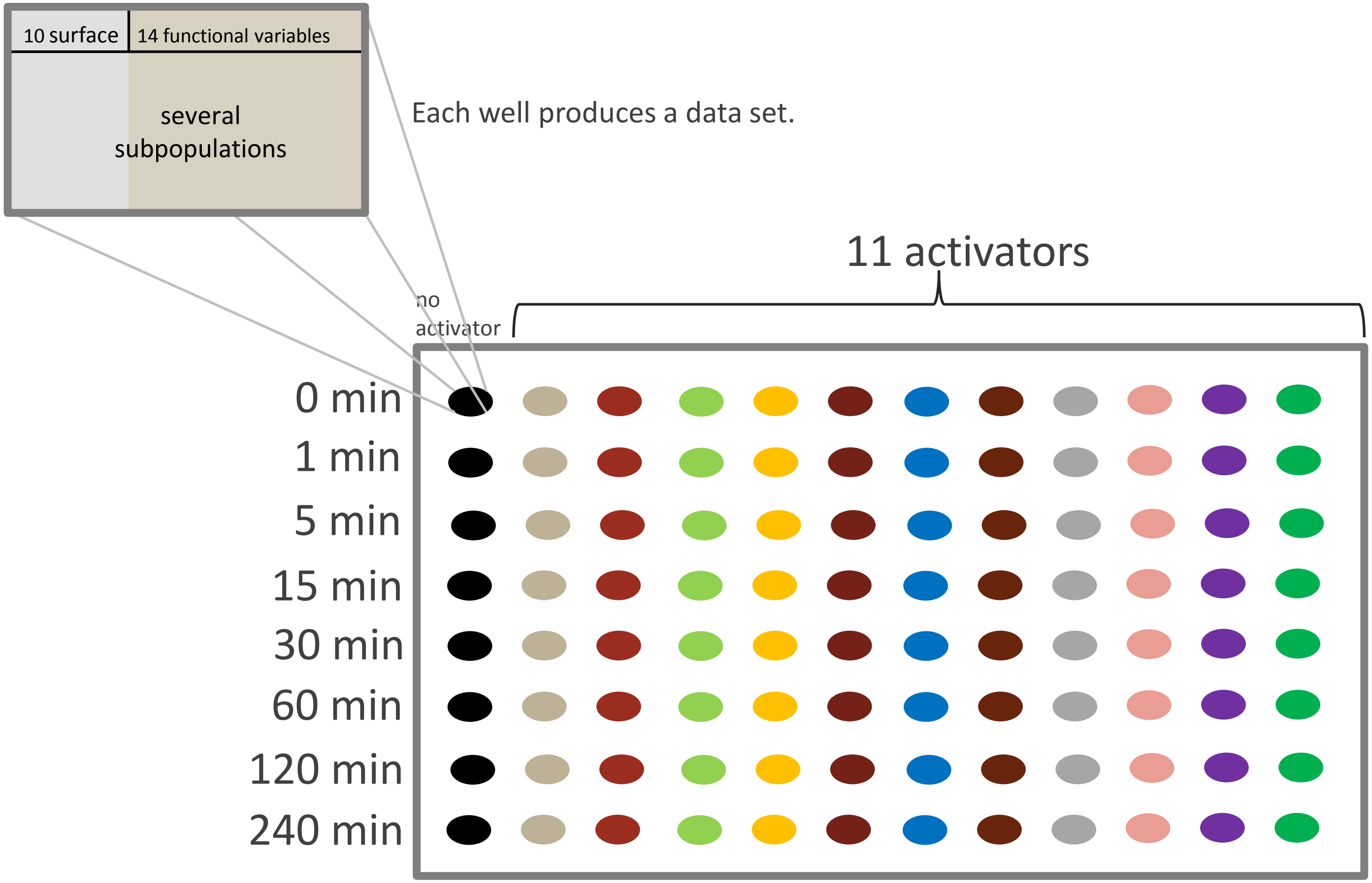
The Public Data

Bendall Data



[**Single-Cell Mass Cytometry of Differential Immune and Drug Responses Across a Human Hematopoietic Continuum, Bendall et al.,** *Science* **332**, 687 (2011)]

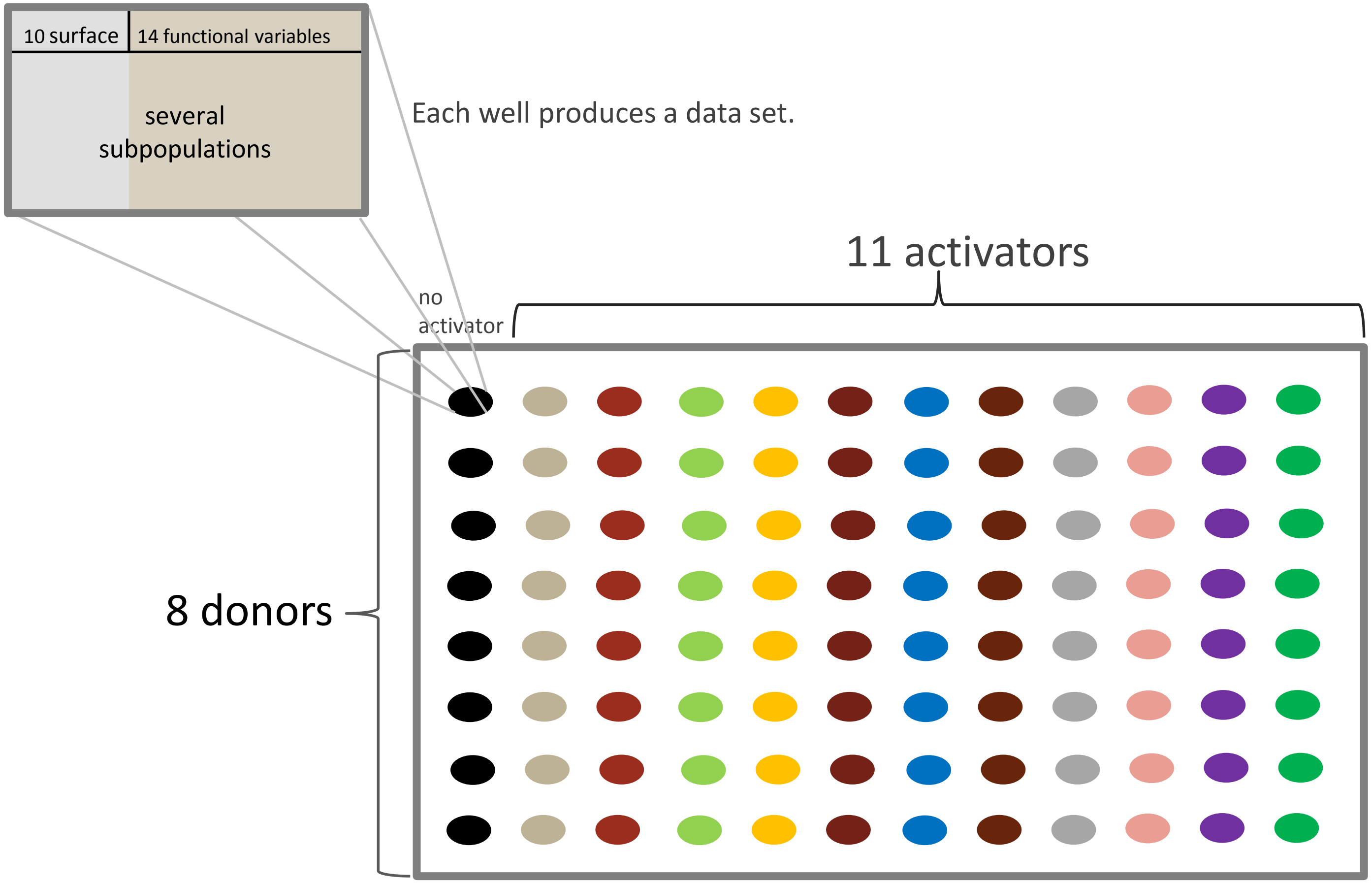
Bodenmiller Data: Time Course



A plate with 96 wells

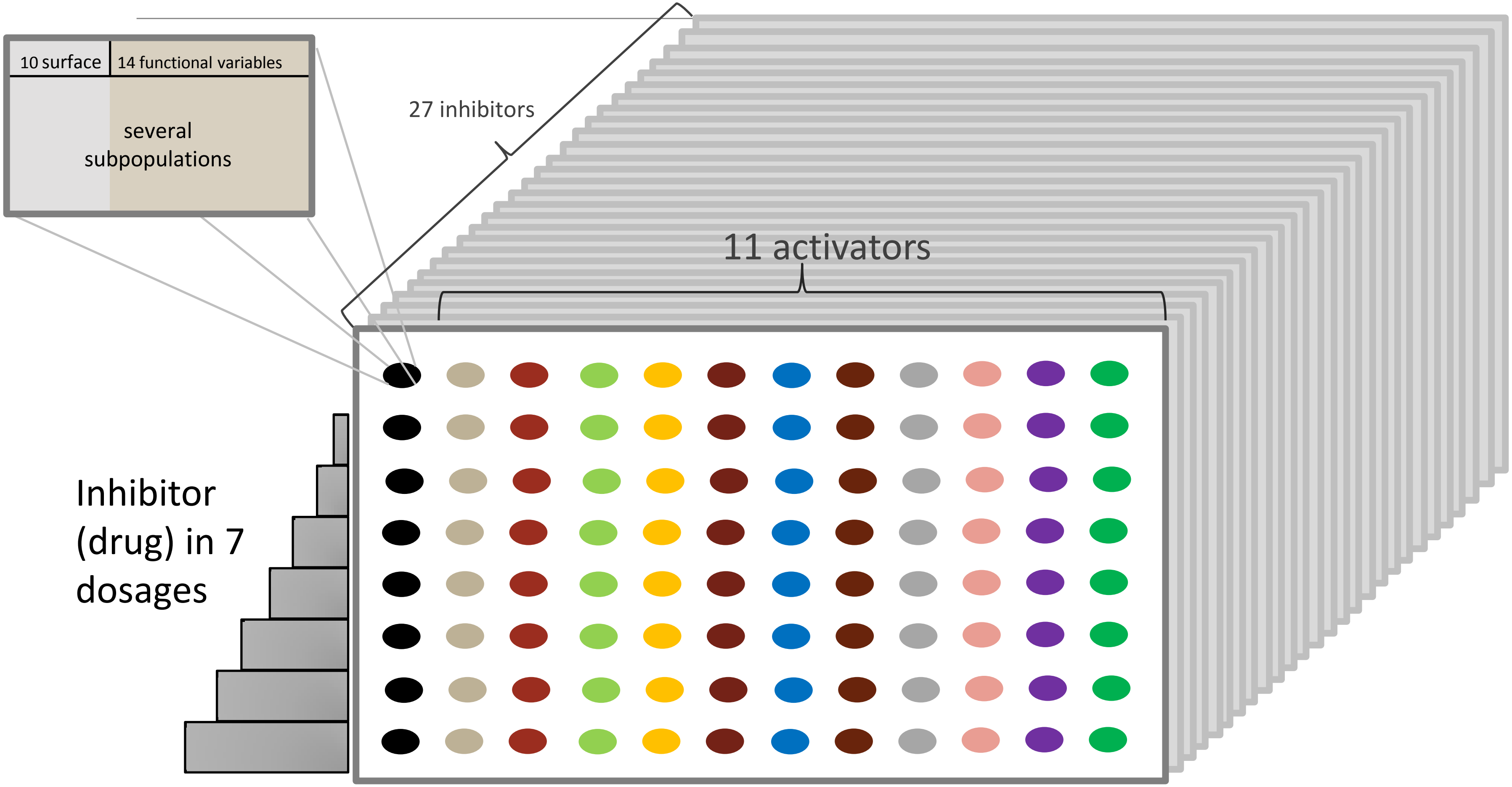
[**Multiplexed mass cytometry profiling of cellular states perturbed by small-molecule regulators, Bodenmiller et al.,** *Nature Biotechnology* **30**, 9 (2012)]

Bodenmiller Data: 8 donors



[**Multiplexed mass cytometry profiling of cellular states perturbed by small-molecule regulators, Bodenmiller et al.,** *Nature Biotechnology* **30**, 9 (2012)]

Bodenmiller Data: Inhibitors



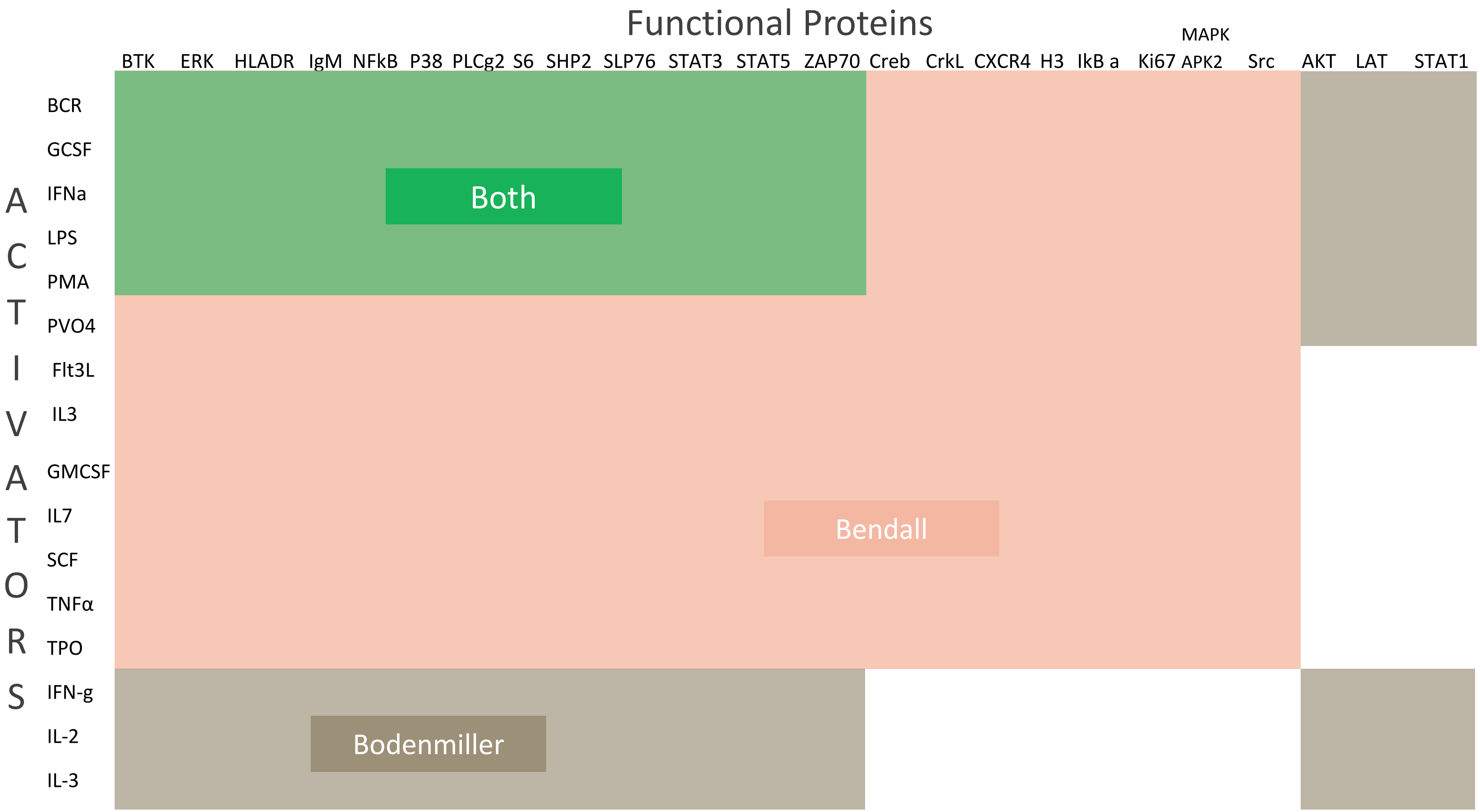
[**Multiplexed mass cytometry profiling of cellular states perturbed by small-molecule regulators, Bodenmiller et al.,** *Nature Biotechnology* **30**, 9 (2012)]

Data summary

	Bodenmiller data			Bendall data
	Inhibitor data	8donor data	Time course data	
Activators	🟩	🟩	🟩	🟩
Time			🟩	
Donors		🟩		🟩
Inhibitors	🟩			
Subpopulations	🟩	🟩	🟩	🟩
Proteins	🟩	🟩	🟩	🟩

Collection of datasets with :
All activators
1 time point (30')
1 donor
All Inhibitors
All Subpopulations
All 10+14 markers measured

Data Summary



Functional Proteins

ACTIVATORS

	BTK	ERK	HLADR	IgM	NFkB	P38	PLCg2	S6	SHP2	SLP76	STAT3	STAT5	ZAP70	Creb	CrkL	CXCR4	H3	IkB a	Ki67	MAPKAPK2	Src	AKT	LAT	STAT1
BCR	Both	Both	Both	Both	Both	Both	Both	Both	Both	Both	Both	Both	Both	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bodenmiller	Bodenmiller	Bodenmiller
GCSF	Both	Both	Both	Both	Both	Both	Both	Both	Both	Both	Both	Both	Both	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bodenmiller	Bodenmiller	Bodenmiller
IFNa	Both	Both	Both	Both	Both	Both	Both	Both	Both	Both	Both	Both	Both	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bodenmiller	Bodenmiller	Bodenmiller
LPS	Both	Both	Both	Both	Both	Both	Both	Both	Both	Both	Both	Both	Both	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bodenmiller	Bodenmiller	Bodenmiller
PMA	Both	Both	Both	Both	Both	Both	Both	Both	Both	Both	Both	Both	Both	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bodenmiller	Bodenmiller	Bodenmiller
PVO4	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bodenmiller	Bodenmiller	Bodenmiller
Flt3L	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall			
IL3	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall			
GMCSF	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall			
IL7	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall			
SCF	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall			
TNFα	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall			
TPO	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall	Bendall			
IFN-g	Bodenmiller	Bodenmiller	Bodenmiller	Bodenmiller	Bodenmiller	Bodenmiller	Bodenmiller	Bodenmiller	Bodenmiller	Bodenmiller	Bodenmiller	Bodenmiller	Bodenmiller									Bodenmiller	Bodenmiller	Bodenmiller
IL-2	Bodenmiller	Bodenmiller	Bodenmiller	Bodenmiller	Bodenmiller	Bodenmiller	Bodenmiller	Bodenmiller	Bodenmiller	Bodenmiller	Bodenmiller	Bodenmiller	Bodenmiller									Bodenmiller	Bodenmiller	Bodenmiller
IL-3	Bodenmiller	Bodenmiller	Bodenmiller	Bodenmiller	Bodenmiller	Bodenmiller	Bodenmiller	Bodenmiller	Bodenmiller	Bodenmiller	Bodenmiller	Bodenmiller	Bodenmiller									Bodenmiller	Bodenmiller	Bodenmiller

Causal Discovery in Mass Cytometry

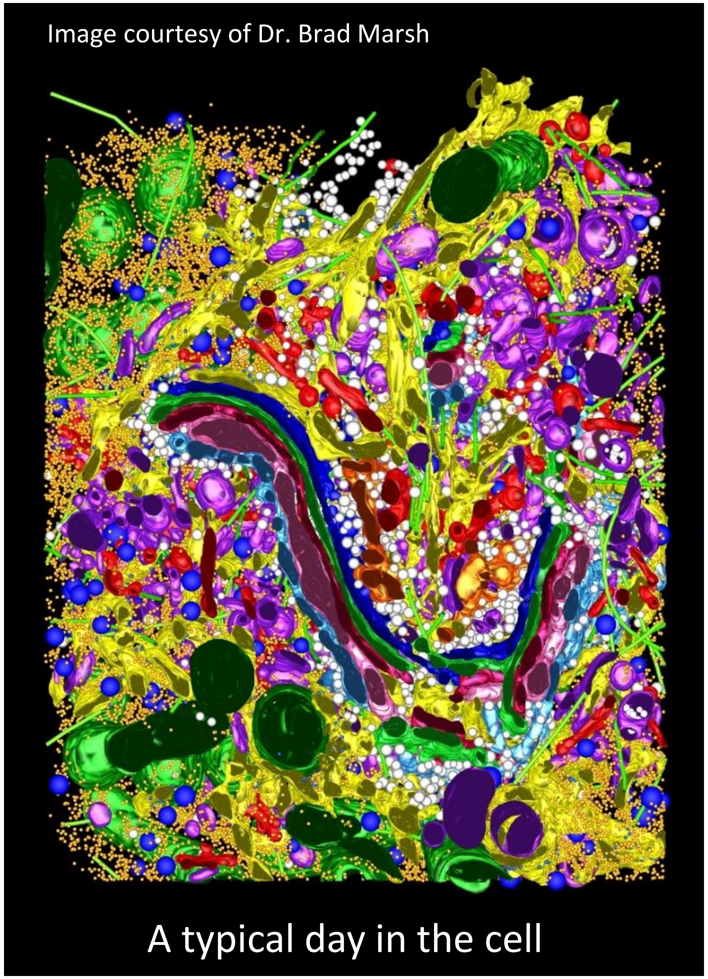


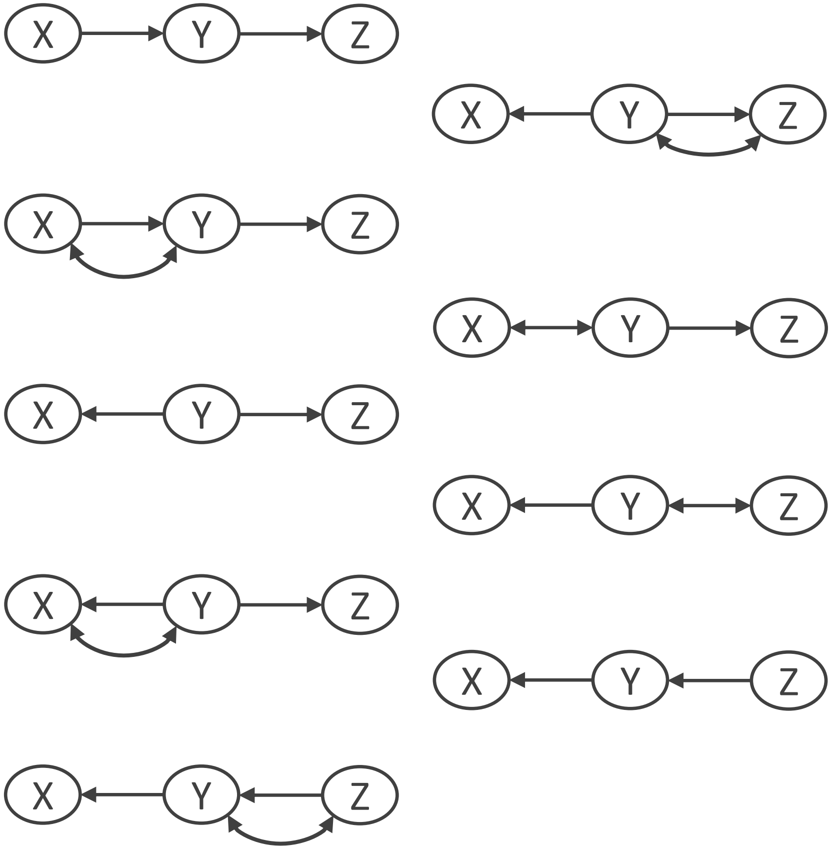
Image courtesy of Dr. Brad Marsh

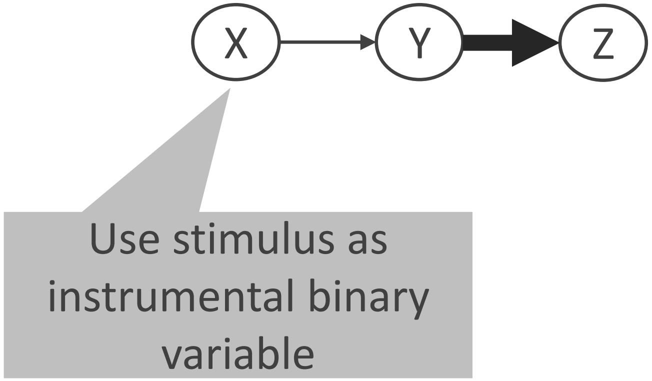
A typical day in the cell

- Feedback loops
- Latent variables
- Non-linear relations
- Unfaithfulness

A Basic Approach

Local Causal Discovery





Use stimulus as instrumental binary variable

Assumptions:

1. Causal Markov Condition
2. Reichenbach’s Common Cause Principle
3. No feedback cycles

$Ind(X,Z|Y)$ + Nothing causes X

Issue #1:
Signaling is Sub-Population Specific

- Gate data
  - Data were gated by the initial researchers in Cytobank.org
- Analyze sub-populations independently
- Gated sub-populations differ between Bodenmiller and Bendall
  - cd4+, cd8+, nk sub-populations in common.

Bodenmiller		Bendall		
cd14+hladr-,	cd14-surf-	Pre-B II	**Mature CD4+ T**	MPP
cd14+hladrhigh	**cd4+**	Mature CD38lo B	**Naive CD4+ T**	HSC
cd14+hladrmid	**cd8+**	Pre-B I	CMP	Megakaryocyte
cd14+surf-	dendritic	Mature CD38mid B	**Naive CD8+ T**	Erythroblast
cd14-hladr-	igm+	Immature B	**Mature CD8+ T**	Platelet
cd14-hladrhigh	igm-	Plasma cell	CD11b- Monocyte	MEP
cd14-hladrmid	**nk**	**nk**	CD11bmid Monocyte	Plasmacytoid DC
		Myelocyte	CD11bhi Monocyte	GMP

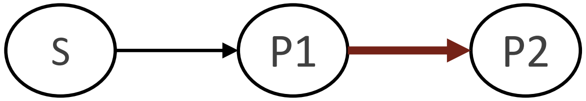
Issue #2:Dormant Relations

- Relations may appear only during signaling
  - Pool together unstimulated and stimulated data

- Different parts of the pathway maybe activated by different activators
  - Analyze data from different activators independently

Issue #3: Testing Independence

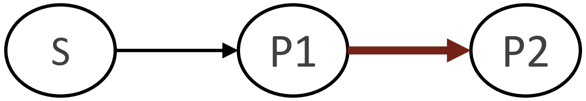
- Check (in)dependencies:
  1. $Dep(X, Y | \boldsymbol{Z})$
  2. $Ind(X, Y | \boldsymbol{Z})$



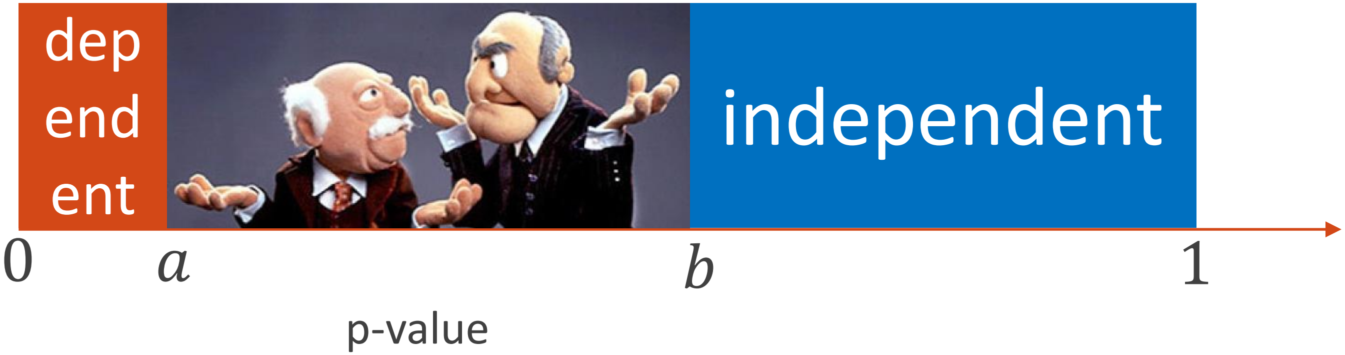
- Choosing a test of conditional independence
  - One binary, two continuous variables
  - Relations typically non-linear
  - Options:
    1. Discretization BUT: does not preserve conditional independencies
    2. Rejected but promising candidates:
       1. Maximal Information Coefficients (Reshef et al., Science 334, 2011)
       2. Kernel-based Conditional Independence test (Zhang et al., UAI 2011)
    3. Fisher z-test of independence + logistic regression

Issue #4
Make Reliable Predictions

- Check **ALL** (in)dependencies:
  1. $Dep(S, P1)$
  2. $Dep(S, P2)$
  3. $Dep(P1, P2)$
  4. $Ind(S, P2|P1)$
  5. $Dep(S, P1|P2)$
  6. $Dep(P1, P2|S)$

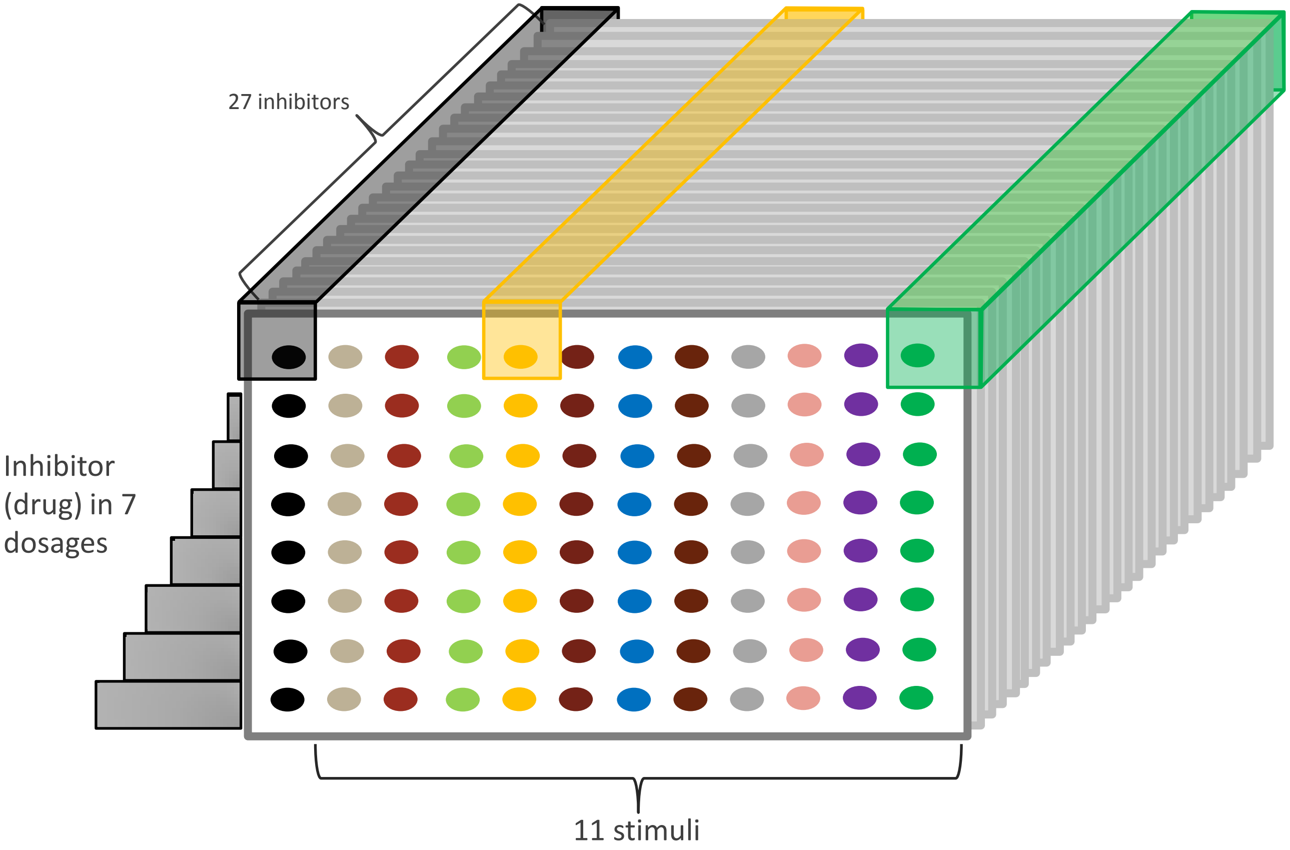


- Two thresholds, a =0.05 for dependence, b =0.15 for independence



Issue #5:
Identify “Outlier” Experiments

- Inhibitor data for “zero” dosage and 8 donor data should represent the same joint distribution
- Do they?



Issue #5:
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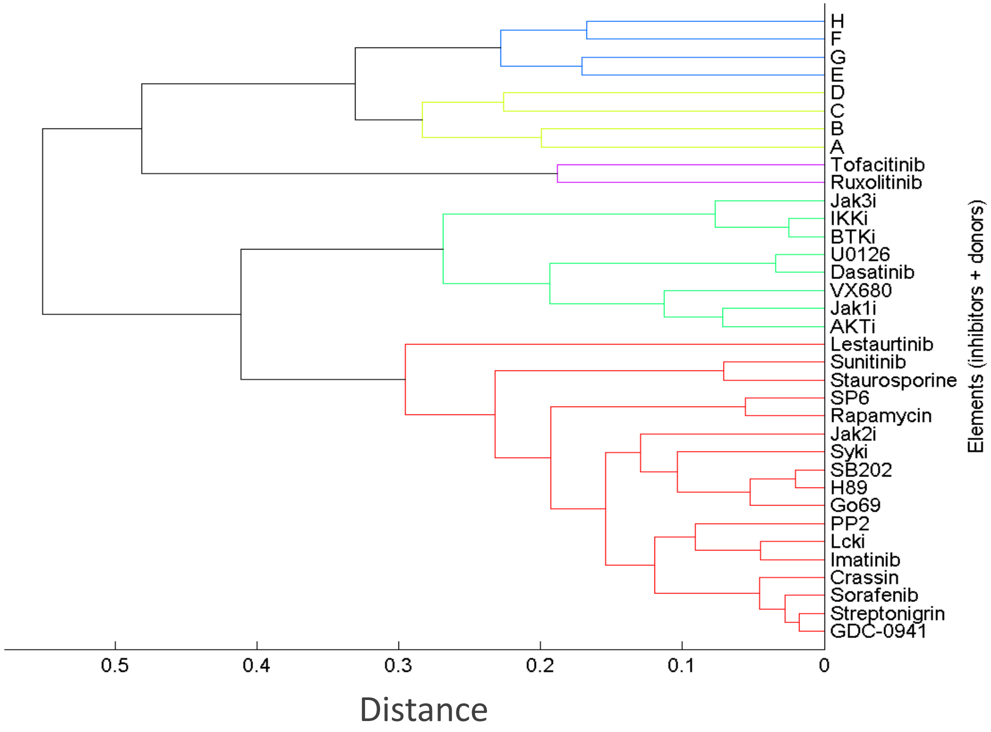
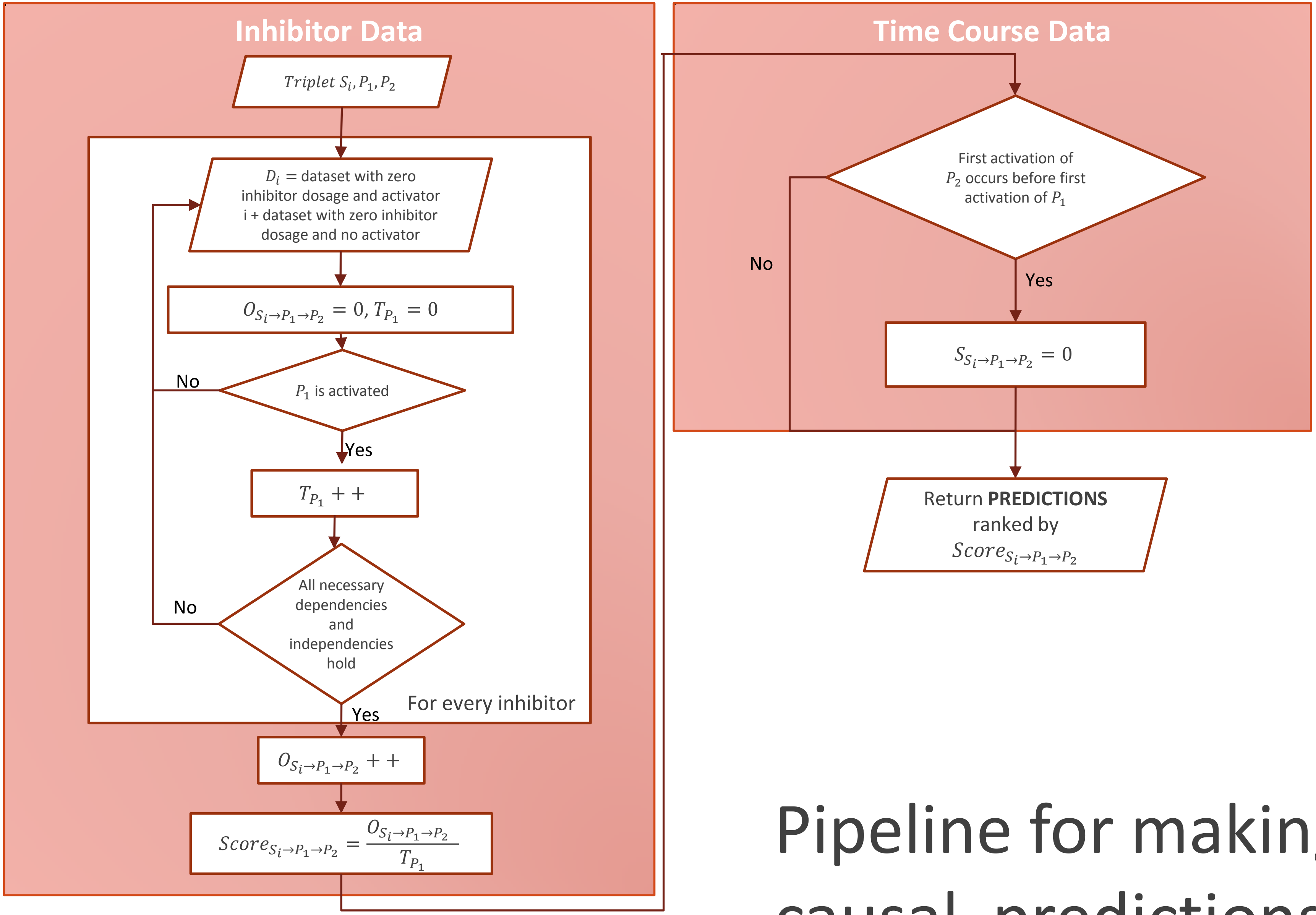


- Given a pair of plates:
  - For each activator, rank correlations (of markers), compute spearman correlation of ranking
  - Distance = 1-min correlation over activators



Inhibitor Data

$Triplet\ S_i, P_1, P_2$

$D_i =$ dataset with zero inhibitor dosage and activator i + dataset with zero inhibitor dosage and no activator

$O_{S_i \to P_1 \to P_2} = 0, T_{P_1} = 0$

P_1 is activated

No

Yes

$T_{P_1} ++$

All necessary dependencies and independencies hold

No

Yes

For every inhibitor

$O_{S_i \to P_1 \to P_2} ++$

$Score_{S_i \to P_1 \to P_2} = \frac{O_{S_i \to P_1 \to P_2}}{T_{P_1}}$

Time Course Data

First activation of P_2 occurs before first activation of P_1

No

Yes

$S_{S_i \to P_1 \to P_2} = 0$

Return **PREDICTIONS** ranked by $Score_{S_i \to P_1 \to P_2}$

Pipeline for making causal predictions

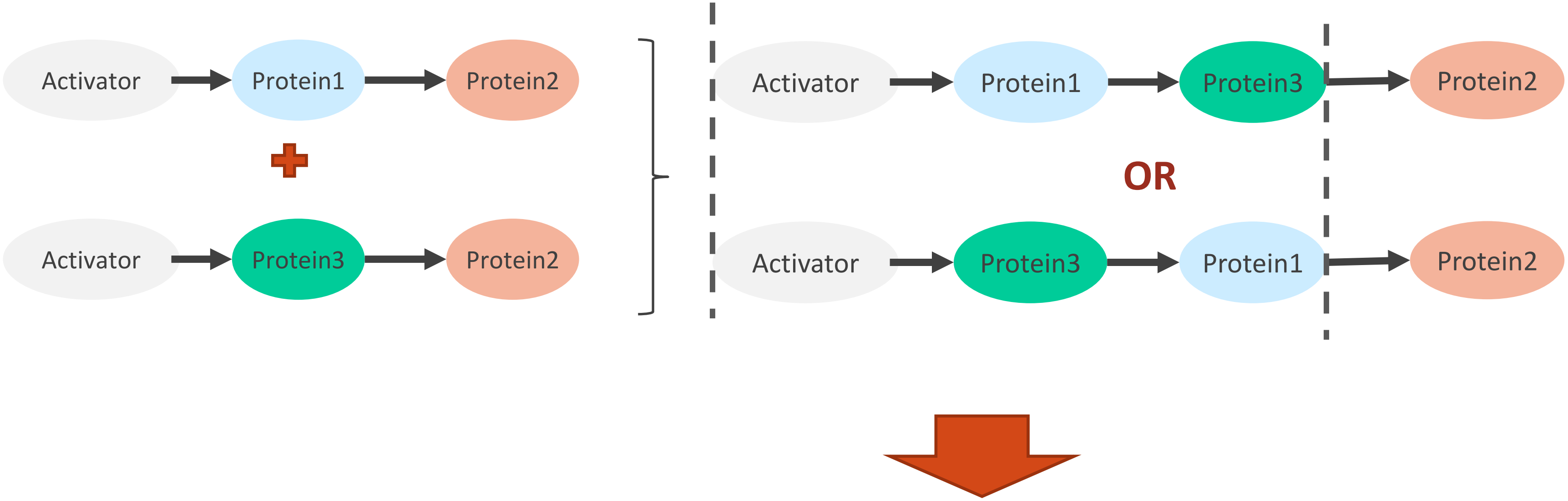
Causal Postulates

PVO_4 → pPlcg2	$\xrightarrow{0.5482}$	pSTAT3	0.875	cd14-hladr-
PVO_4 → pPlcg2	$\xrightarrow{0.5512}$	pZap70	0.8125	cd14-hladrmid
PVO_4 → pSlp76	$\xrightarrow{0.7152}$	pSHP2	0.8125	cd14-hladrmid
PVO_4 → pSHP2	$\xrightarrow{0.6708}$	pSTAT3	0.7857	dendritic
PVO_4 → pPlcg2	$\xrightarrow{0.8526}$	pP38	0.75	cd14+hladr-
PVO_4 → pPlcg2	$\xrightarrow{0.6166}$	pZap70	0.75	cd14-hladr-
PVO_4 → pSlp76	$\xrightarrow{0.5688}$	pZap70	0.75	cd14-hladr-
PVO_4 → pSHP2	$\xrightarrow{0.5688}$	pZap70	0.7143	cd14-hladrmid
PVO_4 → pSTAT3	$\xrightarrow{0.4557}$	pBtk	0.7059	cd14-hladr-
BCR → pS6	$\xrightarrow{0.4557}$	pErk	0.7037	igm-

288 predictions in
14 sub-populations

- A list of **predicted causal** pairs, each “tagged” for a **specific population** and **activator,** ranked according to a score quantifying the frequency of appearance.

Internal Validation

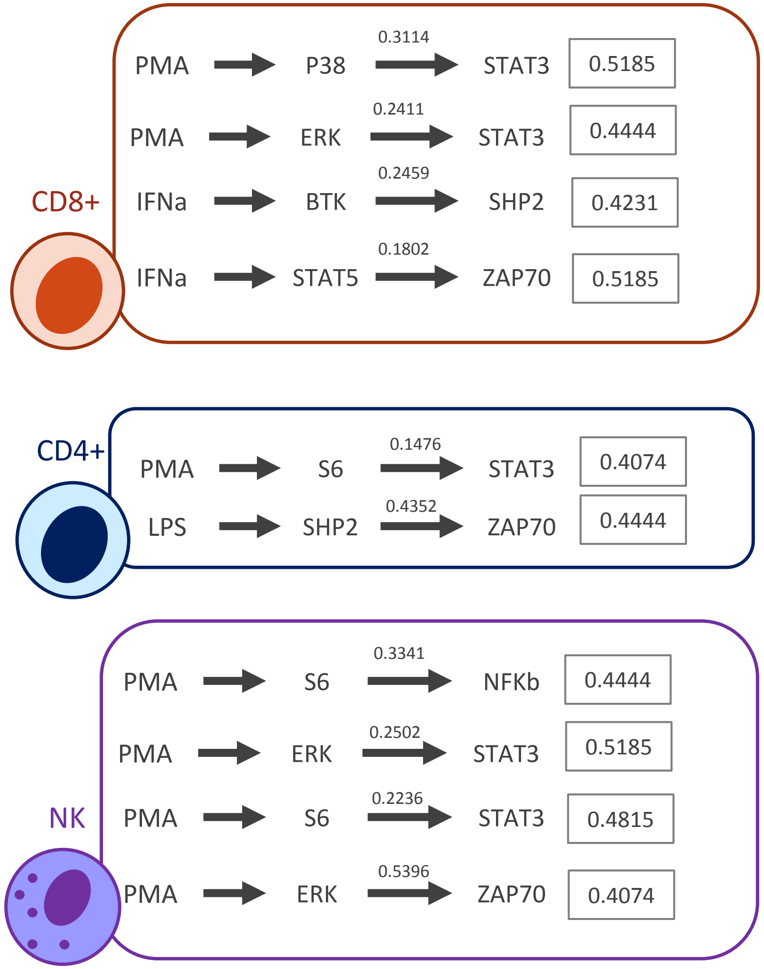


Check whether predicted triplet has also been reported

- 42% of the predicted triplets are also reported
- Despite strict thresholds and multiple testing

- Theory+algorithms: [Tillman et. al. 2008, Triantafillou et. al 2010, Tsamardinos et. al 2012]

Validation on Bendall Data



CD8+

PMA → P38 $\xrightarrow{0.3114}$ STAT3 0.5185

PMA → ERK $\xrightarrow{0.2411}$ STAT3 0.4444

IFNa → BTK $\xrightarrow{0.2459}$ SHP2 0.4231

IFNa → STAT5 $\xrightarrow{0.1802}$ ZAP70 0.5185

CD4+

PMA → S6 $\xrightarrow{0.1476}$ STAT3 0.4074

LPS → SHP2 $\xrightarrow{0.4352}$ ZAP70 0.4444

NK

PMA → S6 $\xrightarrow{0.3341}$ NFKb 0.4444

PMA → ERK $\xrightarrow{0.2502}$ STAT3 0.5185

PMA → S6 $\xrightarrow{0.2236}$ STAT3 0.4815

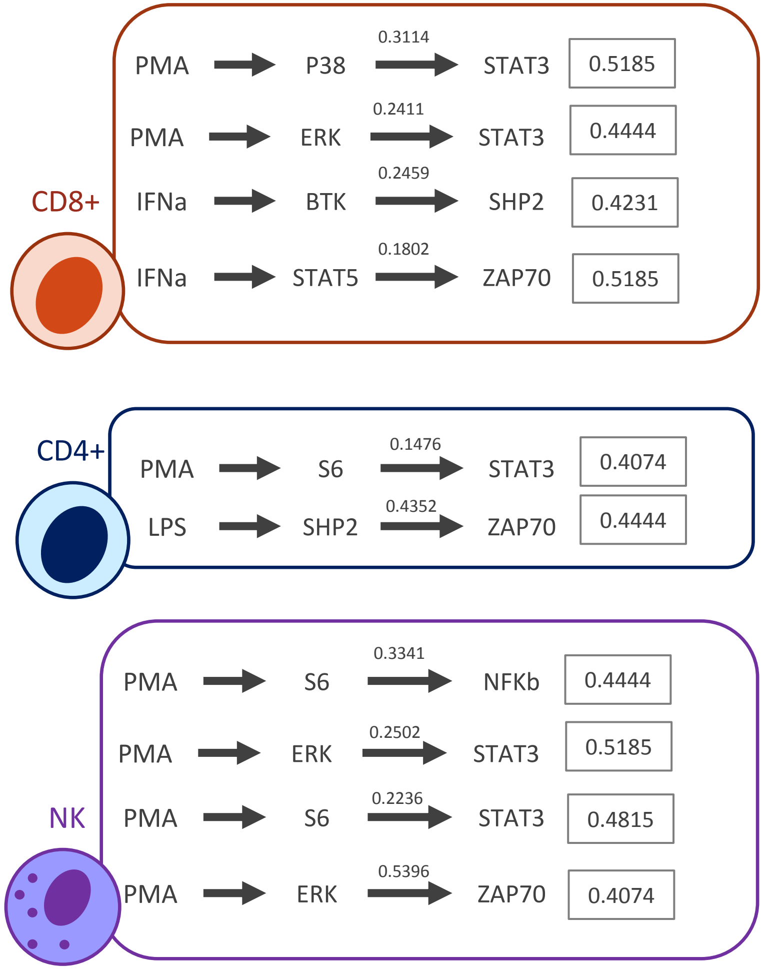
PMA → ERK $\xrightarrow{0.5396}$ ZAP70 0.4074

Bendall Data

- Run FCI with $a = 0.05$
- Bootstrap for robustness
- Report
  - Conflicting structures: Structures where $P_2 \rightarrow P_1$
  - Confirming Structures: Structures where $P_1 \rightarrow P_2$

Measurements in Bendall data are taken 15 minutes after activation

Validation on Bendall Data



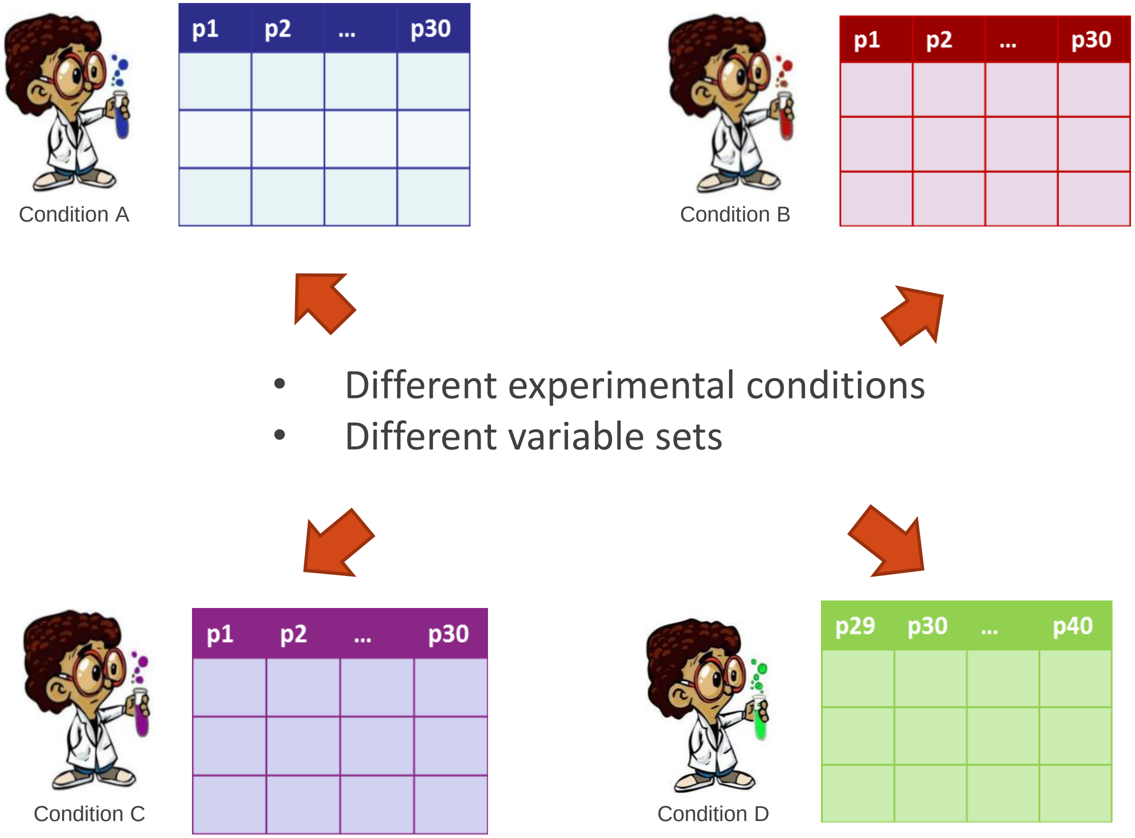
			Conflicting	Confirming	Correlation
CD8+	PMA → P38 $\xrightarrow{0.3114}$ STAT3	0.5185	0.000	0.360	0.2936
	PMA → ERK $\xrightarrow{0.2411}$ STAT3	0.4444	0.130	0.490	0.1561
	IFNa → BTK $\xrightarrow{0.2459}$ SHP2	0.4231	0.050	0.020	0.0628
	IFNa → STAT5 $\xrightarrow{0.1802}$ ZAP70	0.5185	0.020	0.150	0.0793
CD4+	PMA → S6 $\xrightarrow{0.1476}$ STAT3	0.4074	0.000	0.240	0.2221
	LPS → SHP2 $\xrightarrow{0.4352}$ ZAP70	0.4444	0.000	0.050	0.1113
NK	PMA → S6 $\xrightarrow{0.3341}$ NFKb	0.4444	0.010	0.350	0.1843
	PMA → ERK $\xrightarrow{0.2502}$ STAT3	0.5185	0.130	0.360	0.2498
	PMA → S6 $\xrightarrow{0.2236}$ STAT3	0.4815	0.050	0.450	0.1758
	PMA → ERK $\xrightarrow{0.5396}$ ZAP70	0.4074	0.230	0.230	0.1891

Results

- Hundreds of predictions to-be-tested; Experiments under way!
- Internal validation using non-trivial inferences
- Promising validation on another collection of dataset (Bendall)
- Evidence of batch effects and/or biological reasons of variability
- Method based on the most basic causal discovery assumptions

A Not So Basic Approach

Co-analyzing data sets from different experimental conditions with overlapping variable sets



Condition A

p1	p2	…	p30

Condition B

p1	p2	…	p30

- Different experimental conditions
- Different variable sets

Condition C

p1	p2	…	p30

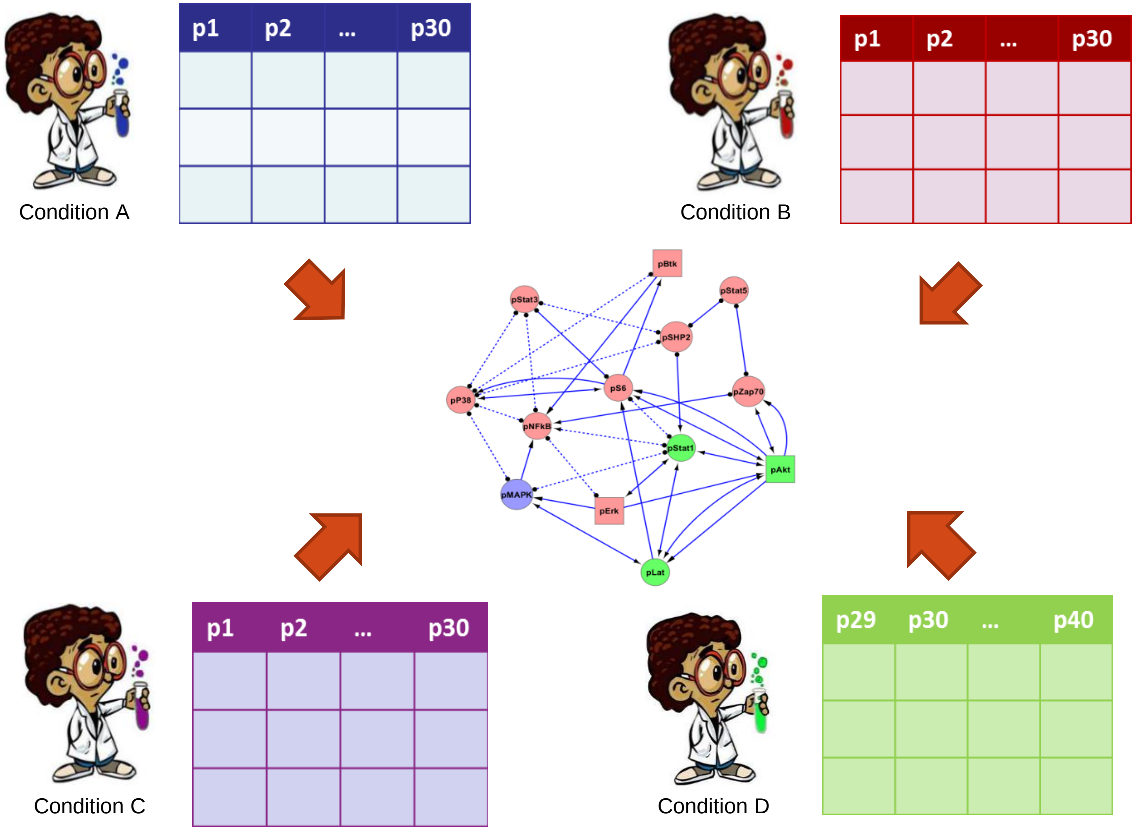
Condition D

p29	p30	…	p40

- Data can not be pulled together because they come from different distributions

- Principles of causality links them to the underlying causal graph

Co-analyzing data sets from different experimental conditions with overlapping variable sets



p1	p2	...	p30

p1	p2	...	p30

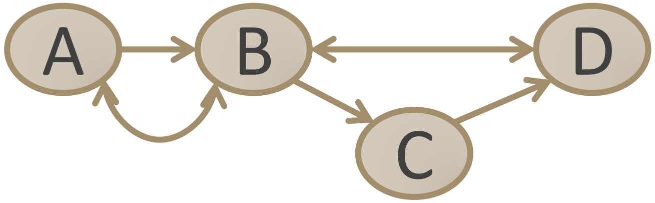
p1	p2	...	p30

p29	p30	...	p40

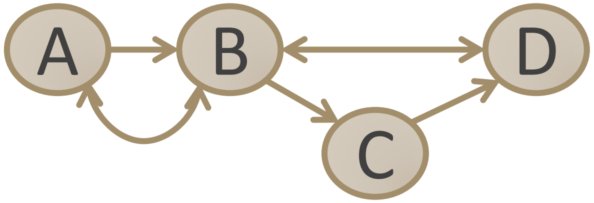
Identify **a single causal** graph that simultaneously fits all data

What type of causal graph?

- Semi-Markov causal models.
- $X \rightarrow Y$: X causes Y directly in the context of observed variables.
- $X \leftrightarrow Y$: X and Y share a latent common cause.
- Under faithfulness, m-separation entails all and only conditional independencies that stem from Causal Markov Condition.
- No learning algorithm.



Manipulations in SMCMs

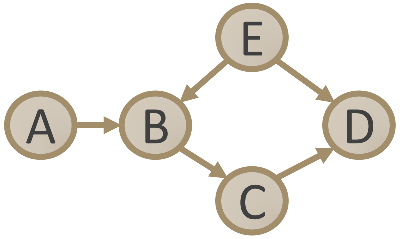


Graph (SMCM) S

- Values of B are set solely by the manipulation procedure
- Graph surgery: Remove all edges into the manipulated node.

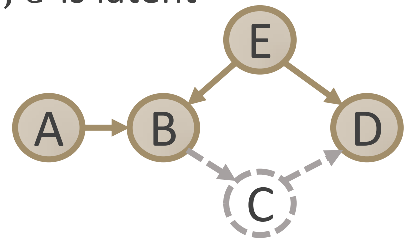
Reverse Engineering

S



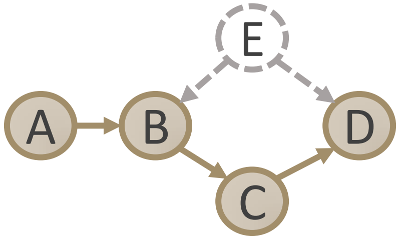
Unknown True SMCM S

S, C is latent



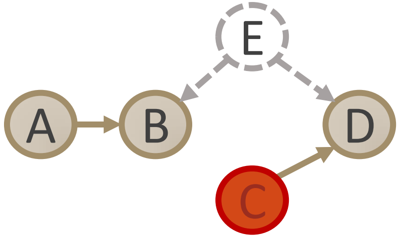
$Dep(A, D|\emptyset)_{D_1}$
$Dep(A, D|B)_{D_1}$
$Dep(A, D|E)_{D_1}$
$Ind(A, D|B, E)_{D_1}$
$Dep(A, B|\emptyset)_{D_1}$
…

S, E is latent



$Dep(A, D|\emptyset)_{D_2}$
$Dep(A, D|B)_{D_2}$
$Dep(A, D|E)_{D_2}$
$Dep(A, D|C)_{D_2}$
$Dep(A, D|B, C)_{D2}$
…

S^C, E is latent



$Ind(A, D|\emptyset)_{D_3}$
$Dep(A, B|\emptyset)_{D_3}$
$Dep(A, B|C)_{D_3}$
$Dep(A, B|D)_{D_3}$
$Dep(A, D|Cd\ D)_{D_3}$
$Ind(B, C|\emptyset)_{D_3}$
…

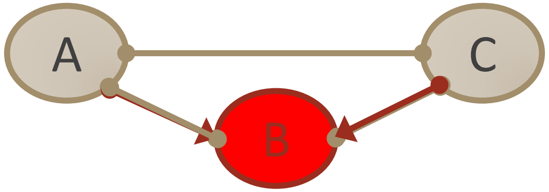
S under manipulation
and marginalization

Observed (in) dependencies

Independencies as constraints

- Suppose you don’t know anything about the structure S of the three variables.
- You find out that in S^B : $Ind(A, C|\emptyset)$
- In path terms: $\nexists$ path in S^B that is m-connecting A and C given $\emptyset$
- In SAT terms:

$$\neg edge(A,C) \wedge$$
$$[\neg edge(A,B) \vee arrow(A,B) \vee edge(B,\mathrm{C}) \vee arrow(C,B)]$$



A-C does not exist

AND

(A-B does not exist

OR

A-B is into B

OR

B-C does not exist

OR

B-C is into B)

Statistical errors

- Constraints correspond to *
  1. Dependencies $Dep(A, B|\boldsymbol{Z})_{D_i}$
  2. Independencies $Ind(C, D|\boldsymbol{W})_{D_i}$

  - *e.g.*, $Ind(A, B|\emptyset)_{D_1} \leftrightarrow \neg edge(A, C) \wedge [\neg edge(A, B) \vee arrow(A, B) \vee edge(B, C) \vee arrow(C, B)]$
- Compare a dependence to an independence
  - How?
  - Low p-value suggests dependence
  - High p-value suggests independence
    (in the respective data set)

Sort constraints!

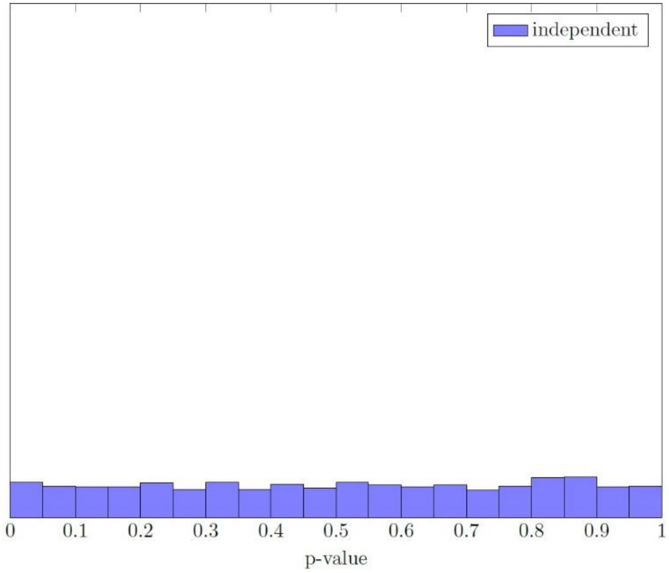
What happens with statistical errors?

Conflicts make SAT instance unsatisfiable!

*well, not really

Comparing p-values

- $H_0 \colon p \sim Beta(1,1)$
- $H_1 \colon p \sim Beta(\xi, 1),\ \xi \in (0,1)$
- $f(p|\pi_o, \xi) = \pi_0 + (1-\pi_0)\xi p^{\xi-1}$, π_0: The proportion of p-values coming from H_0
- If you know $\widehat{\pi_0}$, $\hat{\xi}$ you can find the MAP ratio
- $E_0(p) = \frac{P(H_0|p)P(H_0)}{P(H_1|p)P(H_1)} = \frac{\widehat{\pi_0}}{(1-\widehat{\pi_0})\hat{\xi}p^{(1-\hat{\xi})}}$, $E_1 = 1/E_0$
  - If $E(p) > E(p)^{-1}$, independence is more likely than dependence
- **Sort p-values by max(E_0, E_1)**
- Use (Storey and Tibshirani, 2003) to identify $\widehat{\pi_o}$
- Minimize negative log likelihood of $f(p|\widehat{\pi_0}, \xi) = \widehat{\pi_0} + (1-\widehat{\pi_0})\xi p^{\xi-1}$ to identify $\hat{\xi}$.
- Rank constraints according to MAP ratio and satisfy them if possible in the given order.

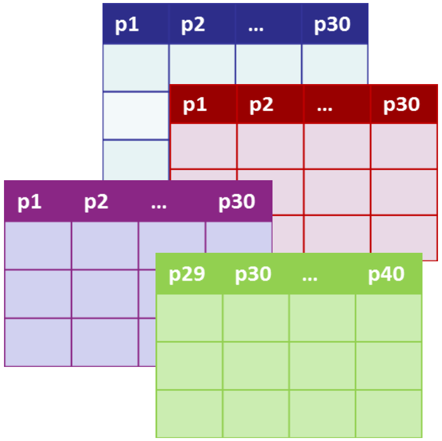


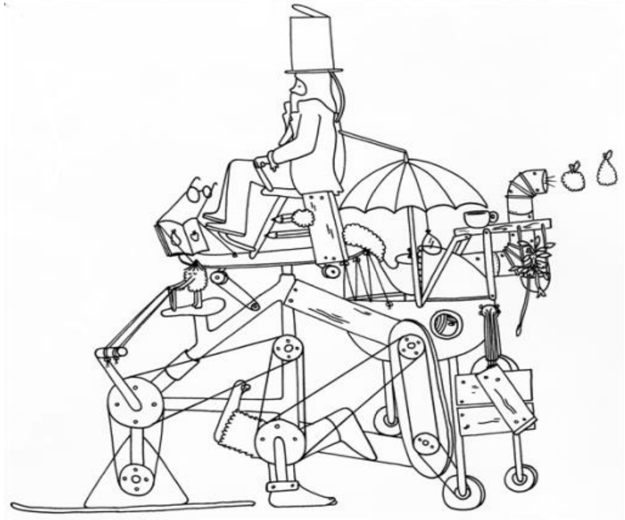
“COmbINE” Algorithm

Data sets D_i measuring overlapping variables under different experimental conditions

COmbINE
Algorithm that transforms independence constrains to SAT instance

Summary of semi Markov Causal models that best fits all data sets simultaneously





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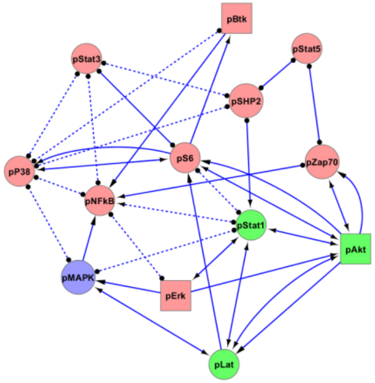
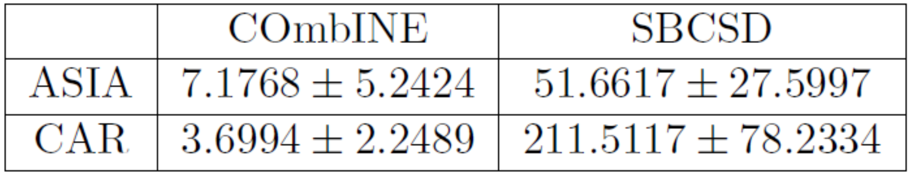


Similar Algorithms

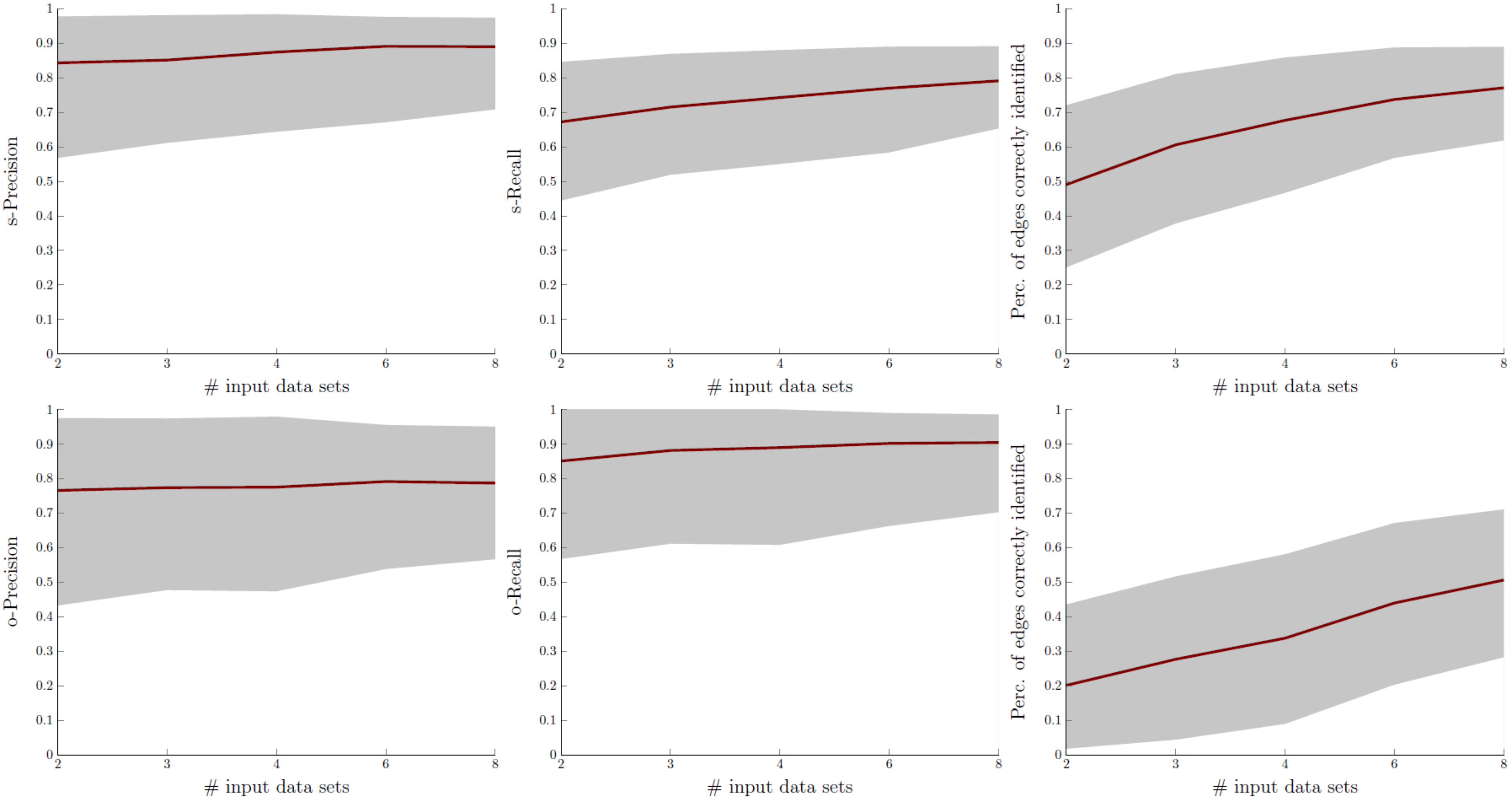
- SBCSD: [Hyttinen et al., UAI, 2013]
  - Inherently less compact representation of path constraints.
  - Does not handle conflicts; non applicable to real data.
  - In addition, it admits cycles.
  - Scales up to 14 variables
- Lininf [Hyttinen et al., UAI 2012, JMLR 2012]
  - Linear relations only.
  - Scales up poorly (6 variables in total with overlapping variables, 10 without).
  - In addition, it admits cycles.



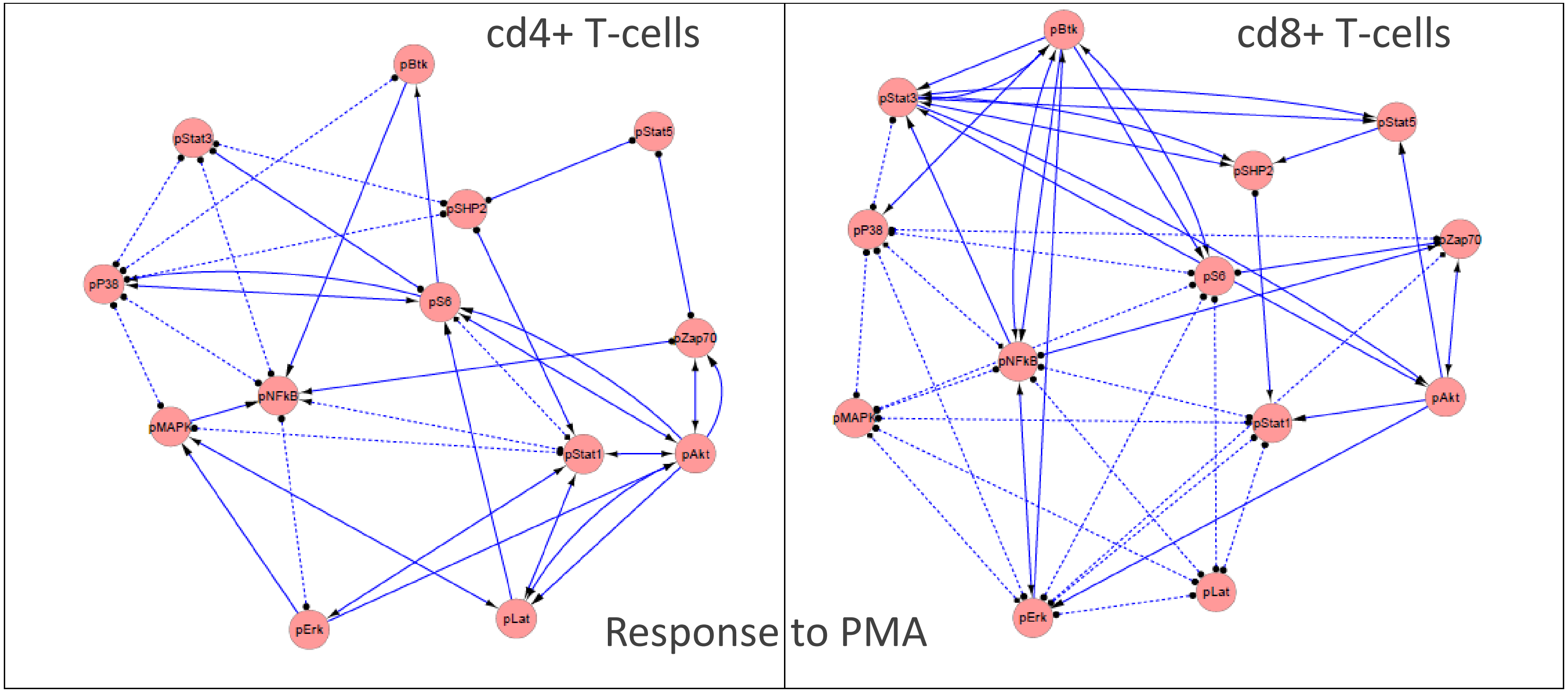
	COmbINE	SBCSD
ASIA	7.1768 ± 5.2424	51.6617 ± 27.5997
CAR	3.6994 ± 2.2489	211.5117 ± 78.2334

Execution Time in Seconds

Performance on Simulated Data



Application on Mass Cytometry data



cd4+ T-cells

cd8+ T-cells

Response to PMA

Data set	Source	$\mathbf{L_i}$	$\mathbf{I_i}$	Donor
$\mathbf{D_1}$	Bodenmiller et al. (2012)	pMAPK	pAkt	1
$\mathbf{D_2}$	Bodenmiller et al. (2012)	pMAPK	pBtk	1
$\mathbf{D_3}$	Bodenmiller et al. (2012)	pMAPK	pErk	1
$\mathbf{D_4}$	Bendall et al. (2011)	pAkt, pLat, pStat1	pErk	2
$\mathbf{D_5}$	Bendall et al. (2011)	pAkt, pLat, pStat1	pErk	3

Summary and Conclusions

- Mass Cytometry data a good domain for causal discovery
- Hundreds of robust causal postulates
- Approach:
  - Conservative: local discovery, performing all tests, independent analysis of populations
  - Opportunistic: using 2 thresholds for (in)dependency
- New algorithm that can handle
  - different experimental conditions
  - overlapping variable subsets
  - deal with statistical errors
- Numerous directions open for future work on this collection of data
  - Experiments under way!

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