Northeastern University
Khoury College of Computer Sciences

Single cell mass spectrometry (MS)-based proteomics coupled with suitable experimental design enables the application of causal inference methods to observational experiments.

Devon Kohler

Khoury College of Computer Sciences, Northeastern University, Boston, MA

Presentation Outline

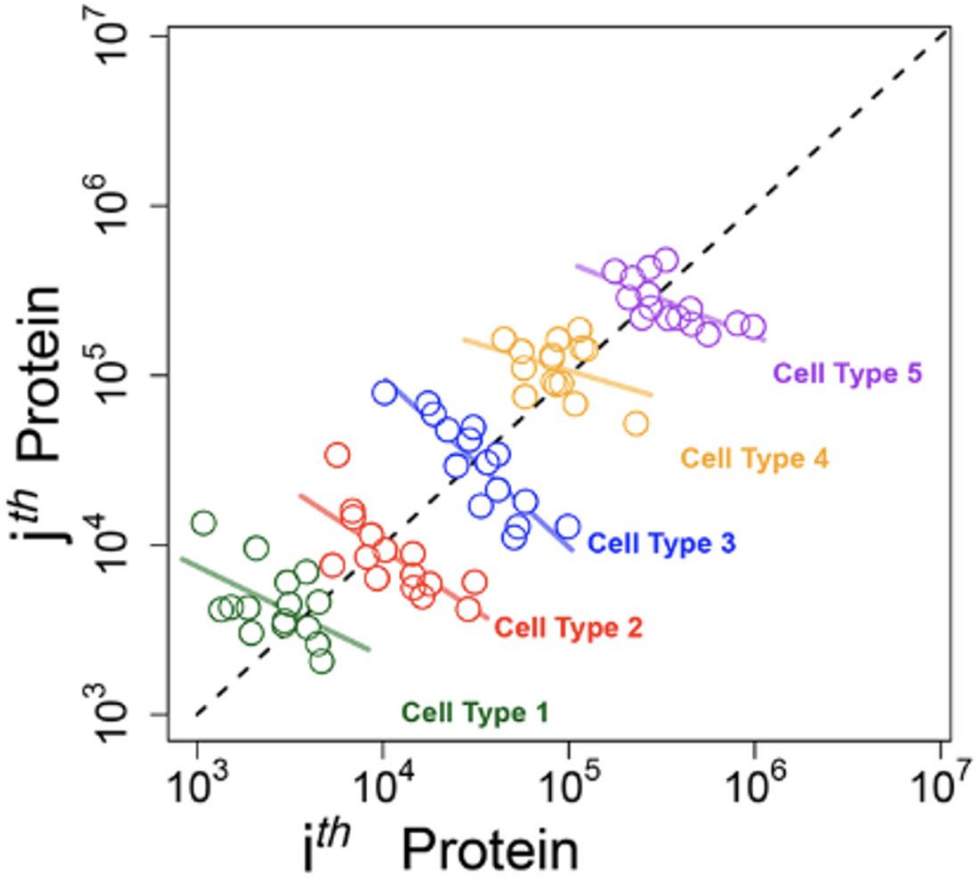
- Problem statement
  - Single cell MS-based proteomics enables estimating the effect of perturbations from observational studies
- Background
- Case studies – Targeted vs Discovery
  - Targeted experiment (simulation)
  - Discovery experiment (biological)

Single cell MS-based proteomics enables estimating the effect of perturbations from observational studies

- Understanding the proteome response to perturbations is an important step towards understanding the protein function
- Causal inference methods allow us to estimate this response from purely observational data (i.e., without performing the perturbation)
- Single cell proteomics allows us to do this better than traditional bulk proteomics

Why do we need single cell?

- Single cell proteomics removes confounding that comes from pooling cell types in bulk proteomics
  - Removes confounding cell effects
  - Differentiate behavior of cellular sub-populations
- Key points to correctly estimate the relationship between proteins



Specht and Slavov (2018). JPR 17(8)

Presentation Outline

- Problem statement
- Background
  - Causal inference
- Case studies – Targeted vs Discovery
  - Targeted experiment (simulation)
  - Discovery experiment (biological)

Estimate the effect of interventions using purely observational data

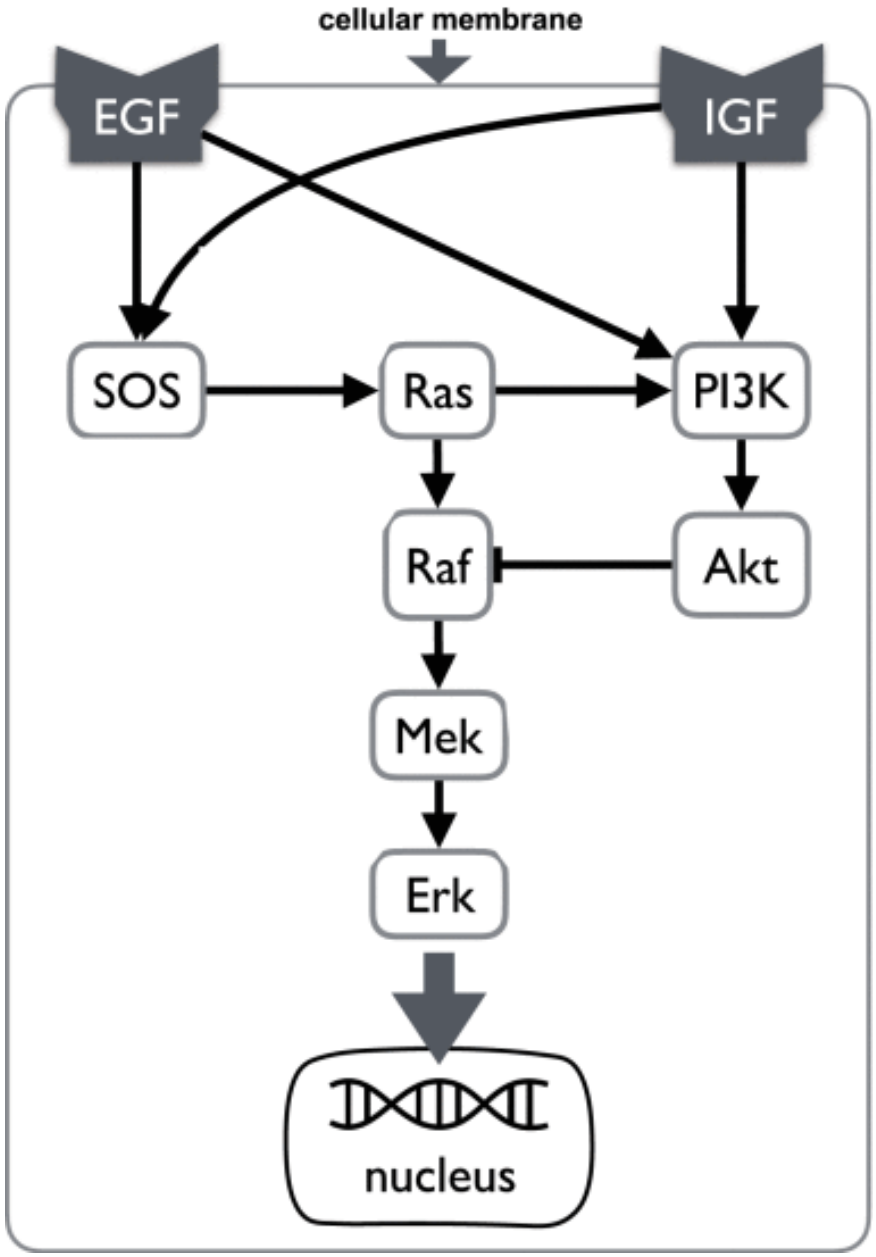
- Requirements for estimating the effect of interventions
  - Observational experimental data
  - Causal network (in the form of a directed acyclic graph (DAG))
    - **Not doing causal discovery**
  - Correct combination of graphical topology and measured proteins

Causal network – IGF signaling pathway

- Insulin-like growth factor (IGF) or epidermal growth factor (EGF) trigger an event including the MAPK signaling pathway
- Well studied with dynamics characterized in ODE/SDE models

- Measured

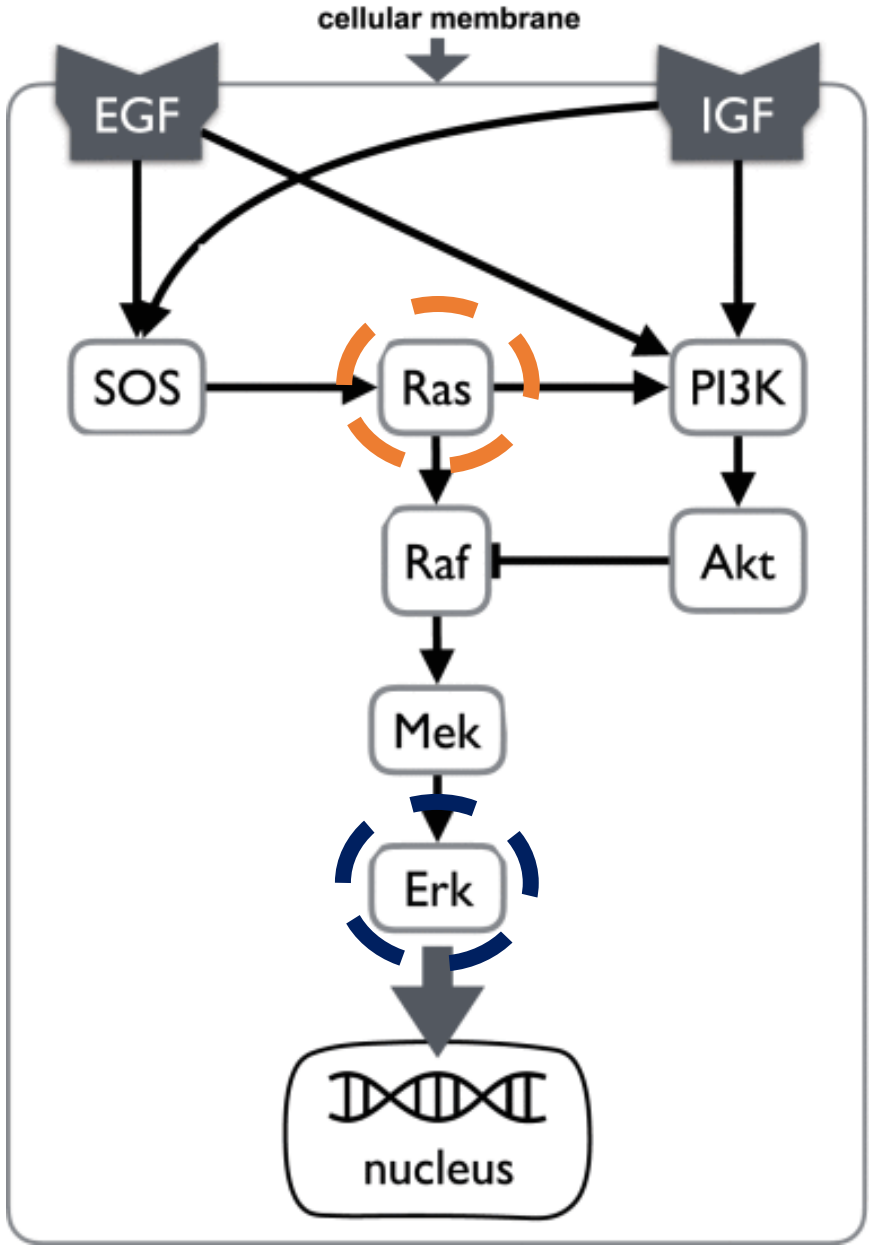
- Latent (unmeasured)



Zucker J. et al. (2021). IEEE Trans. Big Data

IGF signaling system

- Interested in the causal effect of Ras on Erk
- Latent confounder between SOS and PI3K
- P(Erk | do(Ras), SOS) is *identifiable*



- Measured

- Latent

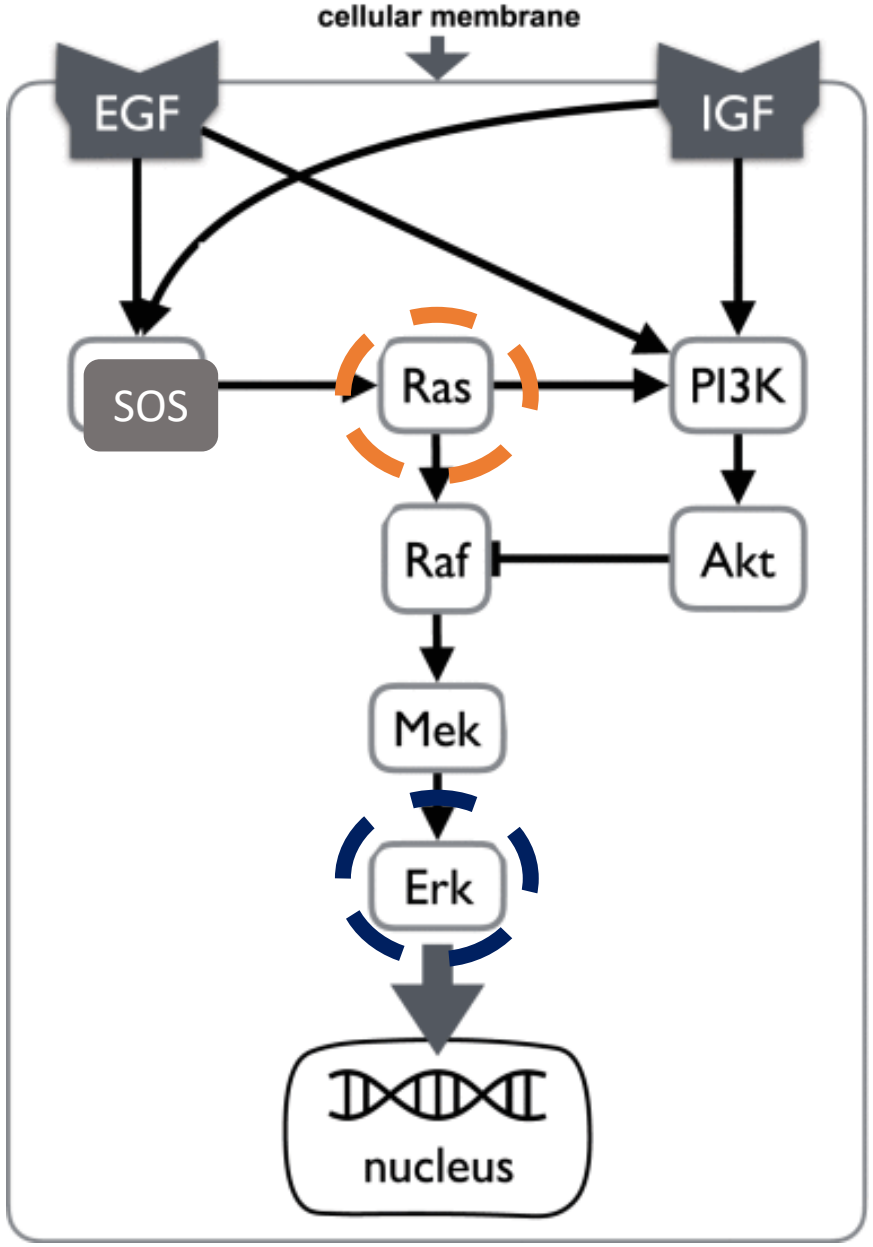
Zucker J. et al. (2021). IEEE Trans. Big Data

IGF signaling system

- Assume SOS was not measured
- Now we cannot close the “back-door” path
- P(Erk | do(Ras)) is not identifiable

- Measured

- Latent



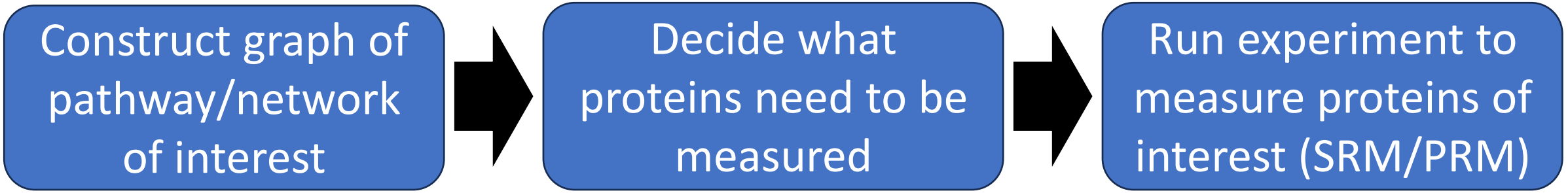
Zucker J. et al. (2021). IEEE Trans. Big Data

Presentation Outline

- Problem statement
- Background
- Case studies – Targeted vs Discovery
  - Targeted experiment (simulation)
  - Discovery experiment (biological)

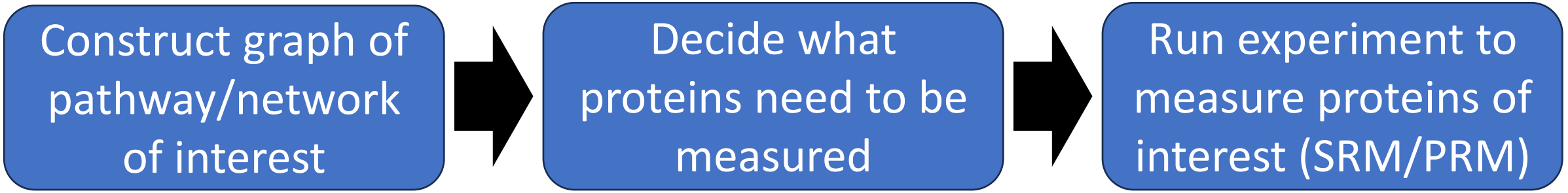
Application of causal inference is dependent on the experimental design and biological question of interest

- Targeted Experiment

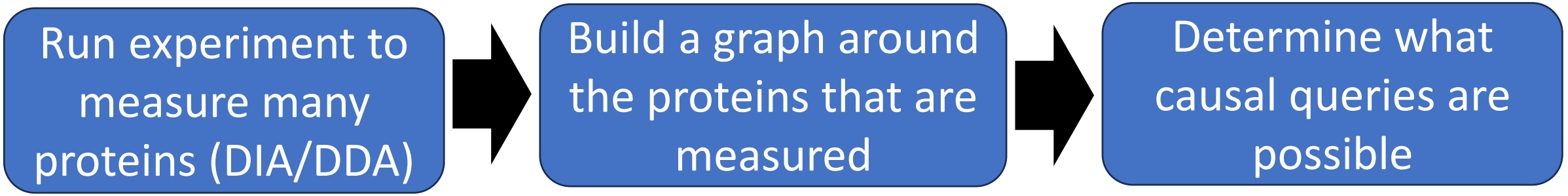


Application of causal inference is dependent on the experimental design and biological question of interest

- Targeted Experiment



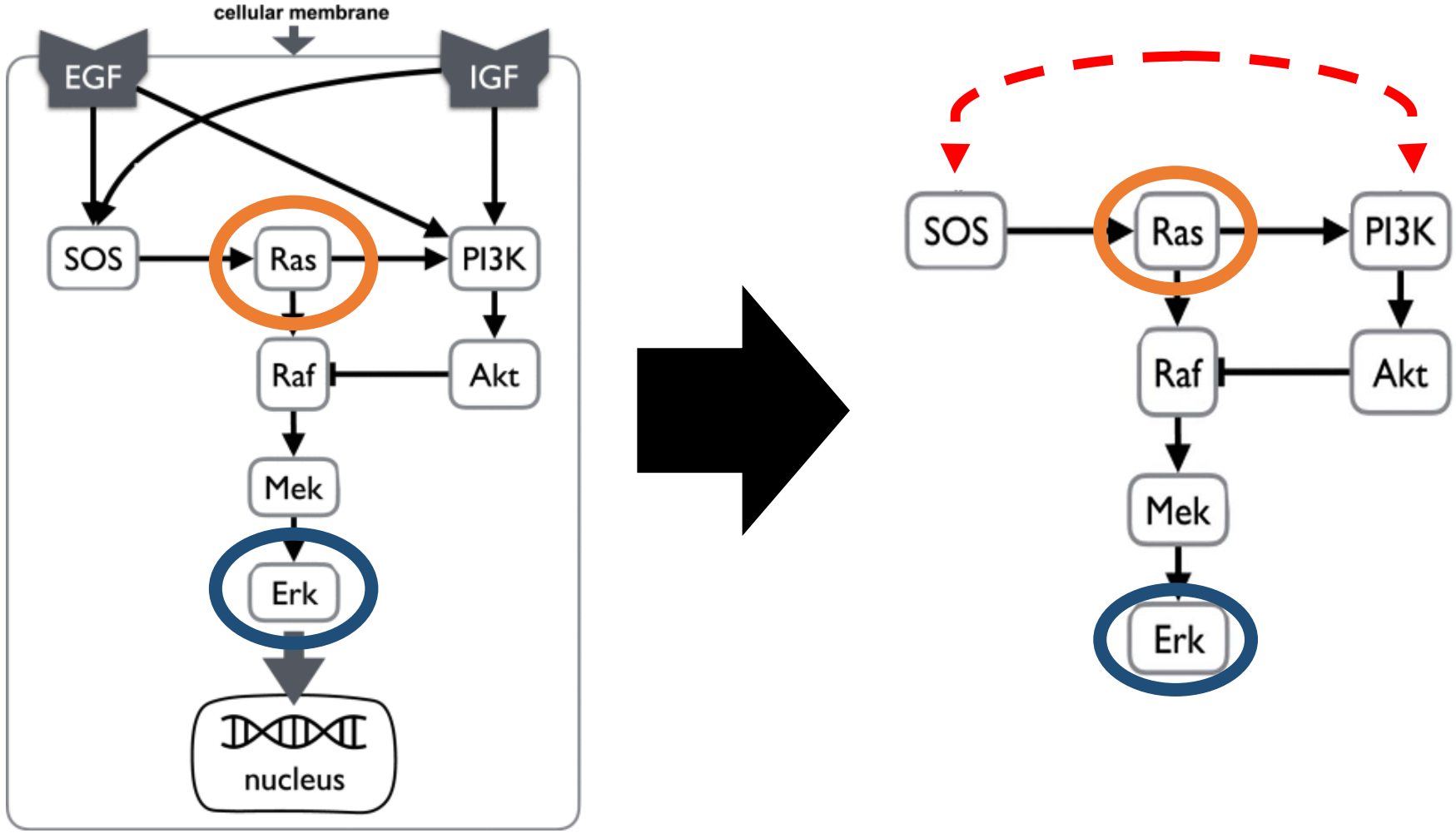
- Exploratory experiment



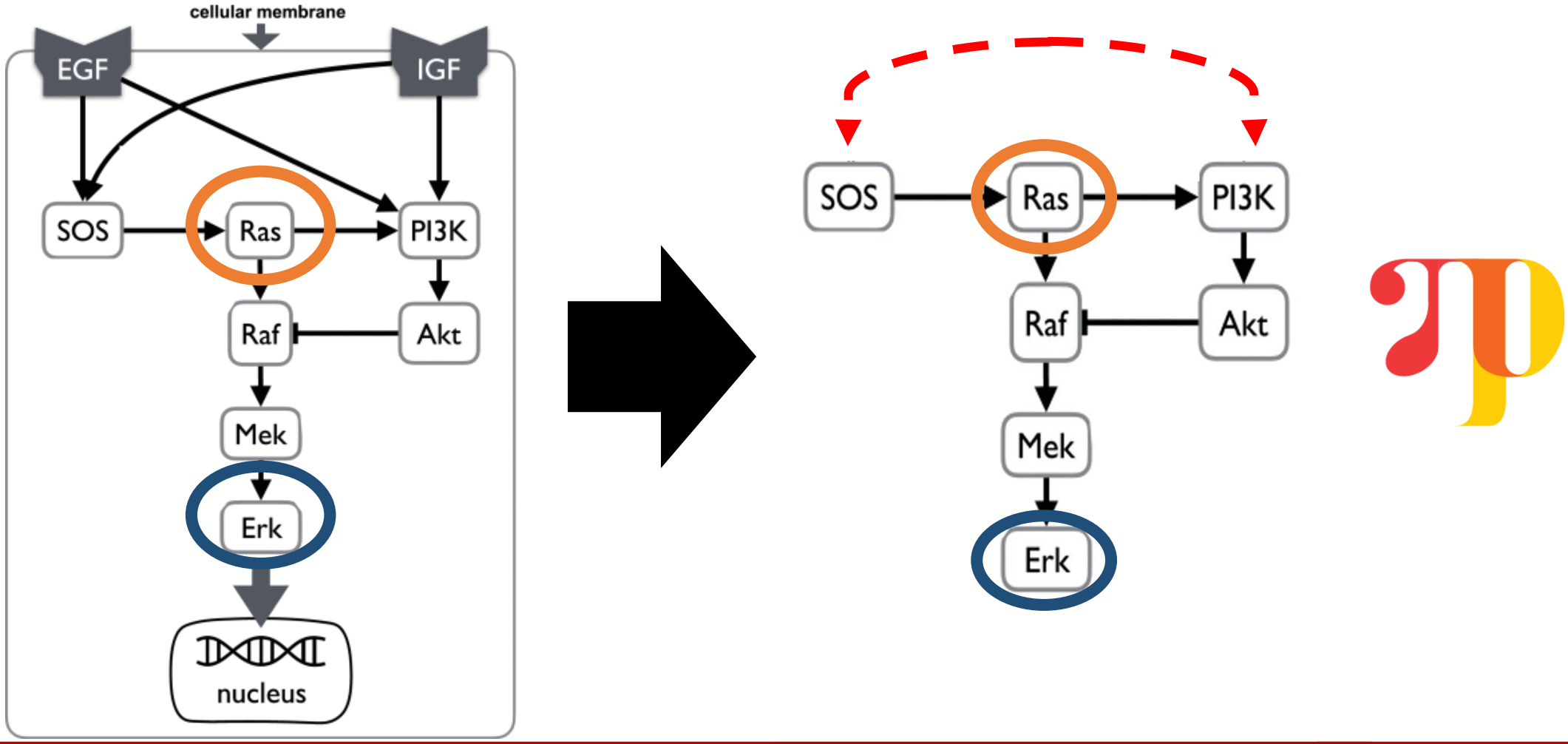
Presentation Outline

- Problem statement
- Background
- Experimental goals and design
  - Targeted experiment (simulation)
    - Bulk vs single cell
    - Replication (e.g., the number of cells)
    - Protein-level imputation
  - Discovery experiment (biological)

Targeted experiments allow us to answer a specific question of interest



Use latent variable model (LVM) to leverage information from unmeasured proteins

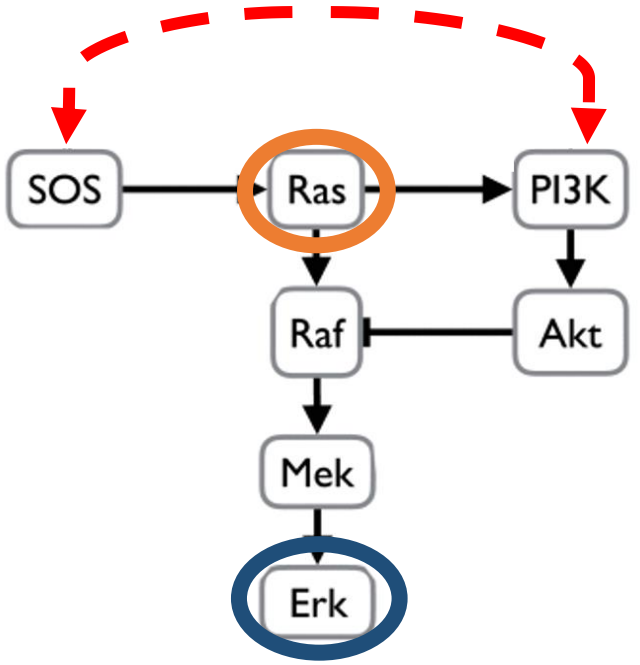


Presentation Outline

- Problem statement
- Background
- Case studies – Targeted vs Discovery
  - Targeted experiment (simulation)
    - Bulk vs single cell
    - Replication (e.g., the number of cells)
    - Protein-level imputation
  - Discovery experiment (biological)

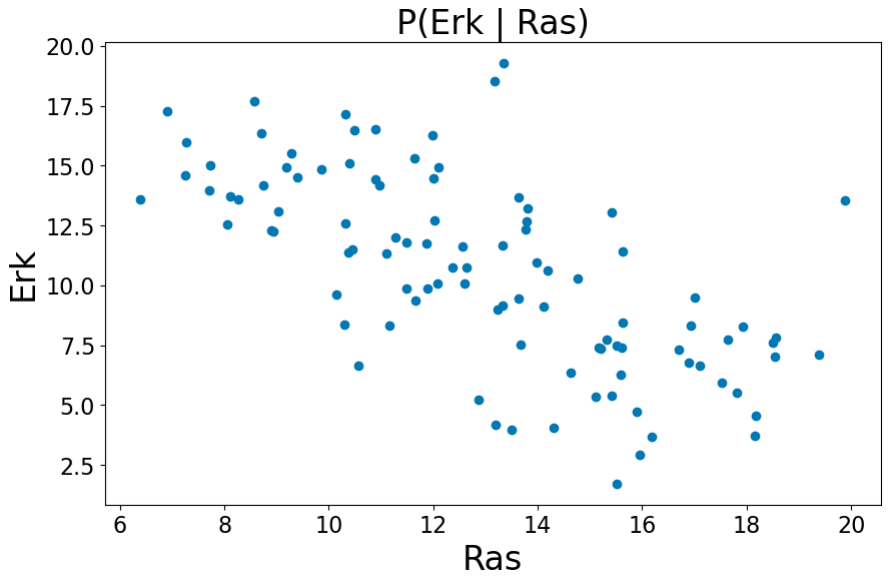
Simulate bulk-MS data

- Linear relationships between proteins
- Peptide ions are missing with some probability being missing at random and missing not at random
- Multiple cell types are mixed



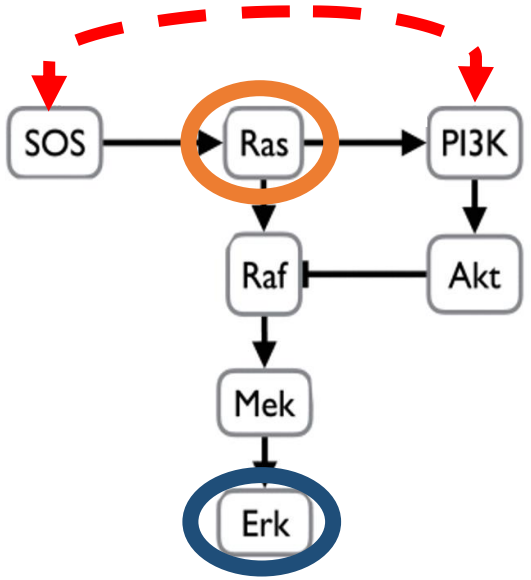
System of linear relationships

$$Ras = \beta_{Ras_0} + \beta_{Ras_1} * SOS + \epsilon_{Ras}$$
$$Raf = \beta_{Raf_0} + \beta_{Raf_1} * Ras + \beta_{Raf_2} * Akt + \epsilon_{Raf}$$
$$Mek = \beta_{Mek_0} + \beta_{Mek_1} * Raf + \epsilon_{Mek}$$
$$Erk = \beta_{Erk_0} + \beta_{Erk_1} * Mek + \epsilon_{Erk}$$



Simulate bulk-MS data

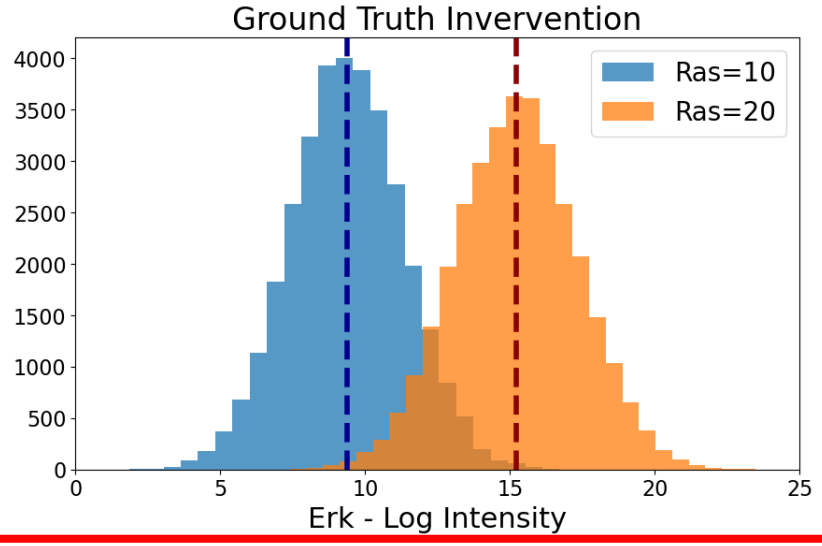
- Linear relationships between nodes
- Peptide ions are missing with some probability of being missing at random and missing not at random
- Multiple cell types are mixed



Ground truth average causal effect (ACE)

Average effect of increasing the $\log_2$ intensity of Ras by 10 on Erk is 5.85

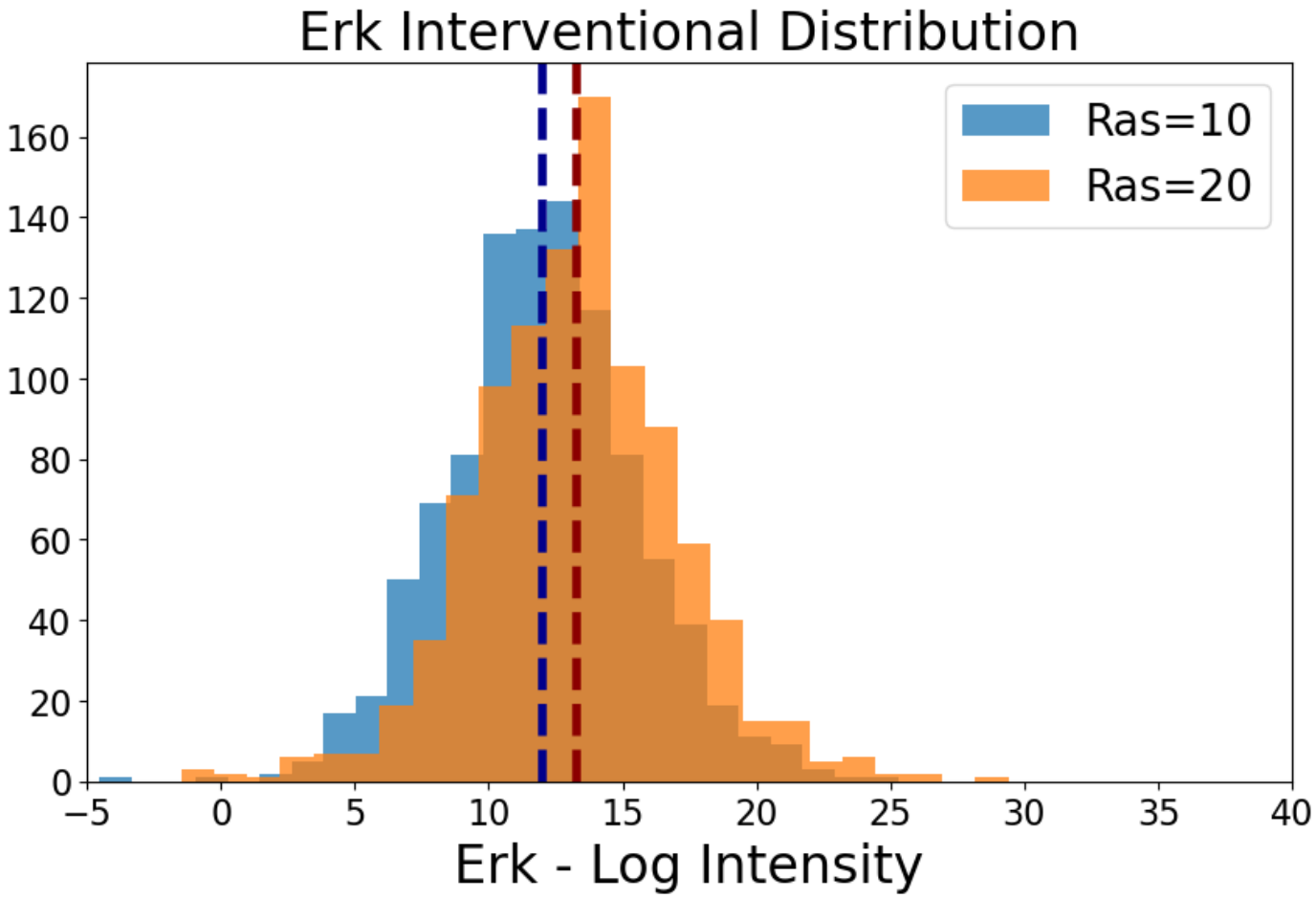
$$P(Erk \mid do(Ras = 20)) - P(Erk \mid do(Ras = 10)) = 5.85$$



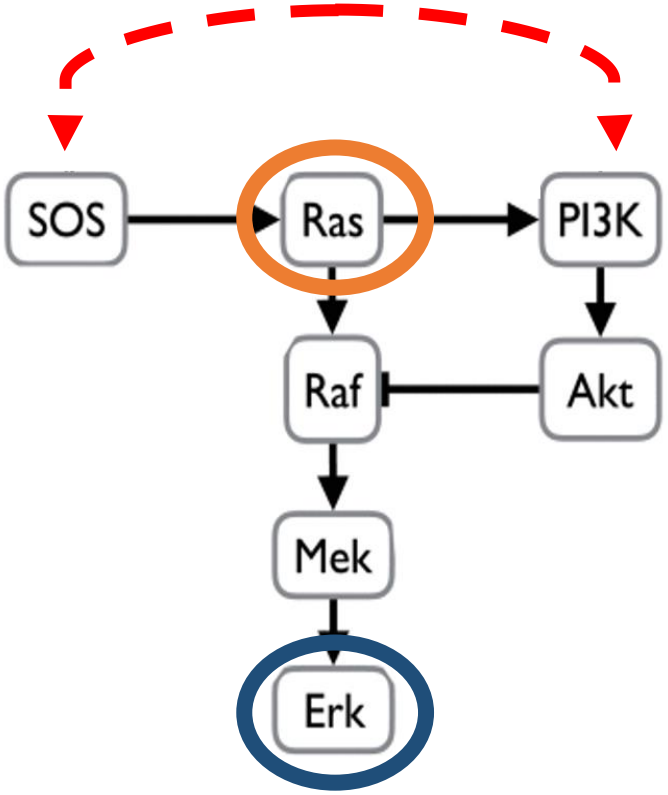
Interventional results are very different from true effect

- Compare two interventions
  - P(Erk | do(Ras = 10)
  - P(Erk | do(Ras = 20)
- Average causal effect (ACE)
  - 1.3

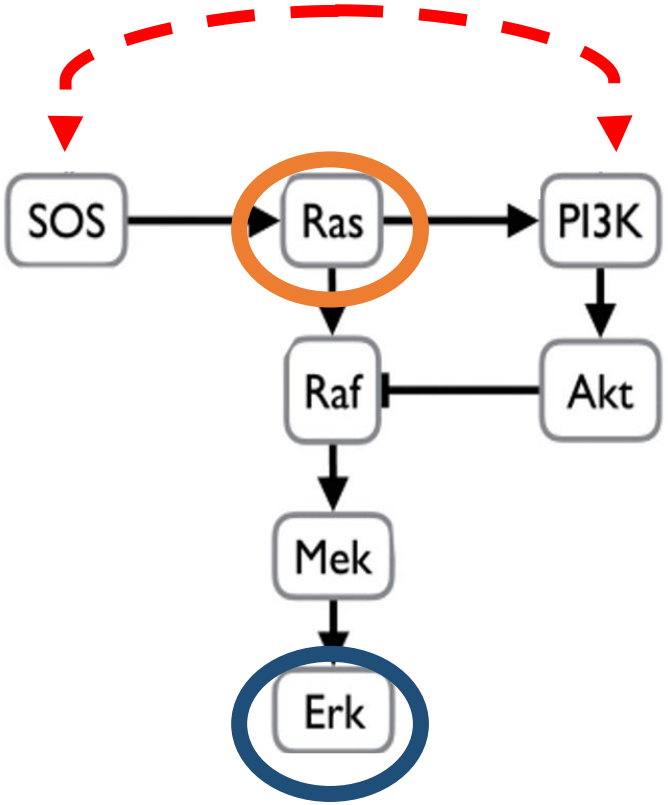
True ACE:
5.85

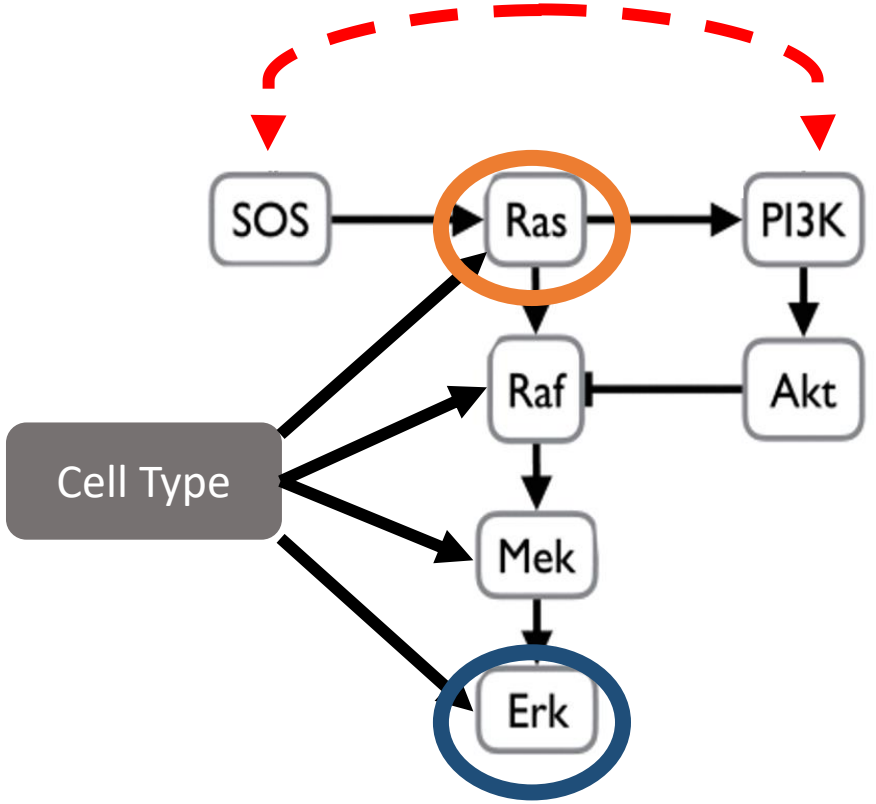


Why is the estimation incorrect?



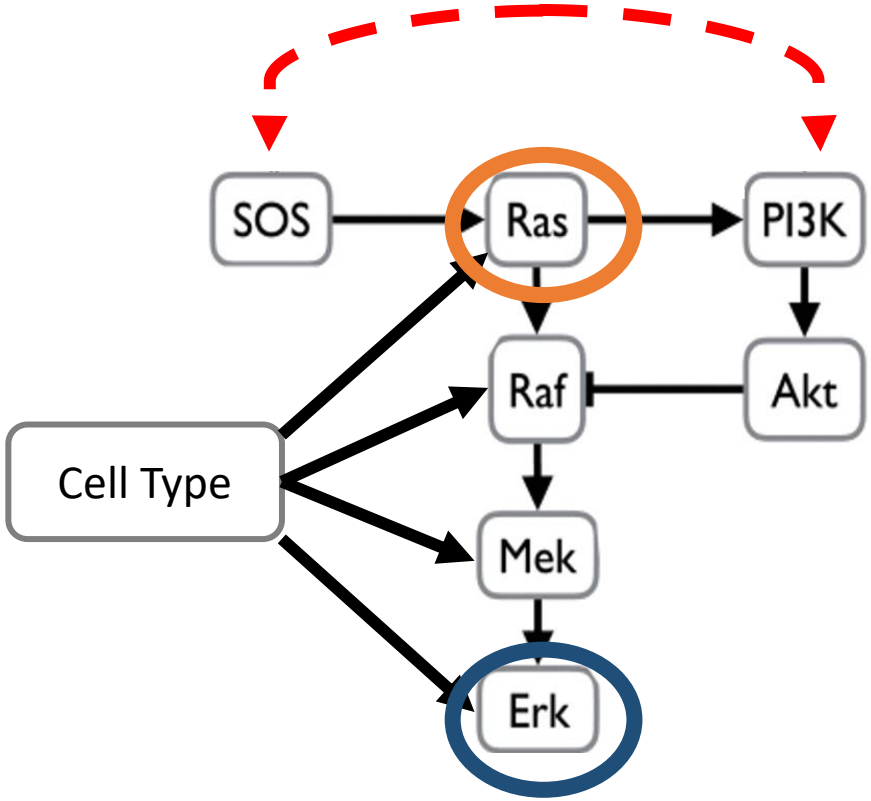
Multiple cell types mixed confounded the inference





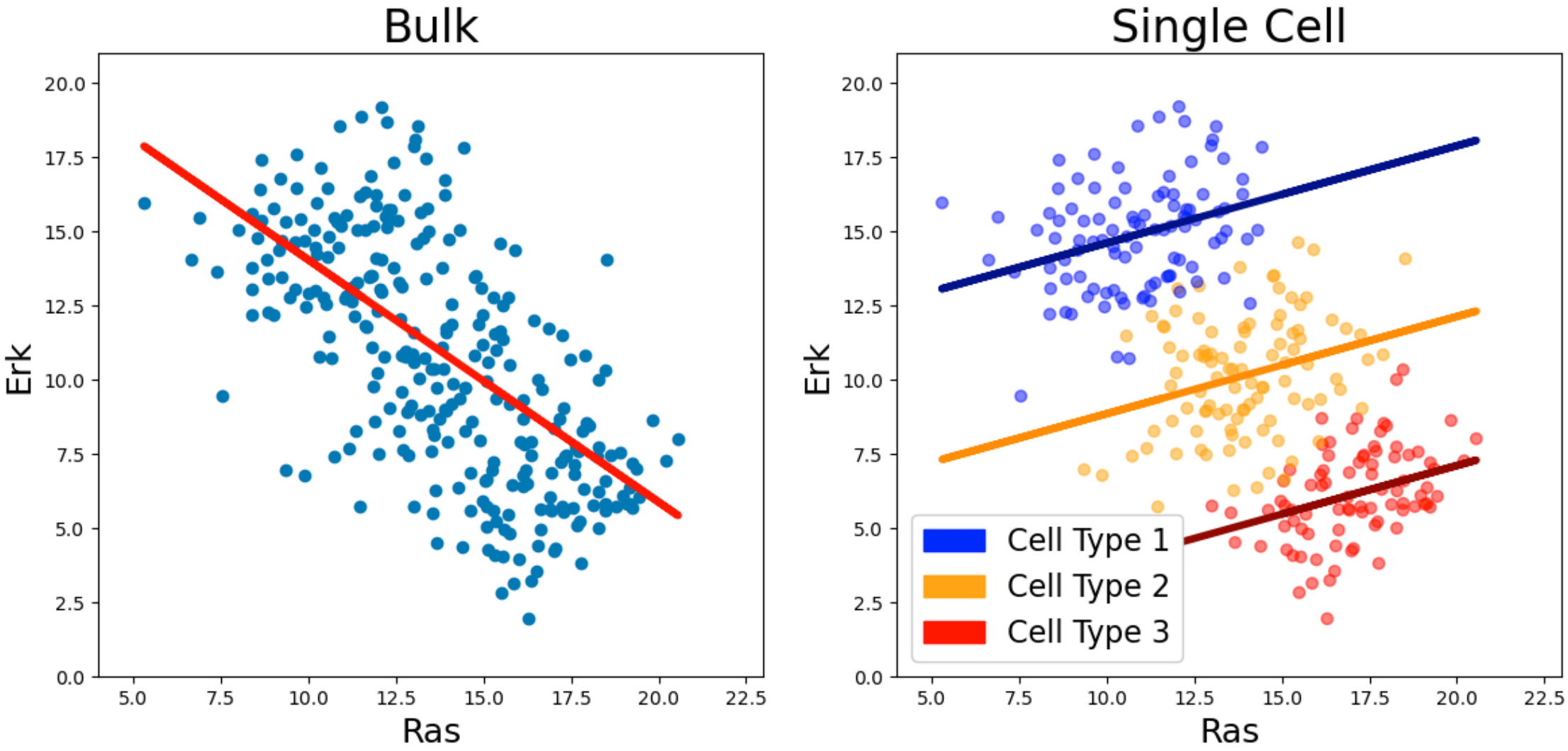
P(Erk | do(Ras)) is not identifiable

Using single cell data, we can observe cell type

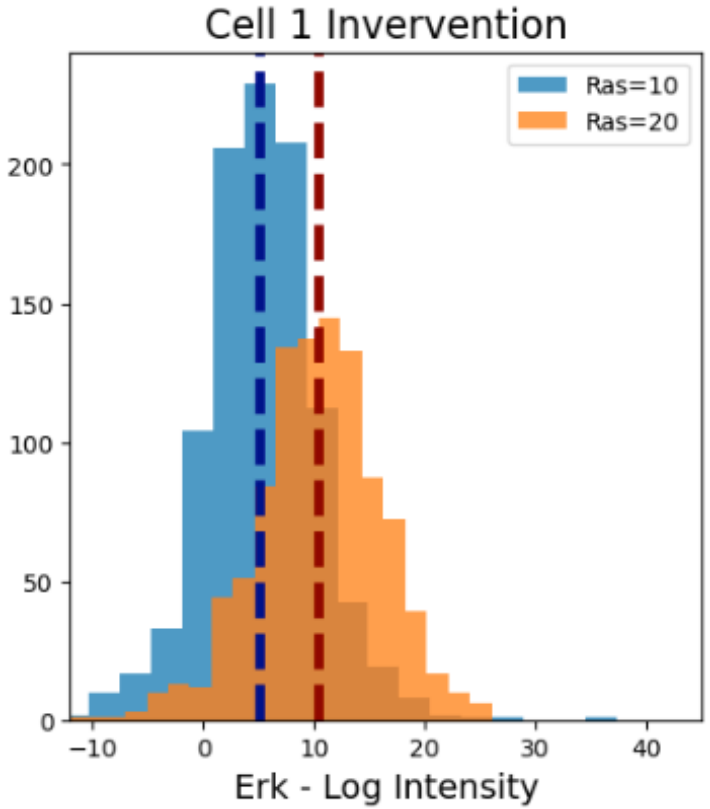


P(Erk | do(Ras), Cell Type) is identifiable

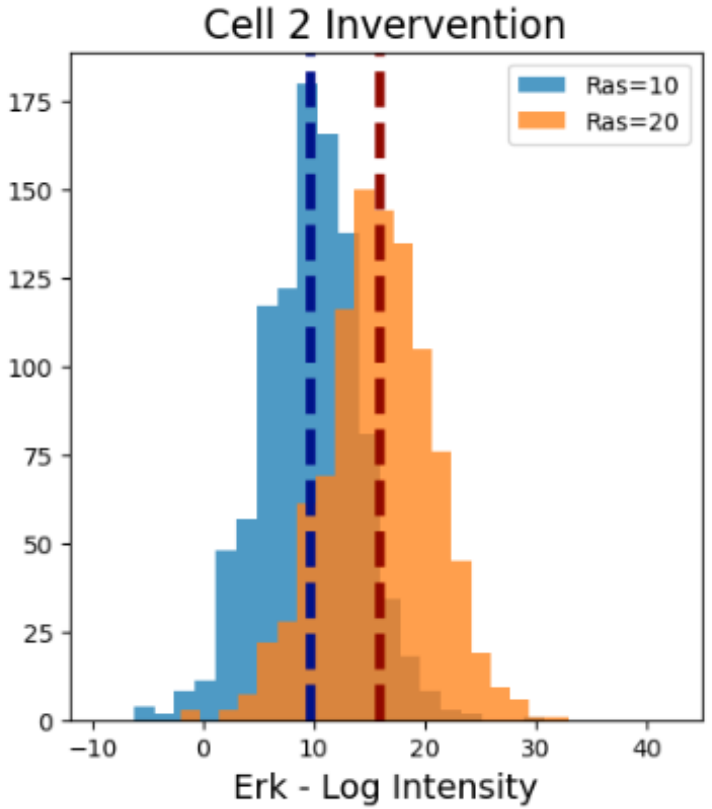
Using single cells show the true relationship



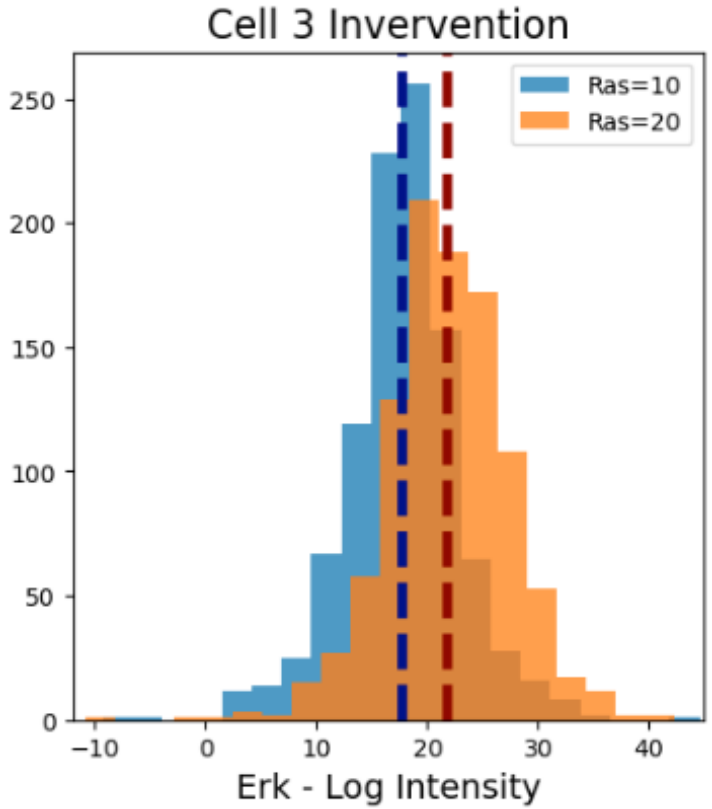
Splitting models up by cell result in a more accurate ACE estimate



ACE=5.25



ACE=6.18



ACE=4.04

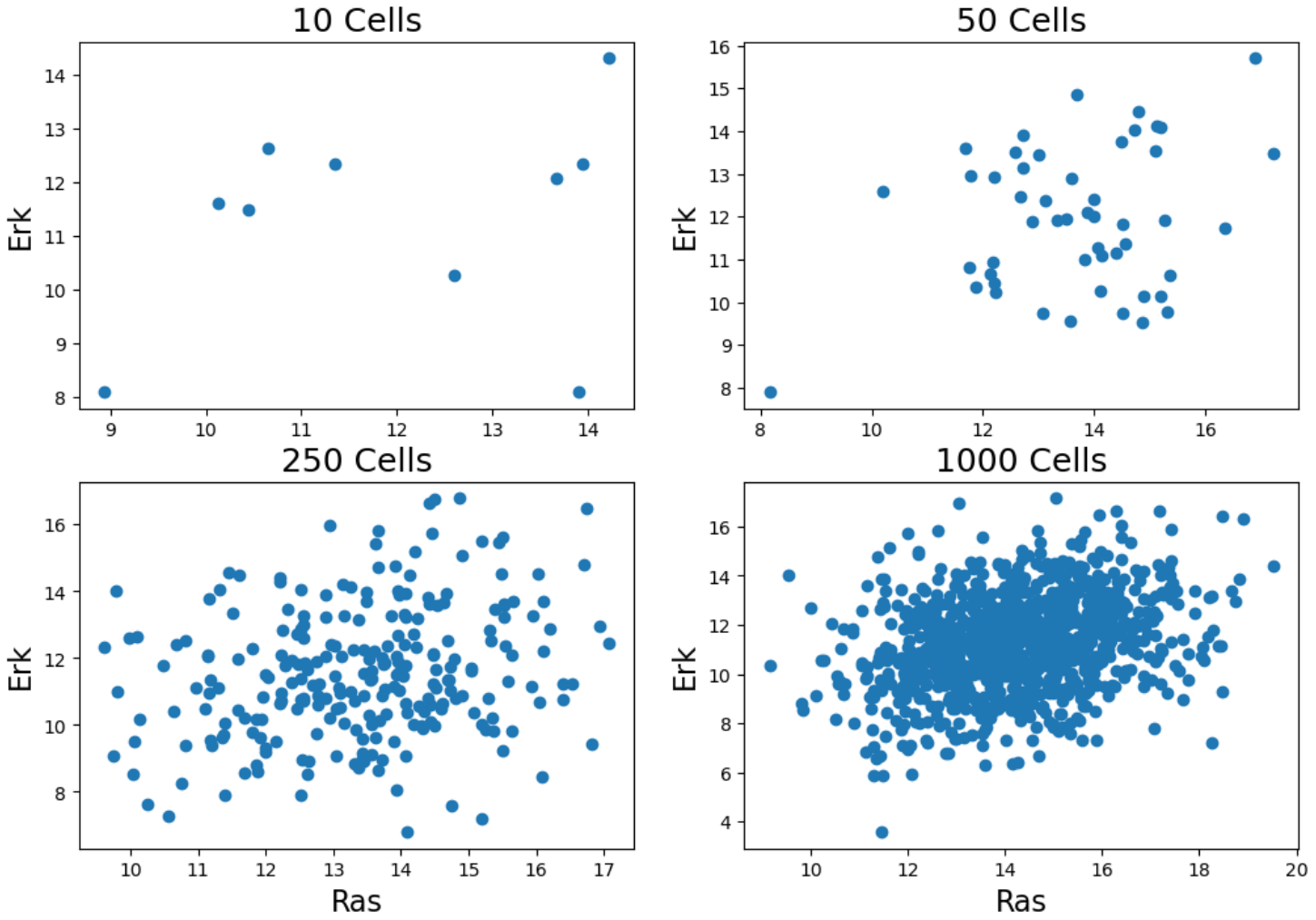
True ACE = 5.85

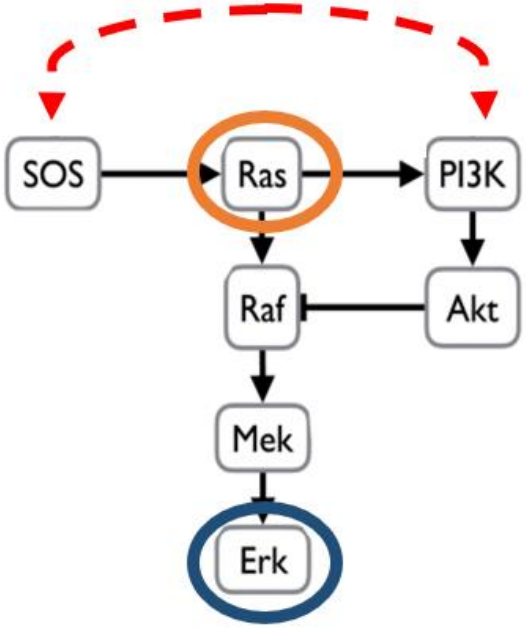
Presentation Outline

- Problem statement
- Background
- Case studies – Targeted vs Discovery
  - Targeted experiment (simulation)
    - Bulk vs single cell
    - Replication (e.g., the number of cells)
    - Protein-level imputation
  - Discovery experiment (biological)

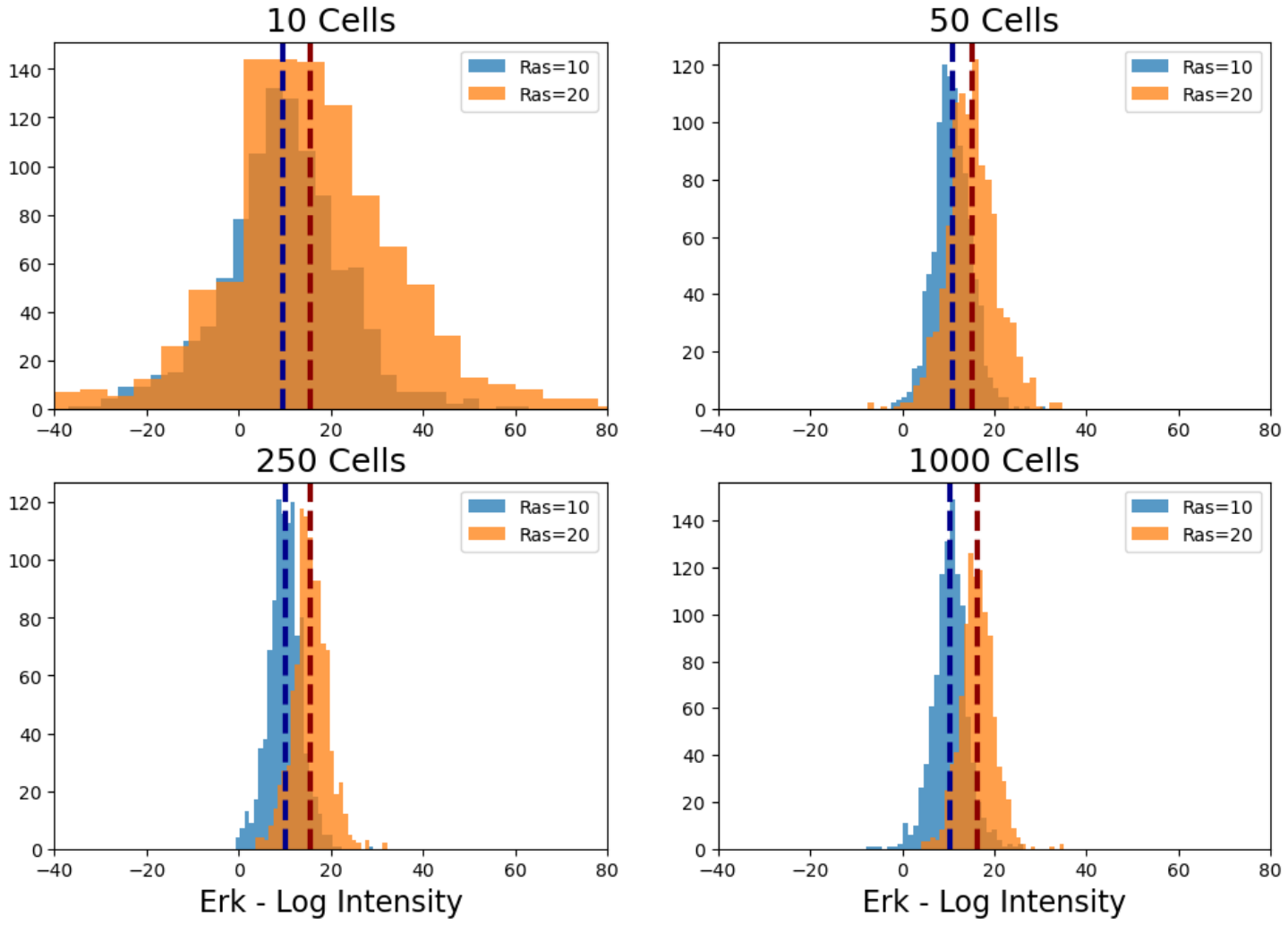
Even when using single cell data, we still need sufficient replicates

1 cell type measured



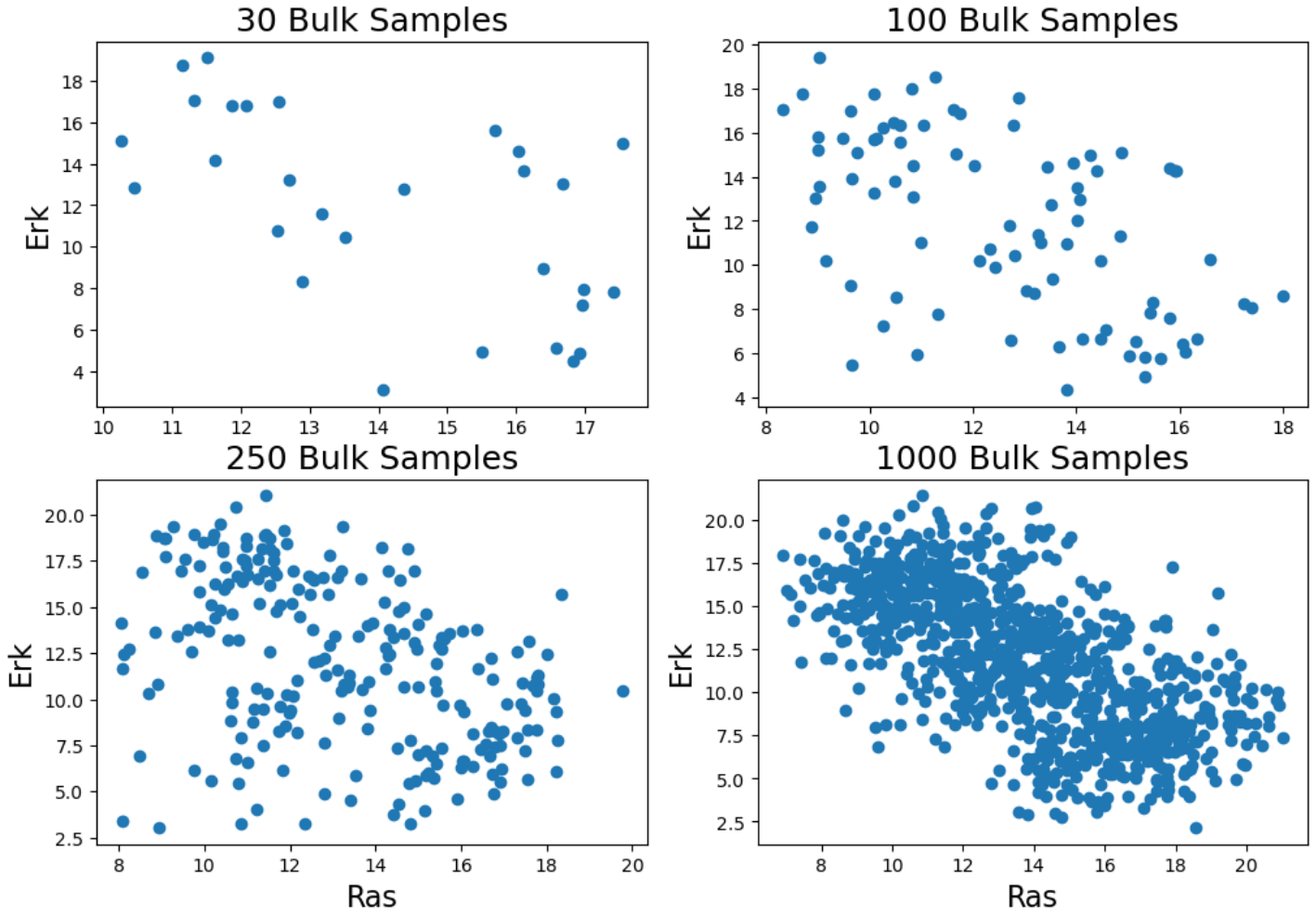


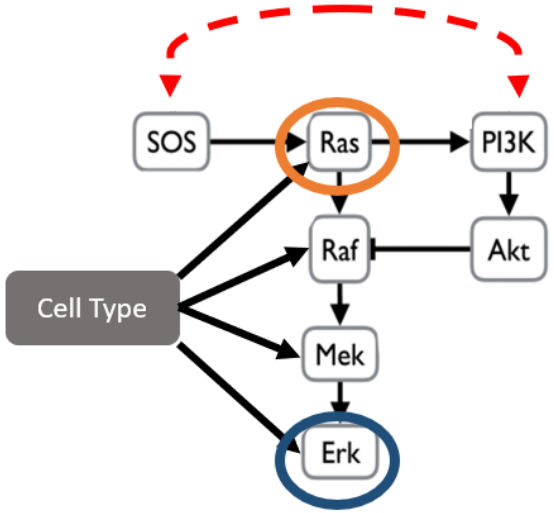
Even when using single cell data, we still need sufficient replicates



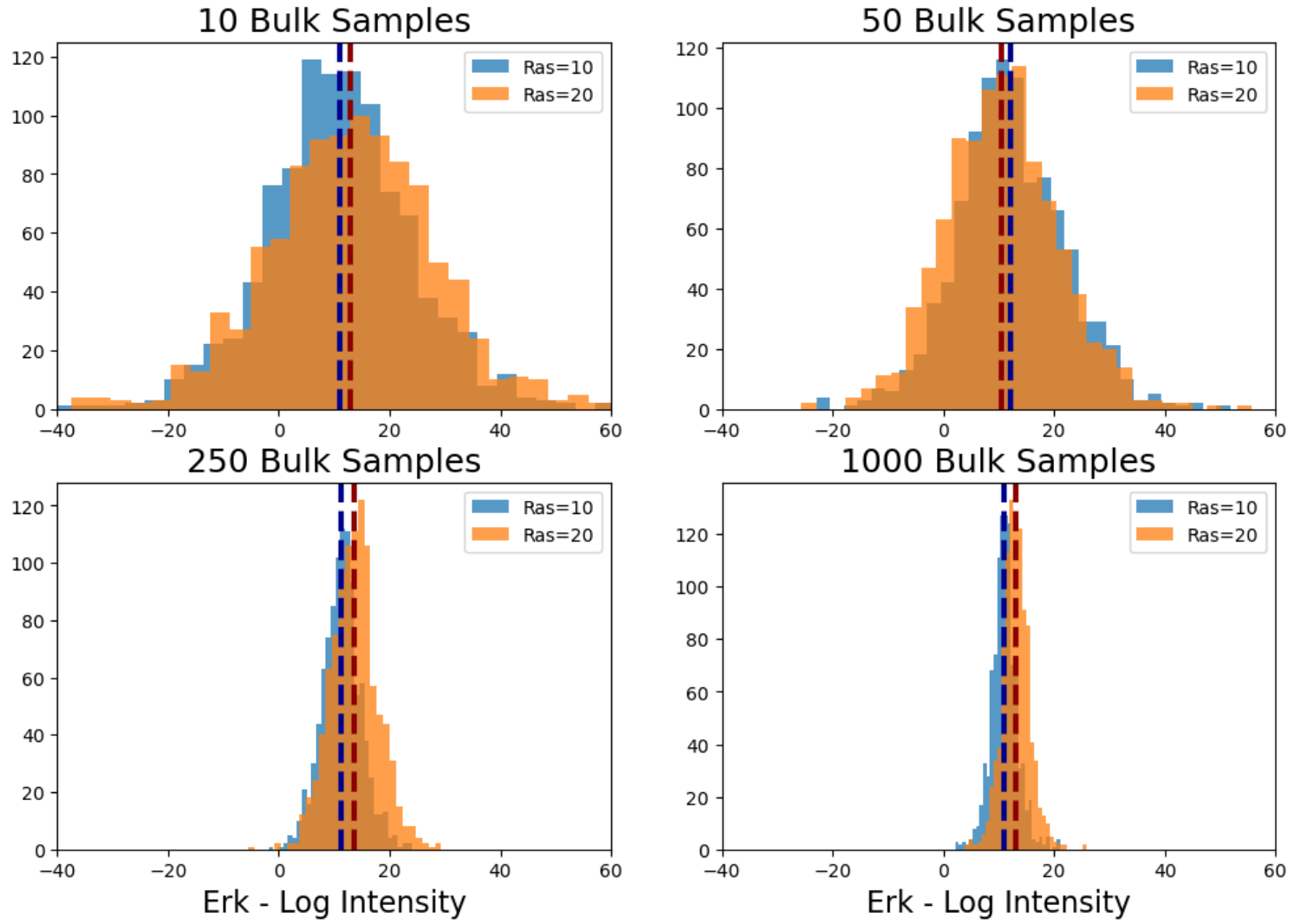
In the presence of latent confounders (e.g., bulk proteomics) no number of replicates can recover the ACE

3 cell types measured in each bulk sample





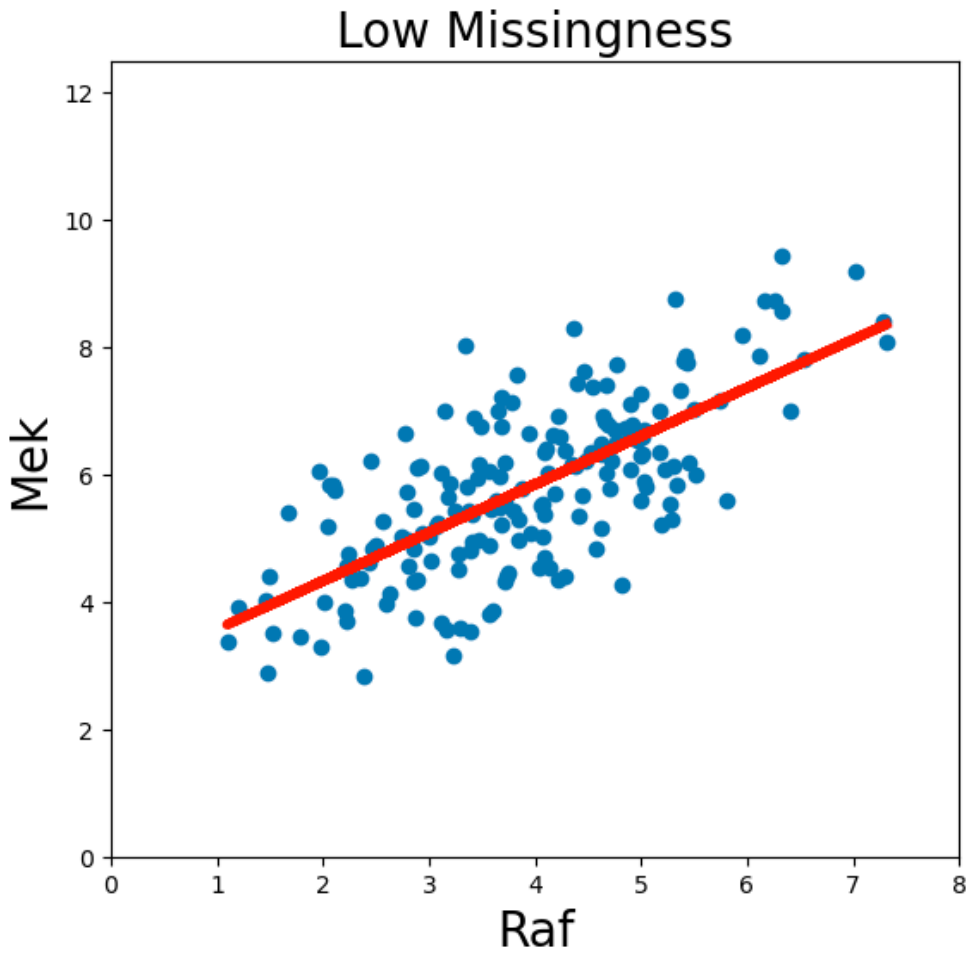
In the presence of latent confounders (e.g., bulk proteomics) no number of replicates can recover the ACE

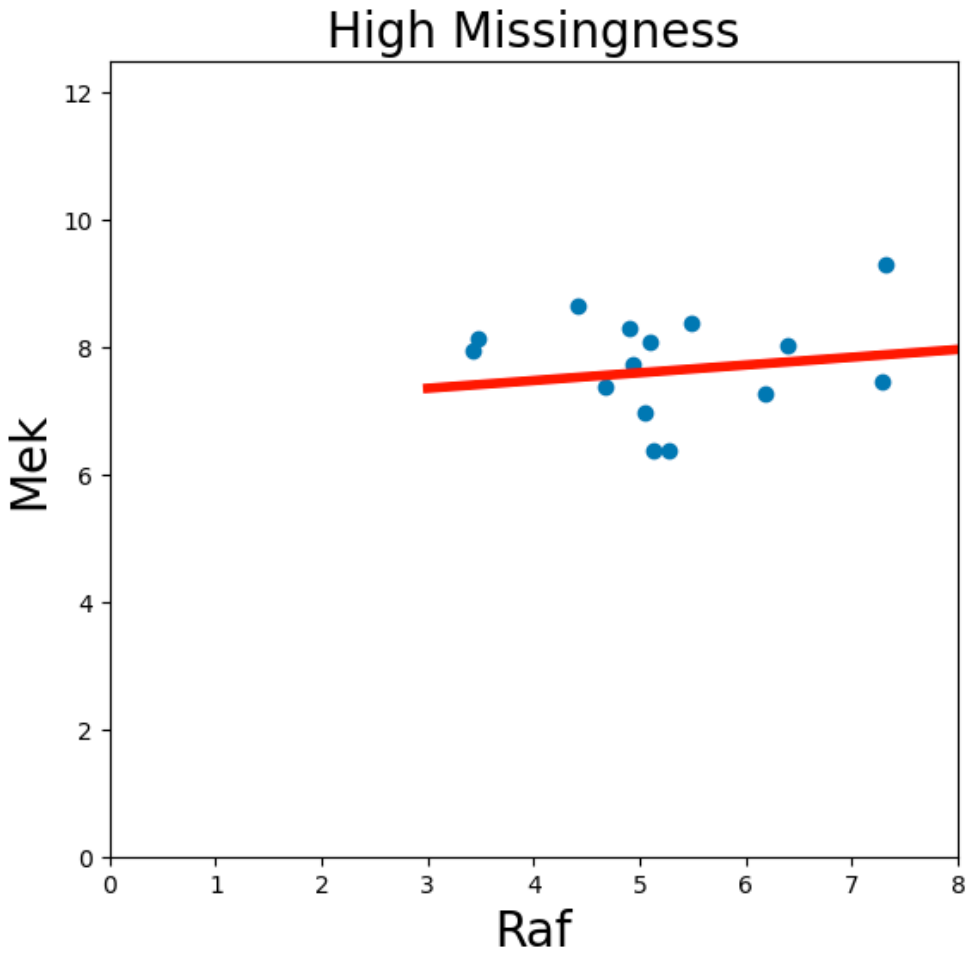


Presentation Outline

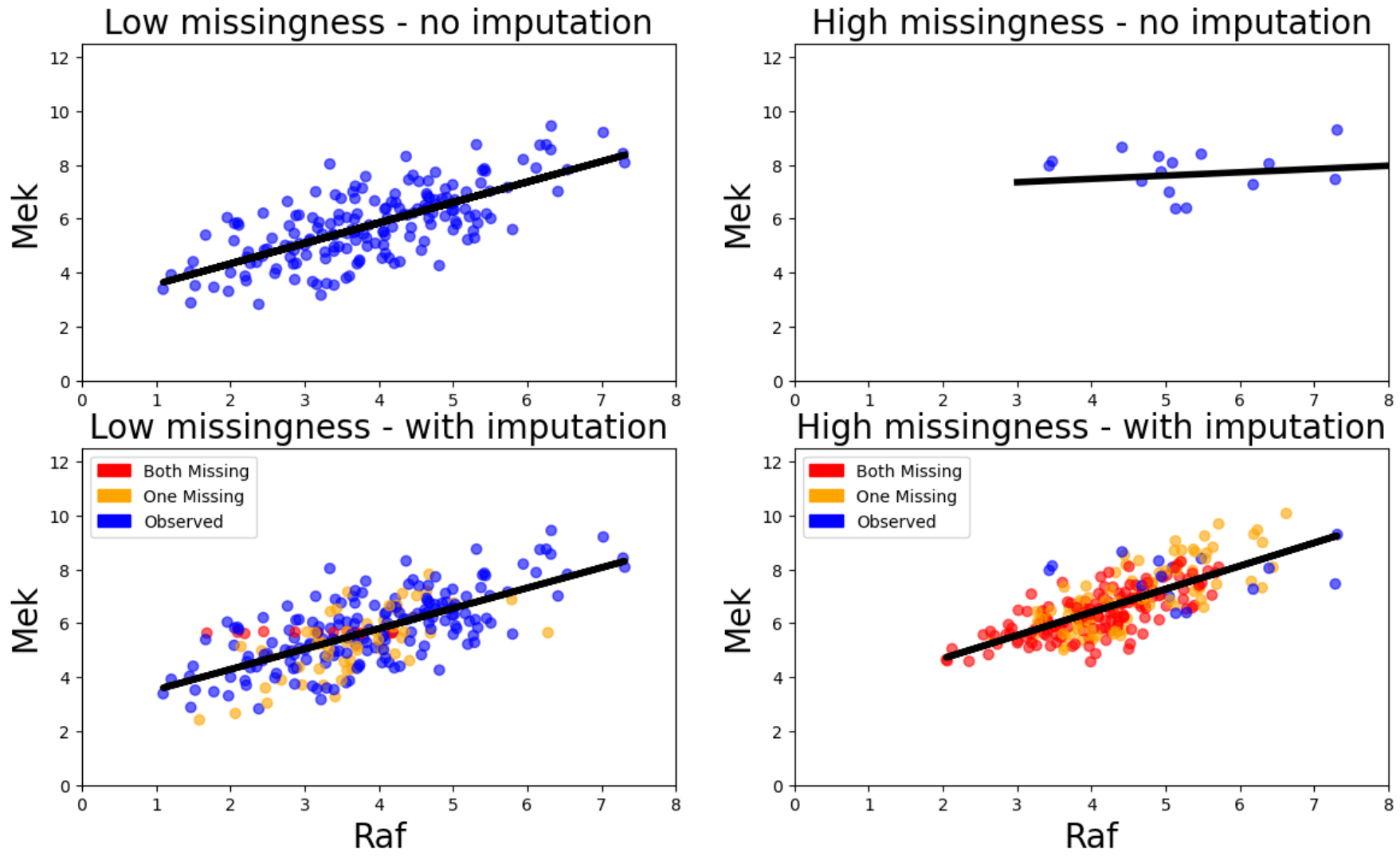
- Problem statement
- Background
- Case studies – Targeted vs Discovery
  - Targeted experiment (simulation)
    - Bulk vs single cell
    - Replication (e.g., the number of cells)
    - Protein-level imputation
  - Discovery experiment (biological)

When observations are MNAR the true correlation/causal effect is masked





Causal imputation correctly recovers causal effect in the presence of missing data



Presentation Outline

- Problem statement
- Background
- Case studies – Targeted vs Discovery
  - Targeted experiment (simulation)
  - Discovery experiment (biological)

Single Cell experiment - Leduc et al, 2022

- 1556 single cells prepared by nPOP method and acquired with TMT 18-plex
- Melanoma and monocyte cell types
- 2844 proteins identified and quantified with MaxQuant
- Data processed with methods in MSstatsTMT

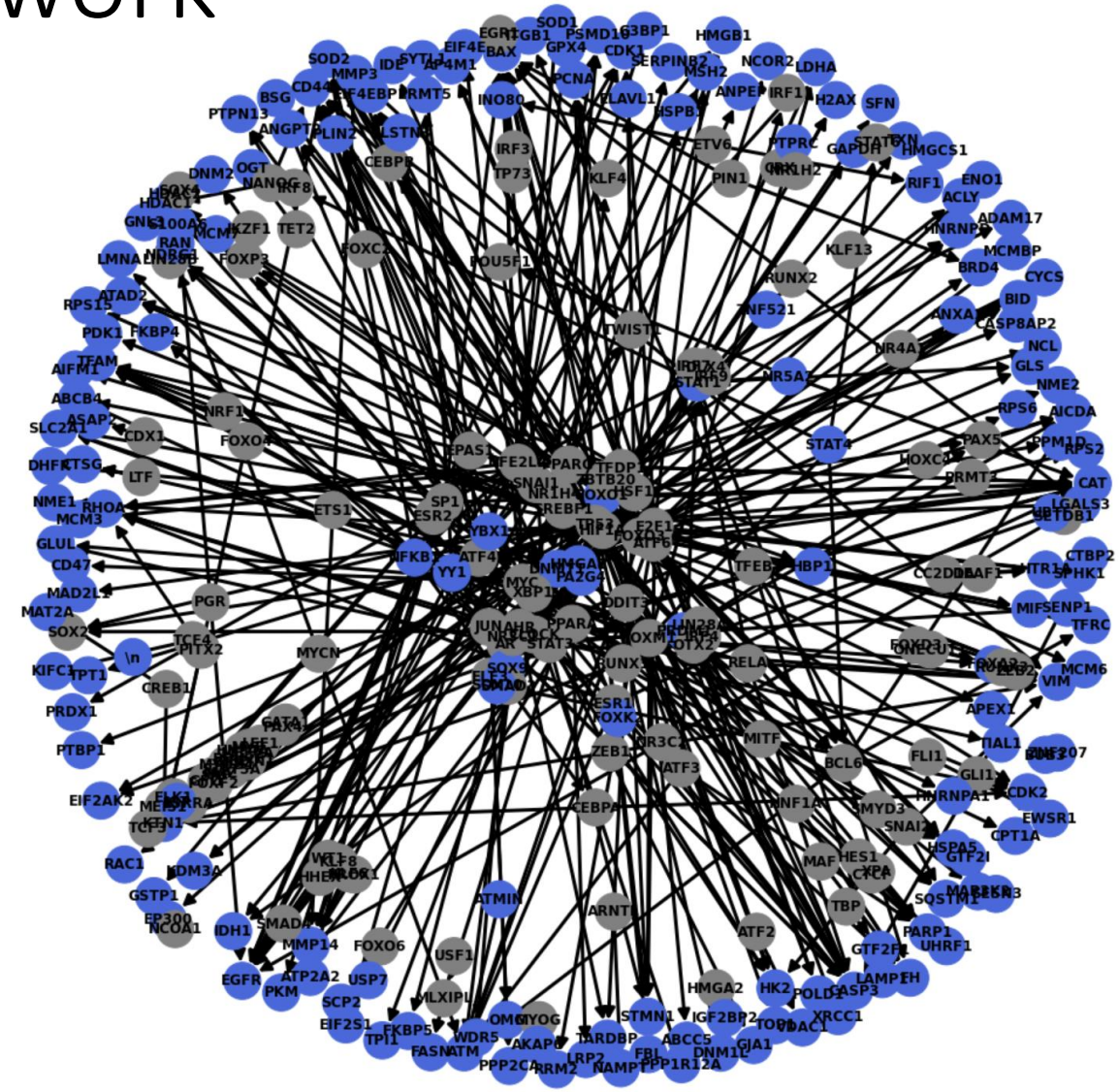
Leduc A, et al. (2023) Exploring functional protein covariation across single cells using nPOP, Genome Biol

Building a causal network around measured proteins

- Creating a hand tailored network across thousands of proteins is very challenging
- Leverage biological databases to extract causal relationships between proteins in the system
- We use the INDRA database, which includes causal information between proteins

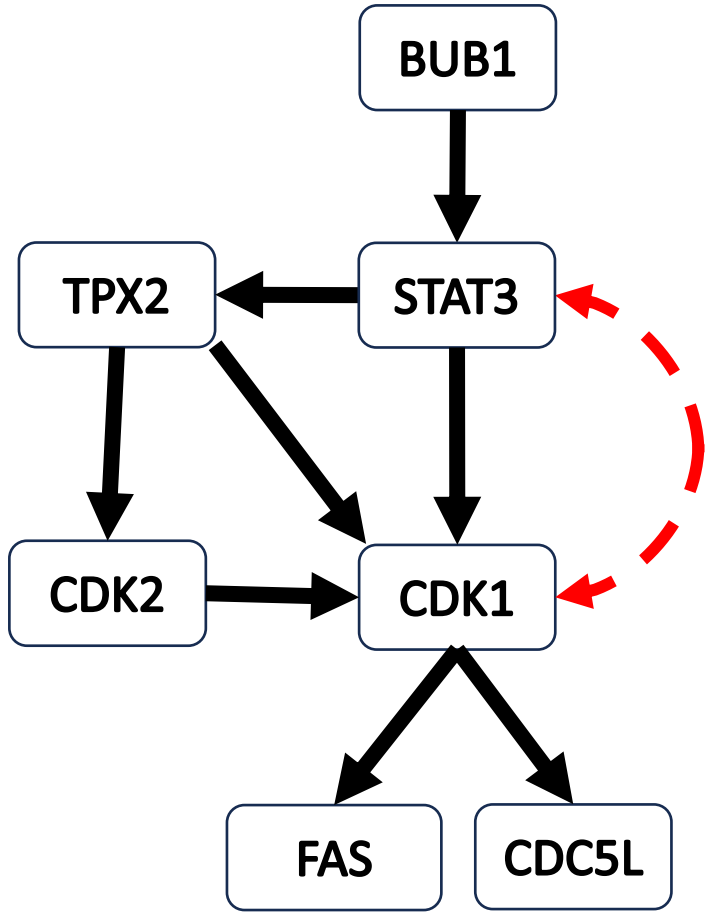
Naïve network extraction results in unusable and uninterpretable network

- Many types of connections may not be relevant
- Some edges have very low evidence
- Not all edges are applicable to the biological question of interest



Thoughtful queries results in reasonable networks

- Focus on abundance events
- Look at specific pathways of interest that show correlation in data
- Filter for edges with high confidence
- Filter for biologically relevant questions



Final causal model

Targeting CDK2 overcomes melanoma resistance against BRAF and Hsp90 inhibitors

Alireza Azimi, Stefano Caramuta, Brinton Seashore-Ludlow, Johan Boström, Jonathan L Robinson, Fredrik Edfors, Rainer Tuominen, Kristel Kemper, Oscar Krijgsman, Daniel S Peeper, Jens Nielsen, Johan Hansson, Suzanne Egyhazi Brage, Mikael Altun, Mathias Uhlen, Gianluca Maddalo

Author Information

Molecular Systems Biology (2018) 14: e7858 | https://doi.org/10.15252/msb.20177858

The FASEB Journal

RESEARCH ARTICLE

Discovery of novel CDK2 inhibitors using multistage virtual screening and in vitro melanoma cell lines

Lihong Yang, Mukuo Wang, Beibei Li, Shangqin Xu, Jianping Lin

First published: 24 March 2023 | https://doi.org/10.1096/fj.202201217RR

Fas-Mediated Apoptosis of Melanoma Cells and Infiltrating Lymphocytes in Human Malignant Melanomas

Tetsuo Shukuwa M.D., Ichiro Katayama M.D. & Takehiko Koji Ph.D.

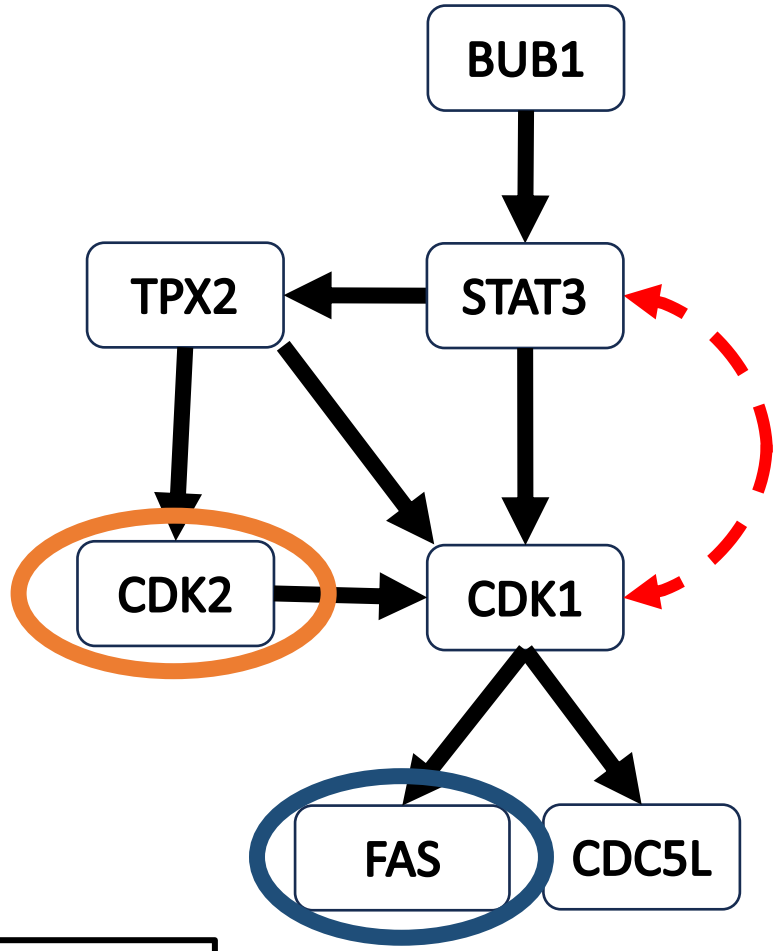
Modern Pathology **15**, 387–396 (2002) | Cite this article

924 Accesses | **17** Citations | Metrics

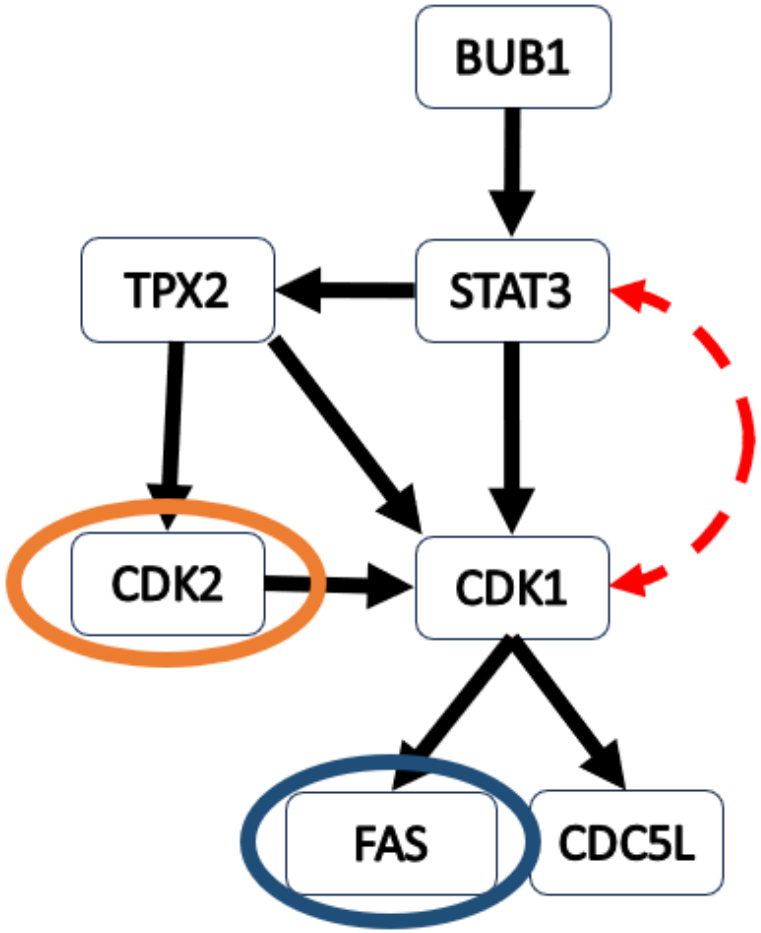
Melanoma Cell Expression of Fas(Apo-1/CD95) Ligand: Implications for Tumor Immune Escape

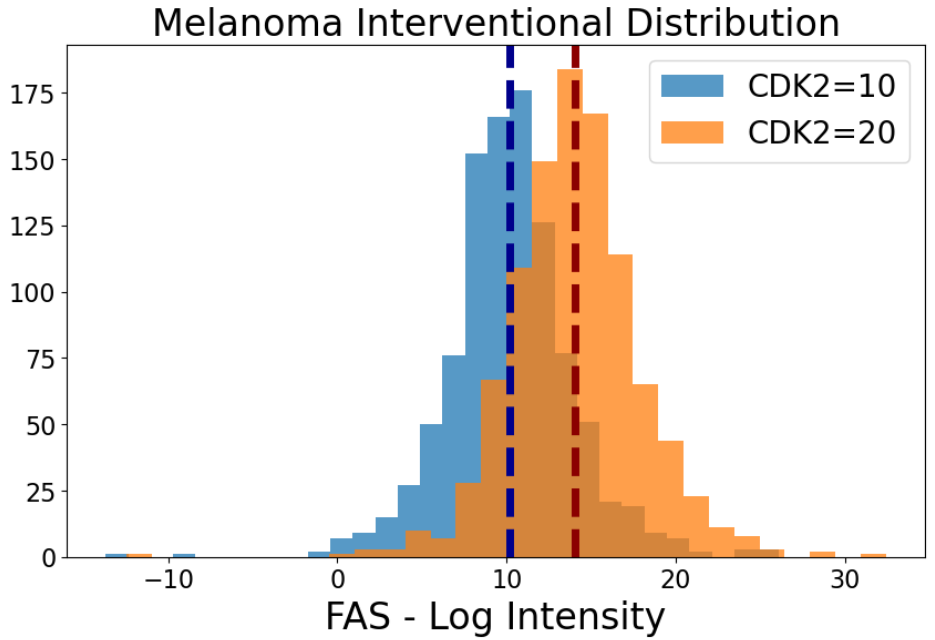
M. HAHNE, D. RIMOLDI, M. SCHRÖTER, P. ROMERO, M. SCHREIER, L. E. FRENCH, P. SCHNEIDER, T. BORNAND, A. FONTANA, [...], AND J. TSCHOPP +2 authors Authors Info & Affiliations

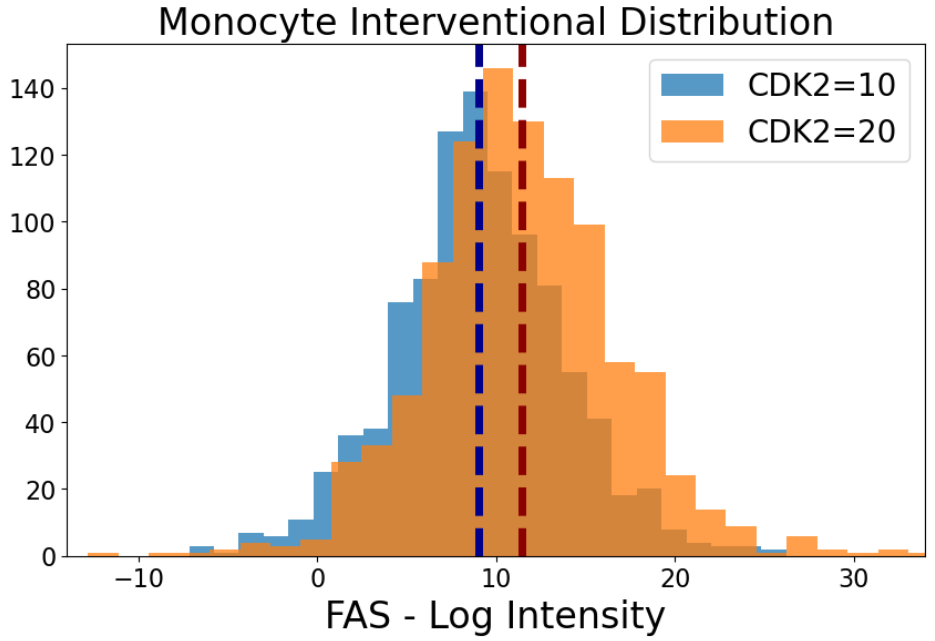
SCIENCE · 22 Nov 1996 · Vol 274, Issue 5291 · pp. 1363-1366 · DOI: 10.1126/science.274.5291.1363



Intervention results







Conclusions

- Estimation of the effect of interventions is possible given observational single cell data
- Targeted and exploratory studies possible, depending on the goal of the experiment

Existing challenges

Near term (computational)

- More work to be done on building causal networks
- Data processing of single cell experiments

Long term (experimental)

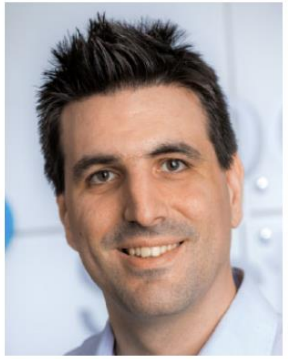
- Post-translational modifications
- Temporal information

Acknowledgements

Northeastern University

OLGA VITEK LAB

Statistical Methods For Studies Of Biomolecular Systems



Ben Gyori
Northeastern

Charlie Hoyt
Northeastern

Jeremy Zucker
PNNL

Lab Members

Kylie Bemis
Sara Mohammad Taheri
Ritwik Anand
Vartika Tewari
Sai Srikanth Lakkimsetty
Sarah Szvetecz
Yinyue Zhu
Mateusz Stankiak

Karen Sachs
NextGen Analytics