Synthesizing Signaling Pathways from Temporal Phosphoproteomic Data

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**Pathway:** Group of proteins that physically interact and work together to execute some function
Example pathway: EGF signaling

- Epidermal growth factor receptor (EGFR) pathway
- Growth, apoptosis, differentiation, etc.
- Perturbed in many cancers
Experimentally measuring pathway activity

- Stimulate receptors
- Observe protein activity changes (e.g. phosphorylation)
Pathway databases do not explain observed phosphorylation changes
How can we generate pathway models from experimental protein phosphorylation data?
Phosphorylation timing reveals information flow in signaling pathways

Mass spectrometry

Measure 1000s of proteins
Phosphorylation timing reveals information flow in signaling pathways

Mass spectrometry

Measure 1000s of proteins
Related approaches using temporal information

• HPN-DREAM network inference challenge (Hill et al. 2016)
  • PropheticGranger (Carlin et al. 2017)
  • FunChisq (Zhang and Song 2013)
• Dynamic Bayesian network (Hill et al. 2012)
• TimeXNet (Patil et al. 2013)
Temporal Pathway Synthesizer (TPS)

- Time series protein phosphorylation
- Protein-protein interactions among phosphorylated and other proteins
- Prior knowledge
Temporal Pathway Synthesizer (TPS)

- Protein-protein interactions among phosphorylated and other proteins
- Temporal Pathway Synthesizer efficiently evaluates all pathway models
- Summarize only the valid pathway models

Time series protein phosphorylation
Temporal Pathway Synthesizer (TPS)

Prune subnetwork with Omics Integrator (Tuncbag et al. 2016)
Program synthesis

def make_odd(input):
    return input * ?? + ??

def make_odd(input):
    return input * 2 + 1

Fill “holes” in program templates
Program synthesis

```
def make_odd(input):
    return input * ?? + ??
```

```
def make_odd(input):
    return input * 2 + 1
```

Fill “holes” in program templates

Fill “holes” in pathways
Formally list constraints for a declarative solver

Satisfiability modulo theories solver
Can’t assess entire pathway directly

Entire pathway

Individual edge queries
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Individual edge queries
Constraint 1: phosphorylation timing eliminates candidate pathways

A first active at 10 minutes
B first active at 2 minutes
Constraint 2: reachability in graph eliminates candidate pathways

Phosphorylated proteins must be reachable from the source protein \( \text{A} \)
Constraint 2: reachability in graph eliminates candidate pathways

Phosphorylated proteins must be reachable from the source protein A

B isn’t reachable

Yes Yes

No No
Applying TPS to study human EGF response
Stimulate engineered cells with EGF

• Measure phosphorylation at 0, 2, 4, 8, 16, 32, 64, 128 min

• 203 significantly differentially phosphorylated proteins
Stimulate engineered cells with EGF

• Measure phosphorylation at 0, 2, 4, 8, 16, 32, 64, 128 min

• 203 significantly differentially phosphorylated proteins
Zooming in on specific kinases
Test predictions with kinase inhibitors

Western blots measure relative phosphorylation

Pathway prediction: ABL2 controls early CRK phosphorylation increase

Inhibiting ABL2 impacts CRK phosphorylation
Recap and next questions for TPS

Pathway databases and phosphoproteomic data give very different views of signaling

TPS uses phosphorylation timing + protein networks to synthesize custom pathways

Future directions
• How to incorporate perturbation data?
• How to move to probabilistic models?
For more information

TPS software:  https://github.com/koksal/tps

Synthesizing Signaling Pathways from Temporal Phosphoproteomic Data. Köksal et al. Cell Reports 2018. doi:10.1016/j.celrep.2018.08.085

Bonus supplement: Köksal et al. figshare 2018. doi:10.6084/m9.figshare.6957461

Mass spectrometry data:  PRIDE PXD006697
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TPS collaborators

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Nathan Camp     Jasmin Fisher
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