Robust small molecule-protein interaction inference reveals unknown drug off-targets

Nils Kurzawa
PhD Student, Savitski Lab, EMBL Heidelberg
ATP

Fumarate
Which proteins interact with certain small molecules?

- Panobinostat
- ATP
- Fumarate
- Caffeine
- Erythromycin
The thermal shift assay

![Diagram showing the thermal shift assay](image)

- Ligand
- +Ligand
- Folded protein
- Aggregated protein
- Stabilization

Martinez Molina et al. (2013) Science; Savitski et al. (2013) Science; Mateus, Määttä & Savitski (2017) Proteome Science
Two dimensional thermal proteome profiling (2D-TPP)

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When is a hit a hit?

clear-cut case!

Temperature

Compound concentration

FC

1.0
1.5
2.0
2.5
3.0
When is a hit a hit?

Clear-cut case!

Yepp!

Temperature

Compound concentration

FC

1.0

1.5

2.0

2.5

3.0
When is a hit a hit?

- clear-cut case!
- yepp!
- I guess...

Compound concentration

Temperature

FC

1.0
1.5
2.0
2.5
3.0

5 09/12/2019
When is a hit a hit?

Compound concentration vs Temperature

- clear-cut case!
- yepp!
- I guess...
- well... maybe?

FC
1.0
1.5
2.0
2.5
3.0
When is a hit a hit?

Temperature

Compound concentration

clear-cut case! yepp! I guess... well... maybe? ehm, no?

FC

- 1.0
- 1.5
- 2.0
- 2.5
- 3.0

5 09/12/2019
How to analyze 2D-TPP datasets with false discovery rate control?
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A functional analysis approach for 2D-TPP data

Null model: protein remains unaffected by treatment

Protein intensity vs. log10(treatment conc.)
A functional analysis approach for 2D-TPP data

Null model: protein remains unaffected by treatment

Alternative model: protein stability is affected by treatment

Protein intensity

\[ \text{log10}(\text{treatment conc.}) \]
Constructing null and alternative models

**Null model**: protein remains unaffected by treatment

**Alternative model**: protein stability is affected by treatment
Constructing null and alternative models

**Null model:** protein remains unaffected by treatment

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Constructing null and alternative models

**Null model:** protein remains unaffected by treatment

**Alternative model:** protein stability is affected by treatment

$$F = \frac{\text{RSS}^0 - \text{RSS}^1}{\text{RSS}^1}$$
α-Actinin 4 revealed as off-target of the BET-inhibitor JQ1

Kurzawa et al. in preparation
α-Actinin 4 revealed as off-target of the BET-inhibitor JQ1

- Previously detected targets are found: BRD2-4 and HADHA
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Kurzawa et al. *in preparation*
Acknowledgements

**EMBL Heidelberg:**
Misha Savitski
Wolfgang Huber
Srishti Dar
Sindhuja Sridharan
Isabelle Becher
André Mateus
Britta Velten
Dorothee Childs

**Cellzome, GSK:**
Marcus Bantscheff
Jessica Perrin
Thilo Werner
Holger Franken
Carola Doce
Maria Fälth-Savitski

**CRUK Cambridge:**
Karsten Bach

**All Savitski and Huber Group members at EMBL**

**ZMBH Heidelberg:**
Simon Anders
Thank you!
Constructing an H1 model (treatment effect)

\[ y_{i,t} = \beta_{i,t}^0 + \epsilon_{i,t} \]

- \( y_{i,t} \): log2 intensity for protein i at temperature t
- \( \beta_{i,t}^0 \): concentration-independent intercept parameter for protein i at temperature t
Constructing an H1 model (treatment effect)

\[ y_{i,t}(c) = \beta_{i,t}^0 + \frac{\alpha_{i,t} \delta_{i}^{\text{max}}}{1 + \exp(\kappa_i(c - \xi(t)_i))} + \epsilon_{i,t,c} \]

- **\( y_{i,t}(c) \)**: log2 intensity for protein i at temperature t, at concentration c
- **\( \beta_{i,t}^0 \)**: concentration-independent intercept parameter for protein i at temperature t (value y will take for \( c = 0 \))
- **\( \delta_{i}^{\text{max}} \)**: maximal stabilization
- **\( \alpha_{i,t} \)**: parameter indicating how much relative stabilization happens at temperature t
- **\( \kappa_i \)**: slope factor
- **\( \xi(t)_i \)**: linear function describing decline of the pEC50 with increasing temperature
Functional analysis of TPP melting curves: NPARC

Null Model

\[ \mu(t) \]

\[ SS^0 = 1.22 \]

Alternative Model

\[ \mu_T(t) \]

\[ SS^1 = 0.08 \]

Fraction non-denatured

Temperature [°C]

SS: sum of squared errors


Method performance on JQ1 lysate dataset

- We currently follow up on ACTN4 as a potential off-target of JQ1
- effects of JQ1 on actin bundle formation have been observed, but were attributed to transcriptional changes via BRDs Qu et al. 2018, Cell Death Discovery
Controlling FDR

- Past experience: F statistic does not lead to valid p-values in melting curve/dose-response setting
  - because residuals are correlated and heteroscedastic
- Approach: bootstrapping null distribution:
  - Fit H0 model for every protein
  - Resample residuals from H0 10 times per protein, fit H1 and compute F statistics
  - Repeatedly ($B$ times) do this and jointly rank results with those from true dataset
- Compute FDR: \[
\text{FDR}_\theta = \frac{\pi_\theta \sum_B \#\{F_{i,b}^0 > \theta\}/(B)}{\#\{F_i > \theta\}}
\]
Thermal proteome profiling (TPP)

2D-TPP data analysis

Cells treated with Panobinostat

2D-TPP data analysis: what's the matter?

- Fitting dose-response models per temperature can be misleading
- Hits defined by manual thresholds
2D-TPP data analysis: what’s the matter?

- Fitting dose-response models per temperature can be misleading
- Hits defined by manual thresholds
- No false discovery rate (FDR) control

→ For experiments with several (expected) targets, FDR estimation is crucial!
Method performance on Panobinostat in-cell dataset

- Previously detected targets are found: HDAC1,2 and 6 and off-targets FADS1, 2, TTC38 and PAH
- New potential off-target found: DHRS1