

Dose-resolved Expression Proteomics

Advanced Omics for Life Sciences
Group 1, Project 5
11-4-2025

By Fernando Sancho Gómez, Matthijs Hamstra,
Marloes van Luik and Jan Paul van Meenen



Table of Contents

1. The Article
2. Graphical Abstract
3. The Dataset
4. Analysis approach
5. Results
6. Conclusions

Decrypting the molecular basis of cellular drug phenotypes by dose-resolved expression proteomics

Stephan Eckert, Nicola Berner, Bernhard Kuster, et al.

- Development of *decryptE*, a method to measure dose-response characteristics for drug-induced protein expression
- Profiling of Drug Effects at Varying Concentrations
- Uncovering new aspects of Drug Mode of Action (MoA) for known medicines
 - Many **off-target affected proteins** were identified



Utrecht
University

Graphical Abstract

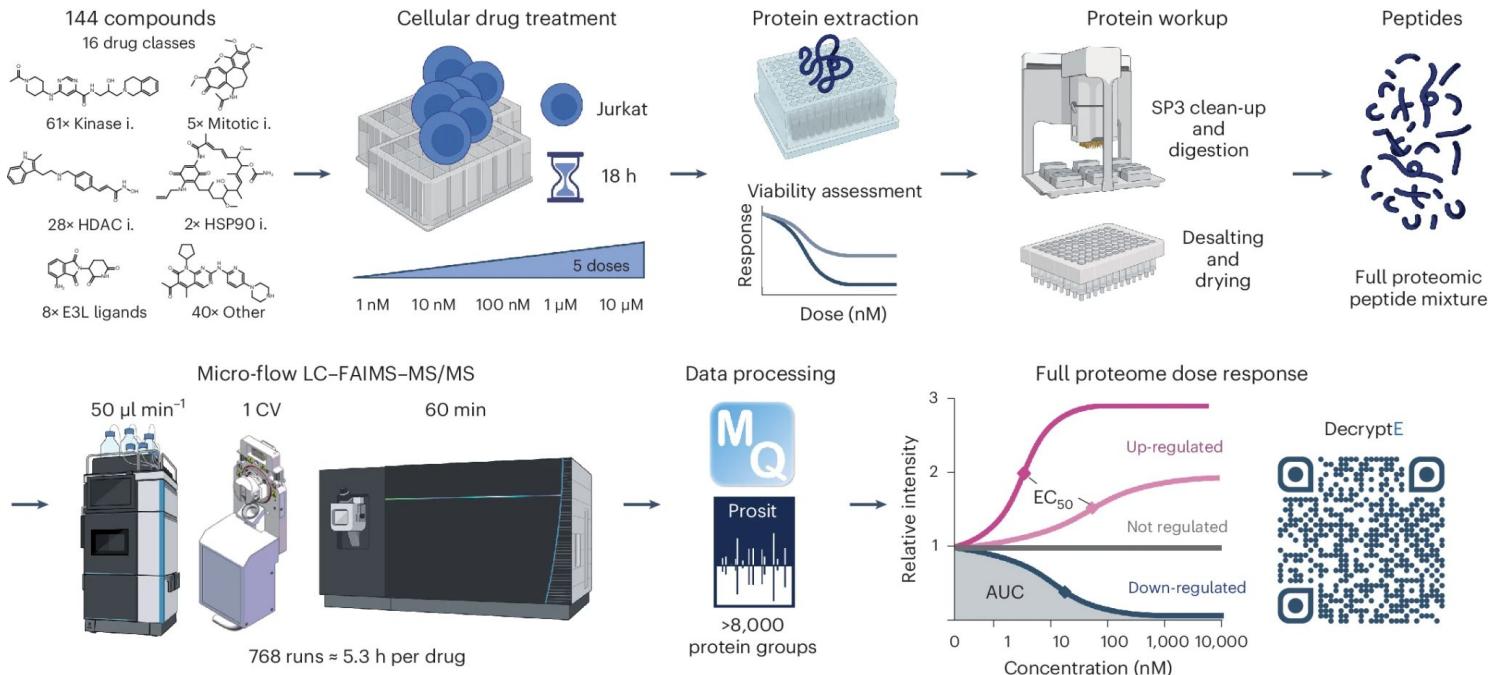


Fig. 1 | DecryptE workflow for the proteome-wide and dose-dependent characterization of drug-induced protein expression changes.

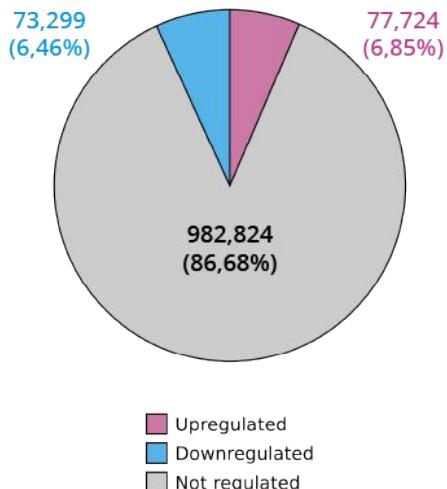


The Dataset

- Generated by **Micro-flow LC-FAIMS-MS/MS**
- 768 instrument-hours
- Spectra database search with FDR of 0.01
- 10x concentration steps:
 - 1nM, 10nM, 100nM, 1µM and 10µM
- Most dose response curves show no effect

Condition	Count
Proteins	8,892
Compounds	144
Dosage Concentrations	5

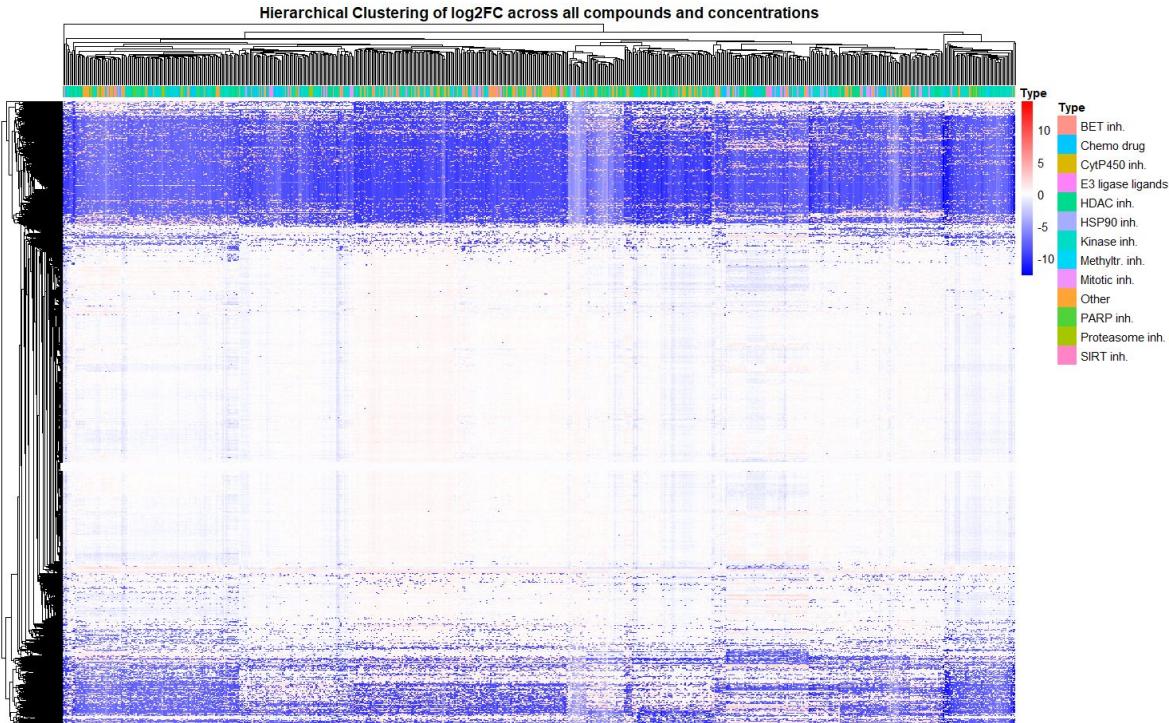
Dose-response curves





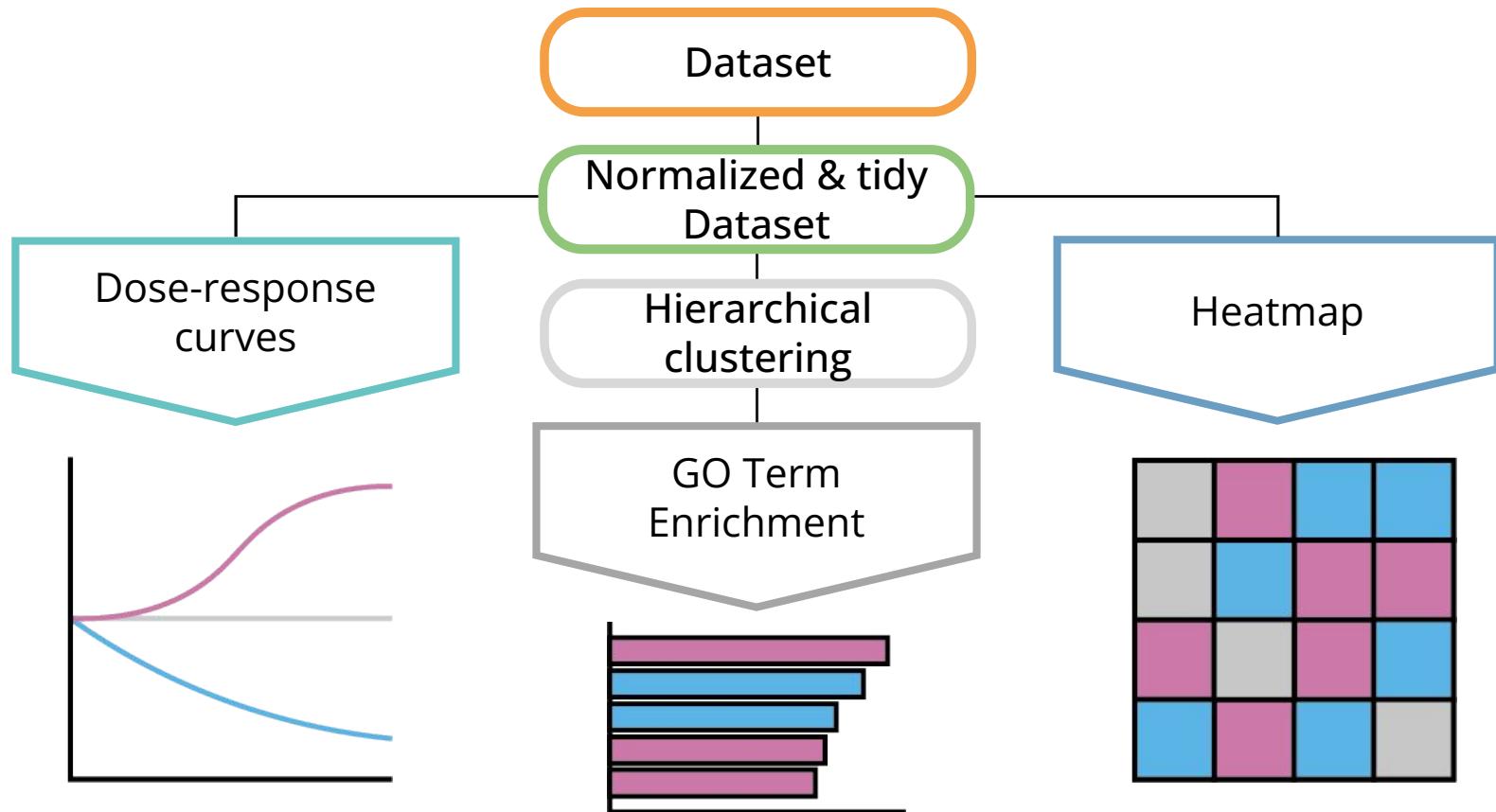
The Dataset (is huge)

- 144 compounds
 - 5 concentrations
 - 8.892 unique proteins
- = 6.402.240 combinations





Analysis Approach



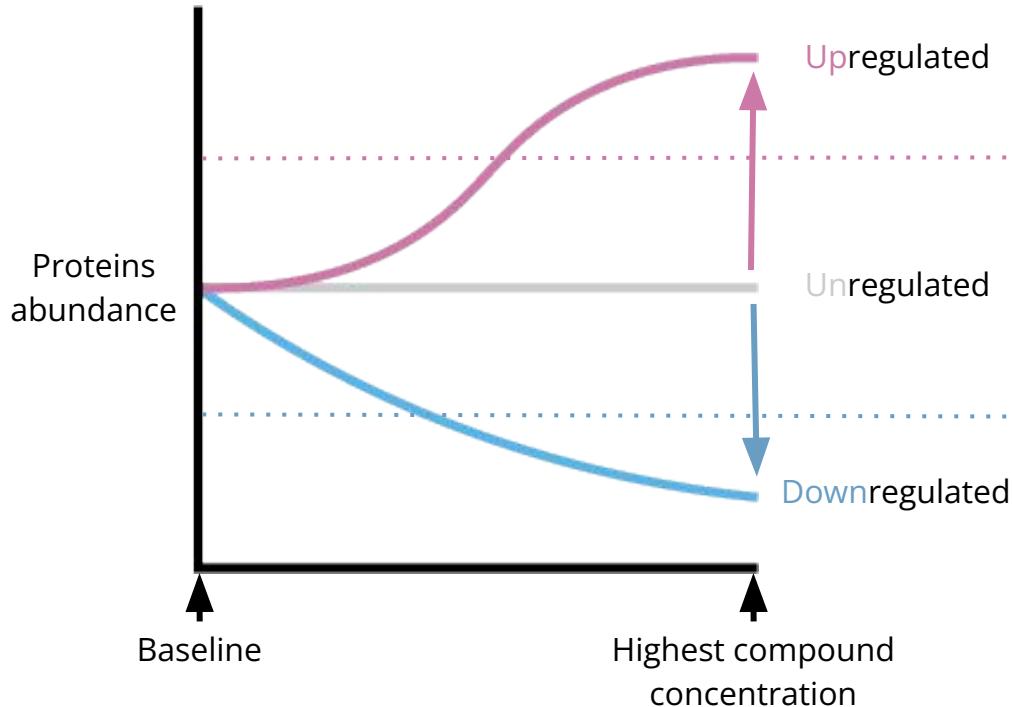


Classification of up- and downregulation

Log2 fold change (Log2FC) protein abundance (highest concentration and the **baseline**)

Regulation cutoffs:

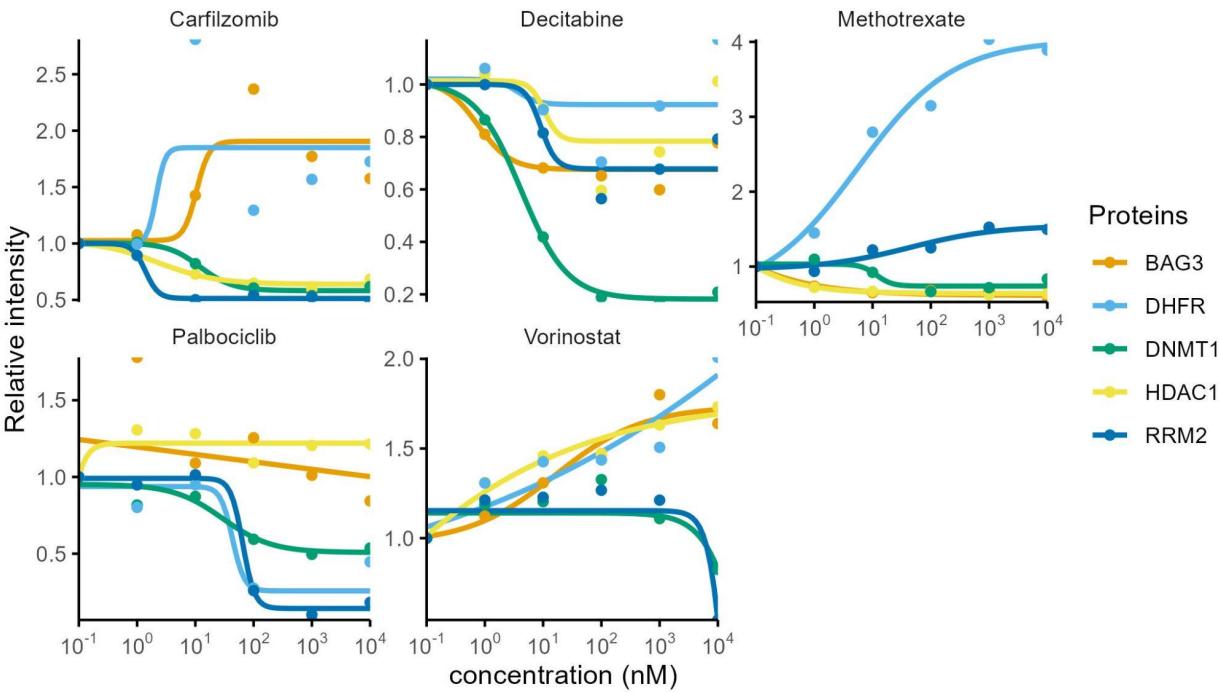
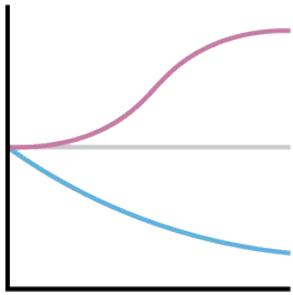
- Up $> \log_2(1.5 \text{ FC})$
- Down $< \log_2(0.7 \text{ FC})$
- Unregulated





Dose-response Curves

Eckert et al. trained classifier
(up-, down-, unregulated)
using dose-response curve
data



Unfortunately,
compound-protein
regulation predictions are
not available



Preprocessing data (round 1)

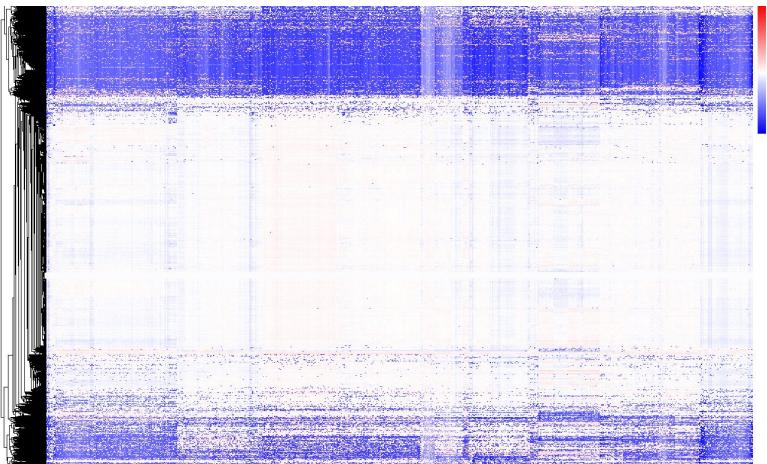
Raw data

- Many missing values & duplicates



(Imputed all `-Inf` and `NA`'s for illustrative purposes)

Proteins

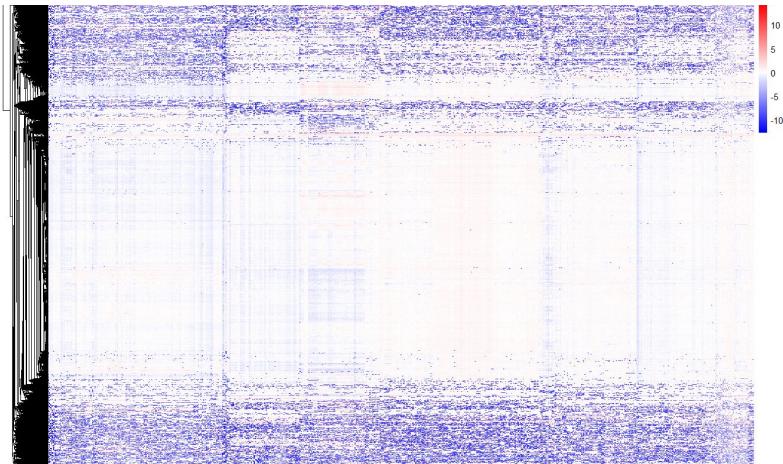


Compound-concentration

Processing (1):

- Filter out:
 - fully missing compound-protein combinations
 - duplicates
- Impute the rest

Proteins



Compound-concentration



Preprocessing data (round 2)

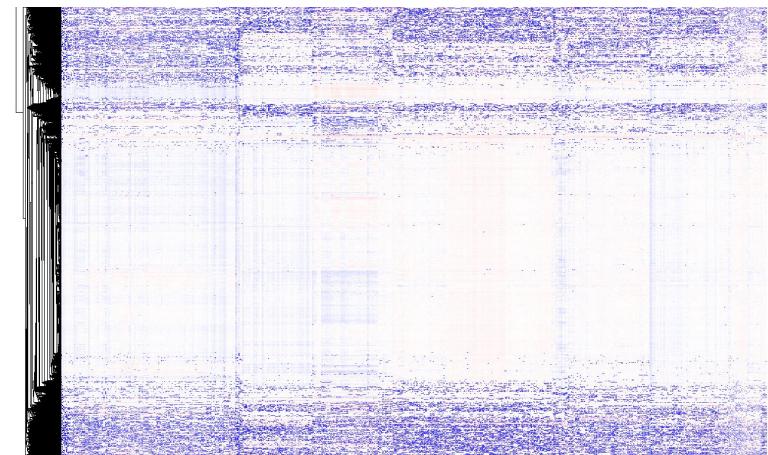
Problem:

- Sparse, many incomplete compound-protein interactions
- Noisy (imputation)
 - Long branch lengths

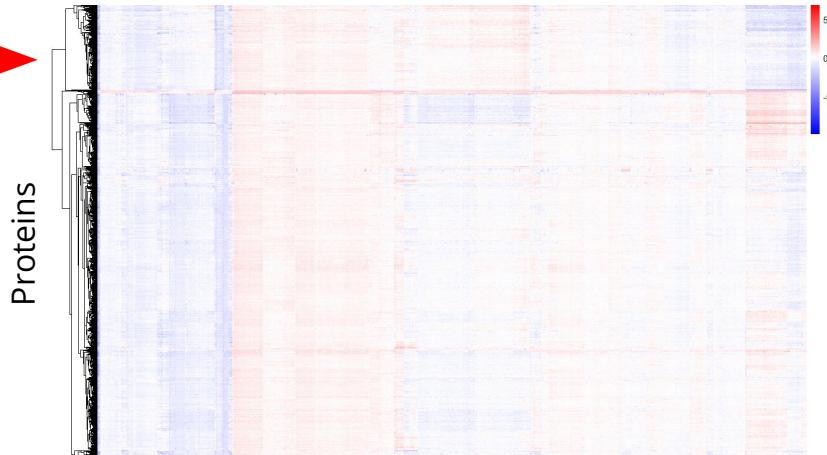


Processing (2):

- Filter out all incomplete proteins (rows)
- Shorter branch lengths
- Clean separation of clusters



Compound-concentration



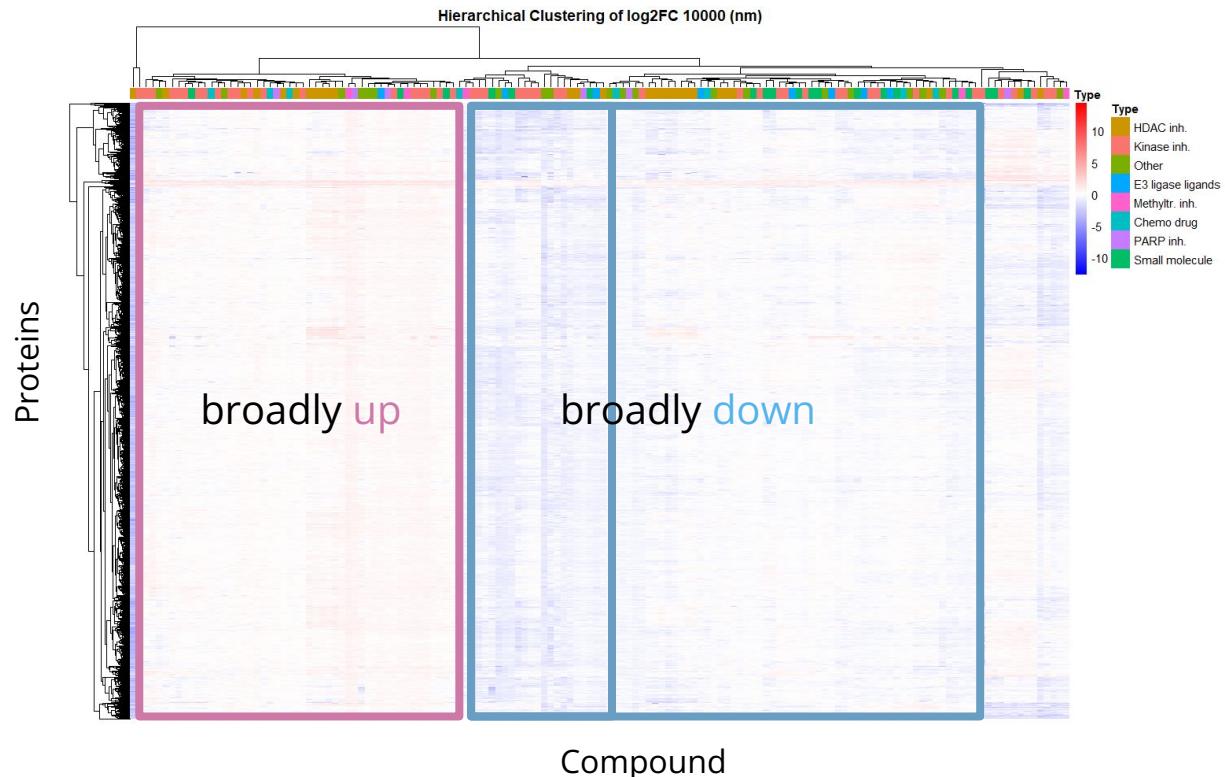
Compound-concentration



Heatmap of all proteins vs all compounds

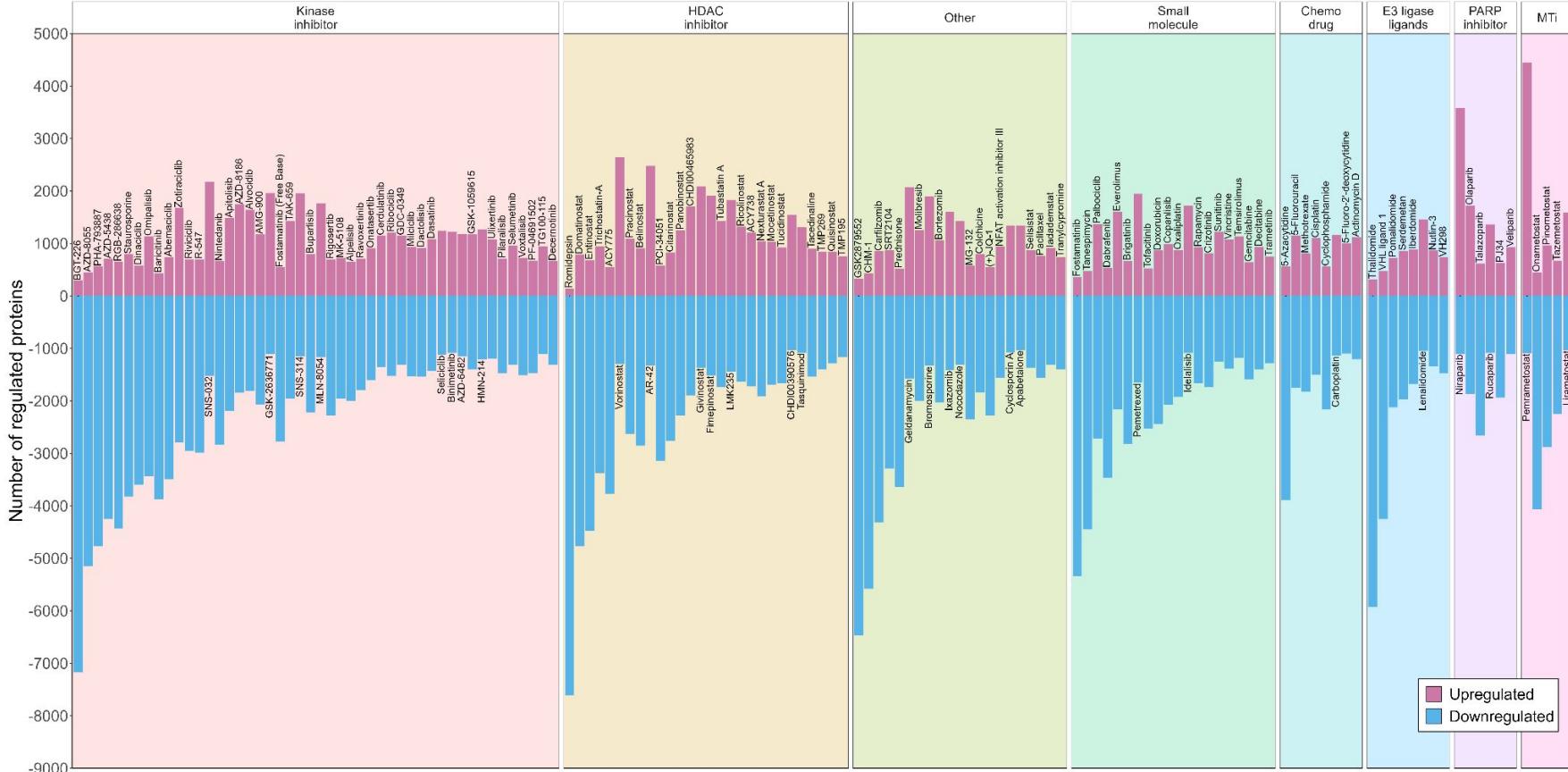
Log2FC protein abundance
(compound concentration
vs baseline concentration)

Mostly within compound
clustering



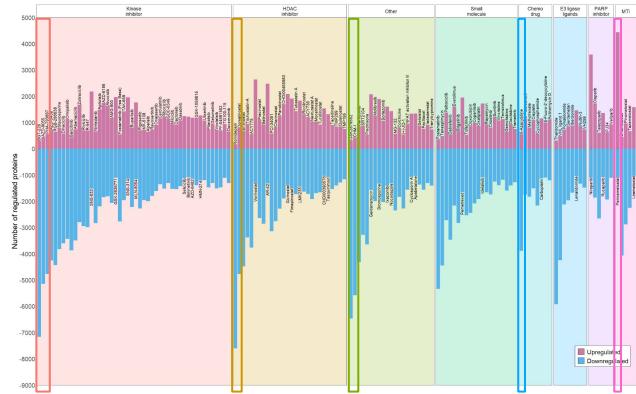
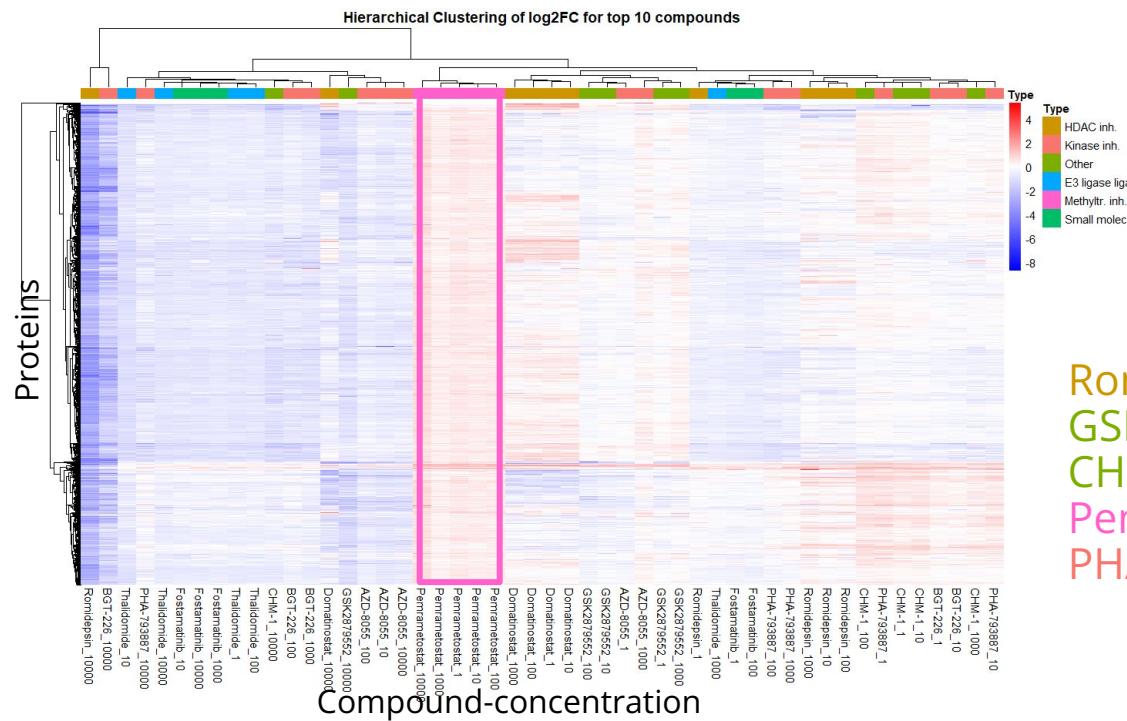


Compound ranking total up & down proteins





Heatmap of top 10 compounds

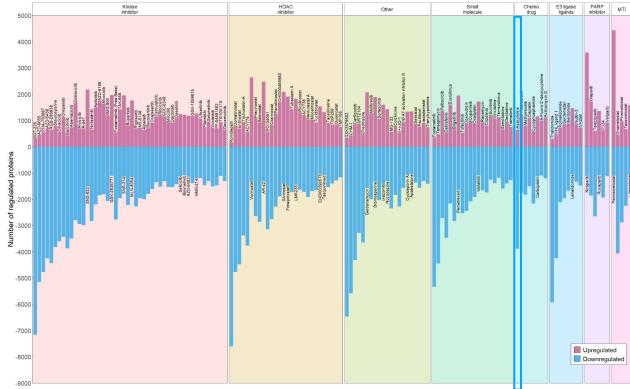
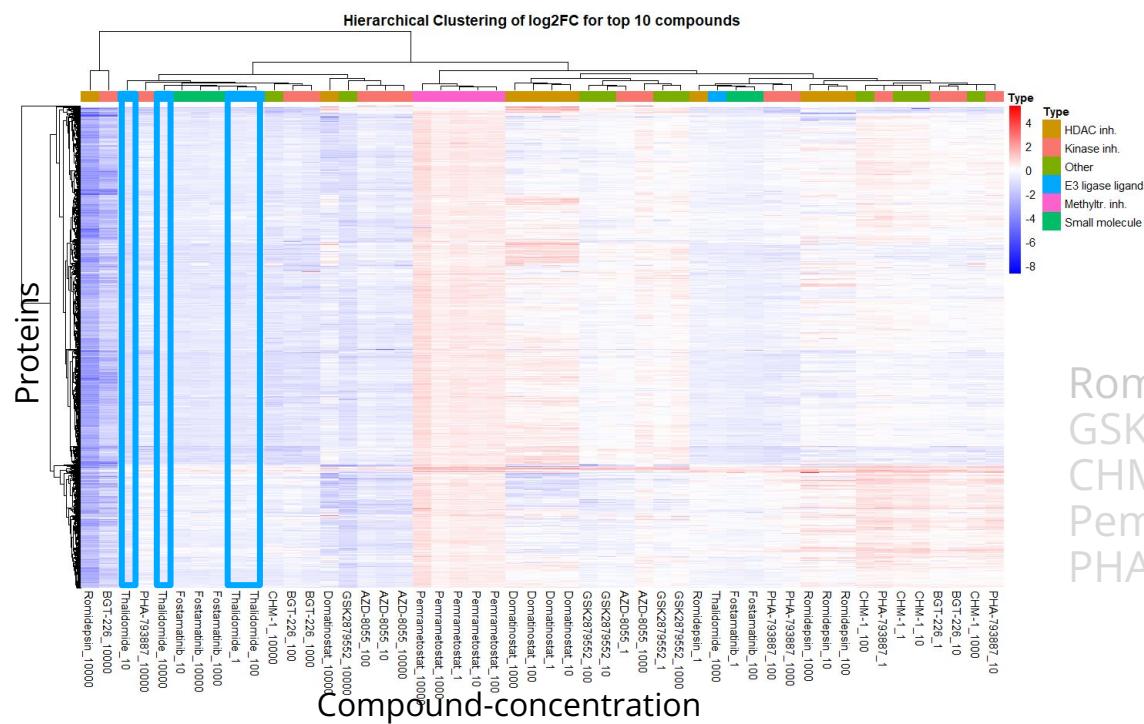


Romidepsin
GSK2879552
CHM-1
Pemrametostat
PHA-793887

BGT-226
Thalidomide
Fostamatinib
AZD-8055
Domatinostat



Heatmap of top 10 compounds

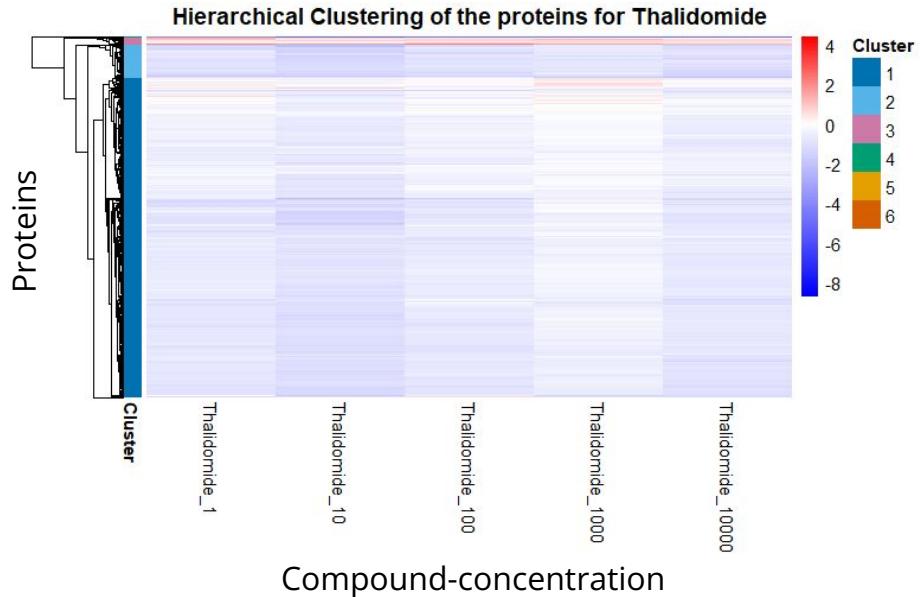


Romidepsin
GSK2879552
CHM-1
Pemrametostat
PHA-793887

BGT-226
Thalidomide
Fostamatinib
AZD-8055
Domatinostat

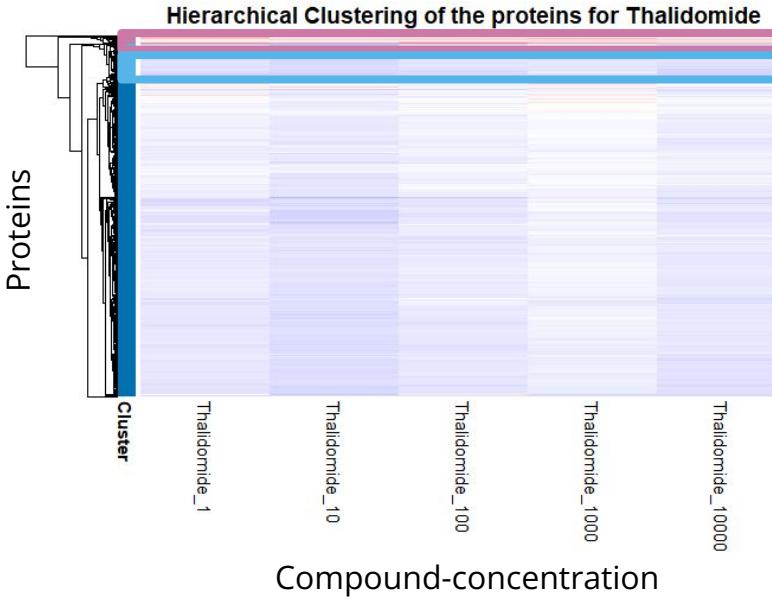


Thalidomide - Heatmap



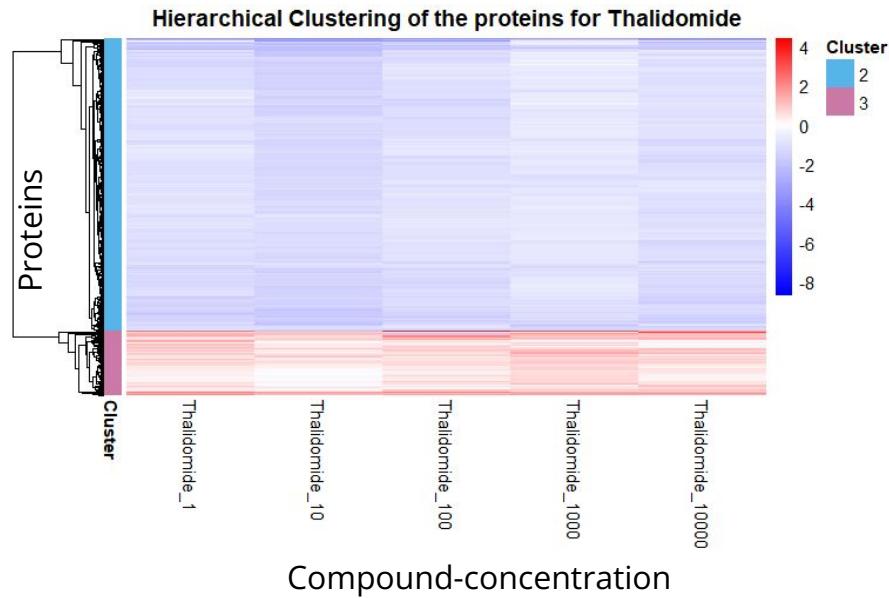


Thalidomide - Heatmap clusters 2 & 3



Cluster 2 strongly downregulated

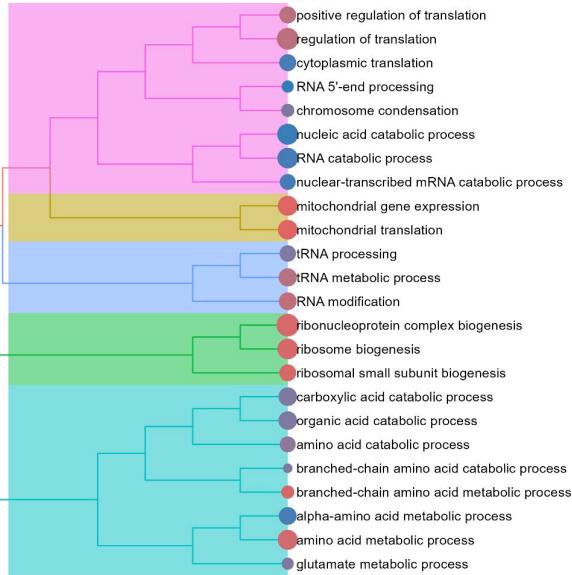
Cluster 3 strongly upregulated





Utrecht
University

GO Cluster 2 (down)



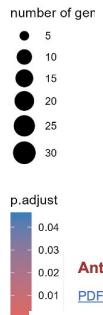
chromosome regulation 5'-end condensation

mitochondrial gene translation expression

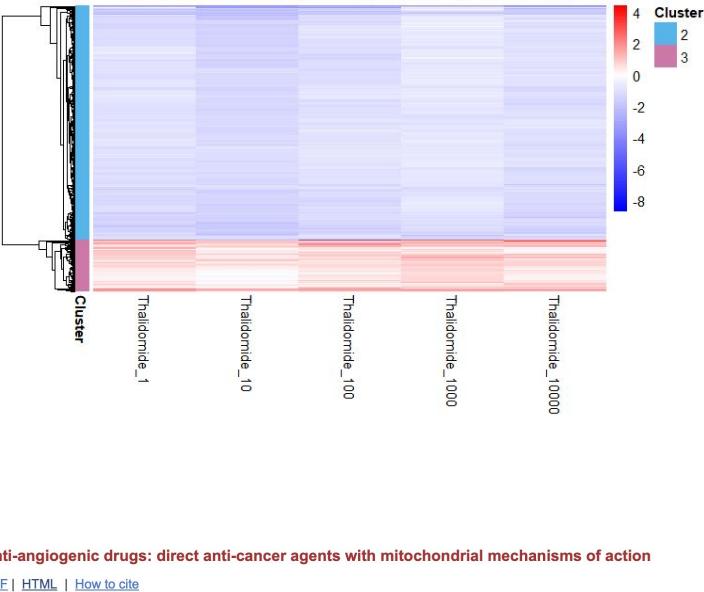
tRNA RNA modification processing

ribonucleoprotein ribosomal complex biogenesis

branched-chain alpha-amino carboxylic amino



Hierarchical Clustering of the proteins for Thalidomide



Anti-angiogenic drugs: direct anti-cancer agents with mitochondrial mechanisms of action
[PDF](#) | [HTML](#) | [How to cite](#)

Internal ribosome entry site of bFGF is the target of thalidomide for IMiDs development in multiple myeloma

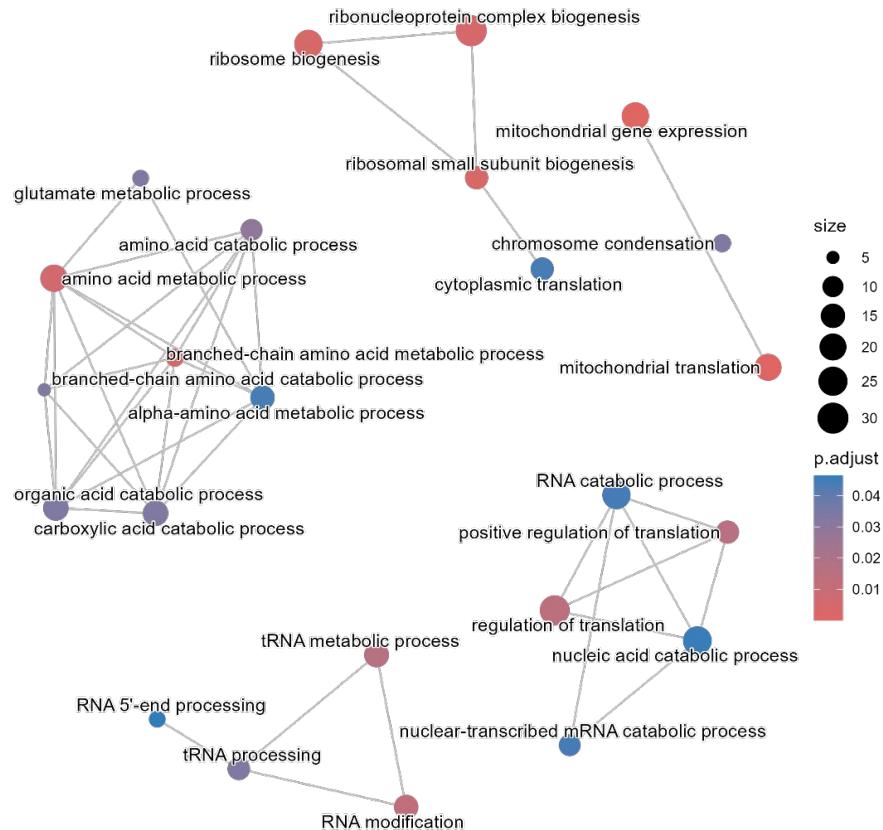
I-Chia Lien ¹, Lin-Yea Horng ², Pei-Lun Hsu ³, Chia-Ling Wu ³, Hui-Ching Sung ³, Rong-Tsun Wu ²

Affiliations + expand

PMID: 25053990 PMCID: PMC4091528 DOI: [10.18632/genesandcancer.11](https://doi.org/10.18632/genesandcancer.11)



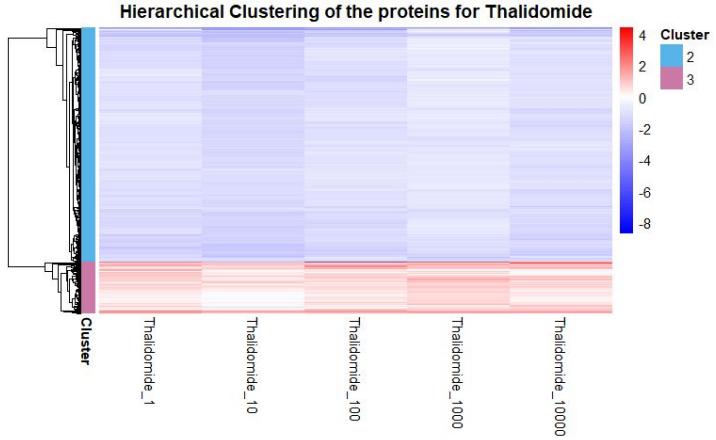
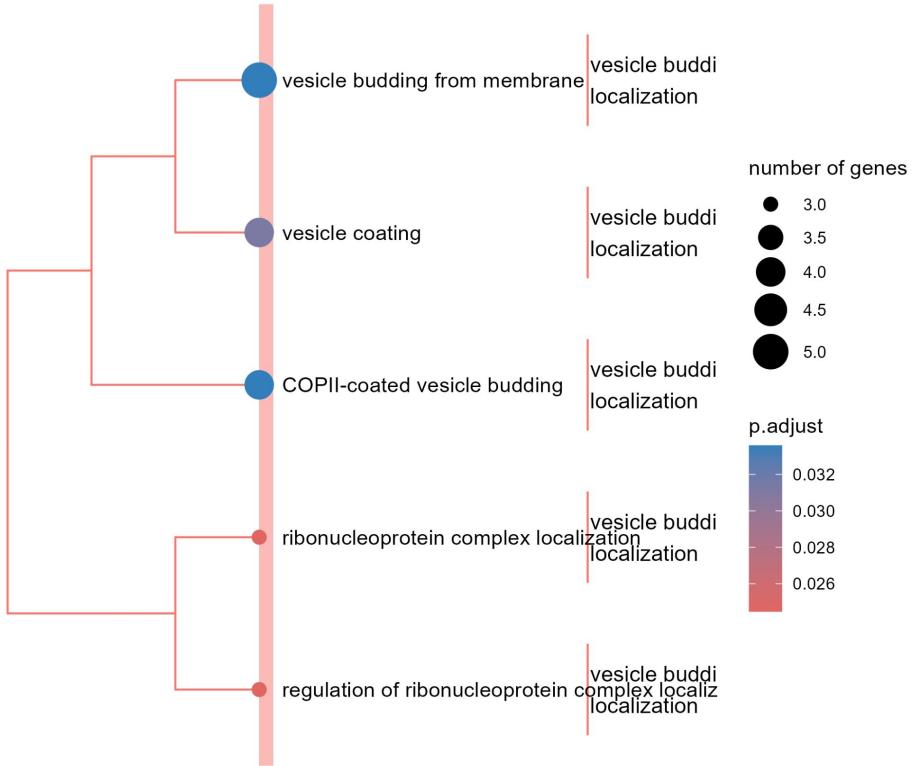
GO Cluster 2 (down)





Utrecht
University

GO Cluster 3 (up)



CUL5 is required for thalidomide-dependent inhibition of cellular proliferation

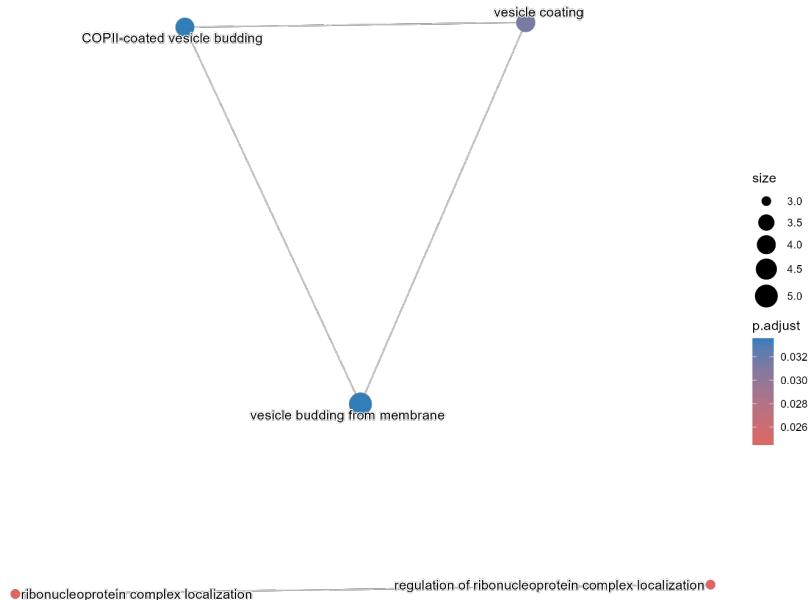
[Bryan Kunkler](#) ¹, [Daniel Salamango](#) ¹, [Zachary J DeBruine](#) ¹, [Caitlin Ploch](#) ¹, [Shirley Dean](#) ¹, [David Grossens](#) ¹,
[Michael P Hledin](#) ¹, [Gabriel A Marquez](#) ¹, [Julie Madden](#) ¹, [Abigayle Schnell](#) ¹, [Michael Short](#) ¹, [Maria A Burnatowska-Hledin](#) ^{1,2,*}

“vesicular trafficking of CUL5 towards the cell membrane was observed at 20 µg/mL of thalidomide”



Utrecht
University

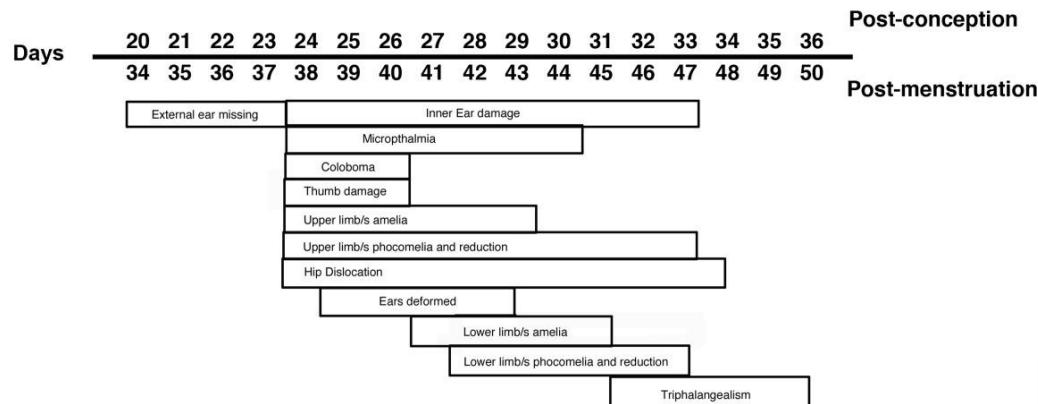
GO Cluster 3 (up)





Consequences of many off-target regulations

Appearance of outward damage caused by thalidomide



From: Lenz and Knapp, 1962, Nowack, 1965, Ruffing, 1973, Smithells and Newman, 1992, Miller and Stromland, 1999





Obstacles in the analysis

- Working with Big Data
 - Processing
- Lack of workflow to identify up- and down-regulated proteins *a priori*
 - Missing classifier
- Many missing proteins for many compounds
 - Missing intended targets of compounds

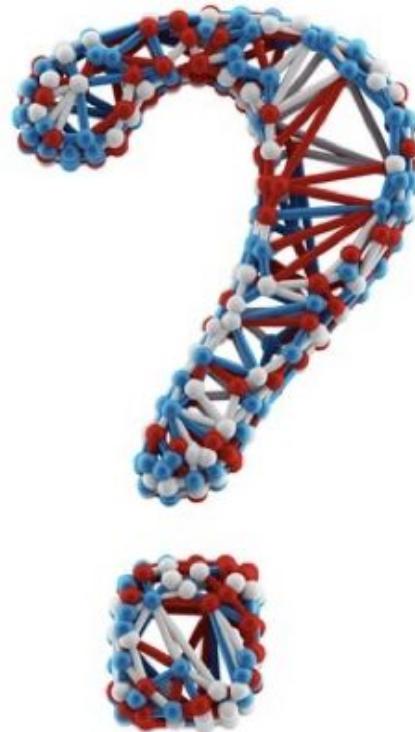


Conclusions

- Drugs can have wide ranging effects on the proteome
- Even drugs with known MoA affect many **off-target** pathways
 - Most off-target effects seem to be down-regulating proteins



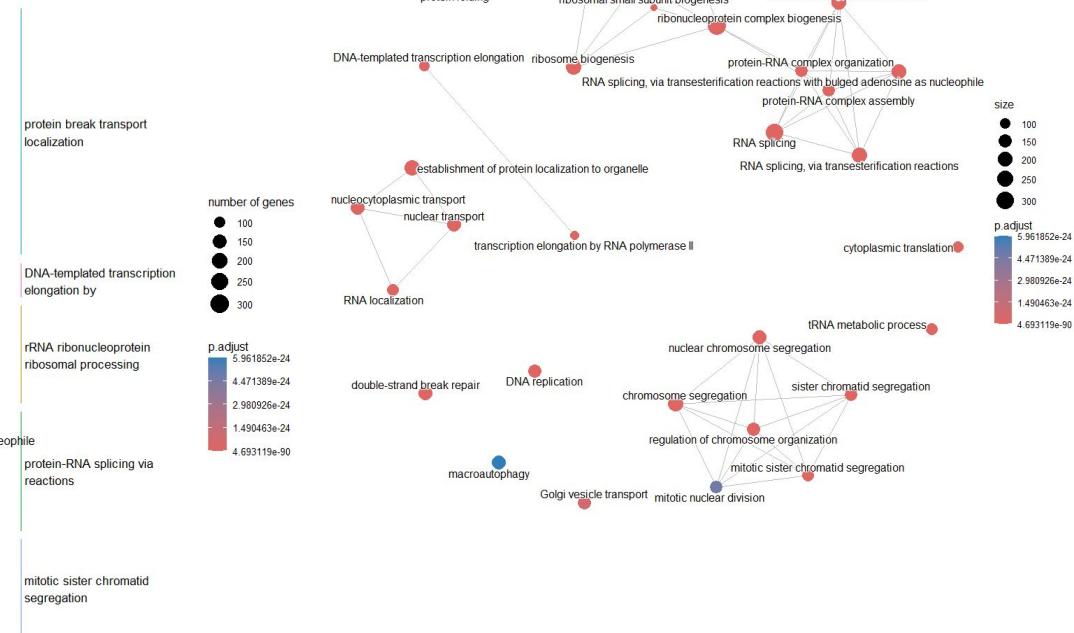
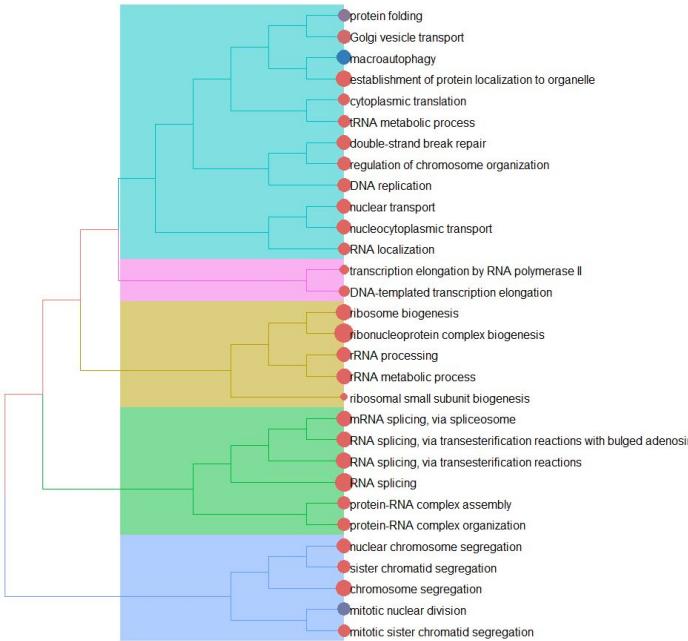
Utrecht
University



<https://github.com/BaronWolfgang/ProjectAOLSProteomics>



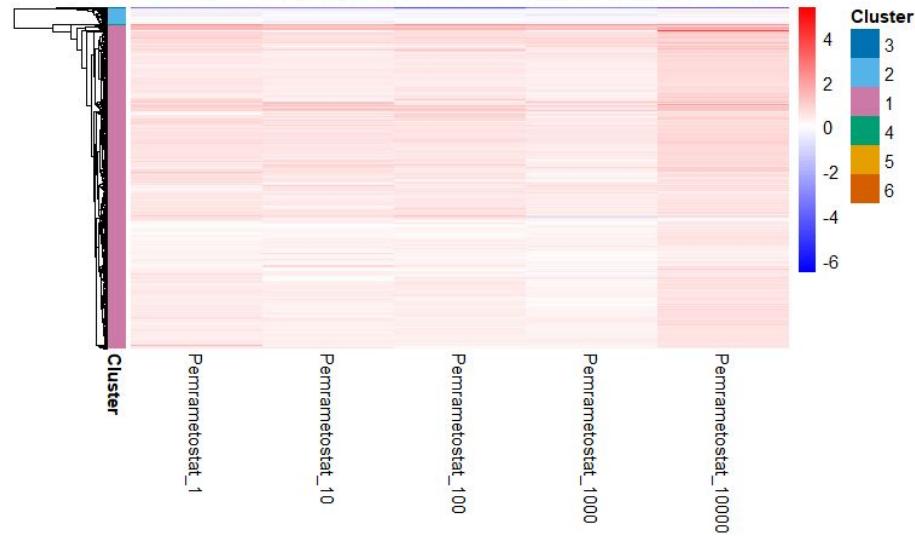
Thalidomide - Cluster 1 (down)





Pemrametostat - Heatmap

Hierarchical Clustering of the proteins for Pemrametostat

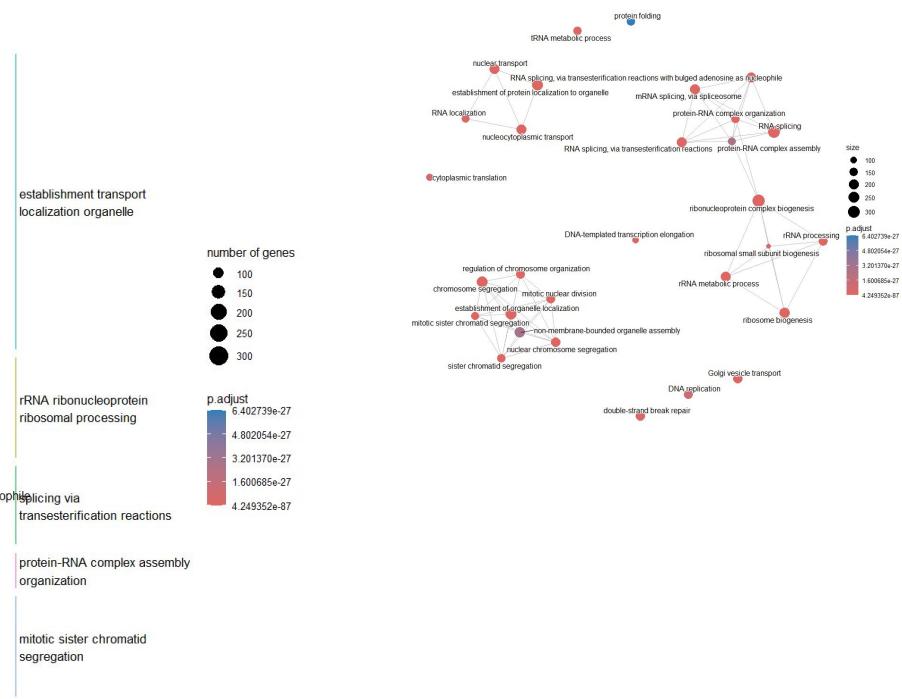
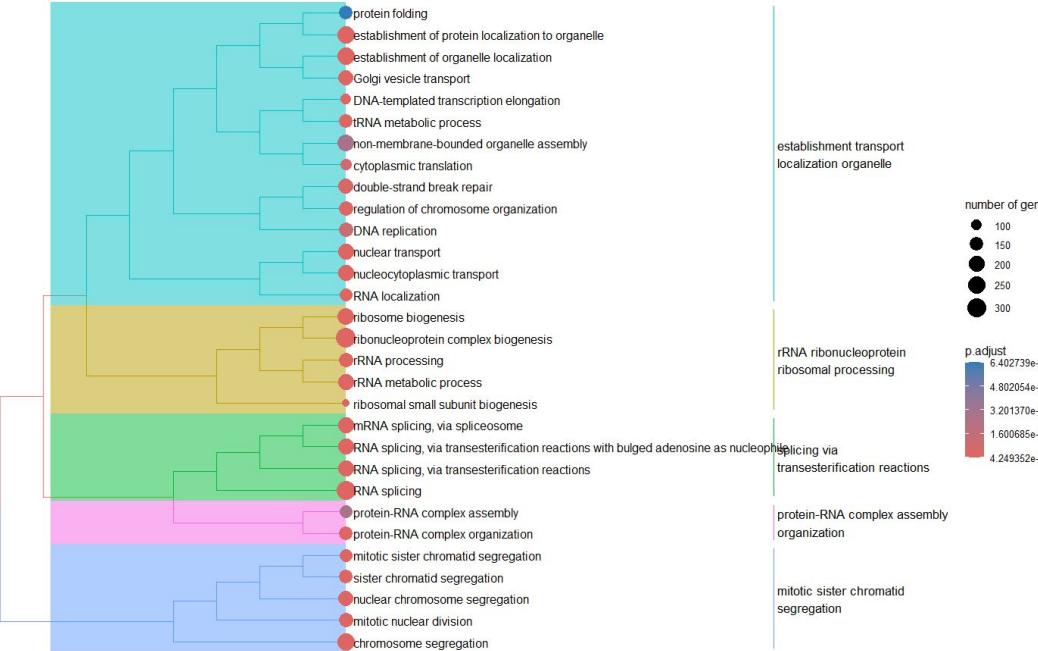


Hierarchical Clustering of the proteins for Pemrametostat





Pemrametostat - Cluster 1





Pemrametostat - Cluster 2

