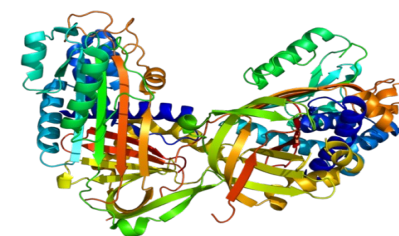




Chemical proteomics in drug design



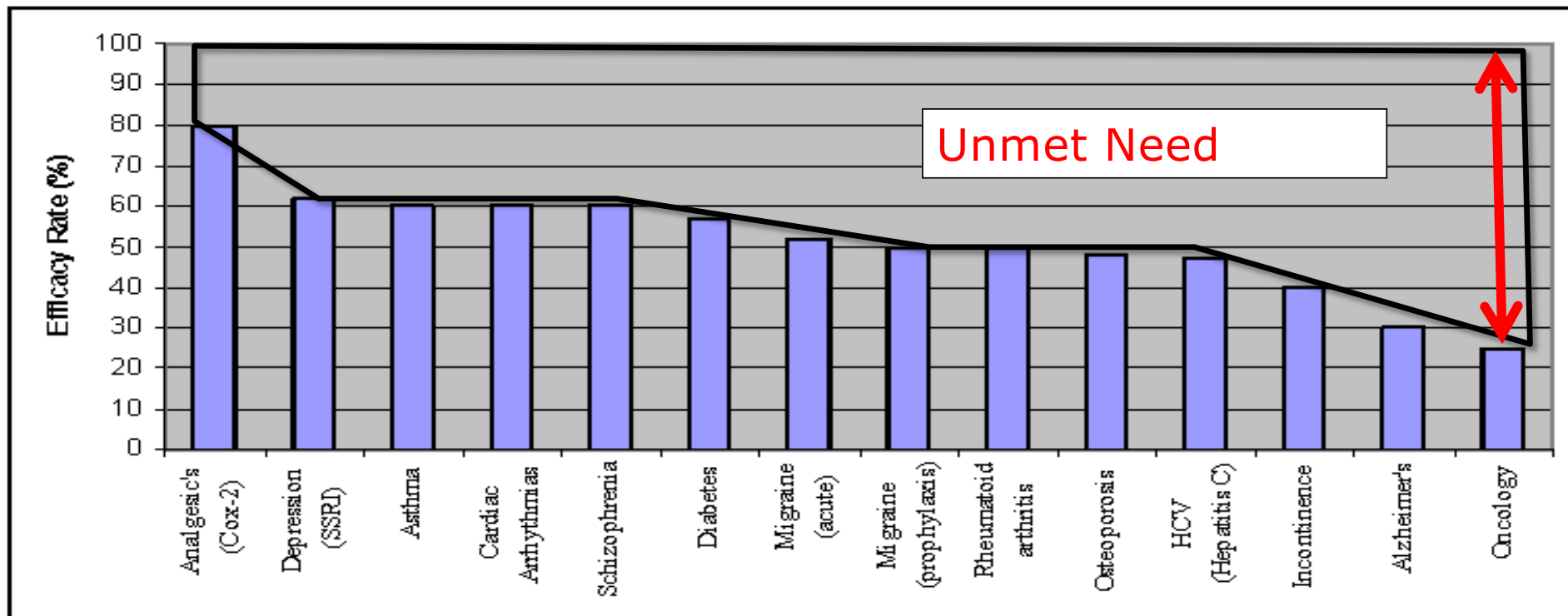
Roman Zubarev

Chemistry I, MBB, Biomedicum, Karolinska Institutet, Stockholm, Sweden

Roman.Zubarev@ki.se

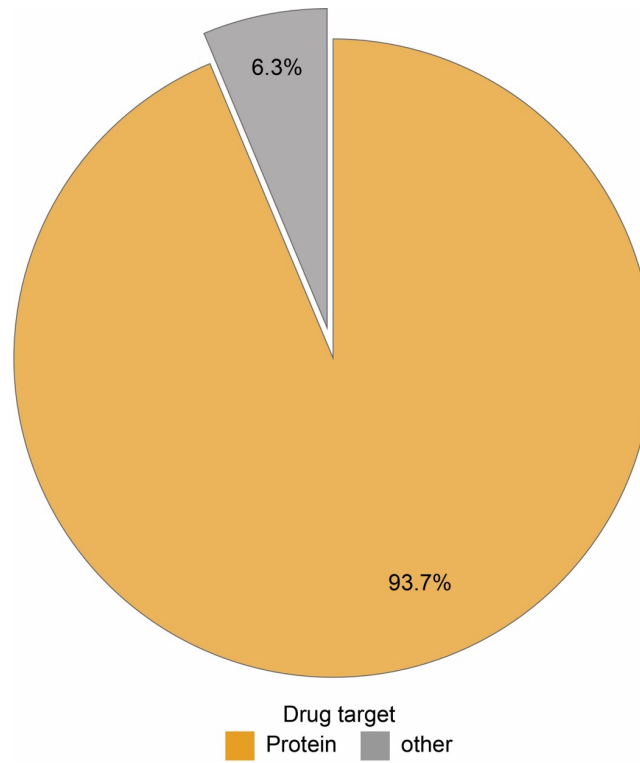


New Medicines Needed for Those Who Do Not Respond to Current Therapy



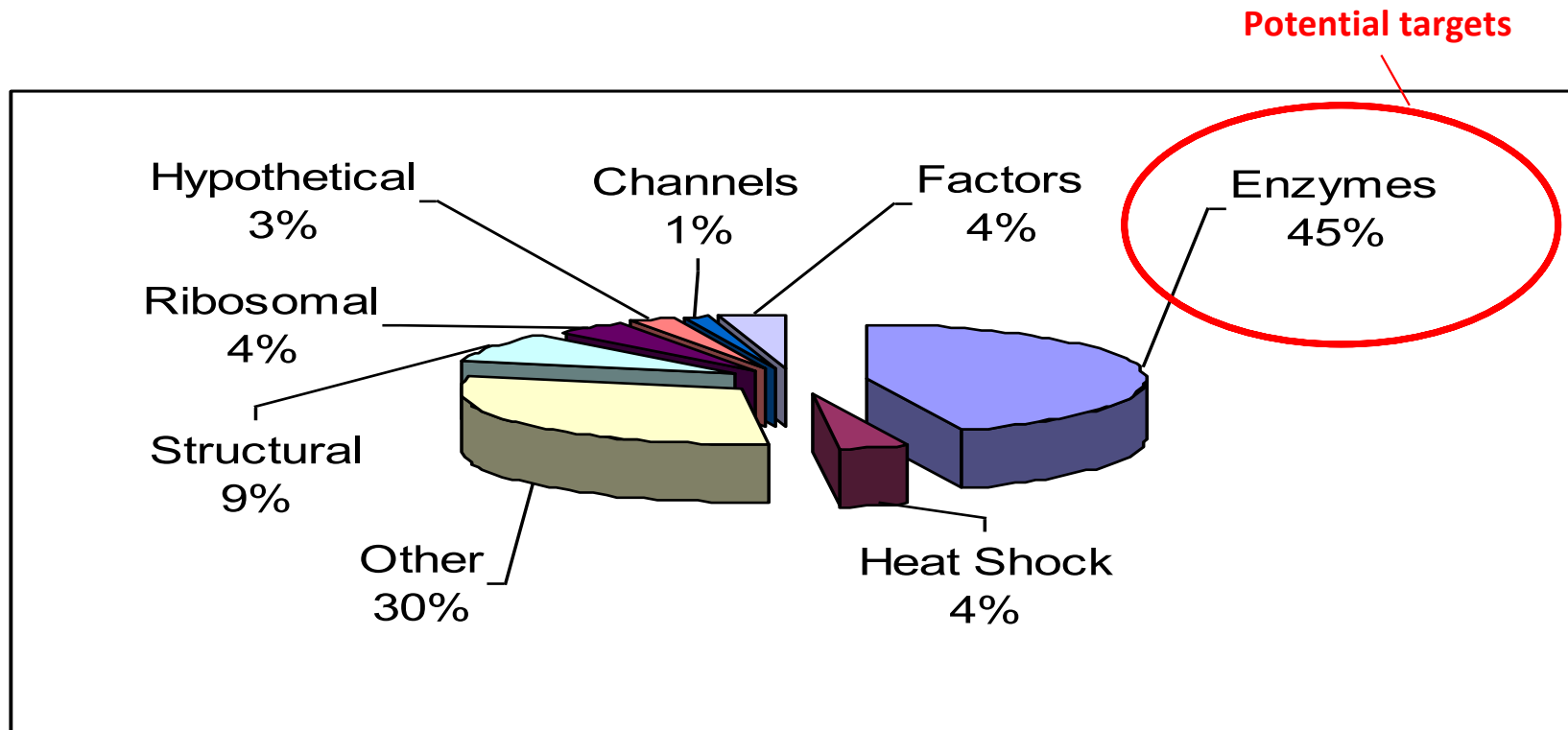
Source: Spear B, et. al. *Trends in Molecular Medicine*, 7(5):201-204, 2001; Eli Lilly internal documents.

Proteins as drug targets



Santos *et al.* *Nature Reviews Drug Discovery* (2016)

Proteins by function



Chemical space is vast – millions of drugs are yet to be discovered



10^{63}

Drug-like small molecules in chemical space

10^{24}

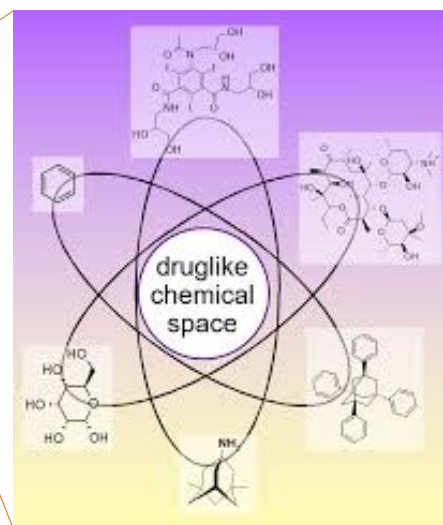
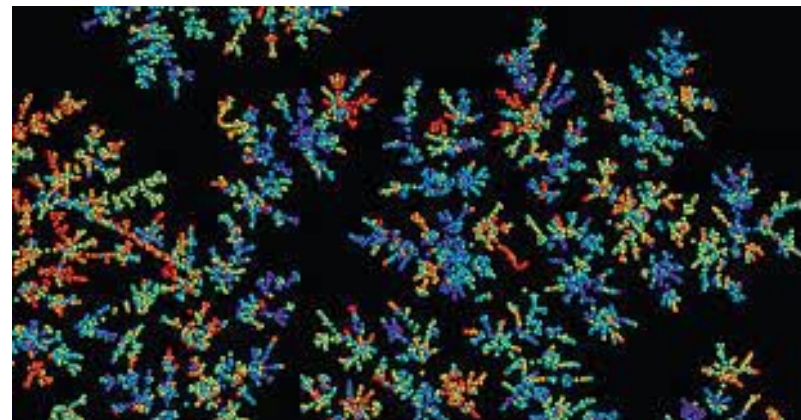
Stars in the universe

10^{20}

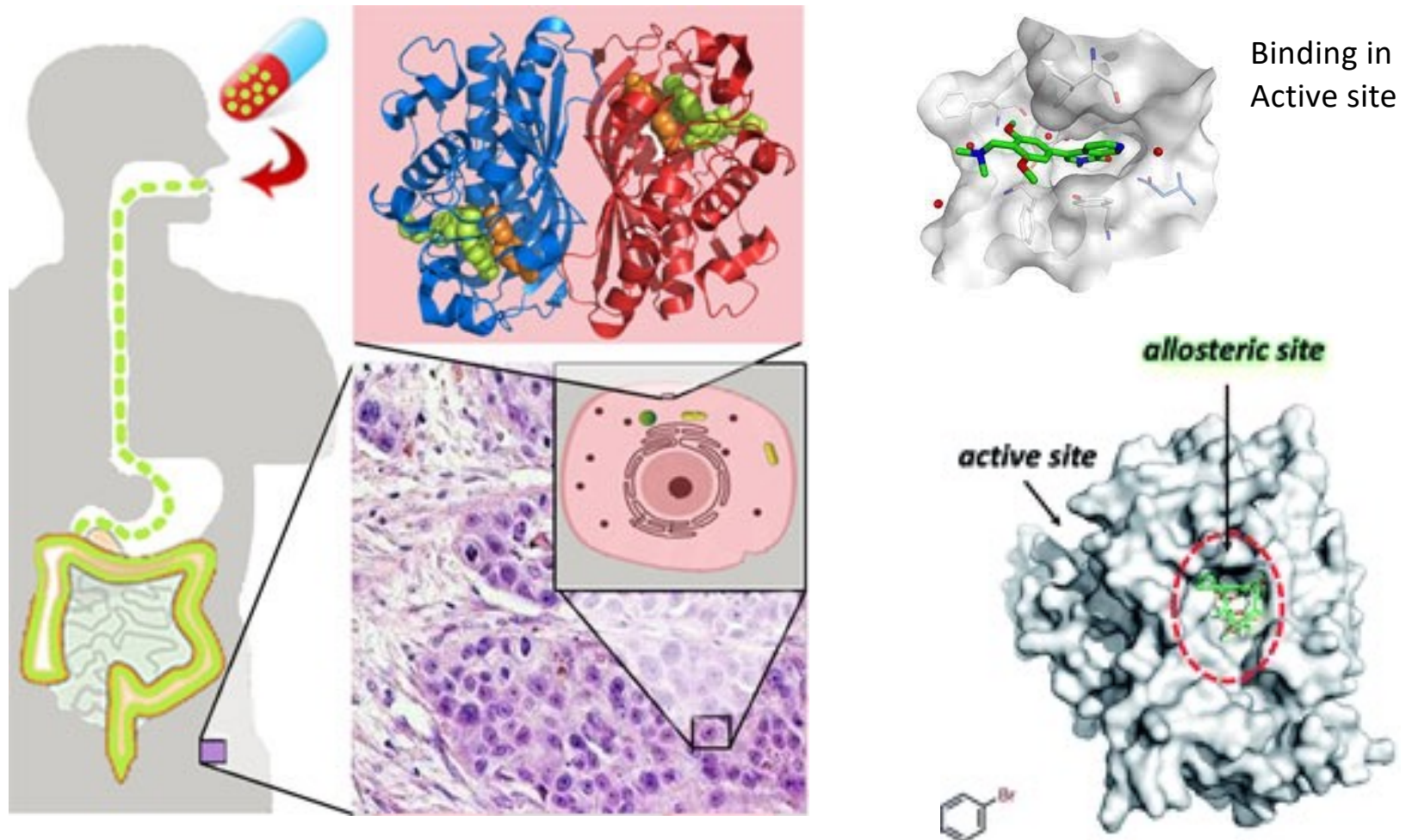
Potential compounds in Merck KGaA's Merck Accessible Inventory (MASSIV)

10^4

Small-molecule drugs



Most small-molecule drugs bind to protein target

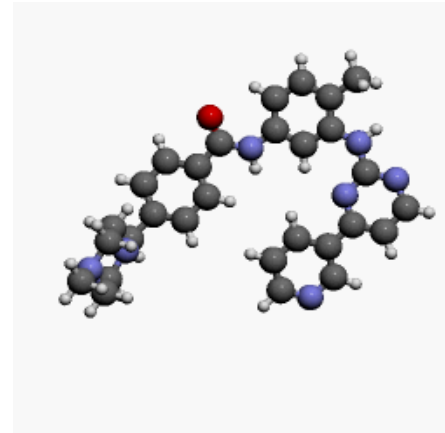


<https://www.medgadget.com/2013/07/new-technology-allows-for-monitoring-of-drug-target-binding-in-cells-and-tissues.html>

Are drugs 'silver bullets' ?...



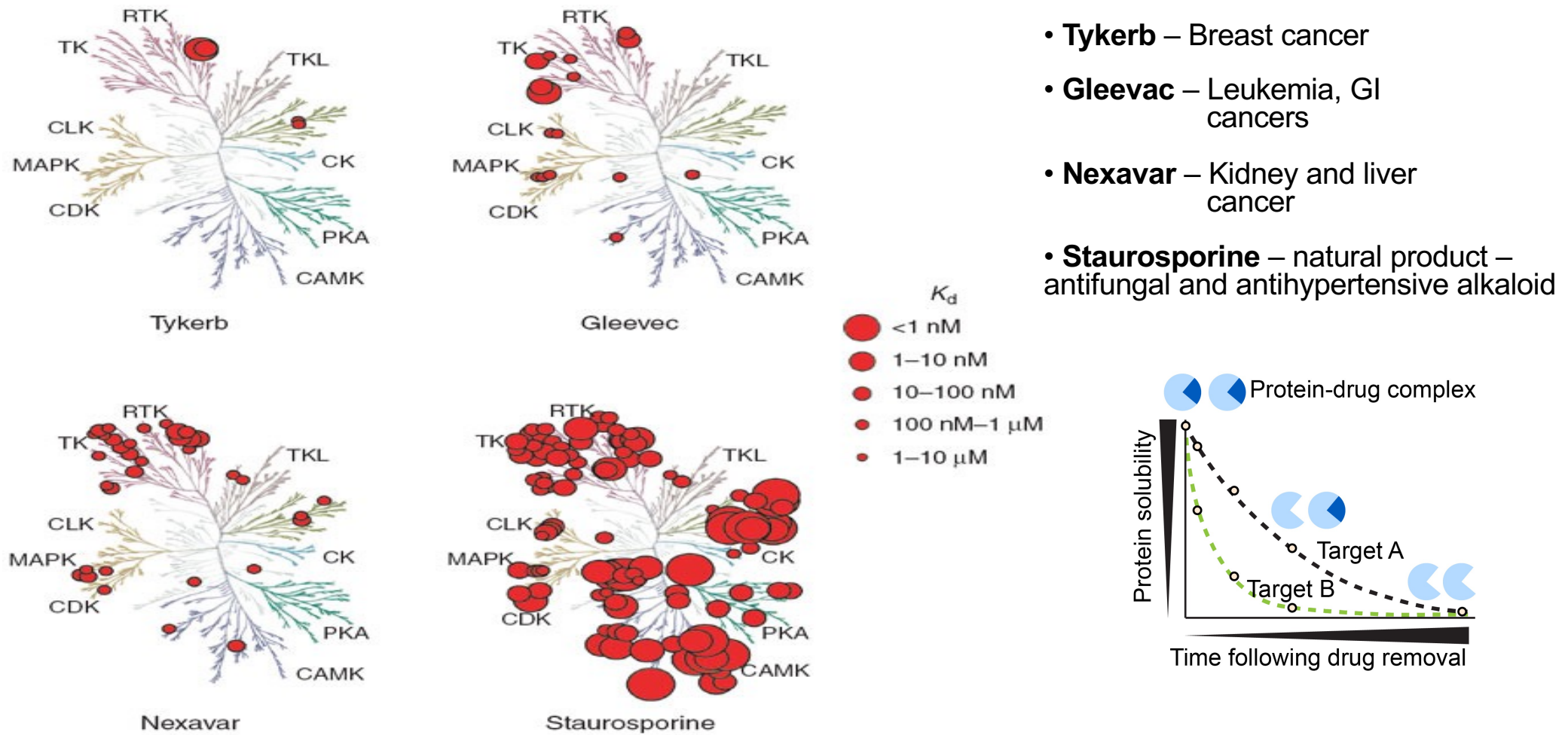
Imatinib (Gleevec)



Inhibits Tyr kinase BCR-Abl

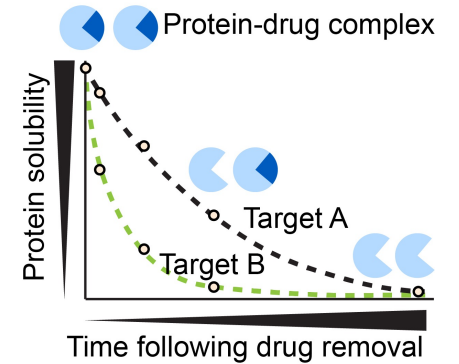
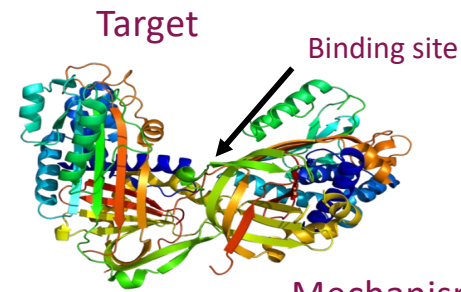


Drugs are usually not very specific



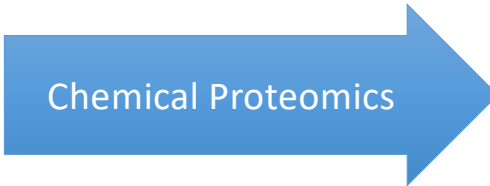
Chemical Proteomics Aims

- Target discovery
- Target engagement
- Thermal profiling
- Redox analysis
- Deuterium exchange analysis

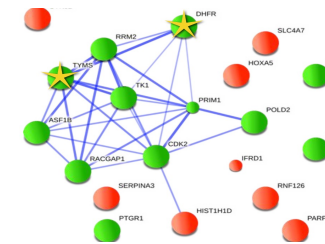


New drug

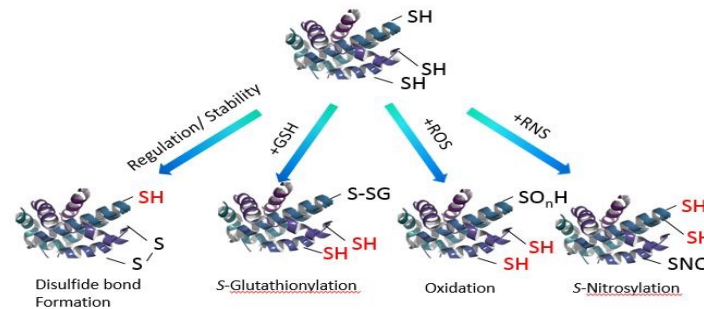
No Chemistry!



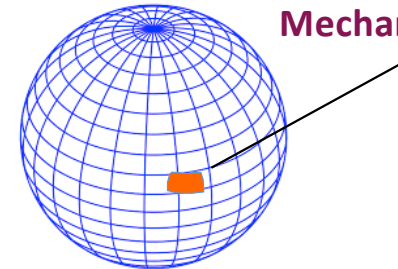
Mechanism of action



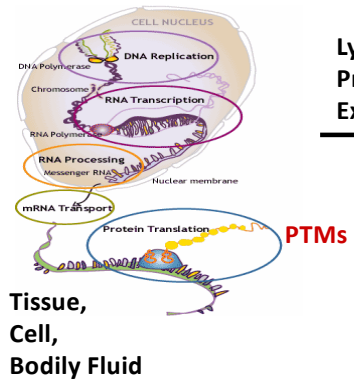
Changes in redox state



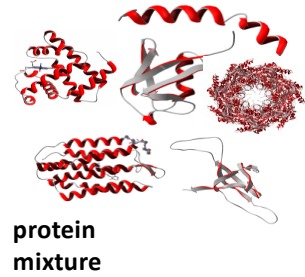
Mechanism of death



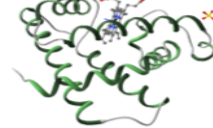
Bottom-Up (Shotgun) Proteomics



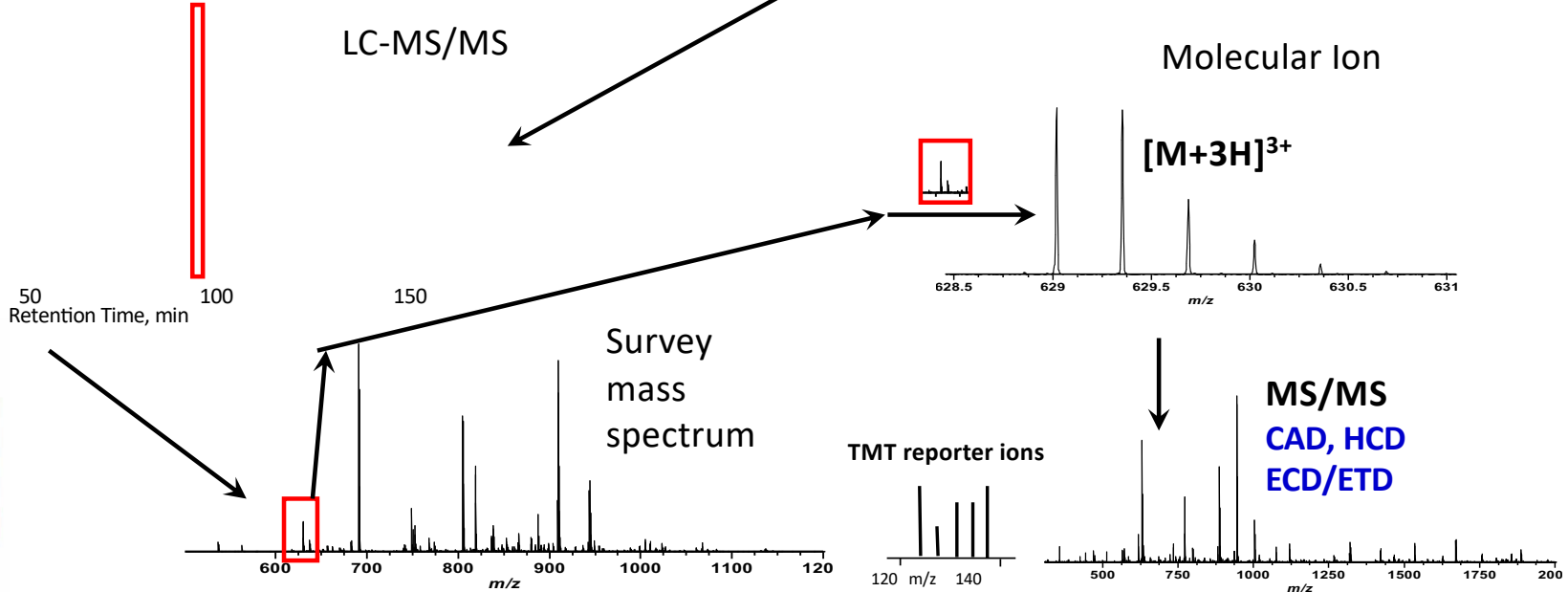
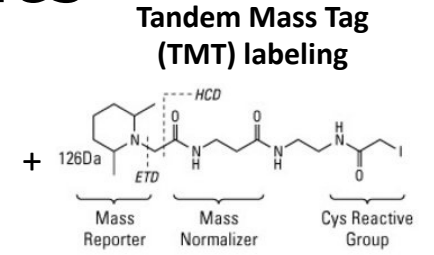
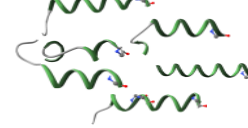
Lysis, Protein Extraction



Protein S-S bond reduction & alkylation



enzymatic cleavage

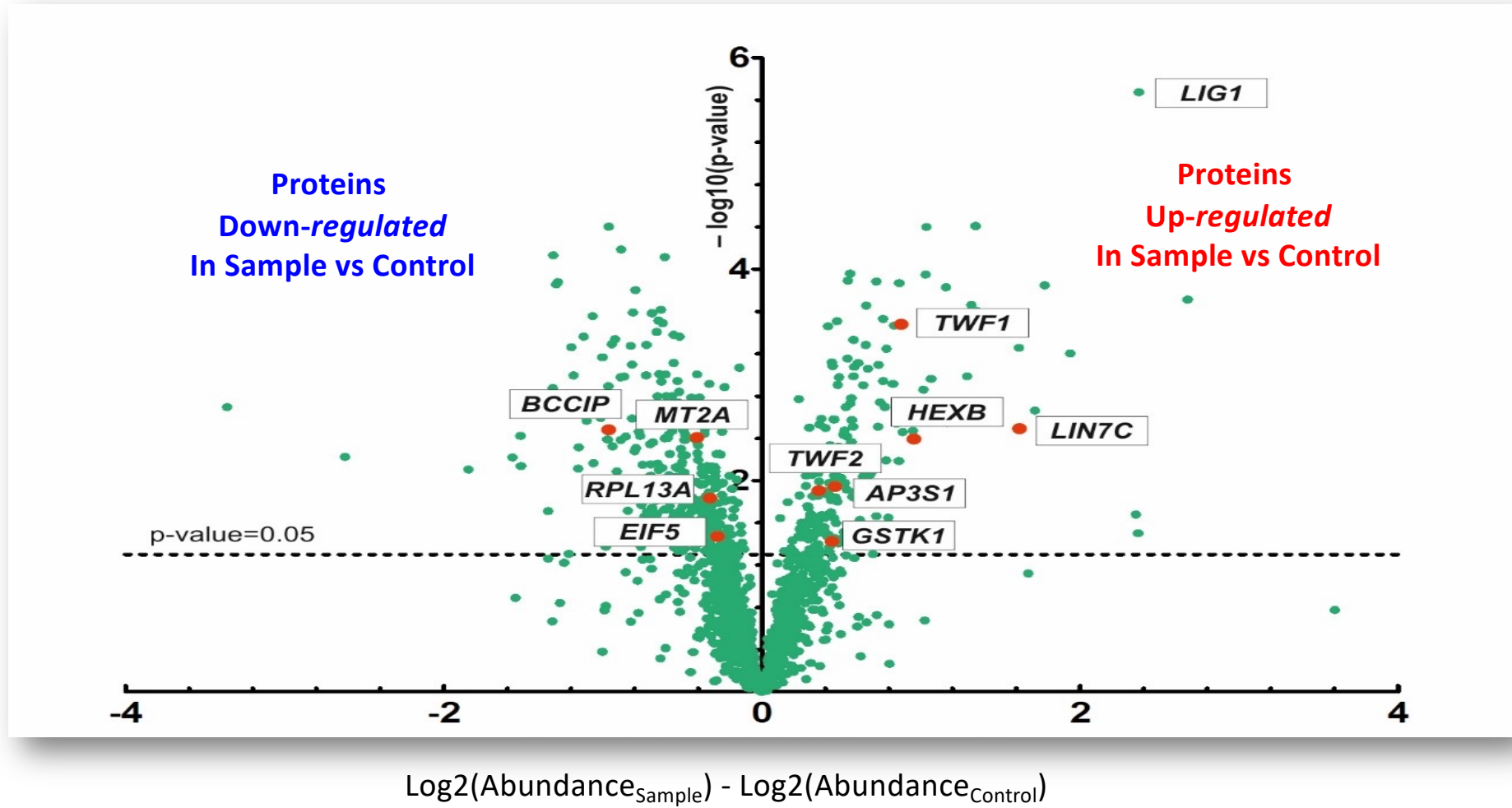


M N I F E M L R I D E G L R L K I Y K D T E G Y Y T I G I G
 H L L T K S P S L N A A K S E L D K A I G R N N G V I T K
 D E A E K L F N Q D V D A A V R G I L R N A K L K P V Y D S
 L D A V R R A L I N M V F Q M G E T G V A G F T N S L R M
 L Q Q K R W D E A A N L A K S R W Y N Q T P N R A K R V I
 T T F R T G T W D A Y K N L

<https://prosightptm.scs.uiuc.edu/>

Peptide ID; Protein ID
 Some modifications

Quantitative Proteomics



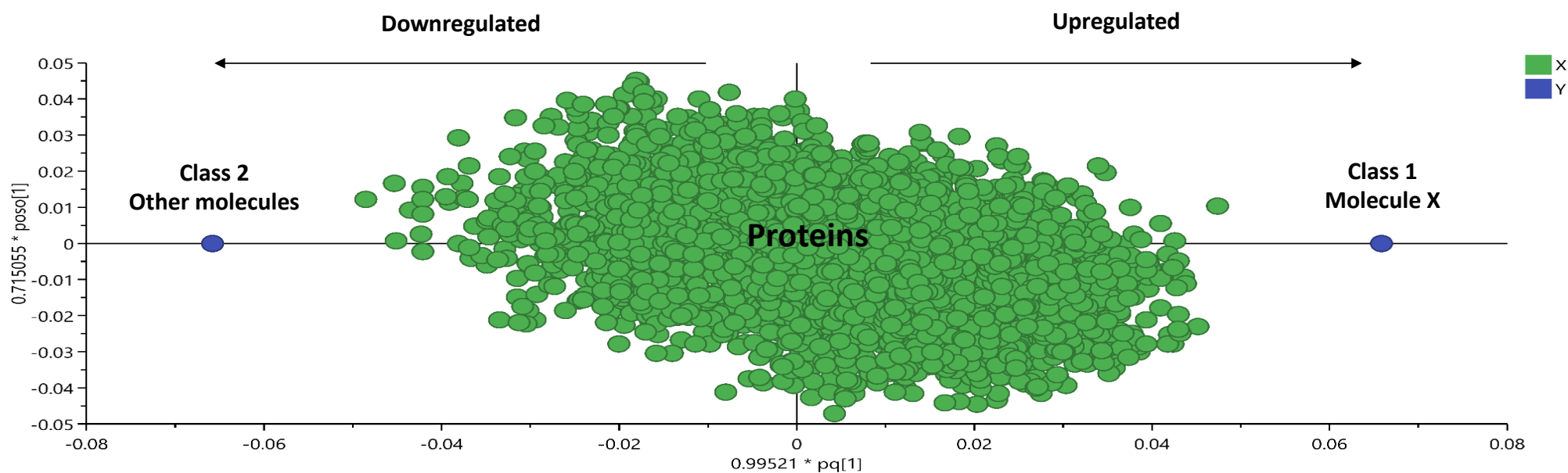


Research Article

OPLS discriminant analysis: combining the strengths of PLS-DA and SIMCA classification[†]

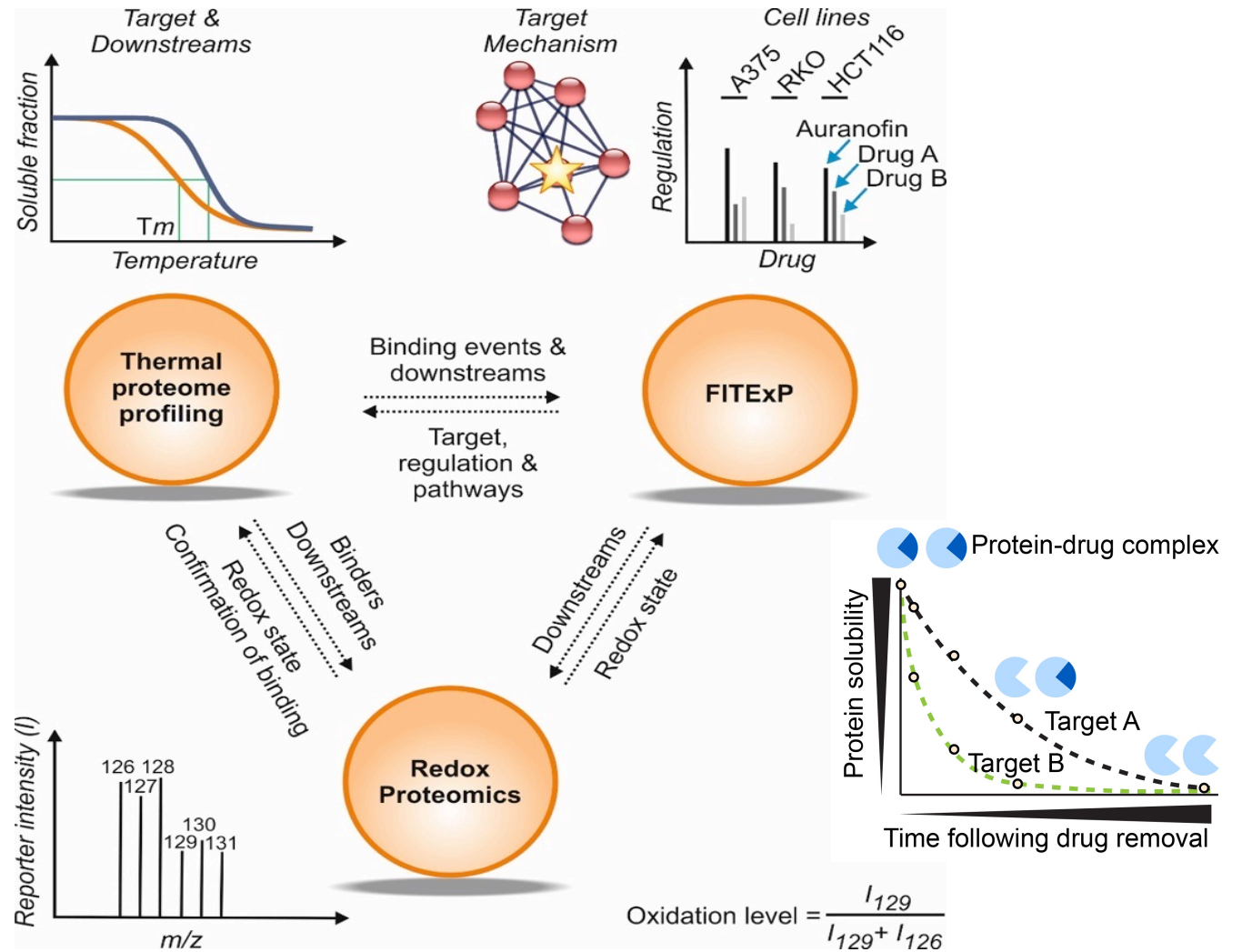
Max Bylesjö, Mattias Rantalainen, Olivier Cloarec, Jeremy K. Nicholson, Elaine Holmes, Johan Trygg

OPLS-DA - Orthogonal [Projection to Latent Structures / Partial Least Square] – Discriminant Analysis

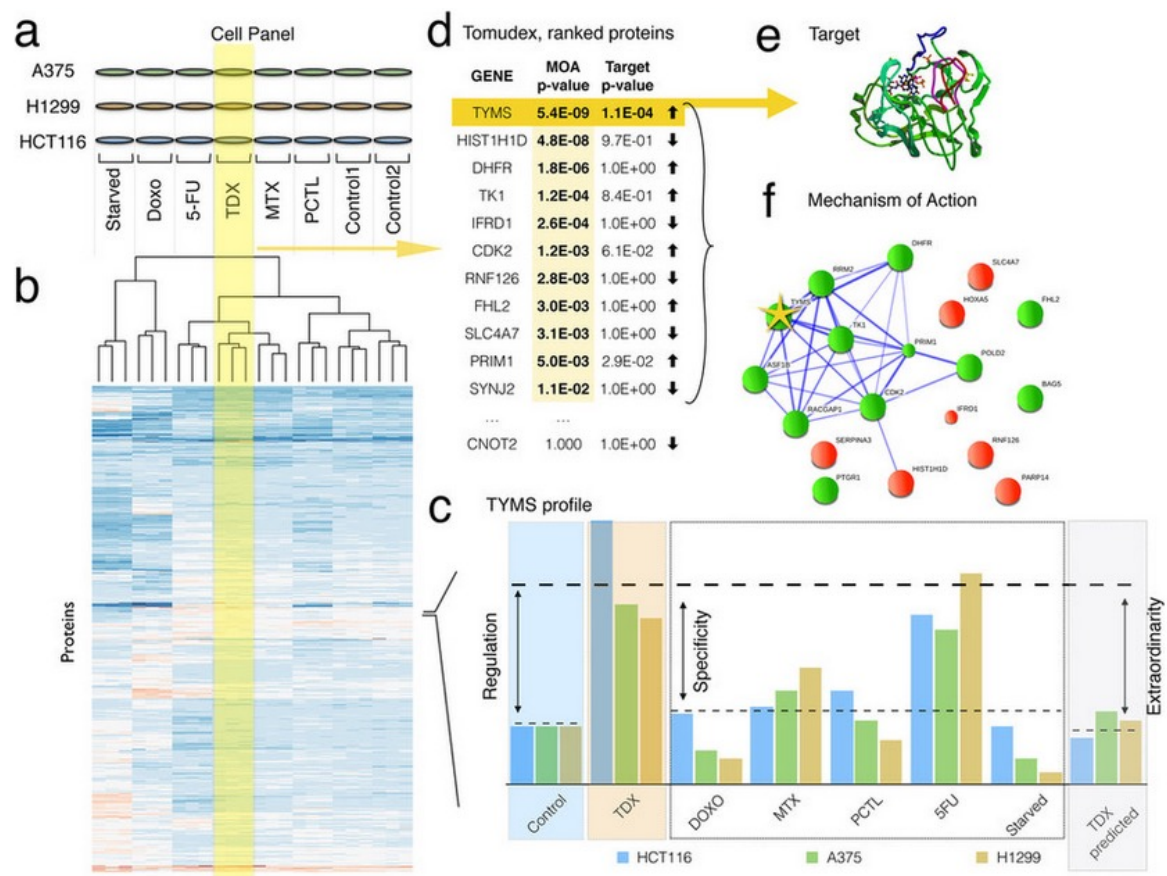


Chemical Proteomics workflow

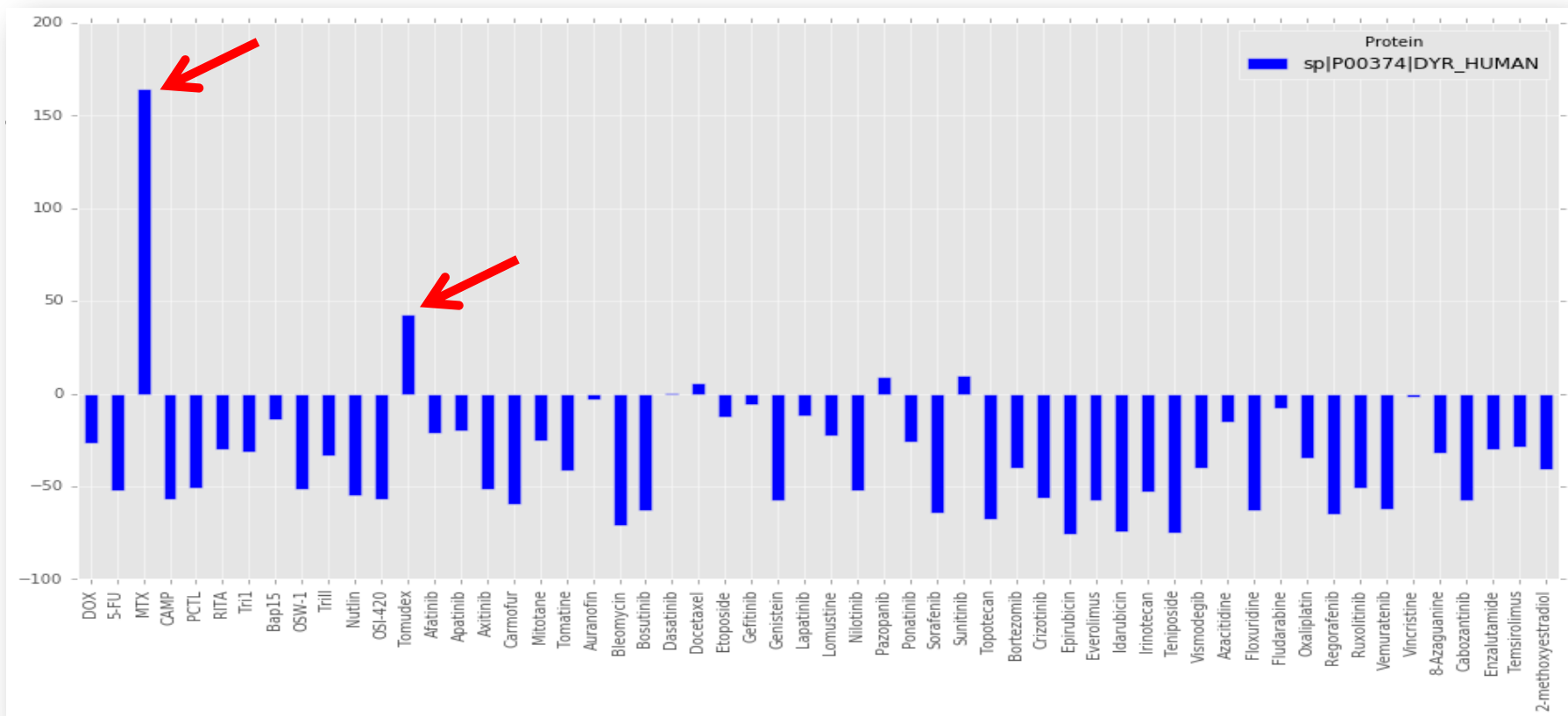
Saei, A. et al., *Redox Biology*, 2020, doi.org/10.1016/j.redox.2020.101491



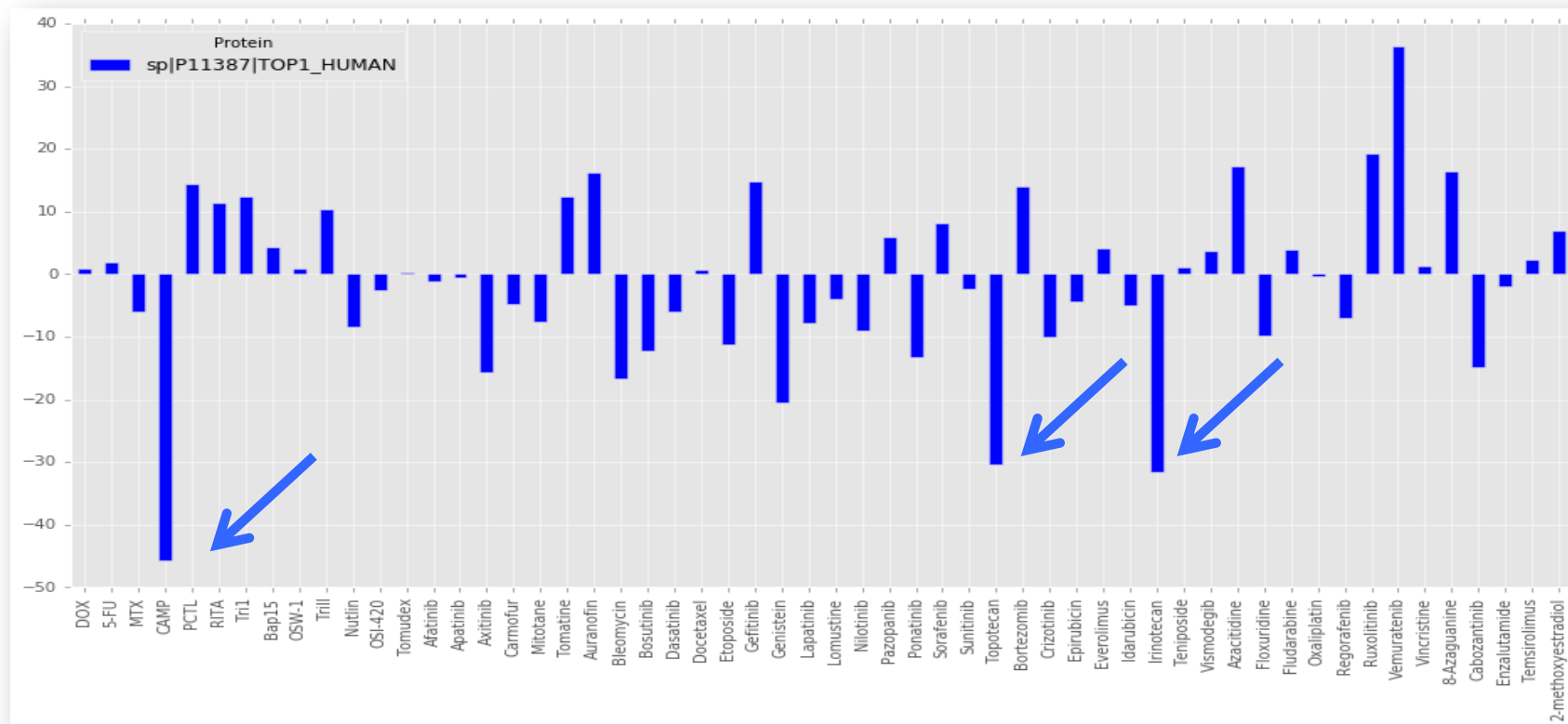
Functional Identification of Targets by Expression Proteomics, FITExP



Protein abundance change: DHFR

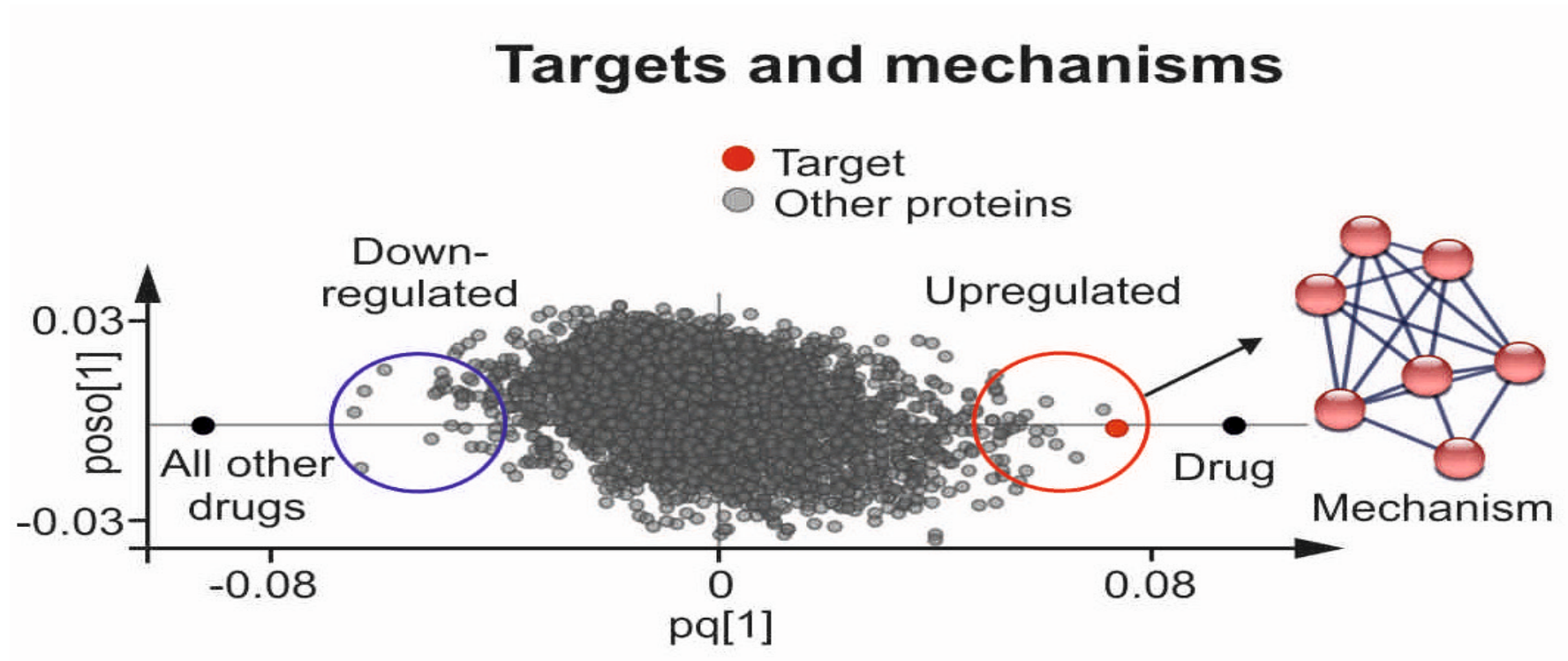


Protein abundance change: TOPI

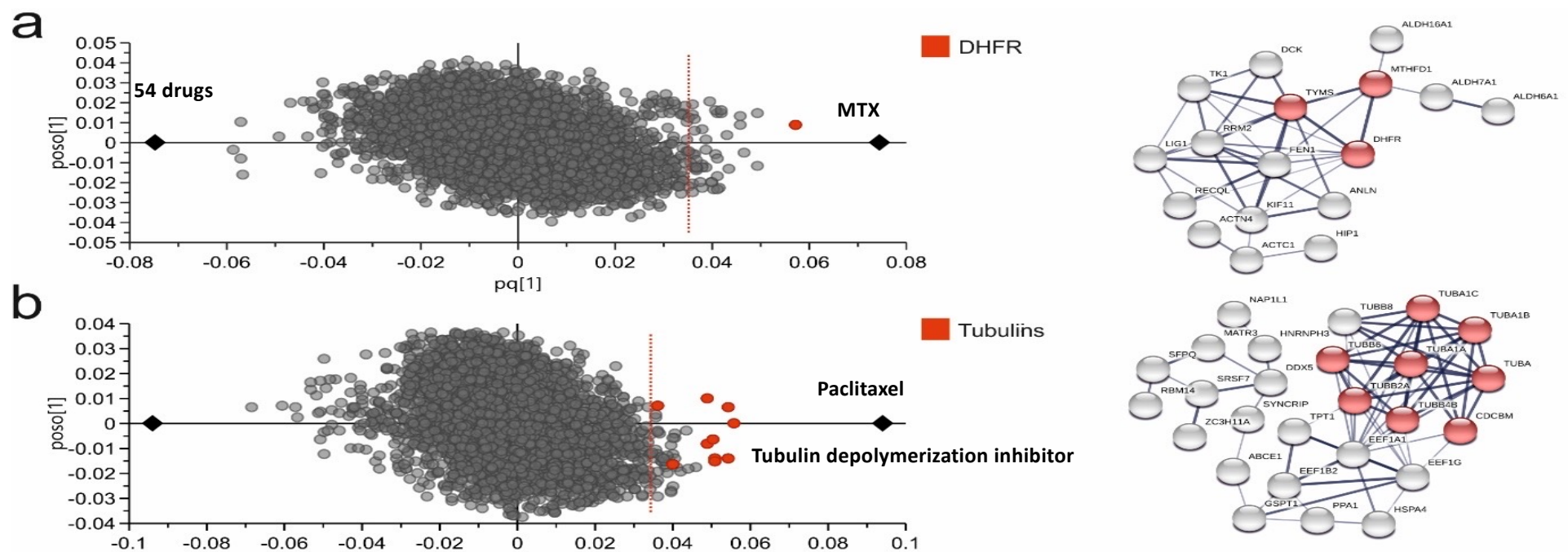


Functional Identification of Targets by Expression Proteomics, FITExP

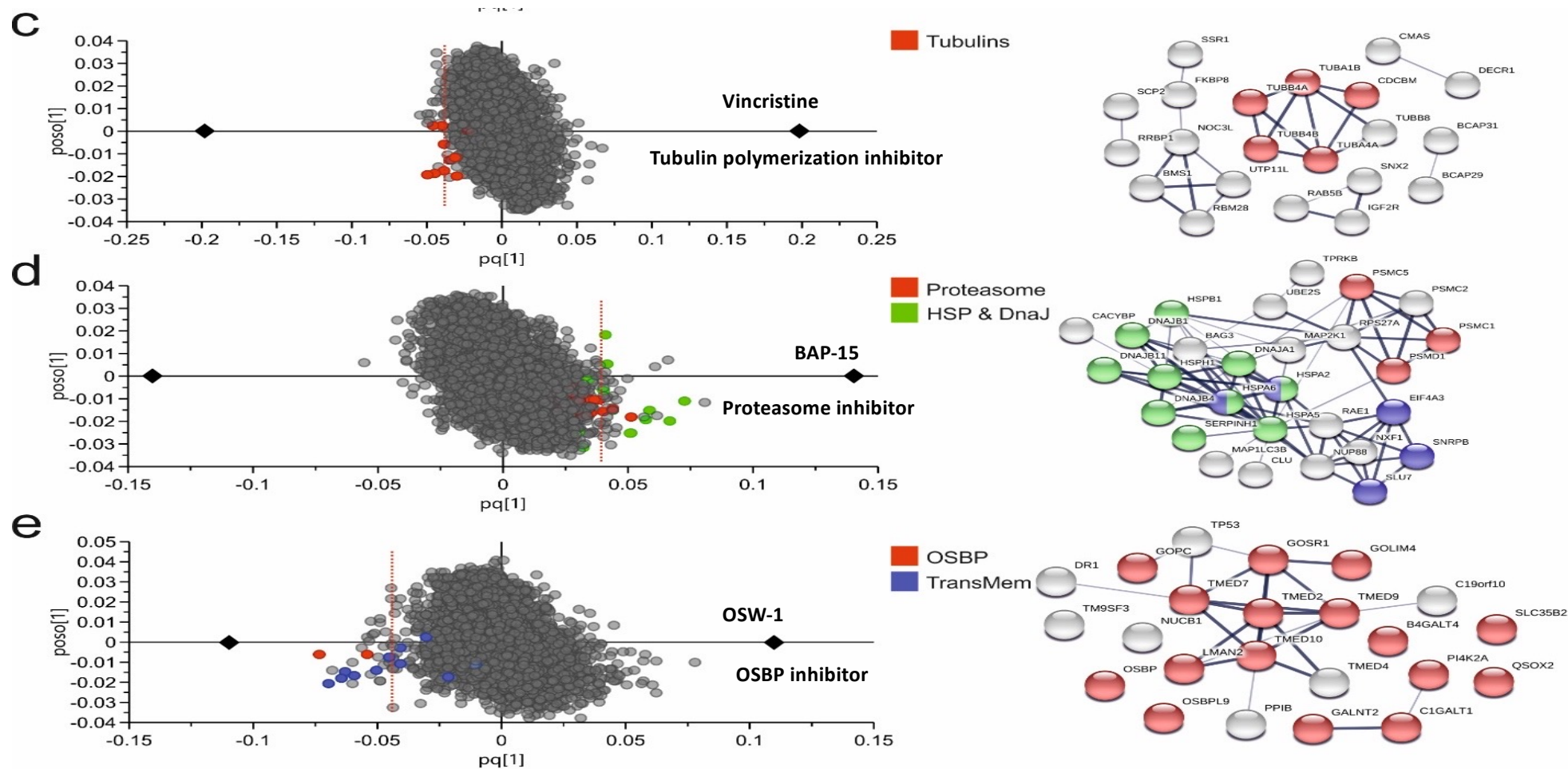
OPLS-DA - Orthogonal [Projection to Latent Structures / Partial Least Square] – Discriminant Analysis



ProTargetMiner: Target and mechanism deconvolution



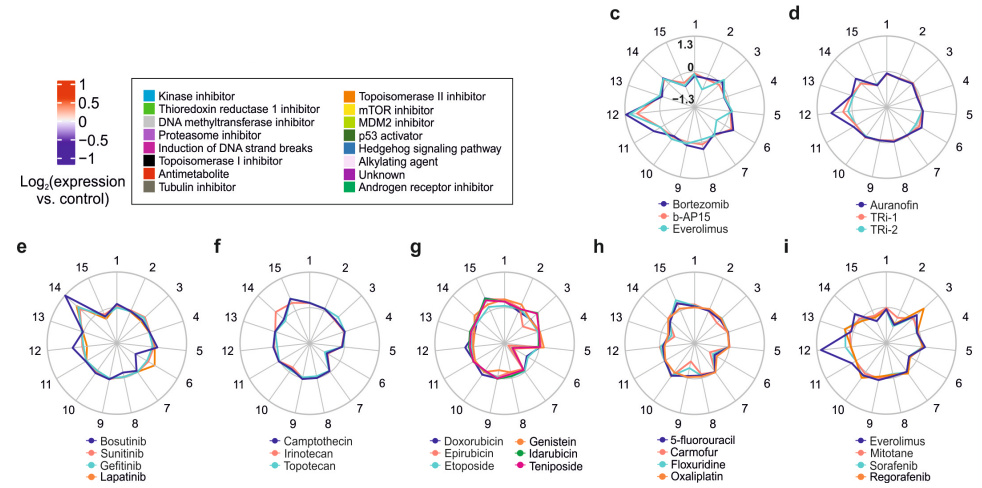
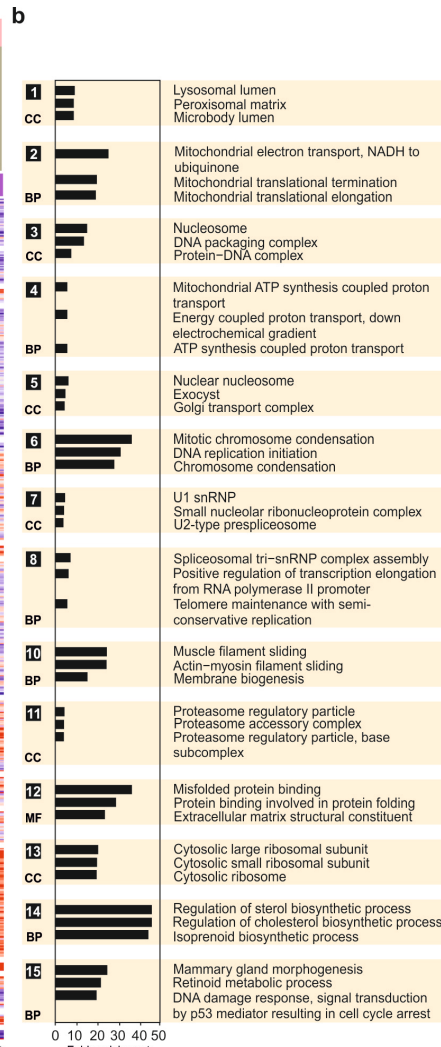
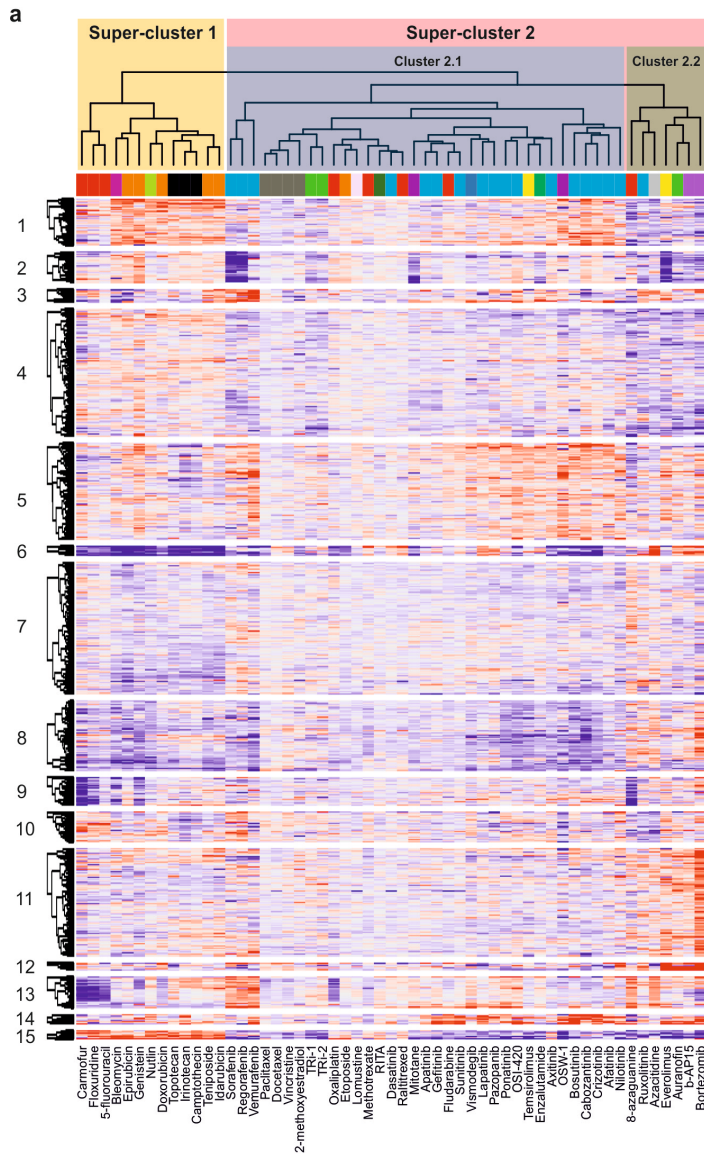
ProTargetMiner: Target and mechanism deconvolution



Saei, A. et al., *Nat. Commun.* 2019, 10, Article number: 5715.

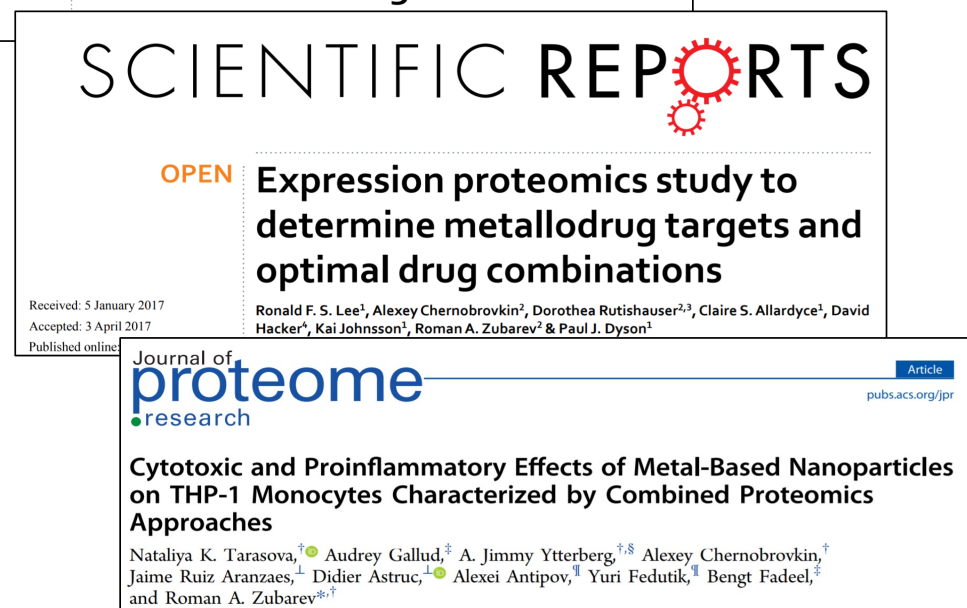
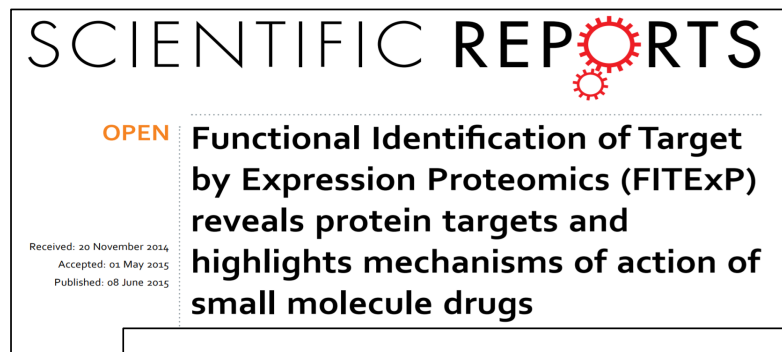
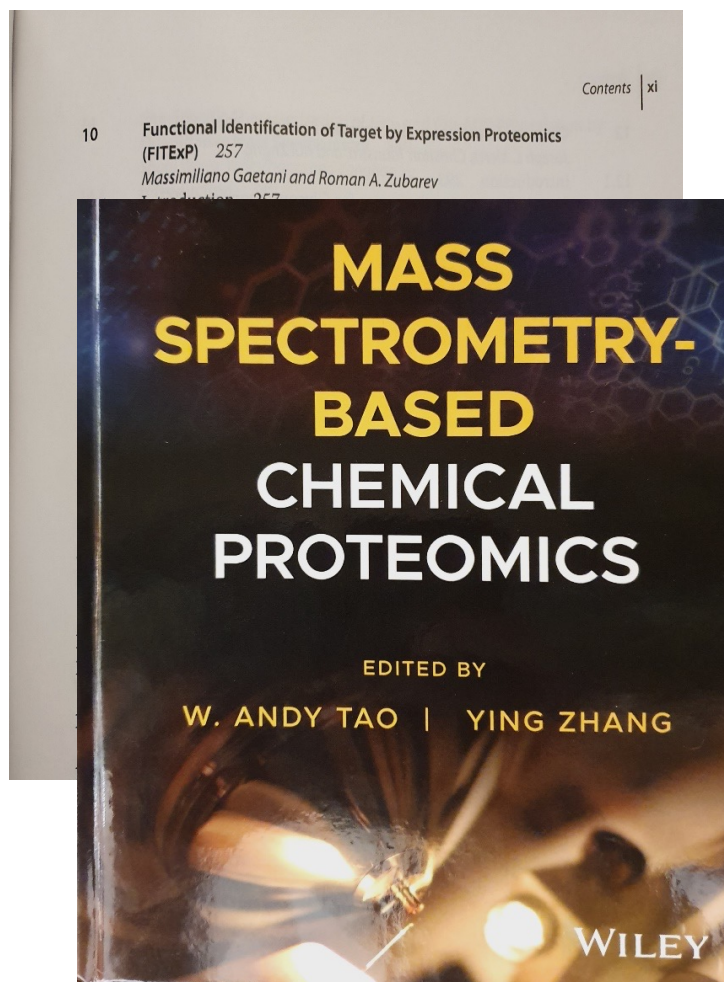
Hierarchical clustering

≈300 LC-MS/MS runs
 7572 proteins quantified
 4557 best-quantified proteins used for mapping



PCA: 13 dimensions (1% cut-off)

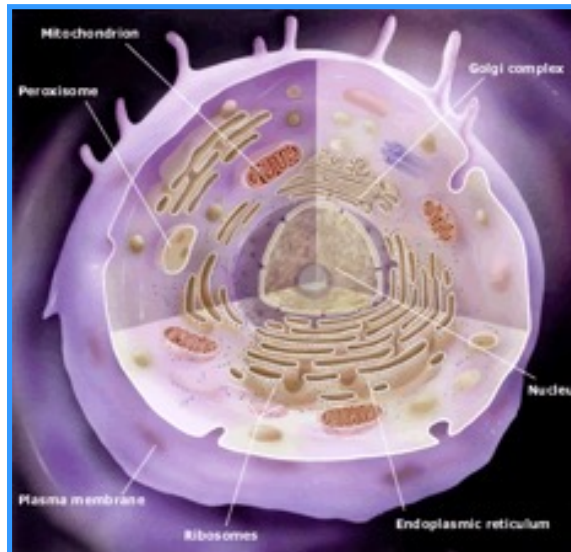
FITEXP: Functional Identification of Target by Expression Proteomics



Challenges of Single Cell Proteomics

- Size = 5-10 μm
- Protein amount = 100-250 pg

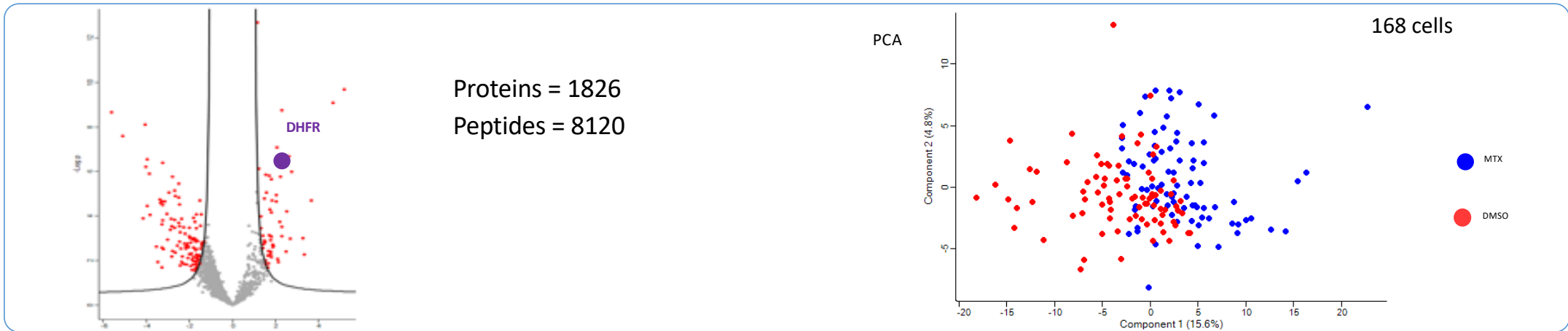
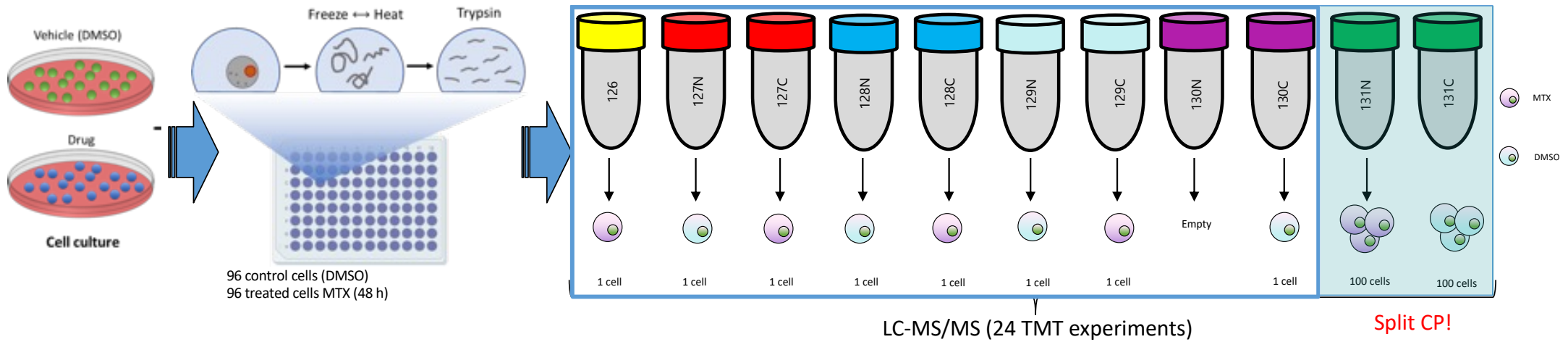
Can we take
Chemical Proteomics
to Single Cell level?...



Dynamic range of proteins in the
cell:
 ≥ 7 orders of magnitude

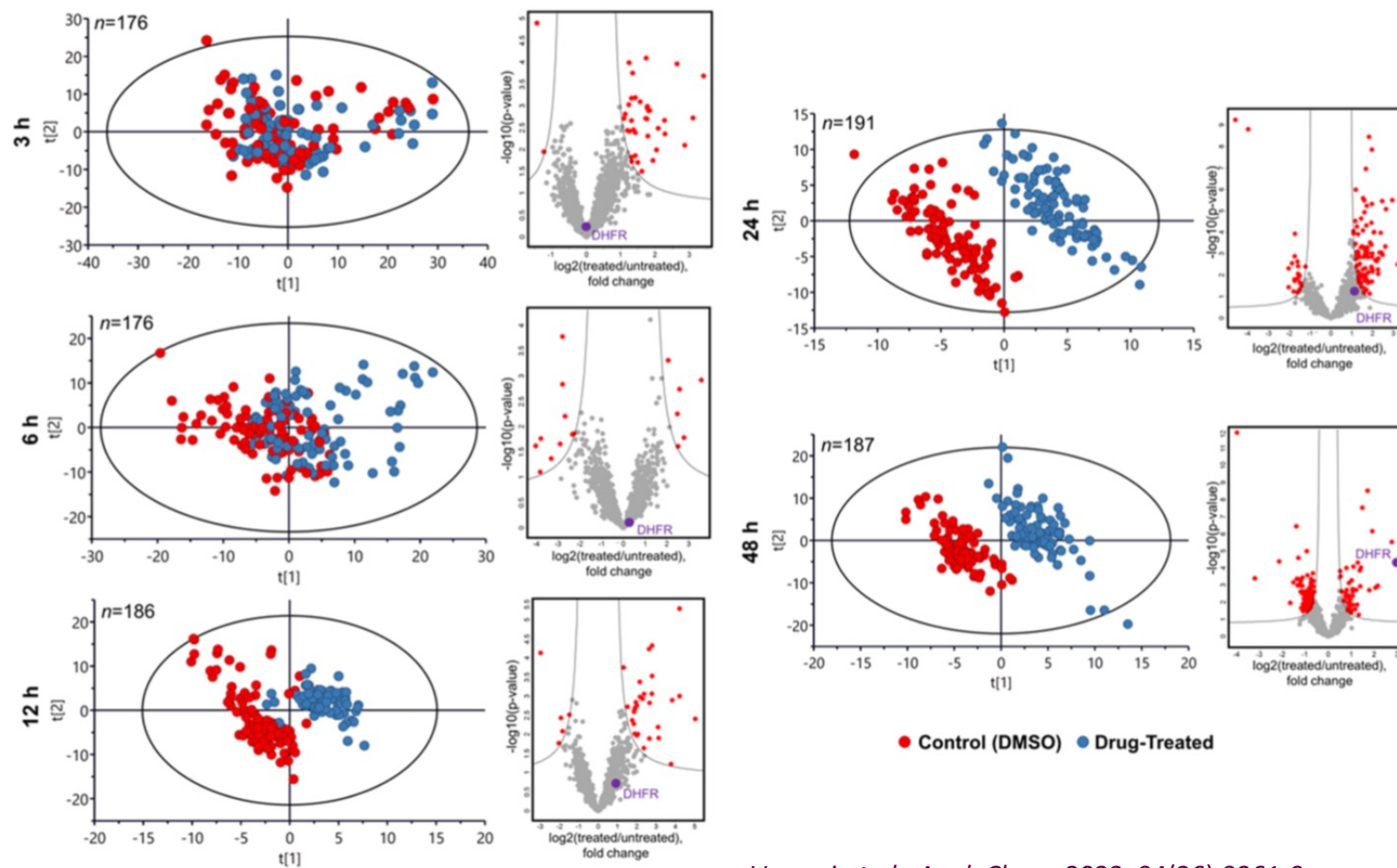
Finding the protein in the right context at the time remains an important obstacle to overcome!

SCoPE-MS enables Single Cell Proteomics



Vegvari *et al.*, *Anal. Chem.* 2022, 94(26):9261-9

96 A549 cells treated with MTX vs 96 untreated cells



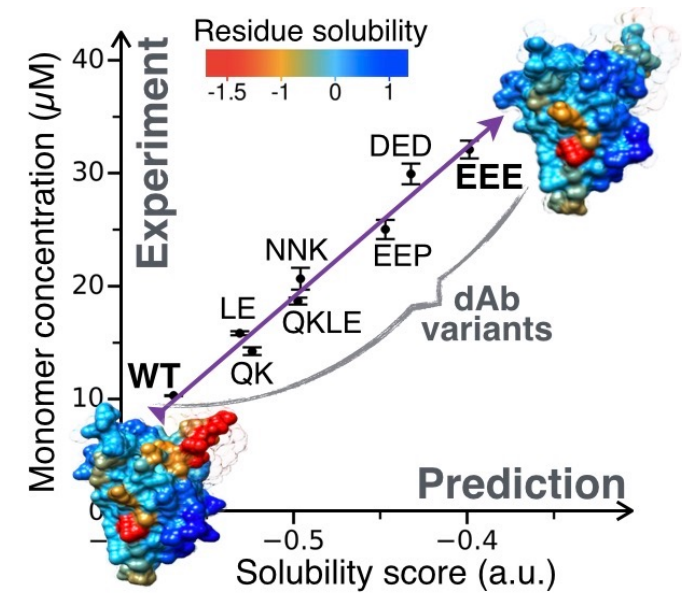
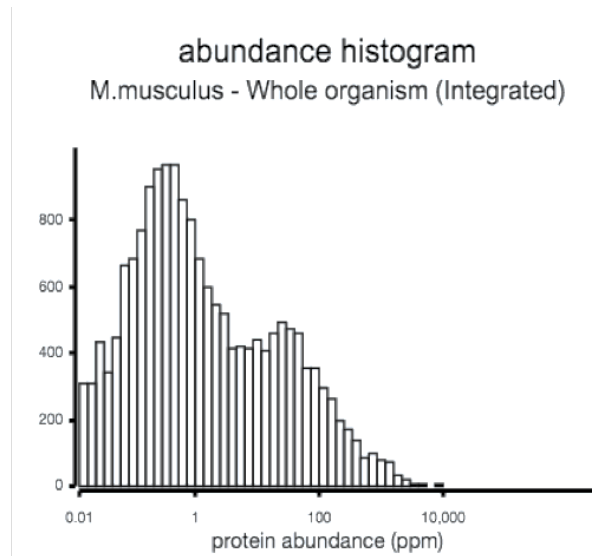
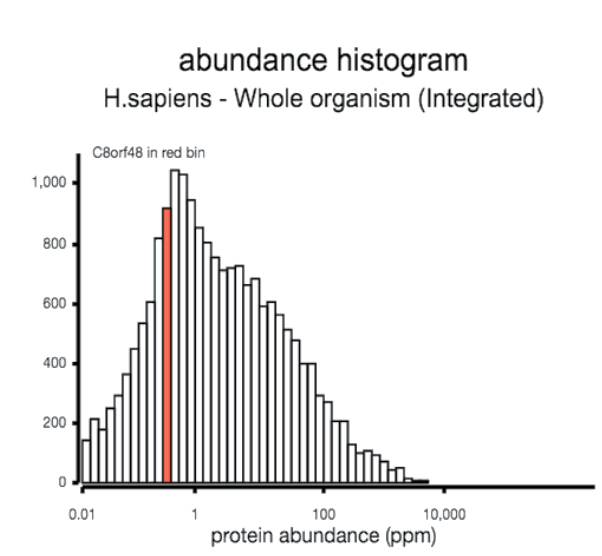
Dihydrofolate reductase

Vegvari et al., *Anal. Chem.* 2022, 94(26):9261-9

Protein Abundance

vs

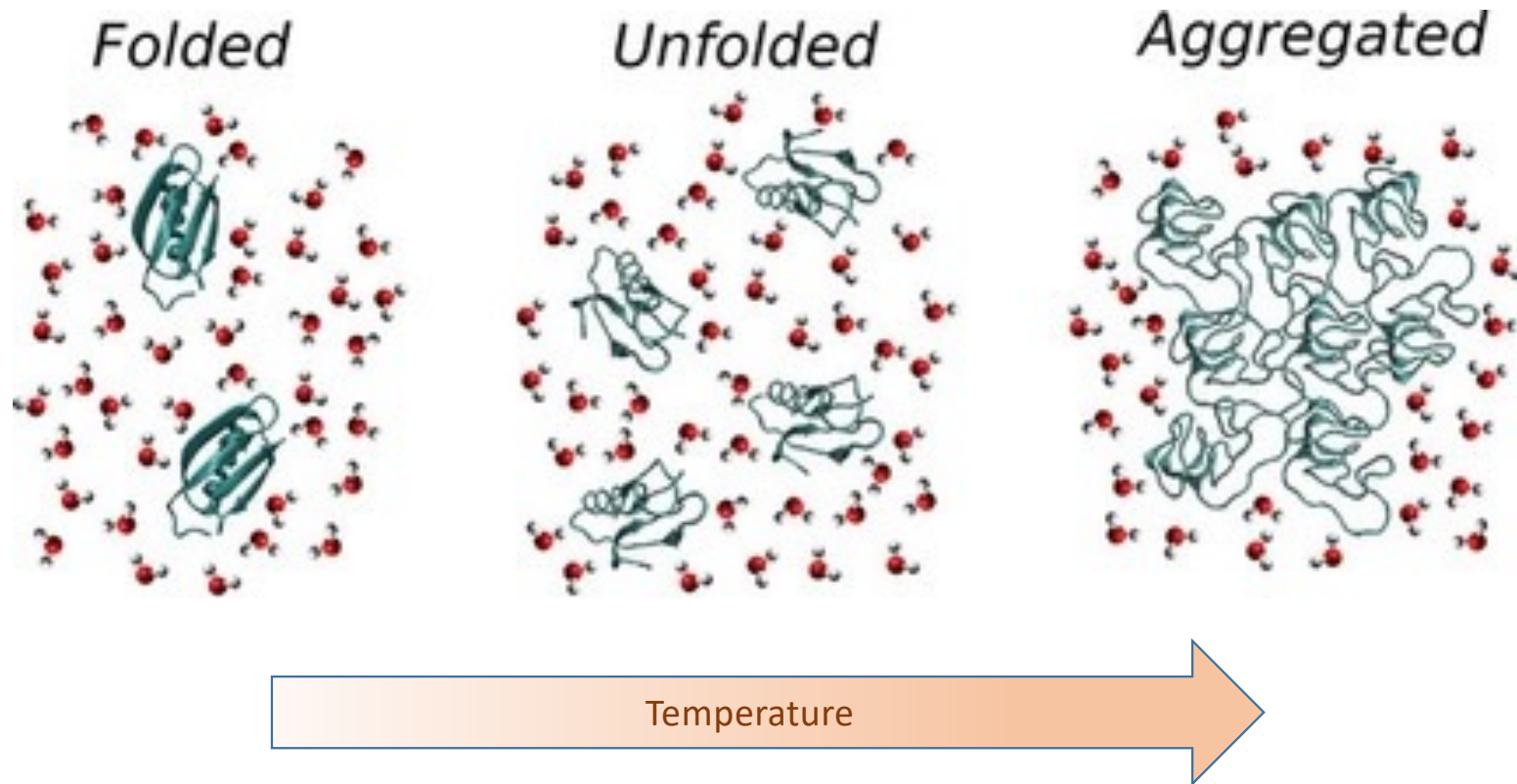
Protein Solubility



https://commons.wikimedia.org/wiki/File:PaxDB_C8ORF48_Protein_Abundance.png

Vendruscolo lab, Cambridge University

Protein Aggregation



Adopted from: <https://doi.org/10.1002/cphc.201900904>

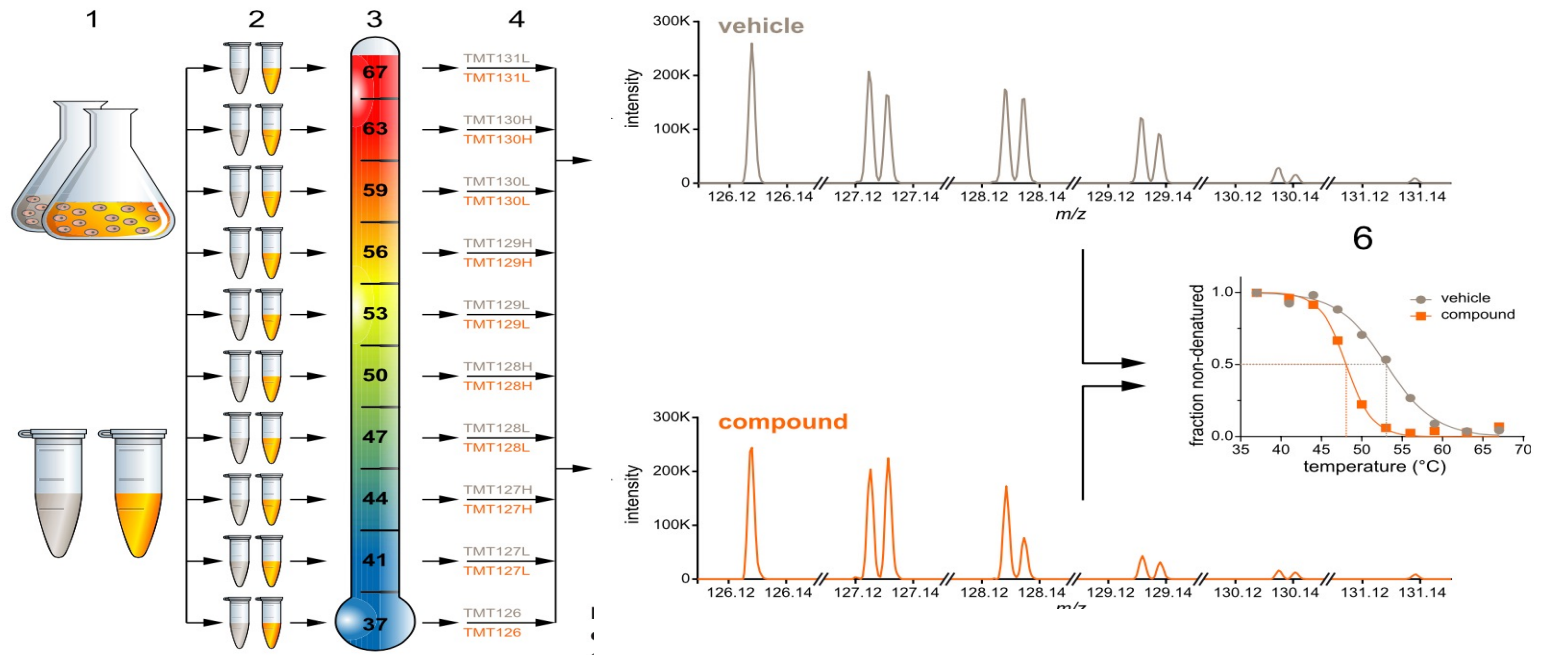
Thermal Proteome Profiling (TPP)

Tracking cancer drugs in living cells by thermal profiling of the proteome

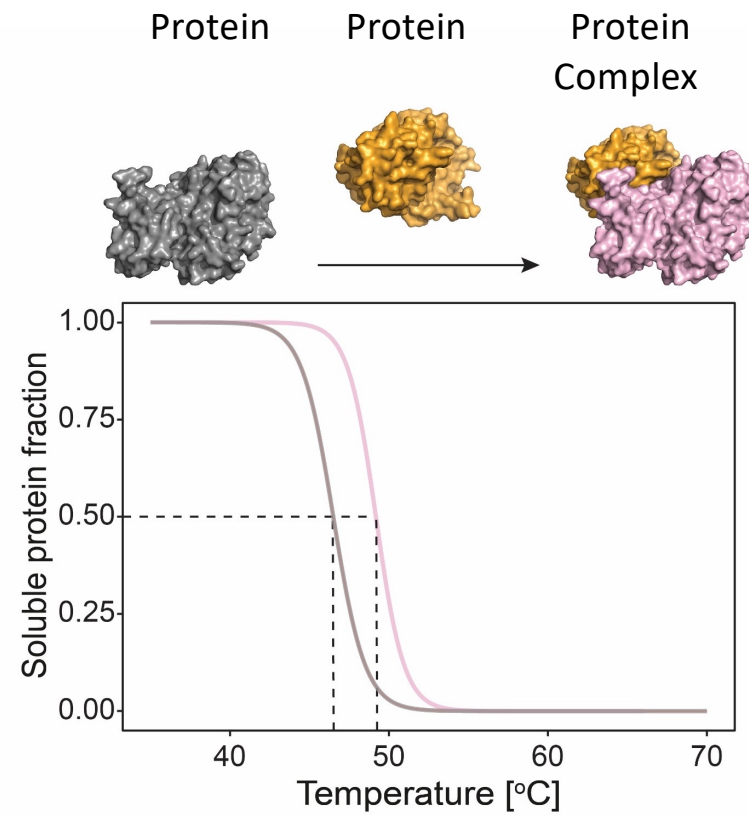
SCIENCE

3 OCTOBER 2014 • VOL 346 ISSUE 6205

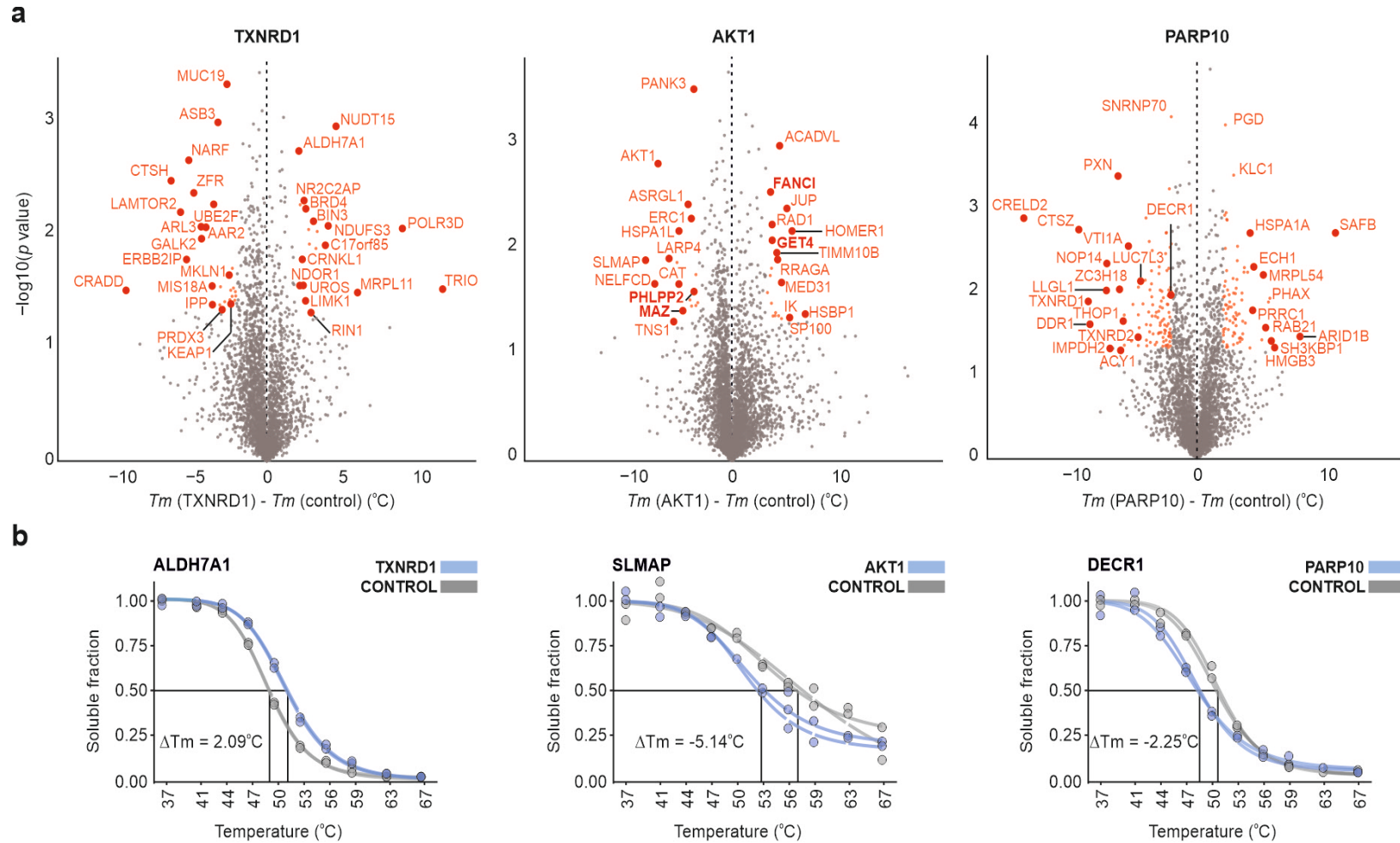
Mikhail M. Savitski,^{1*}† Friedrich B. M. Reinhard,^{1†} Holger Franken,¹ Thilo Werner,¹ Maria Fälth Savitski,¹ Dirk Eberhard,¹ Daniel Martinez Molina,² Rozbeh Jafari,² Rebecca Bakszt Dovega,² Susan Klaeger,^{3,4} Bernhard Kuster,^{3,4} Pär Nordlund,^{2,5} Marcus Bantscheff,^{1*} Gerard Drewes^{1*}



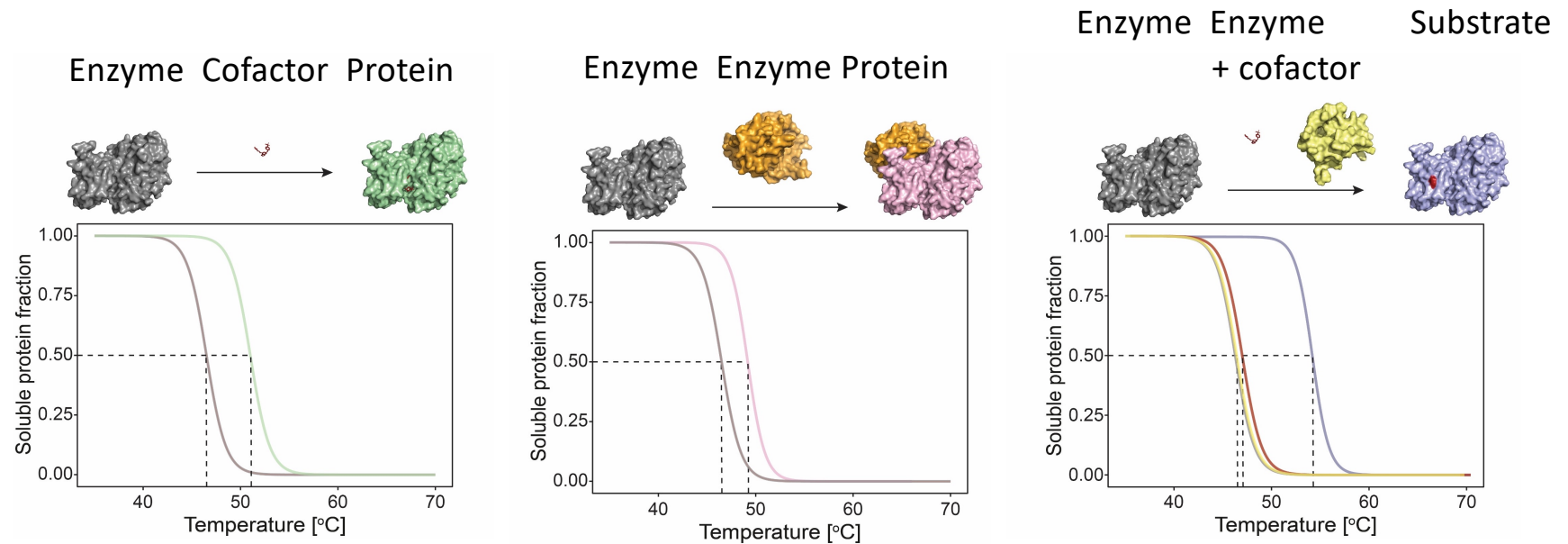
TPP in Protein-protein interaction



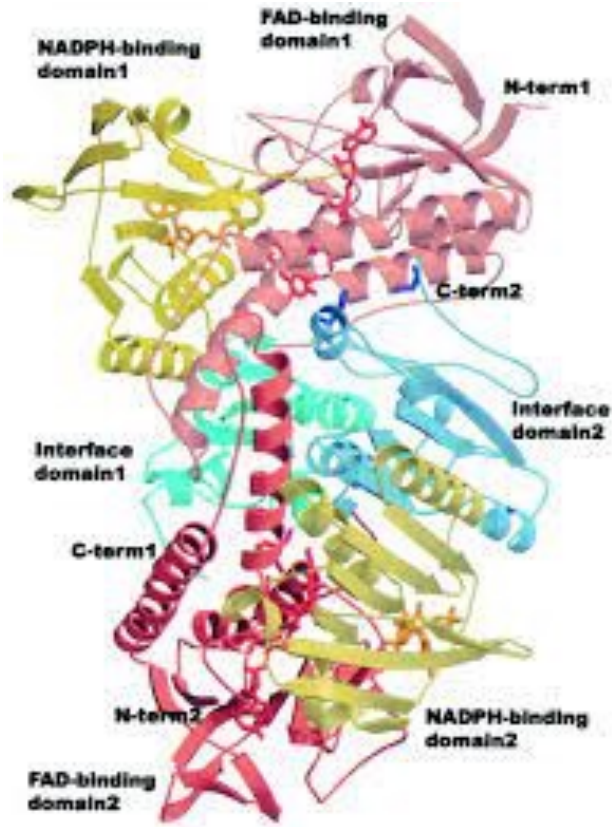
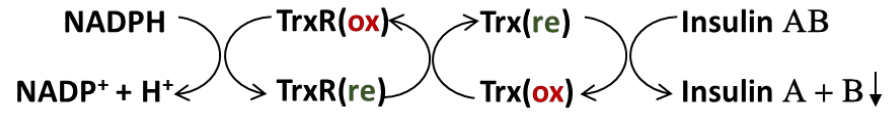
TPP identifies protein interaction partners of proteins added to cell lysates



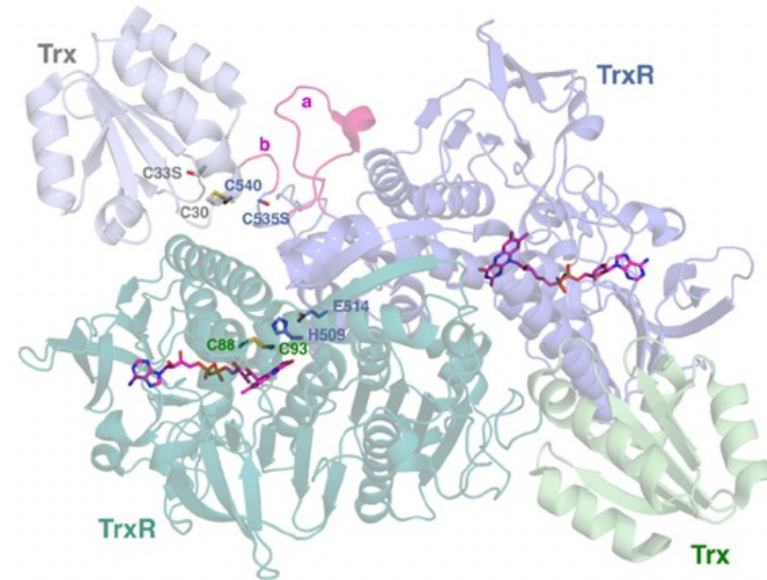
System-wide Identification of Substrates by Thermal Analysis - SIESTA



Thioredoxin reductase TrxR

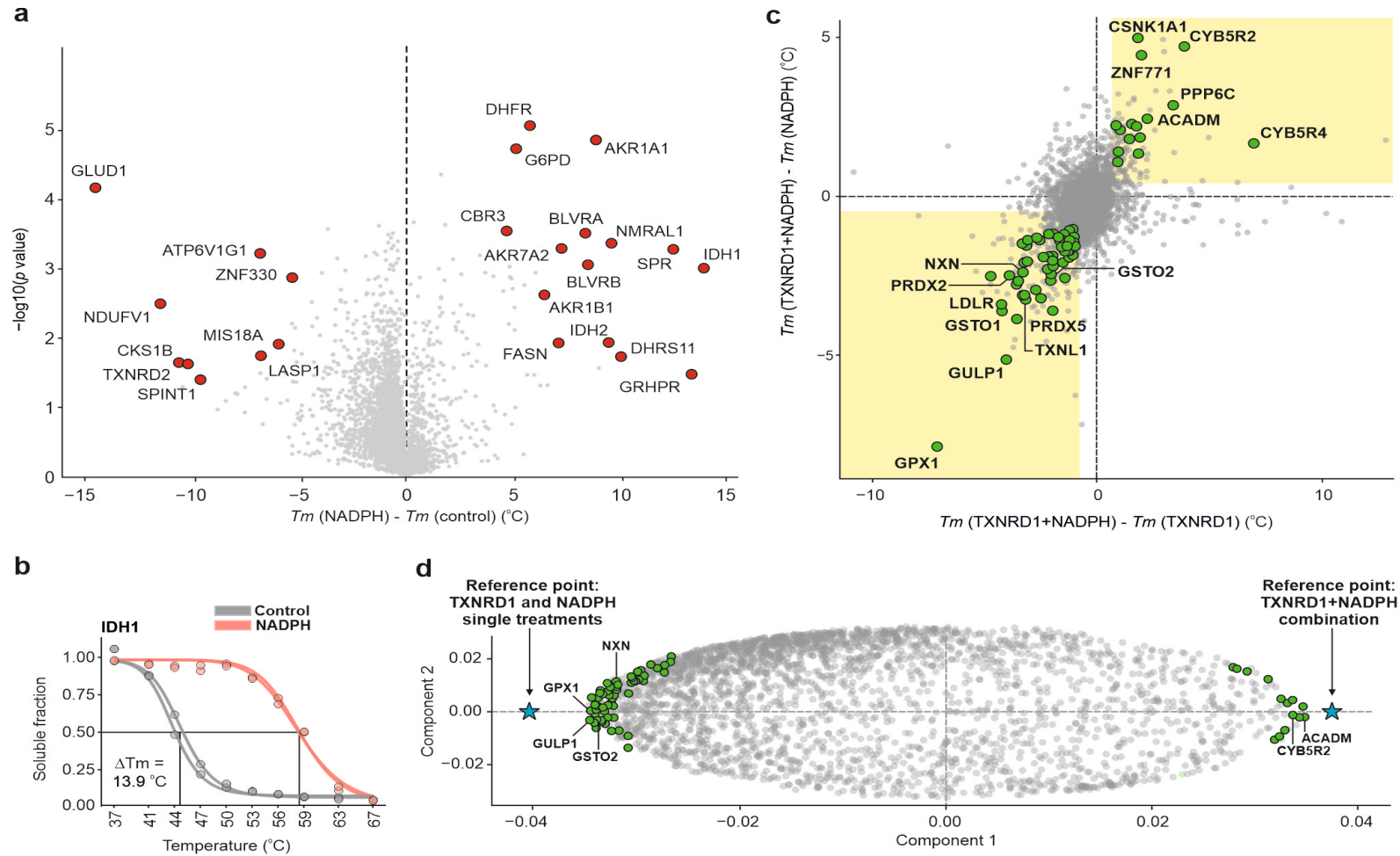


TrxR dimer + Trx



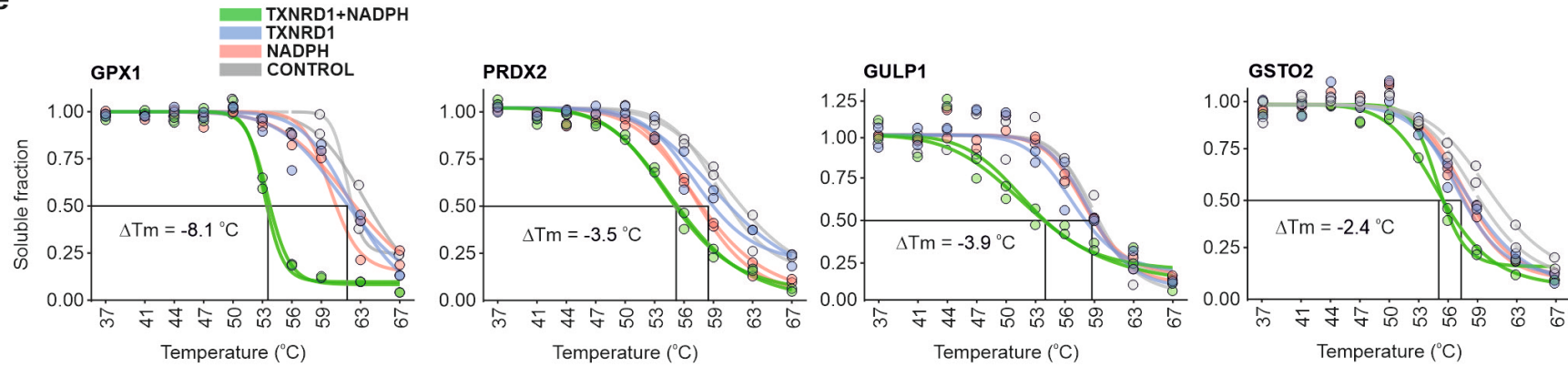
Conserved C-terminal sequence Gly-Cys-SeCys-Gly

System 1: TXNRD1 + NADPH -> S-S bond reduction



System 1: TXNRD1 + NADPH -> S-S bond reduction

e



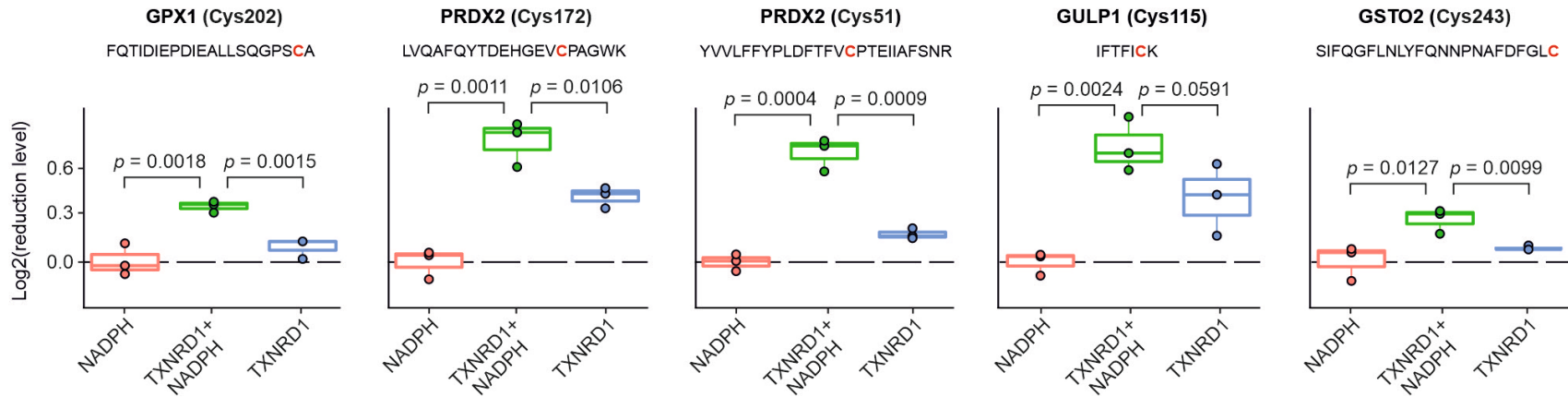
f

Validation: redox proteomics

NADPH

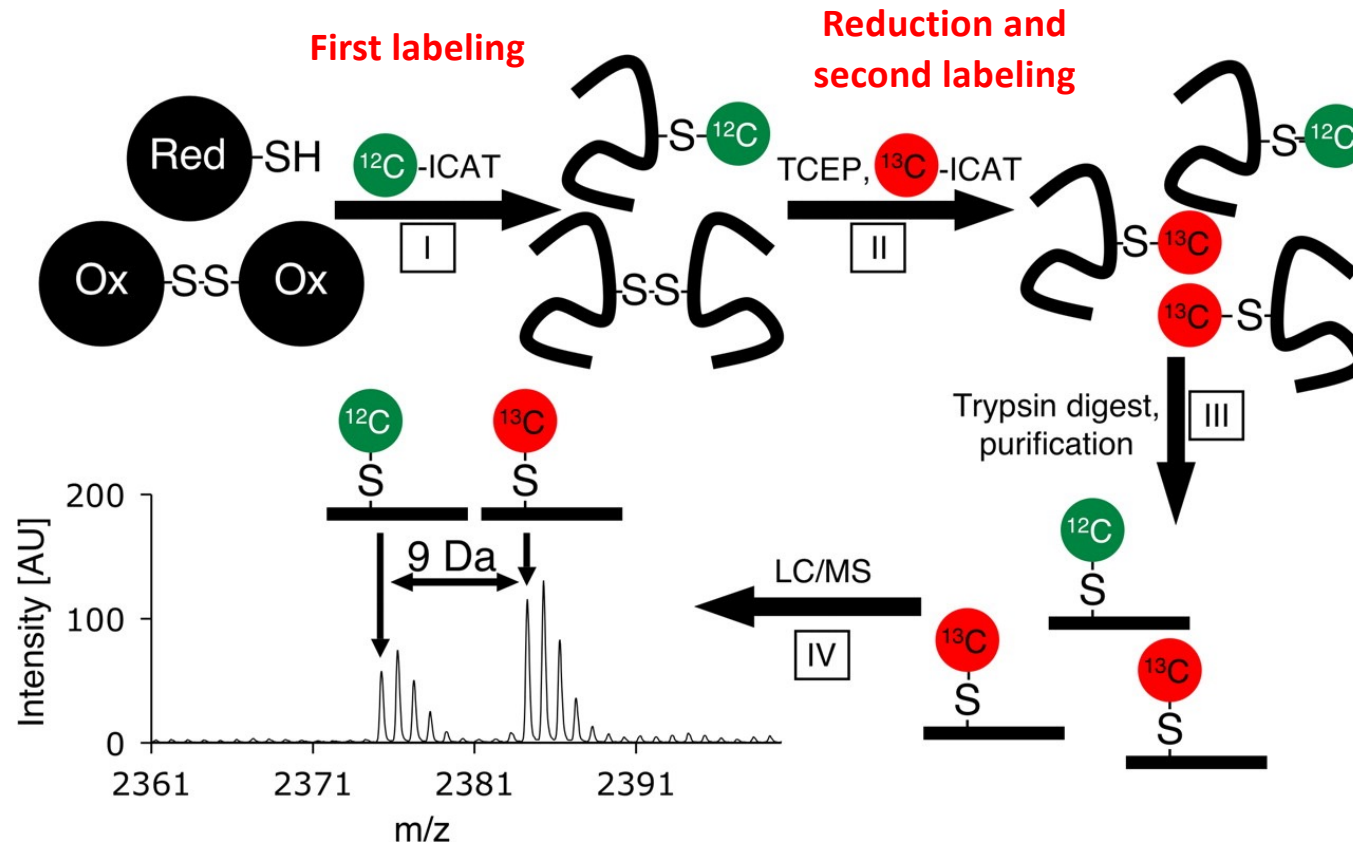
TXNRD1+NADPH

TXNRD1

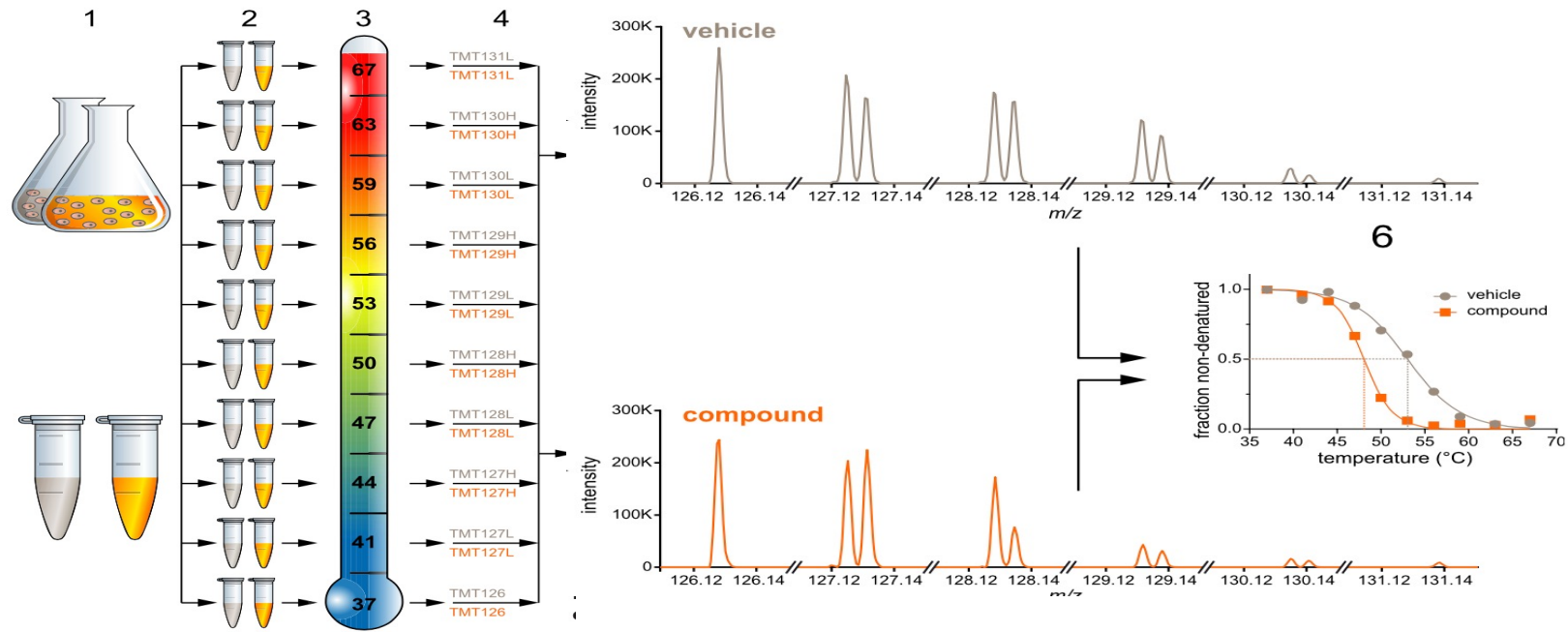


A. Saei et al., *Nat. Commun.* 2021

Redox proteomics

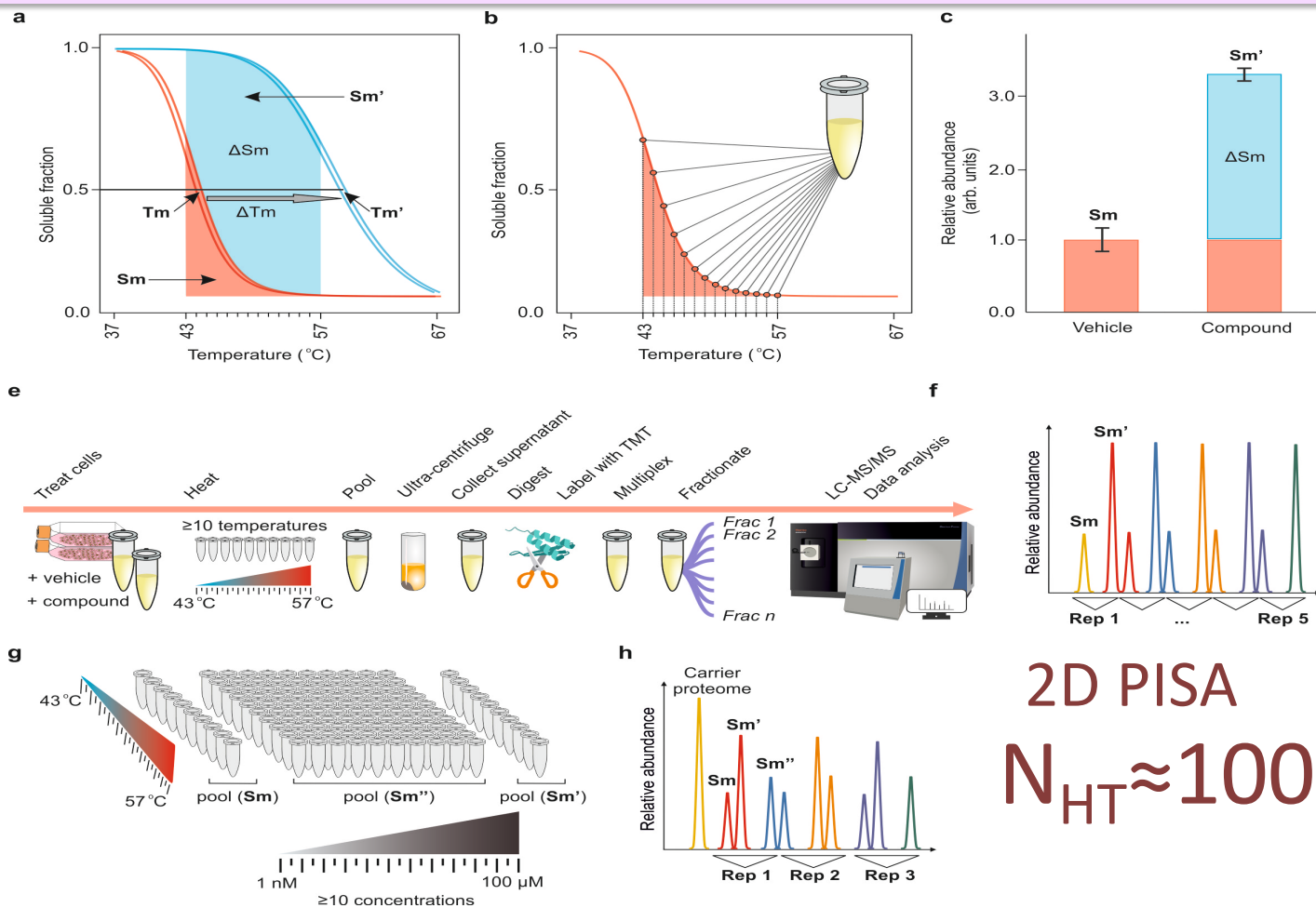


TPP limitations



- Not all proteins aggregate with temperature
- Too time consuming to measure every temperature point

Proteome Integral Solubility Alteration (PISA) Assay



1D PISA

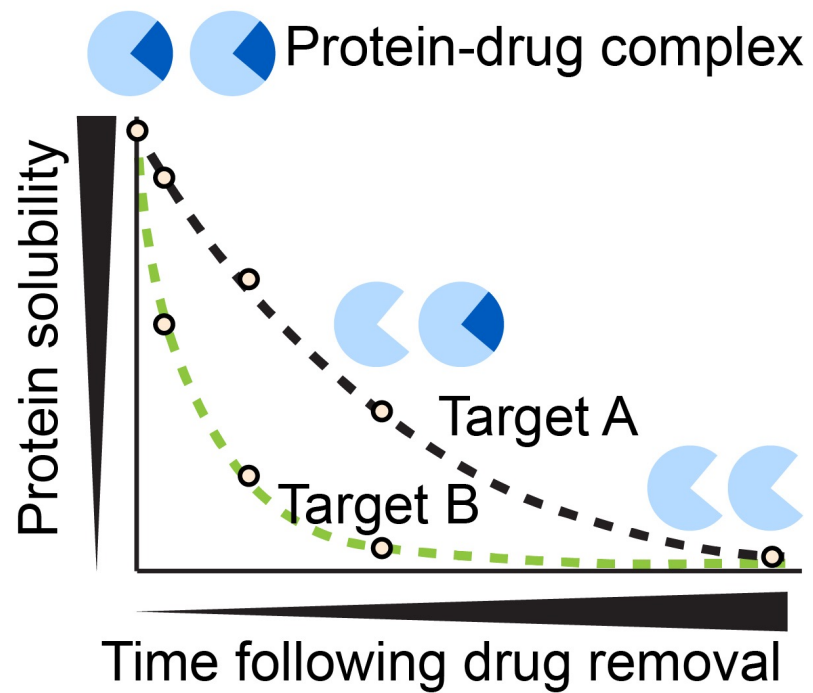
$N_{HT} \approx 10$

2D PISA

$N_{HT} \approx 100$

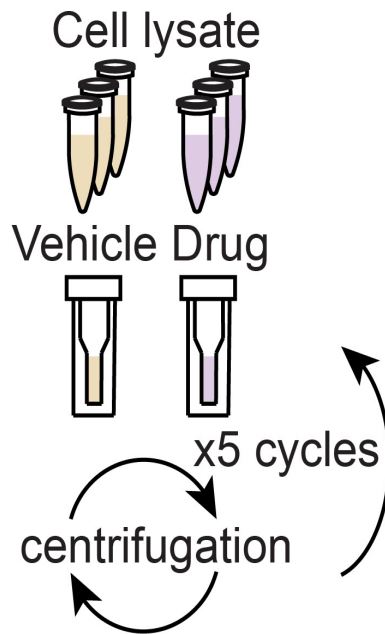
Gaetani M, et al. *J Proteome Res* 2019,
DOI:10.1021/acs.jproteome.9b00500

- Drug binding is not the only parameter of interest;
Residence time of drug on target is of very high significance

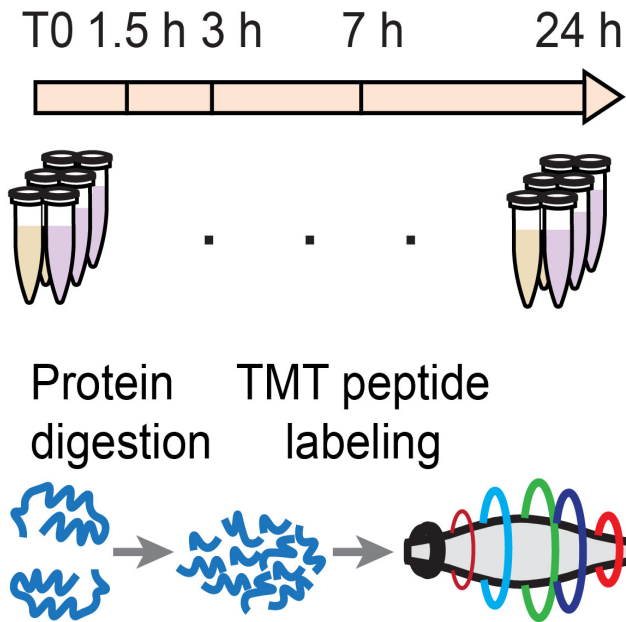


PISA determines drug residence time

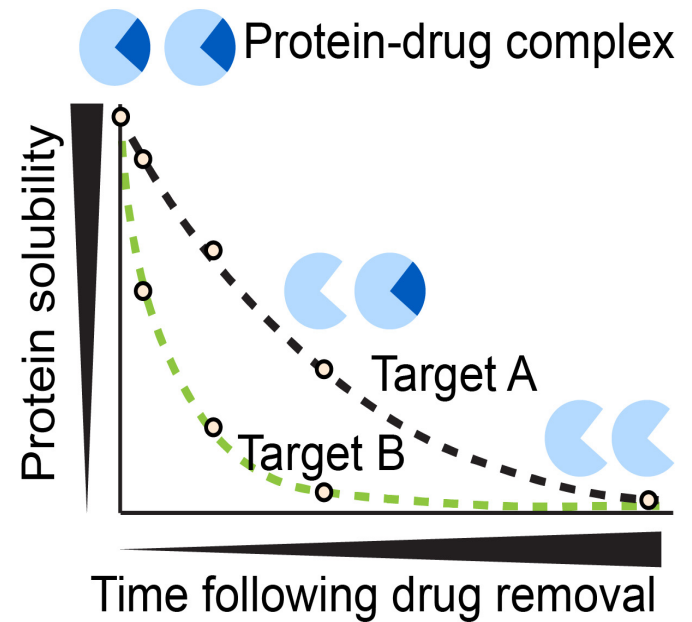
Removal of the drug by filtration



Time-series PISA analysis and LC-MS/MS measurement



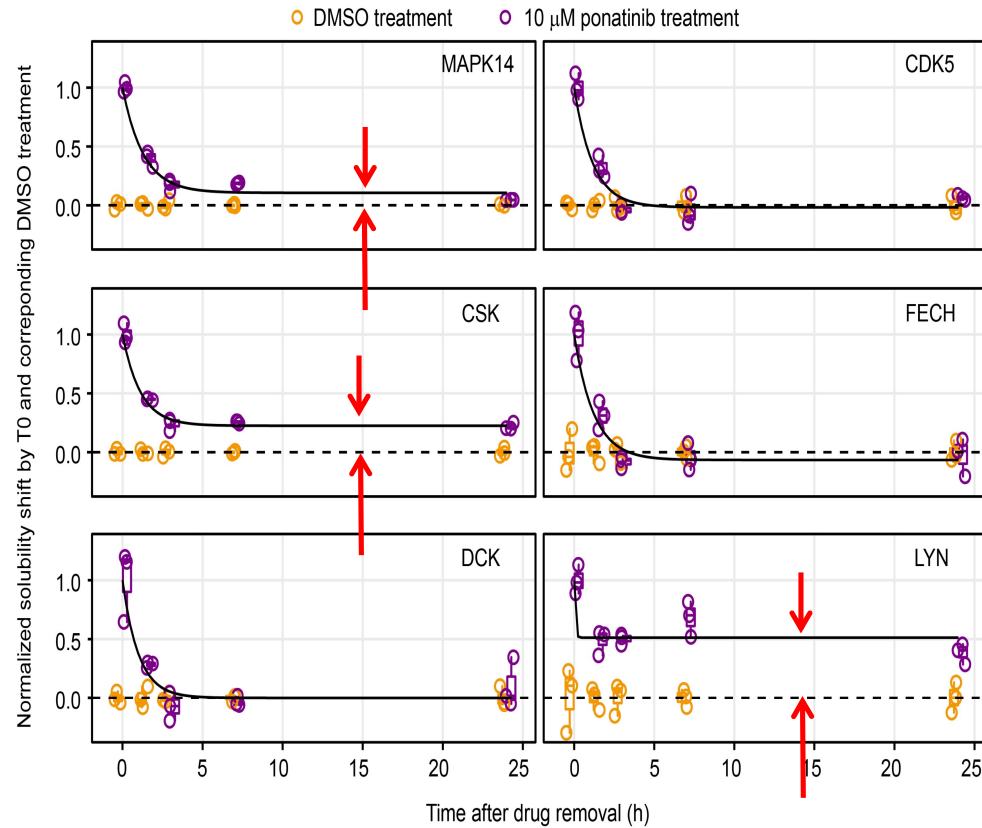
Measurement of drug residence time



PISA determines drug residence time

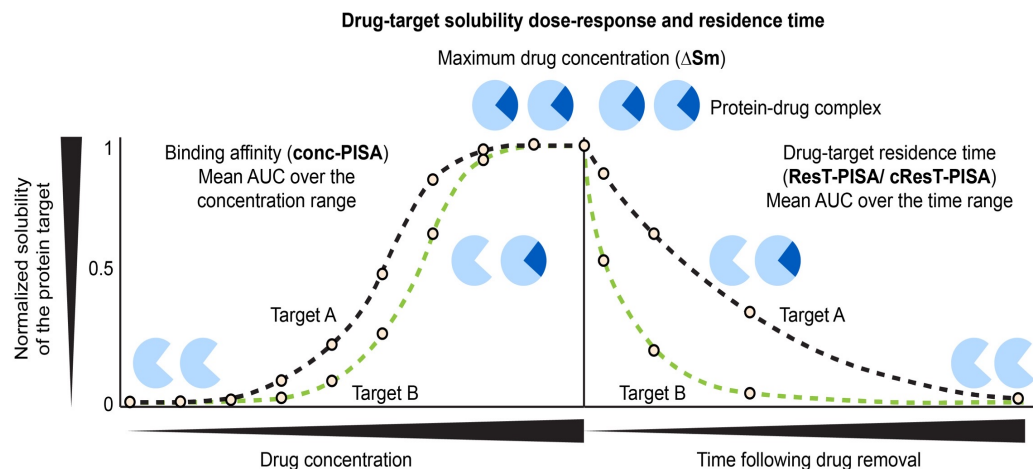
b

kinase inhibitor ponatinib

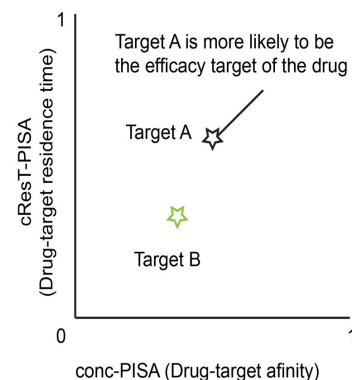


PISA helps ranking targets

a



Prioritization of targets



c

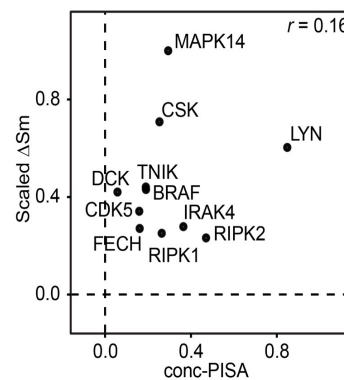
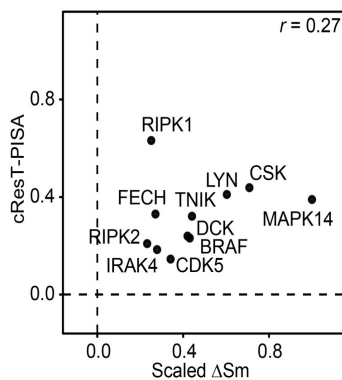
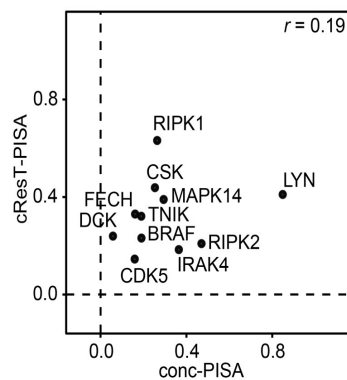
	Rank ΔSm	Rank ΔSm	Rank conc-PISA and conc-PISA and cResT-PISA and cResT-PISA
1	LYN	1 MAPK14	1 LYN
2	MAPK14	2 CSK	2 RIPK1
3	CSK	3 LYN	3 CSK
4	RIPK2	4 RIPK1	4 MAPK14
5	IRAK4	5 TNIK	5 RIPK2
6	TNIK	6 BRAF	6 IRAK4
7	BRAF	7 DCK	7 TNIK
8	RIPK1	8 FECH	8 FECH
9	CDK5	9 CDK5	9 BRAF
10	DCK	10 IRAK4	10 CDK5
11	FECH	11 RIPK2	11 DCK

b

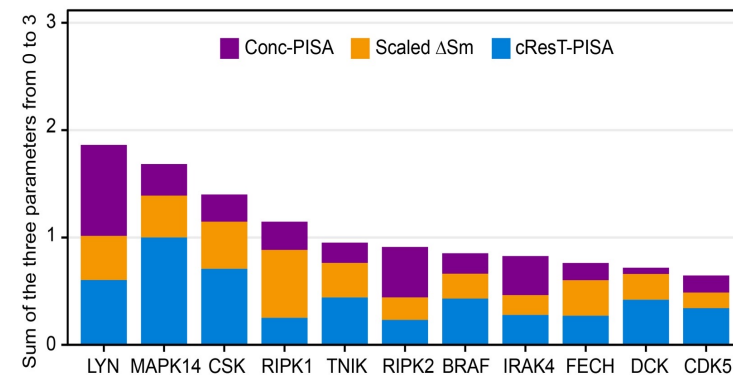
Concentration

Solubility shift

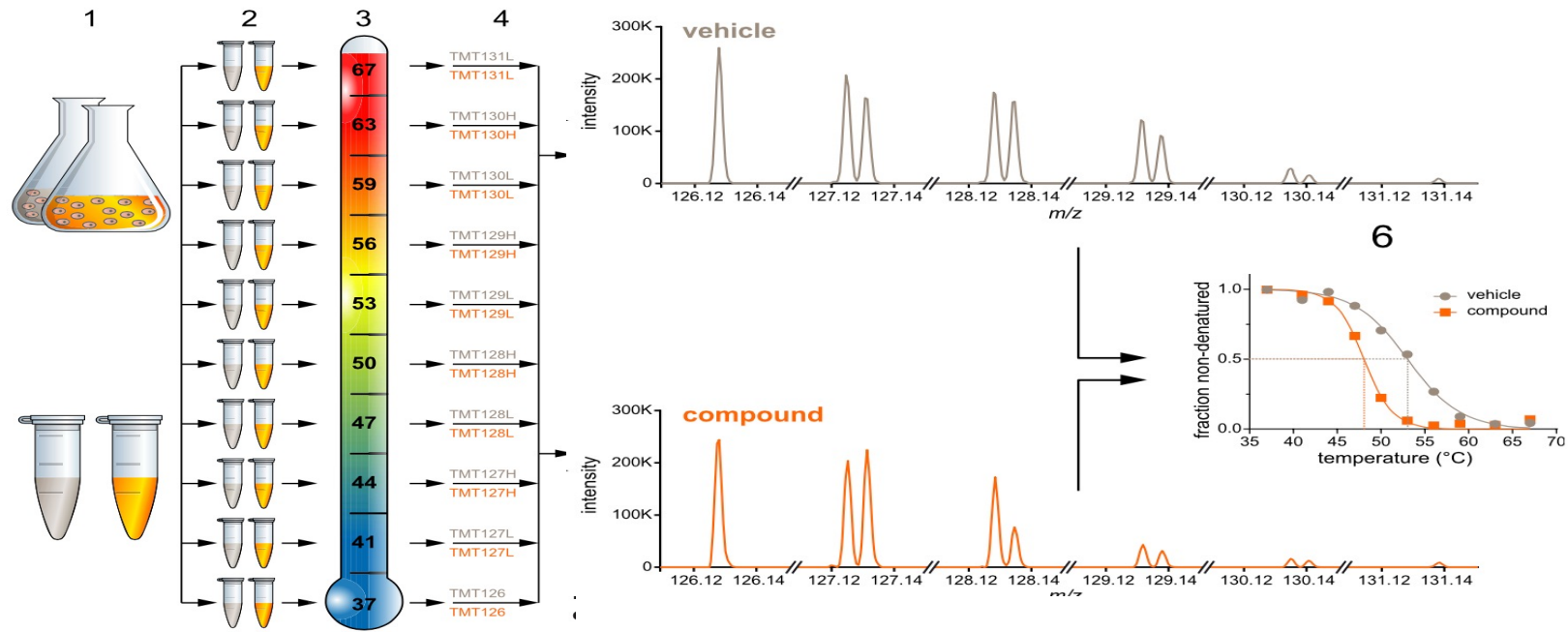
Residence time



d

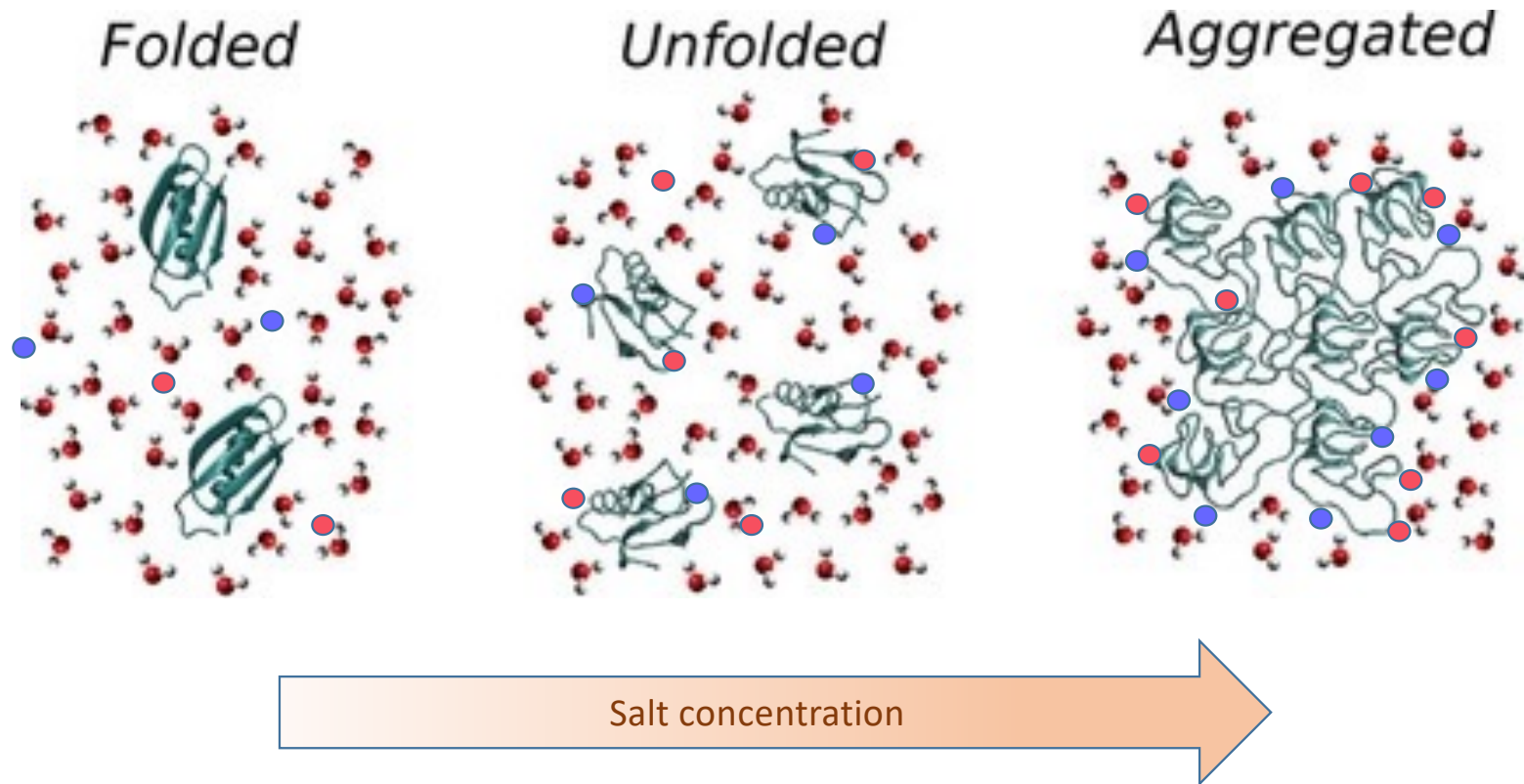


TPP/PISA limitations



- Not all proteins aggregate with temperature
- Too time consuming to measure every temperature point

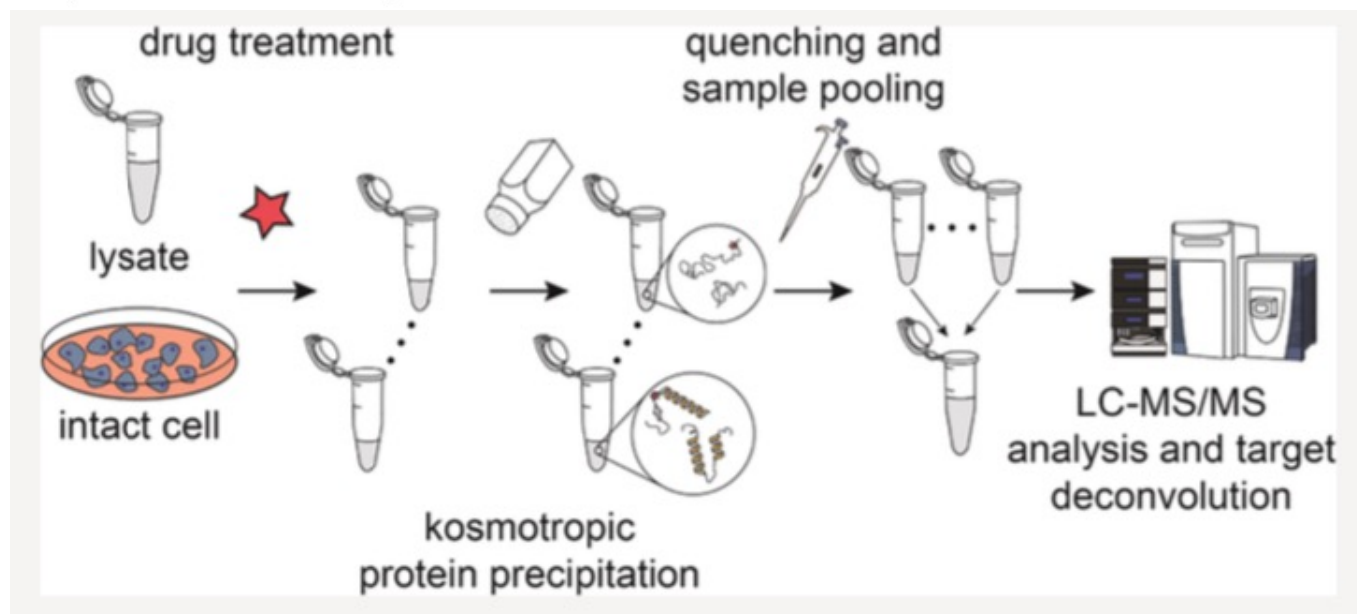
Protein Aggregation



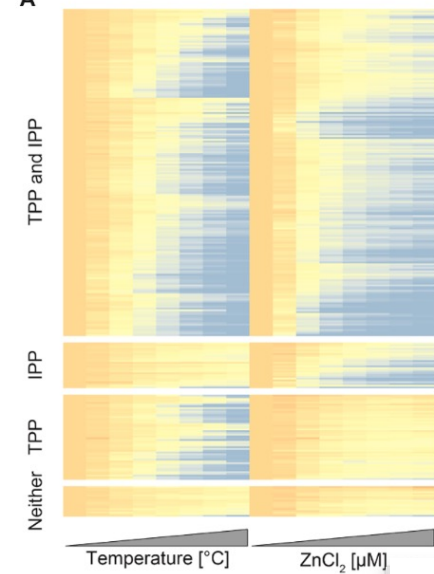
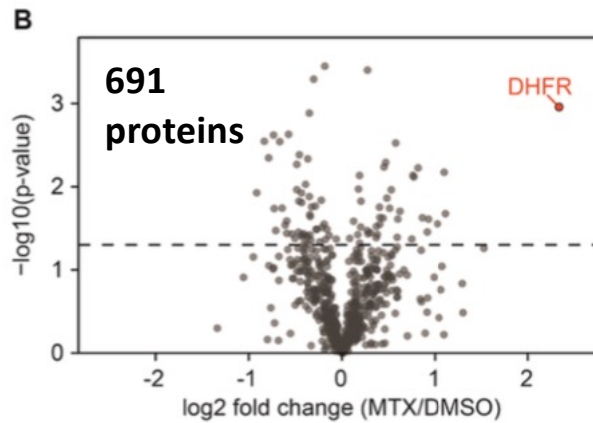
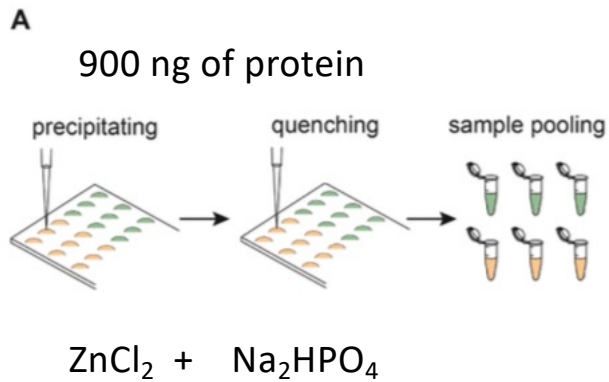
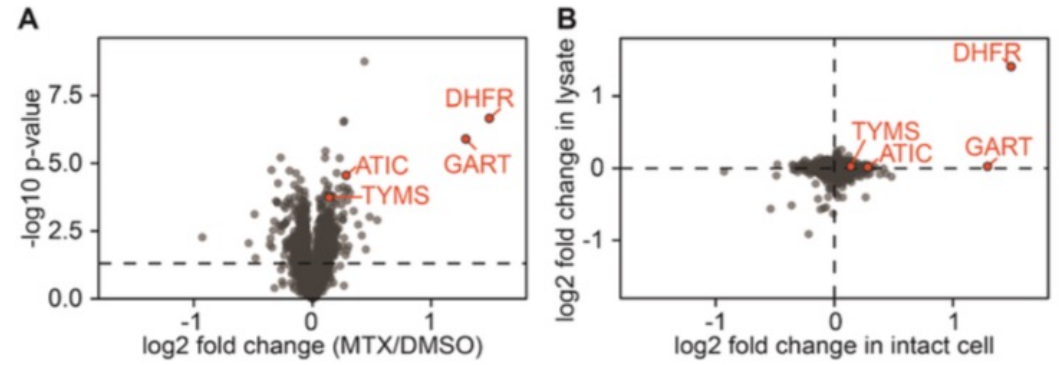
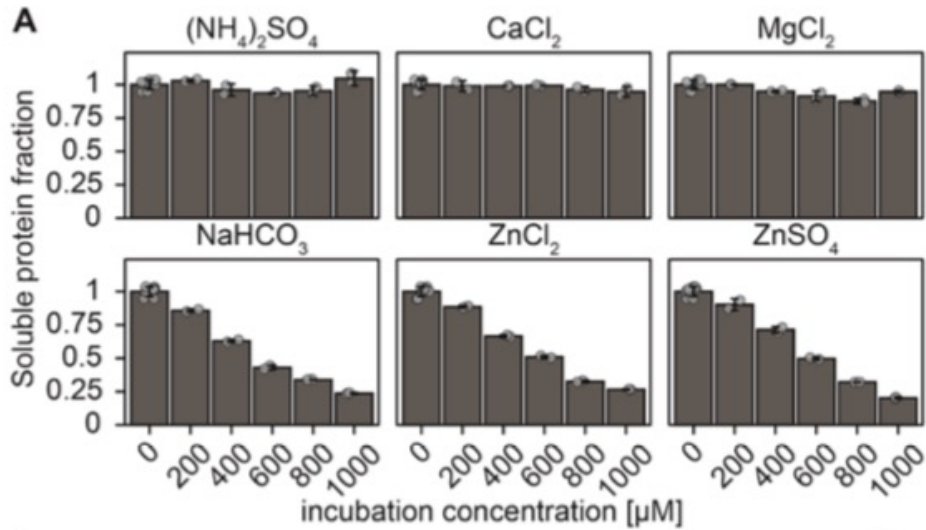
Adopted from: <https://doi.org/10.1002/cphc.201900904>

Ion-Based Proteome-Integrated Solubility Alteration Assays for Systemwide Profiling of Protein–Molecule Interactions

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iPISA works in lysate and cells



C. Beusch et al., *Analyt Chem* 2022

Conclusions:

- Chemical proteomics reveals drug targets without chemical modification of drugs
- FITExP / ProTargetMiner uses expression level changes for drug target deconvolution; is already translated to single cell level
- TPP/PISA performs target deconvolution based on solubility changes
- PISA allows one to easily determine:
 - interacting partners of proteins;
 - enzyme substrates;
 - the residence time of drugs
- Ion-based PISA reaches sub-microgram level of sensitivity

Acknowledgements

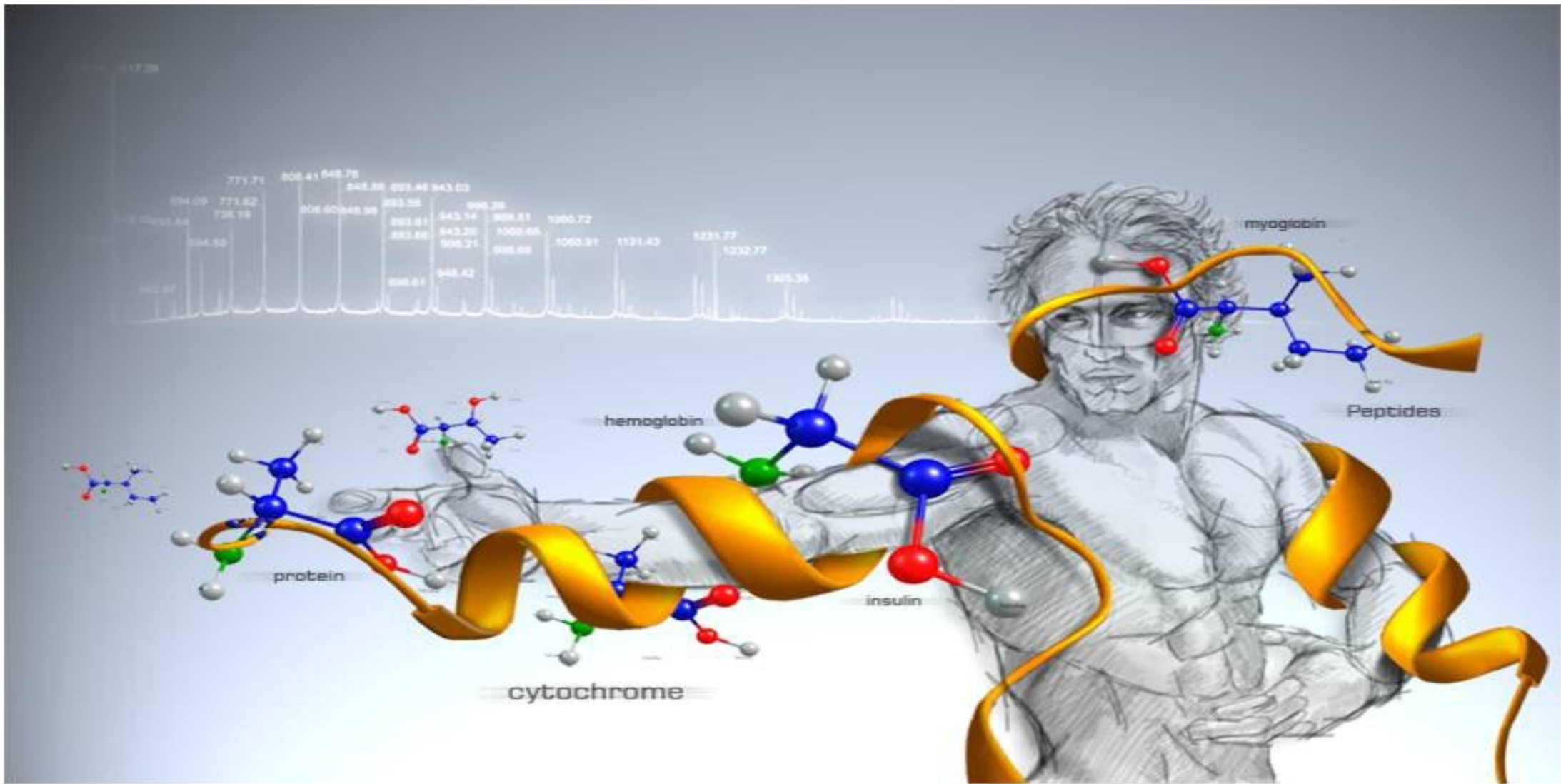
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