Forschungs-Zentrum für Immuntherapie

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Quantitative Proteomanalyse: Grundlagen und Anwendung in der biomedizinischen Forschung

Univ.-Prof. Dr. Stefan Tenzer
Institut für Immunologie

Pharmaforum 2017

Our Group

Core Facility for Mass Spectrometry:

Head: Univ.-Prof. Dr. Stefan Tenzer

Postdoc: Dr. Ute Distler

Bioinformatics: Dr. Jörg Kuharev

Technical Assistant: Ruben Spohrer

Funding: DFG, BMBF, FZI, FTN

Waters Synapt G2-Si

- ion mobility separation
- identification and quantification of up to 5000 proteins

Label-free quantitative Proteomics

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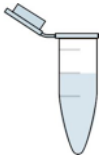
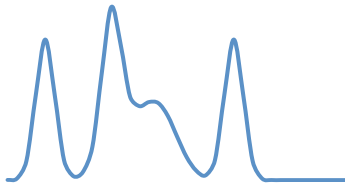


20 µg cellular proteins

→ tryptic digest FASP/SP3 →



nanoUPLC separation

↓

Mass Spectrometric Analysis

Synapt G2-Si HDMS
(25.000 FWHM Resolution)

← PLGS 3.0 ← In-house software ISOQuant

Data processing

<u>Stringent identification criteria</u>:
UniProtKB/Swissprot reference proteome
Reversed decoy database
Minimum 2 peptides/ protein
1% FDR at peptide and protein level

Ion mobility enhanced proteomics

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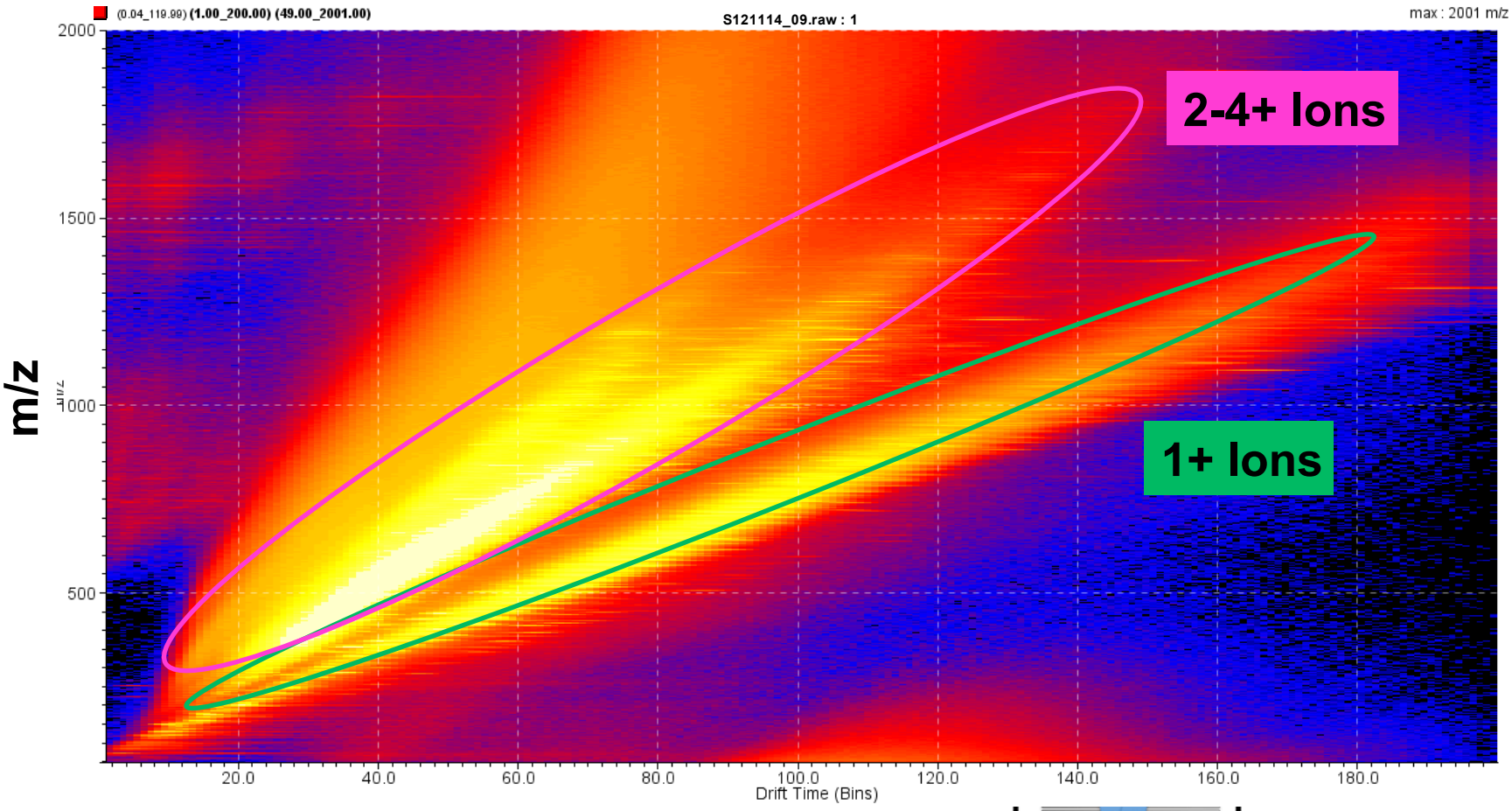

Distler et al. Nat Methods 2014

Distler et al. Nat Protocols 2016

Method Development – MS analysis

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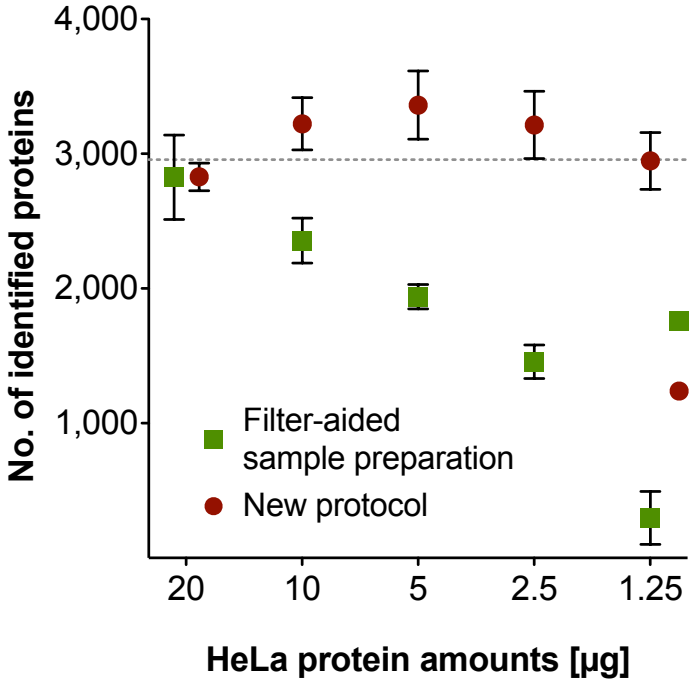
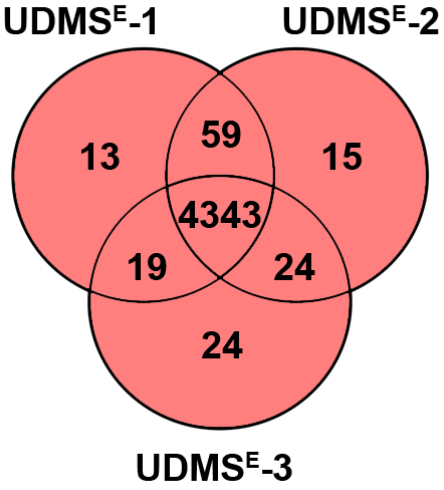
High proteome coverage workflow

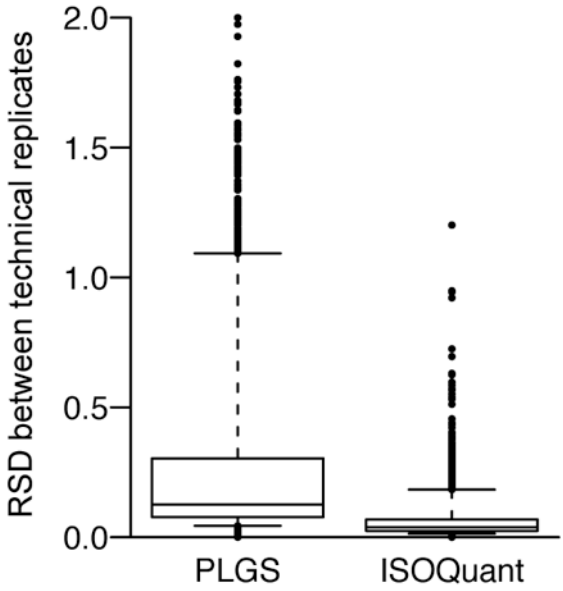
Optimized Sample Preparation



Overlap between technical replicates



High precision label-free quantification



→ over 4000 proteins using 180 min-gradients

→ High reproducibility and quantitative precision

Distler et *al., Nat Protocols* 2016 Distler et al. *Nat Methods* 2014

Selected Applications:

- **Defining the interactions between Nanoparticles and Proteins: The „Protein Corona“**
- **Identification of Protein Interaction Partners**
- **Tumor Biomarker Discovery**
- **Profiling Cellular Reactions to Bioactive Compounds**

Applications: The Protein Corona

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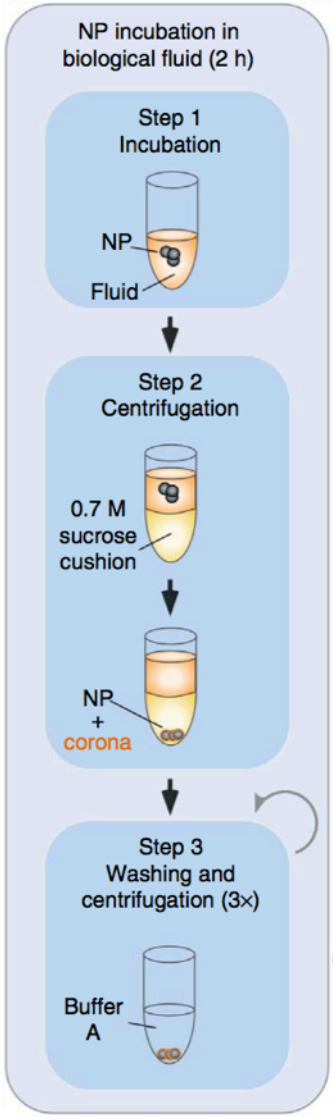
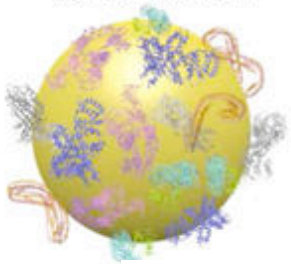
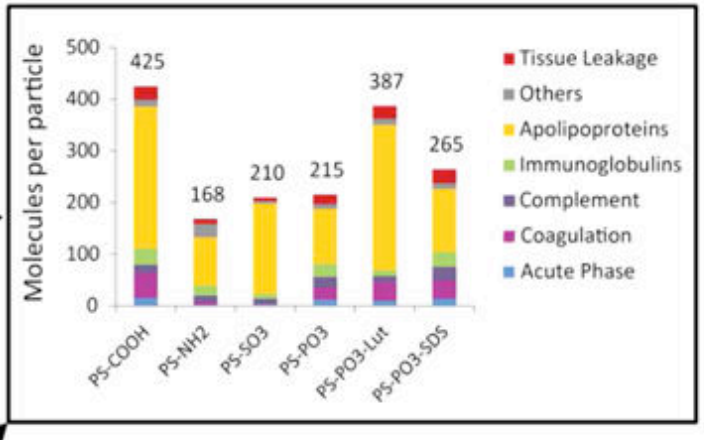


Protein Corona of Nanoparticles

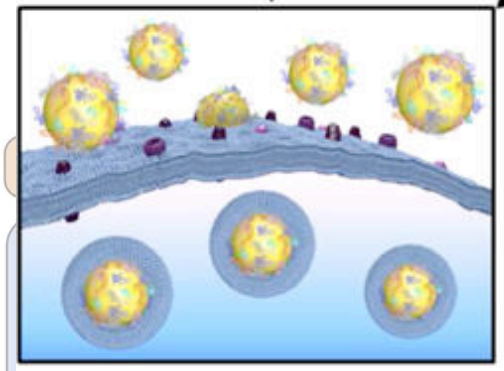


LC-MS Analysis

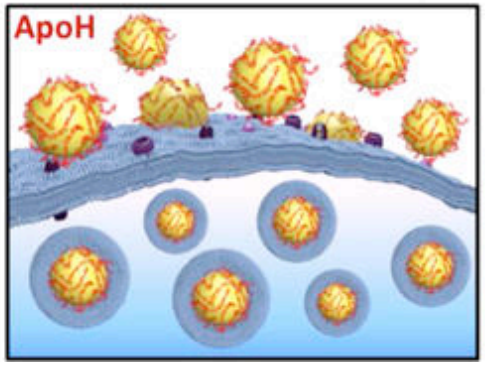


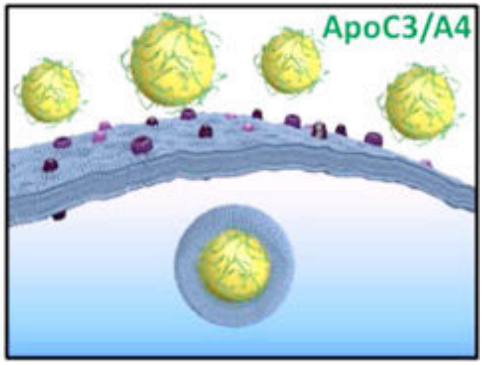
Cellular uptake

Correlation



Identification of single proteins influencing cellular uptake





Tenzer et *al., Nat Nanotechnol* 2013
Ritz et al. *Biomaterials* 2015
Docter et al. *Nat Protocols* 2014

Applications: Protein interactions

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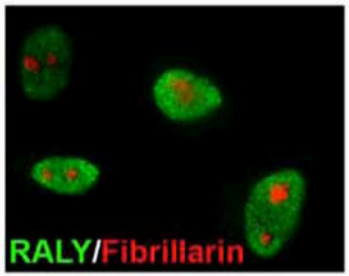

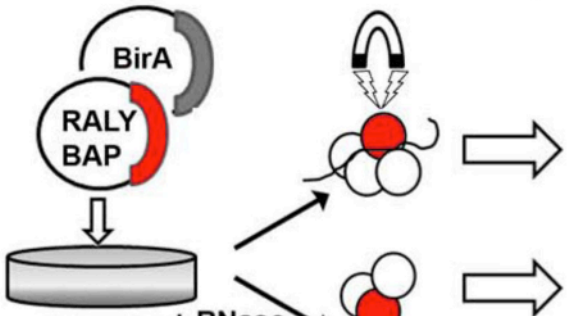

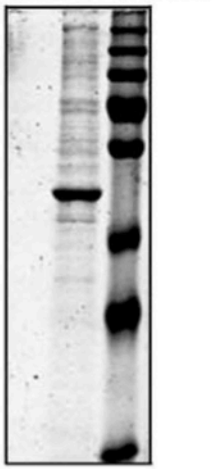

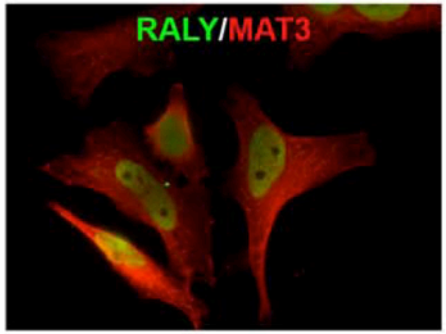

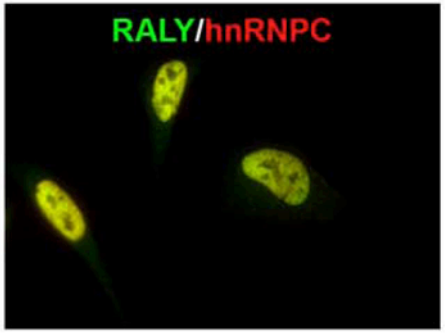

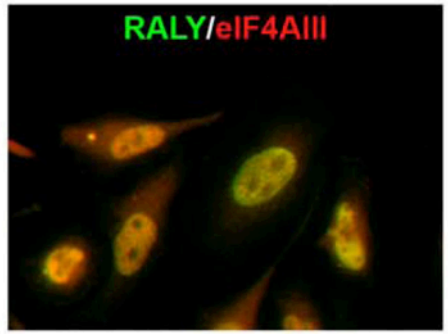

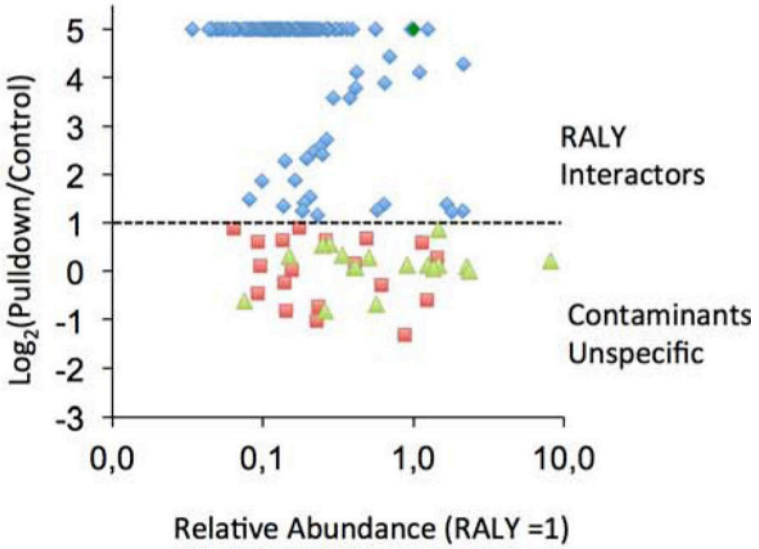

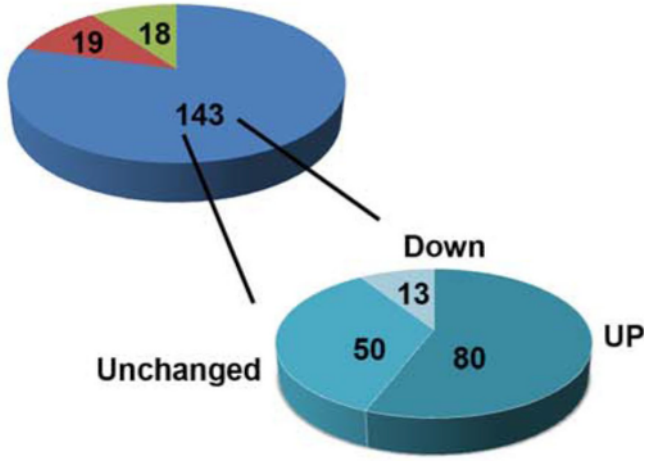

Tenzer et *al., Journal of Proteome Research 2013*

Applications: Tumor Biomarkers

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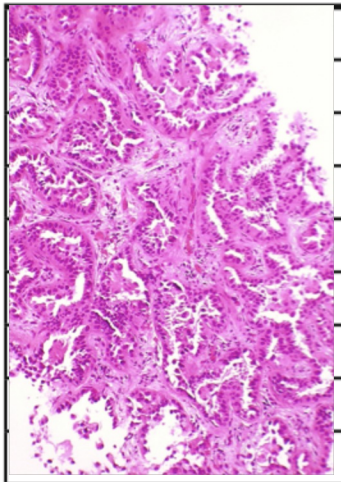
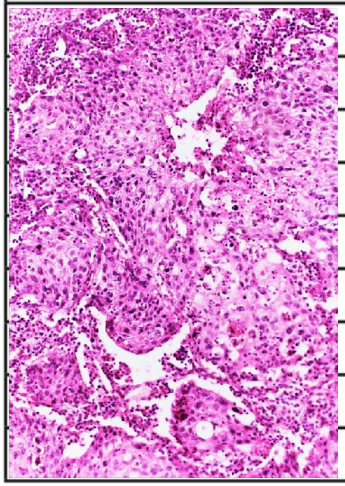
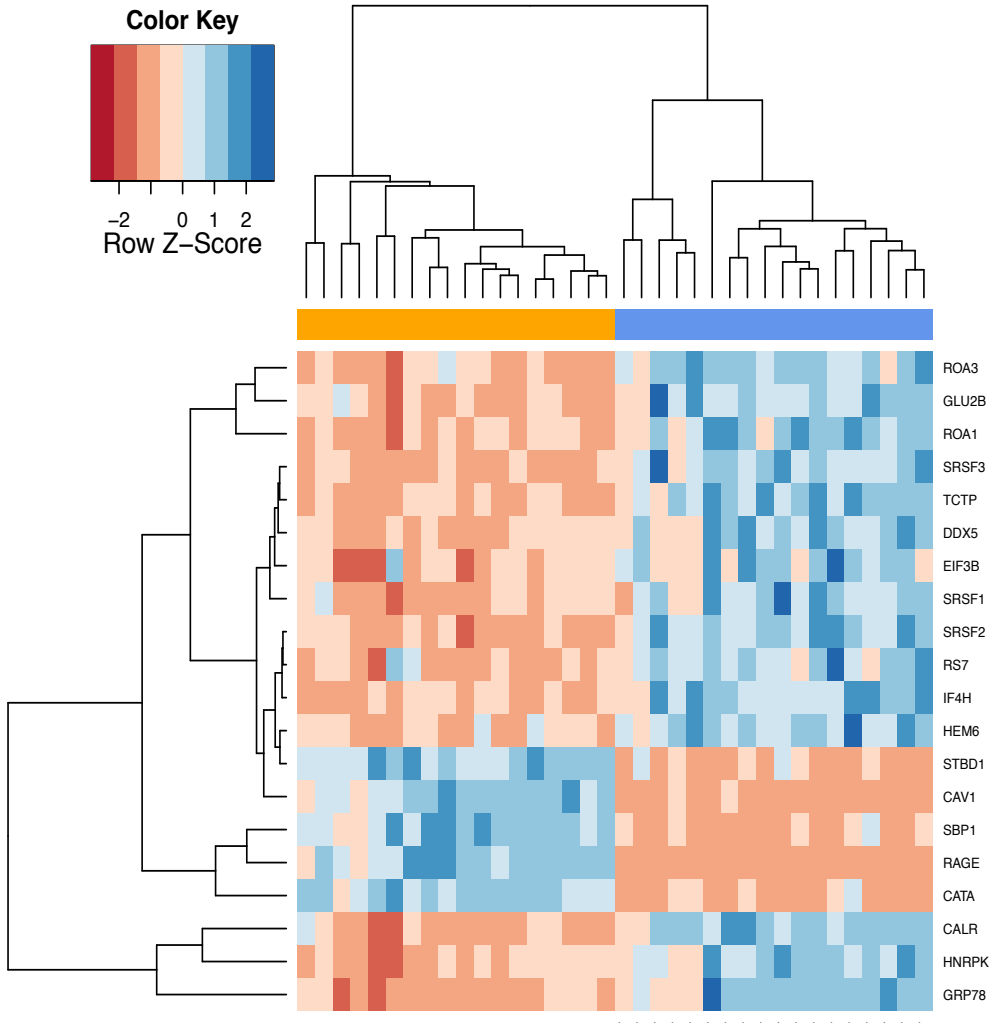




Tumor Type	TNM
Adeno-Ca	T2aN1
Adeno-Ca	T2aN1
Adeno-Ca	T1bN0
Adeno-Ca	T2aN0
Adeno-Ca	T3N0
Adeno-Ca	T2aN0
Adeno-Ca	T2aN1
Adeno-Ca	T3N1
Adeno-Ca	T3N2
SCC	T1aN0
SCC	T1bN0
SCC	T2bN0
SCC	T2aN0
SCC	T2bN1
SCC	T3N0
SCC	T2aN1
SCC	T3N0
SCC	T4N2



- clear separation of lung cancer and control samples

Applications: Effector Proteomics

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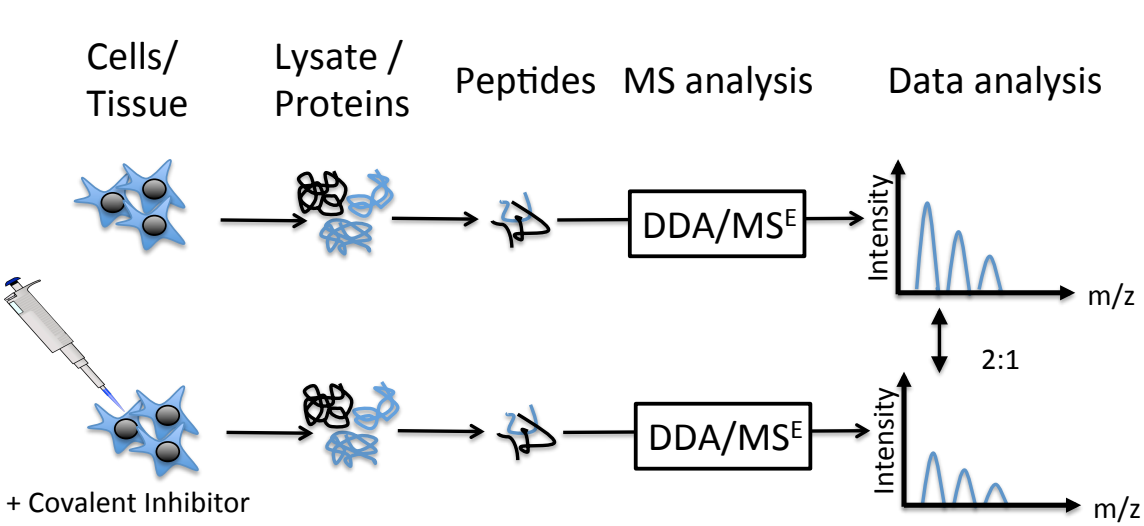
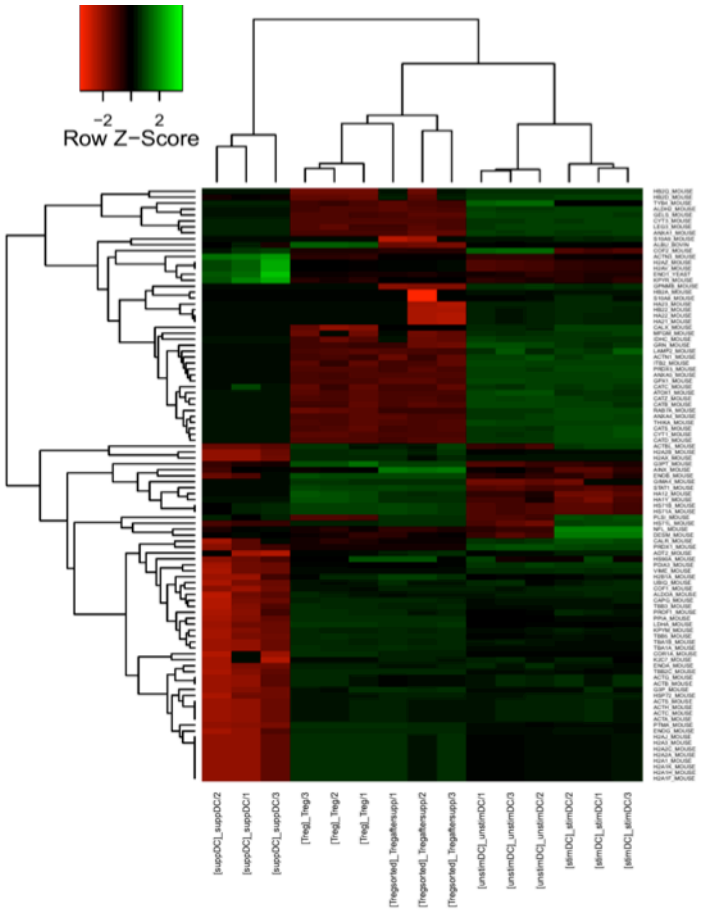
How does a cell react to a pharmaceutical compound?





100 most regulated proteins

Acknowledgements

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Petra Leidinger
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Prof. Paolo Macchi

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FZI Forschungs-Zentrum für Immuntherapie

FTN Forschungszentrum Translationale Neurowissenschaften Mainz

DFG Deutsche Forschungsgemeinschaft

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