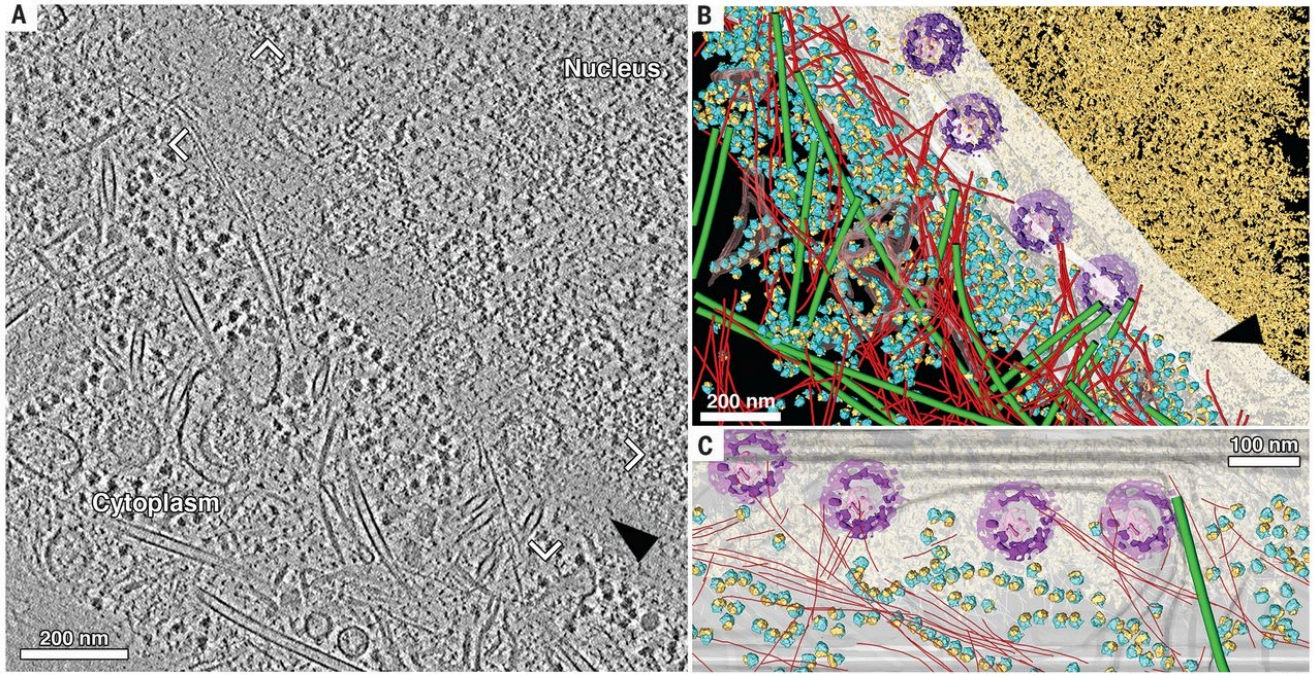
Chemo-proteomics

Target identification

Specificity profiling

Drug repurposing

The Needle in the Haystack



Mahamid *et. al. Science*, **351**, 969-972, (2016)

Porteomics

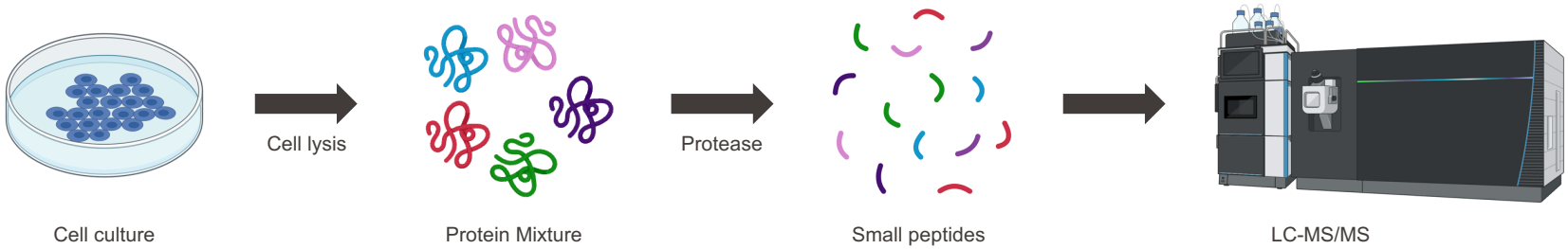
Nicolas Goldbach

How do you analyze all proteins in a cell at once?

Nicolas Goldbach

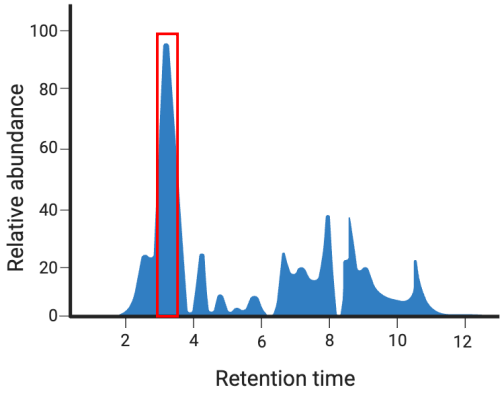
Bottom-up Proteomics

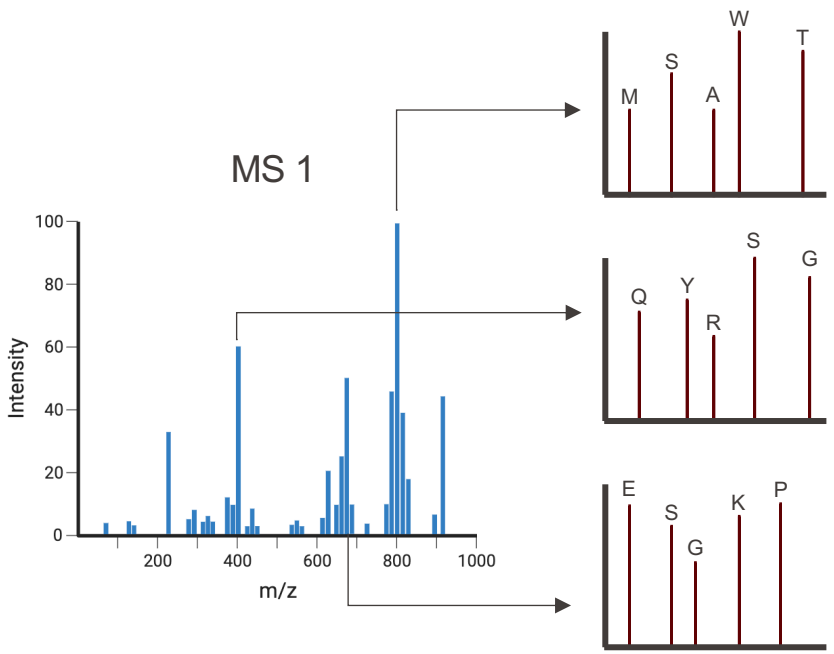
The science of turning an organized biological system into a soup of proteins.
(and sorting out the mess afterwards)

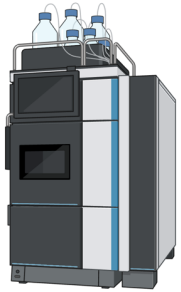


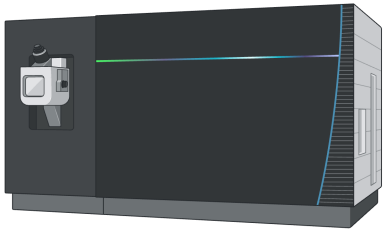
Nicolas Goldbach

LC-MS/MS



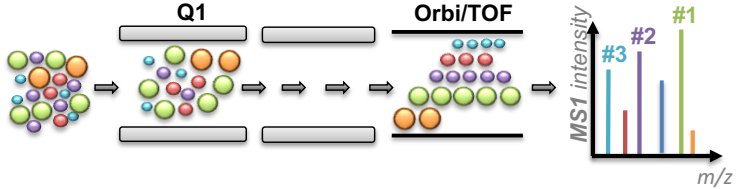






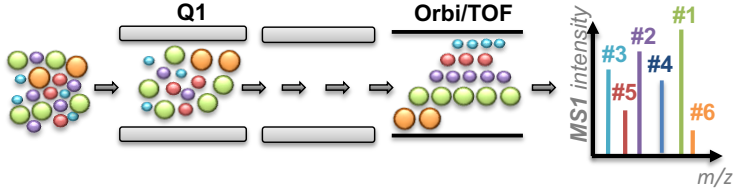
Data dependent acquisition

1. **Precursor (MS1) full scan:**



Data dependent acquisition

1. Precursor (MS1) full scan:



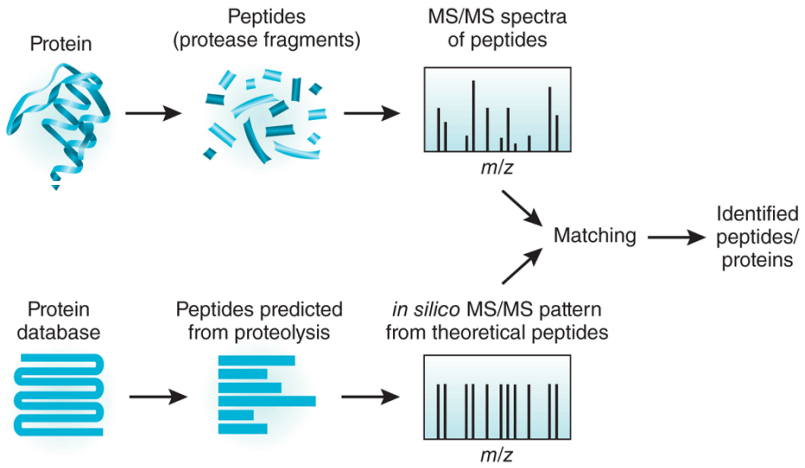
Exclusion list for 30 seconds:

m/z #1
m/z #2
m/z #3

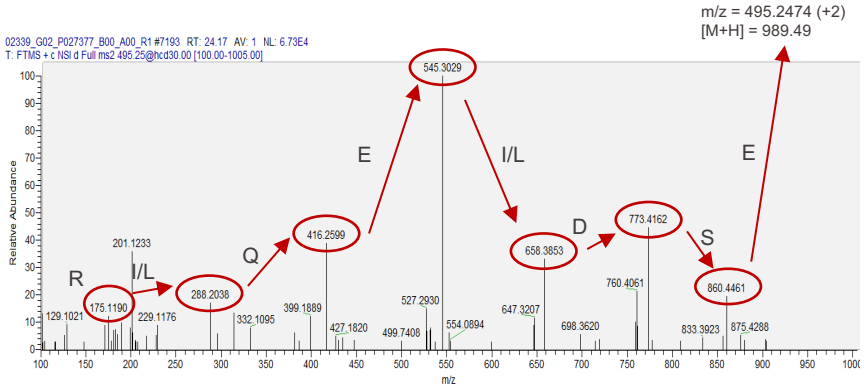
Nicolas Goldbach

Data analysis: Sorting out the mess we made

MS 1



MS 2



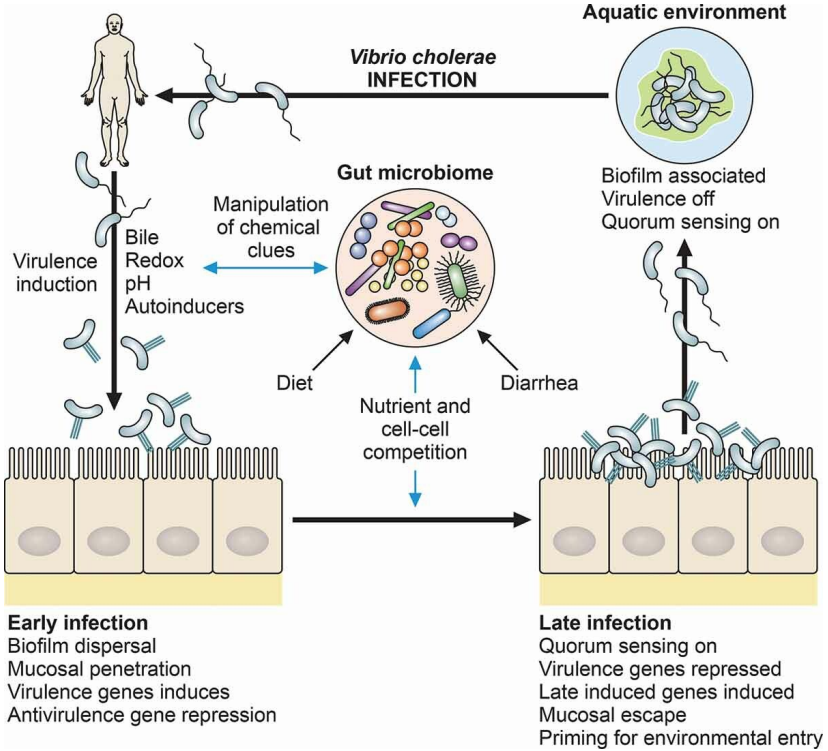
Nature Biotechnology **28**, 659–664 (2010)

Nicolas Goldbach

Vibrio cholerae

- Extracellular bacterium causing diarrhea
- Secretes serine hydrolases to shape ist intestinal niche

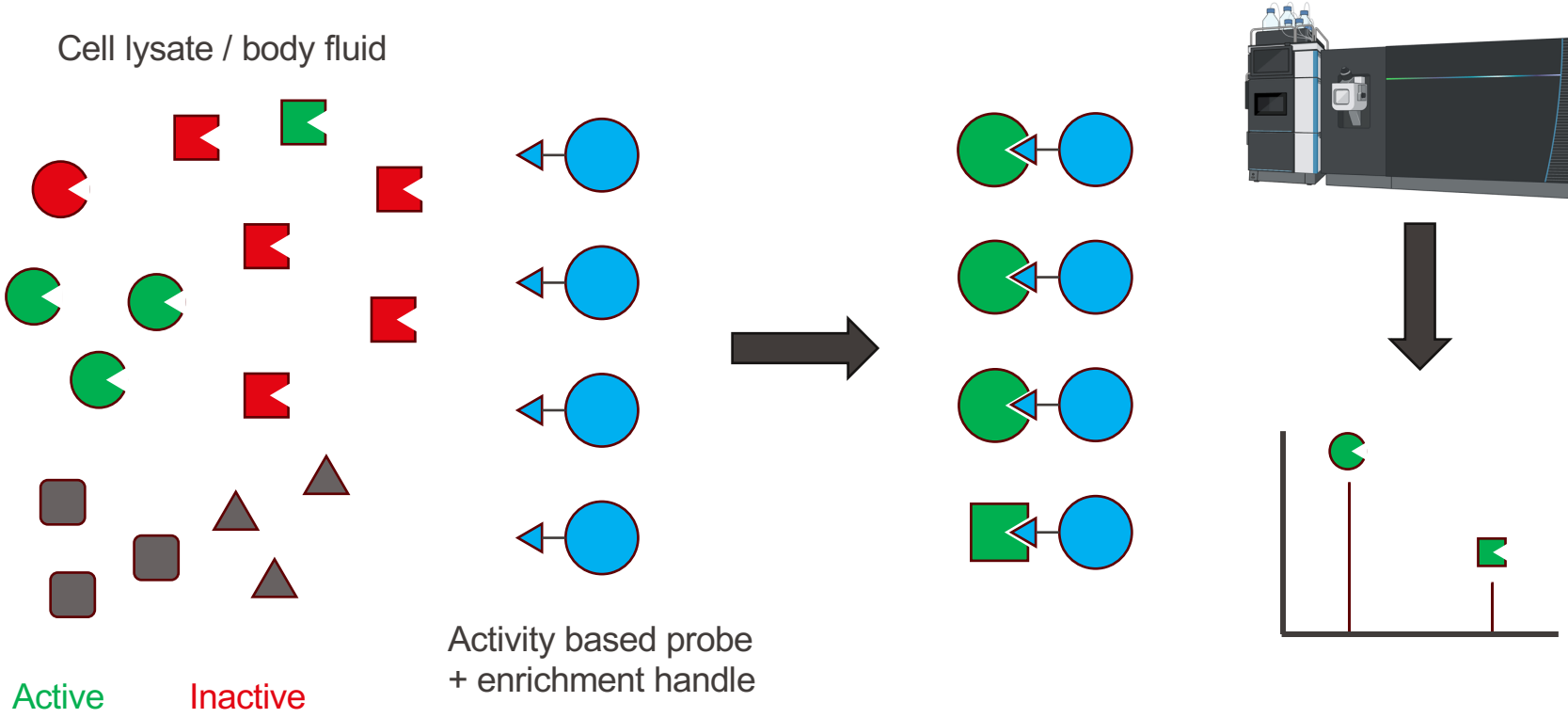
Which properties would you require to identify a hydrolase as a promising drug target?



Ansel Hsiao & Jun Zhu (2021), Virulence, 11:1, 1582-1599

Nicolas Goldbach

Activity-based Protein Profiling (ABPP)



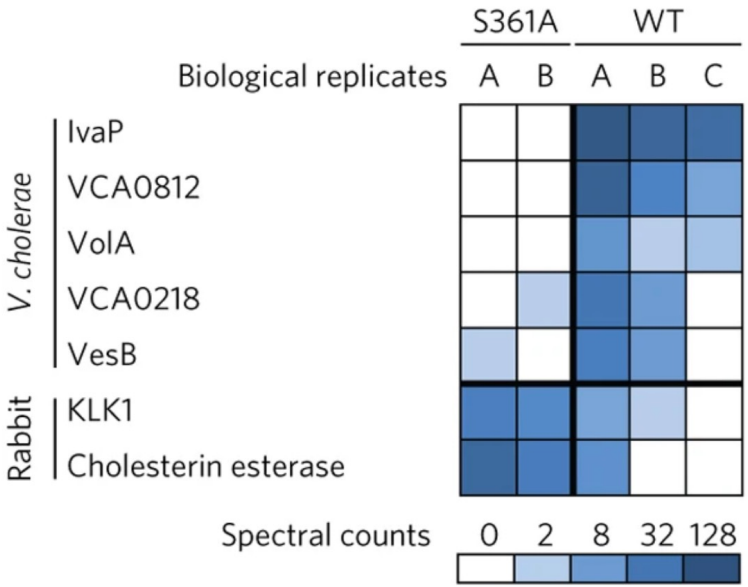
Nicolas Goldbach

V. Cholerae: Hydrolase targets

		Sample A[a]		Sample B		Sample C	
UniProt ID	***V. cholerae* Proteins in Rabbit Cecal Fluid**	**FPB**	**DMSO**	**FPB**	**DMSO**	**FPB**	**DMSO**
Q9KVI8	Alkaline serine protease IvaP (VC0157)	76	0	128	0	30	0
Q9KLE3	Serine protease VesA (VCA0803)	13	0	4	0	6	0
Q9KSQ6	Trypsin-like serine protease VesB (VC1200)	12	0	7	0	4	0
Q9KLD4	Leucine aminopeptidase-related protein VCA0812	6	0	8	0	8	0
Q9KUF5	Protease DO VC0566	23	0	4	0	0	0
P01556	Cholera enterotoxin subunit B ctxB	4	0	2	0	0	0
Q9KMV0	Thermolabile hemolysin VCA0218	8	0	0	0	7	0
Q9KUB9	Putative uncharacterized protein VC0603	11	0	0	0	0	0
Q9KLG1	Arylesterase VCA0783	6	0	0	0	0	0
Q9KQJ6	Putative uncharacterized protein VC2002	4	0	0	0	0	0
Q9KUG7	Protease, insulinase family VC0554	4	0	0	0	0	0
P01555	Cholera enterotoxin subunit A ctxA	3	0	0	0	0	0
P15493	Lipase lipA	0	0	5	0	0	0
Q9KLP3	Chitodextrinase VCA0700	0	0	0	0	2	0

Nature Chemical Biology **12**, 268–274 (2016)

IvaP: key modulator of infection



Nature Chemical Biology **12**, 268–274 (2016)

Drug Discovery

Nicolas Goldbach

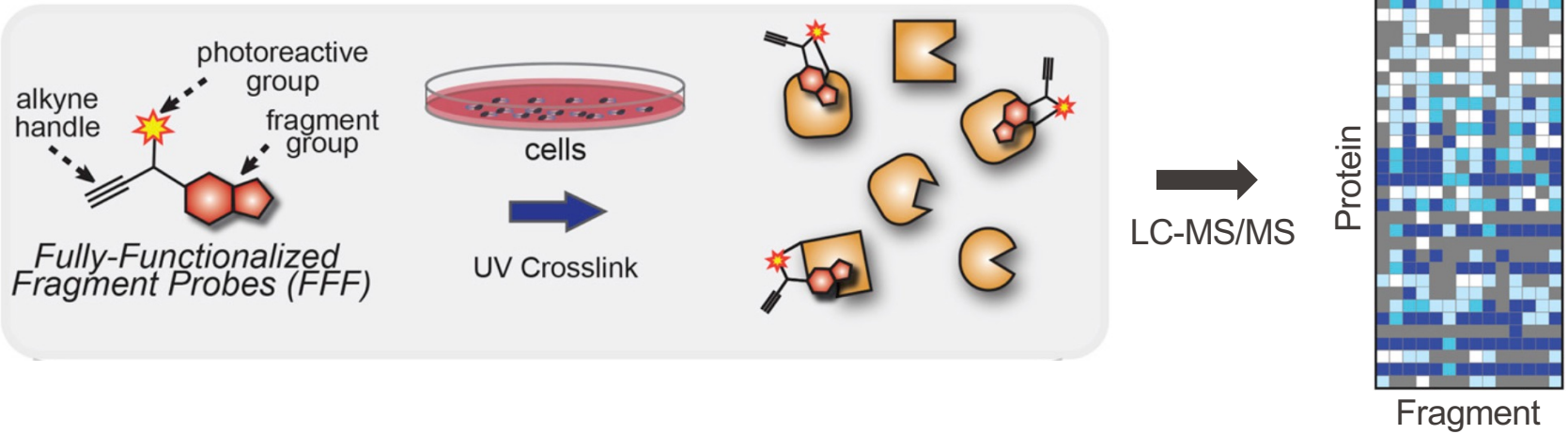
After identifying a promising drug target, how can we quickly identify a starting compound for preclinical research?

We can screen known drugs (or fragments thereof)
for binding to our target.

Nicolas Goldbach

Proteomics aided Drug Discovery

Can we search for drug-like fragments in a proteome-wide manner to identify drug candidates?

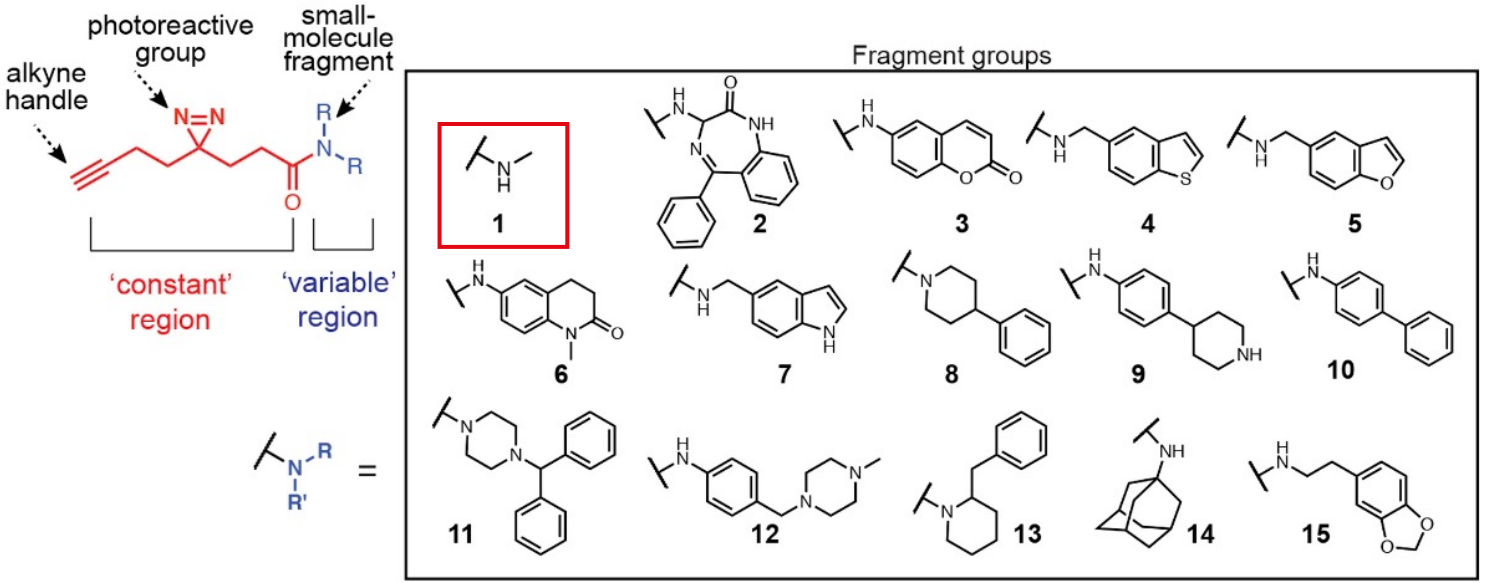


Cell **2017**, *168*, 527.

Why do you need to crosslink the fragment probes with the proteins?

To identify transient interactions, which would be disrupted during enrichment

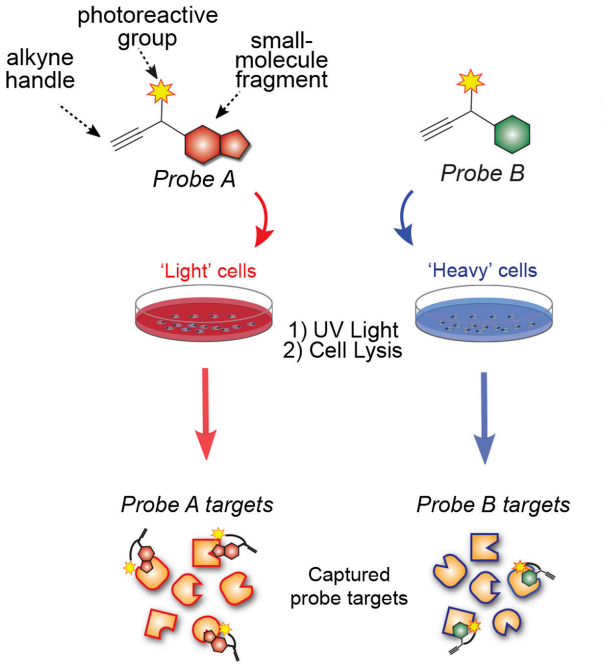
Proteomics aided Drug Discovery

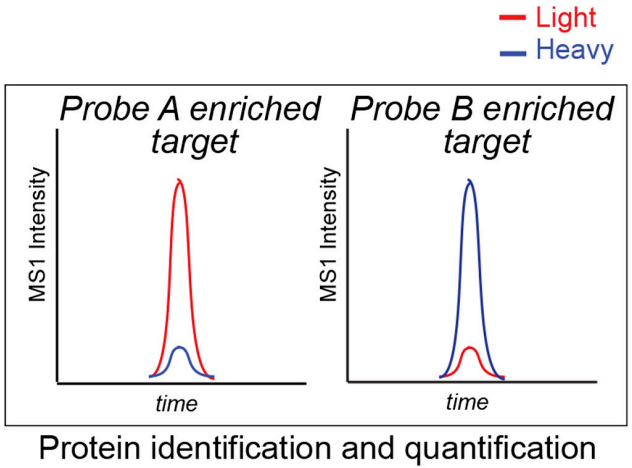


Cell **2017**, *168*, 527.

SILAC

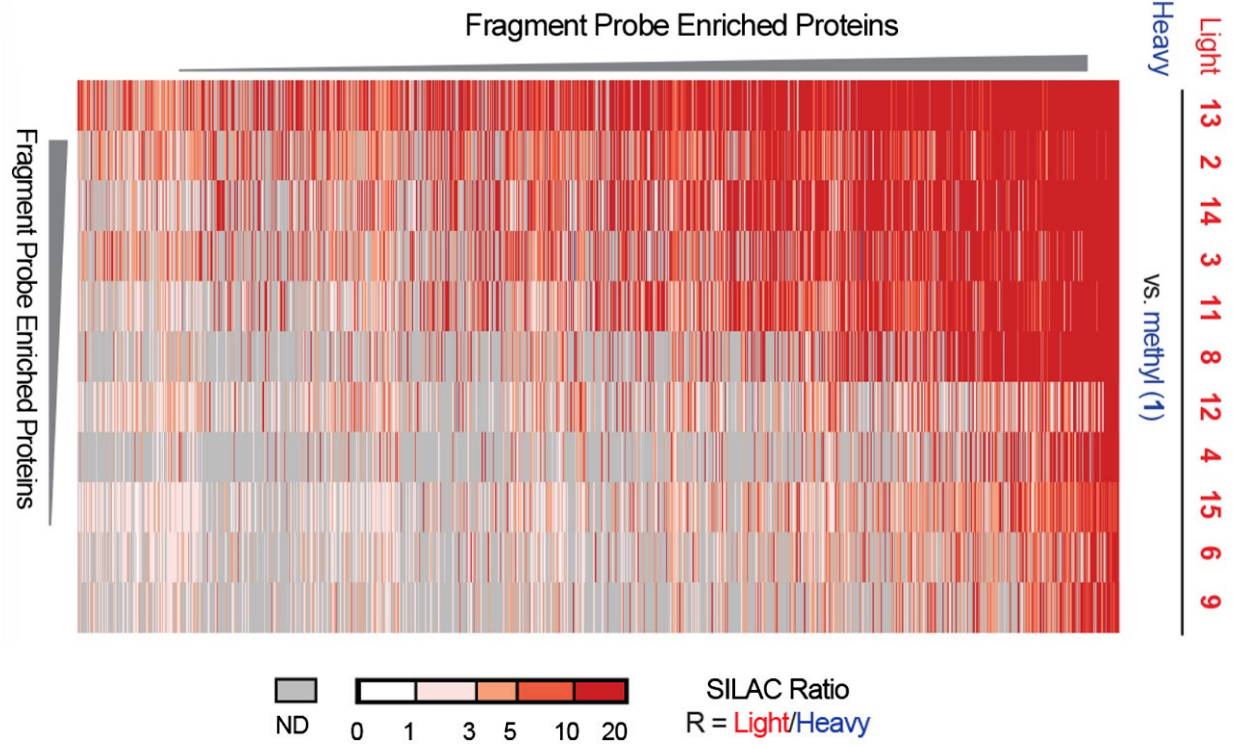
stable isotope labeling by/with amino acids in cell culture





Protein identification and quantification

Targets enriched from HEK293T cells



Cell **2017**, *168*, 527.

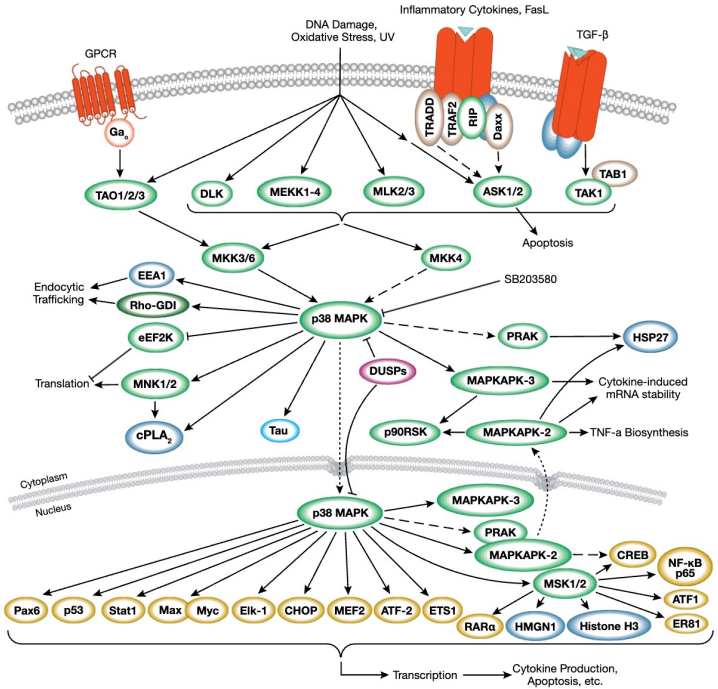
Which information is crucial for early stage drug development?
(beside proof that the molecule binds the drug target)

We need to identify potential off-targets to
determine the specificity of the candidate!

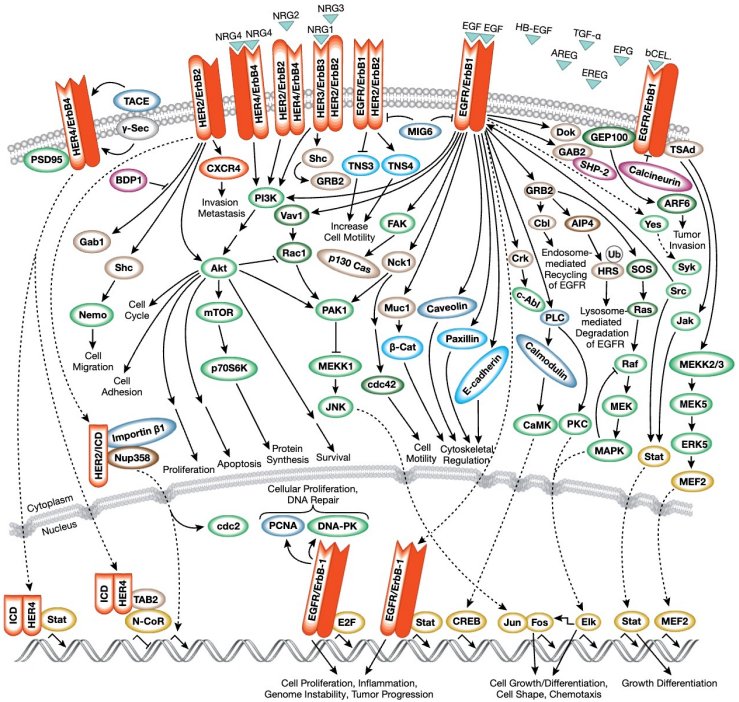
Nicolas Goldbach

Kinases:
Key players of cellular signal transduction

p38 MAPK



ErbB signaling

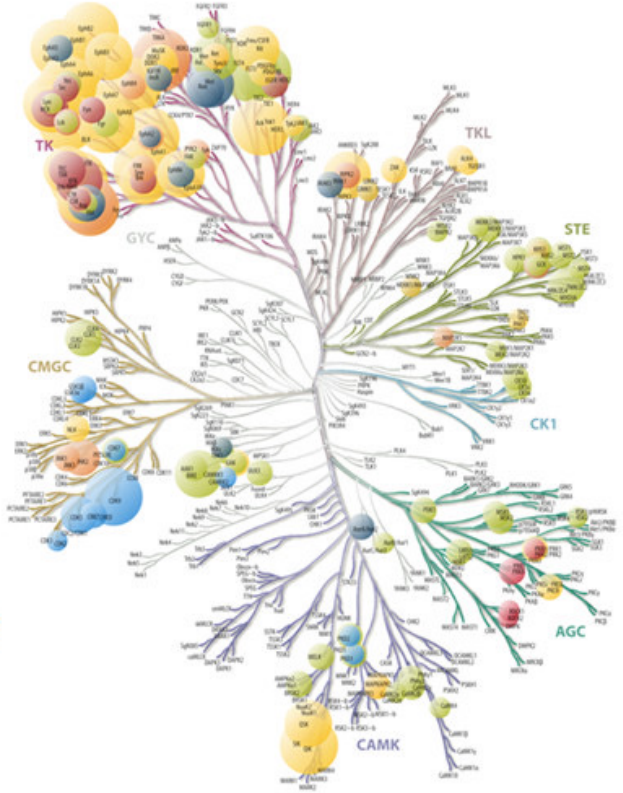


Kinase

Kinase Inhibitors

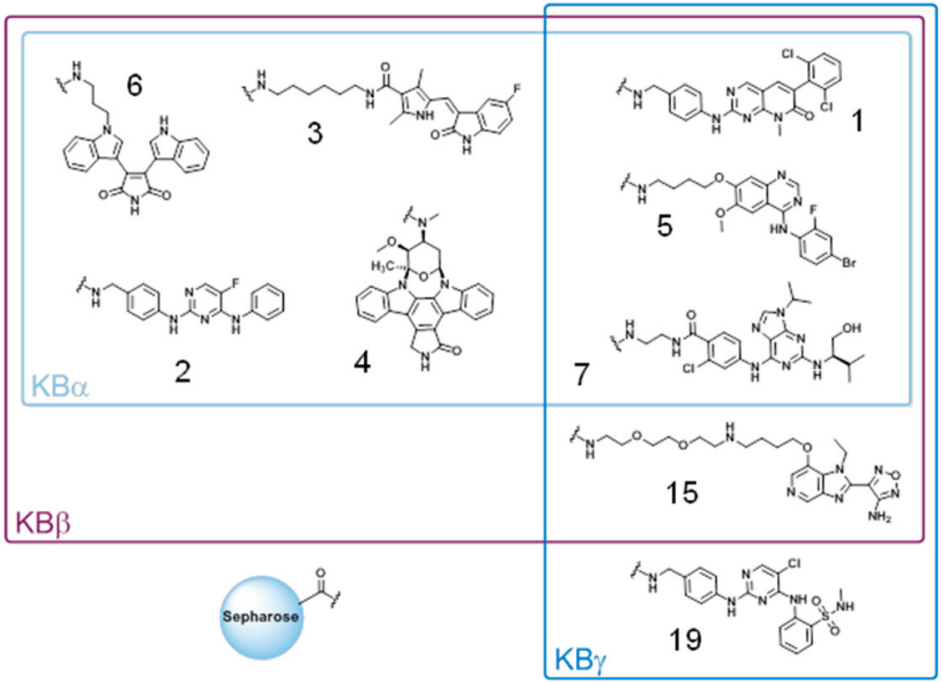
- 50% of human kinases are linked to diseases
- About 120 kinase inhibitors approved to date, 30% R&D costs spent on kinase inhibitors
- Kinase inhibitors usually target the conserved ATP pocket causing severe side effects

We need a fast and reliable method to profile the target landscape of kinase inhibitors



Nicolas Goldbach

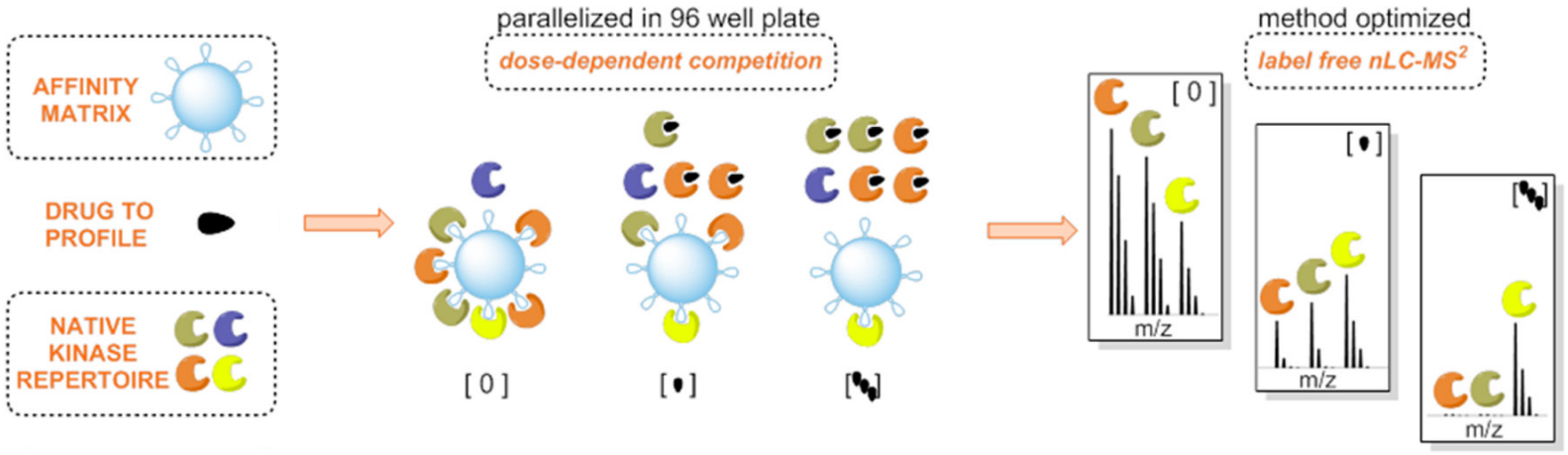
Kinobeads:
affinity matrix to enrich the “kinome”



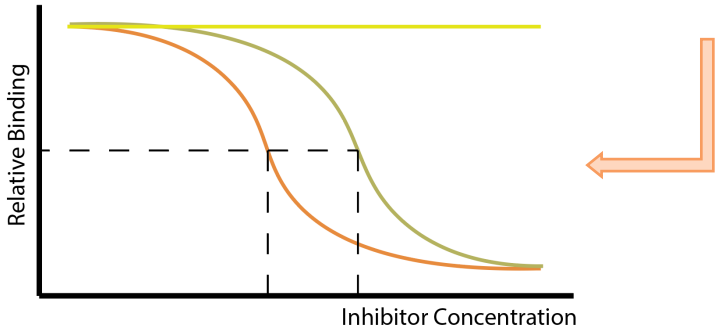
~ 260 kinases

Medard et. al. JPR 2015

Selectivity Profiling with Kinobeads



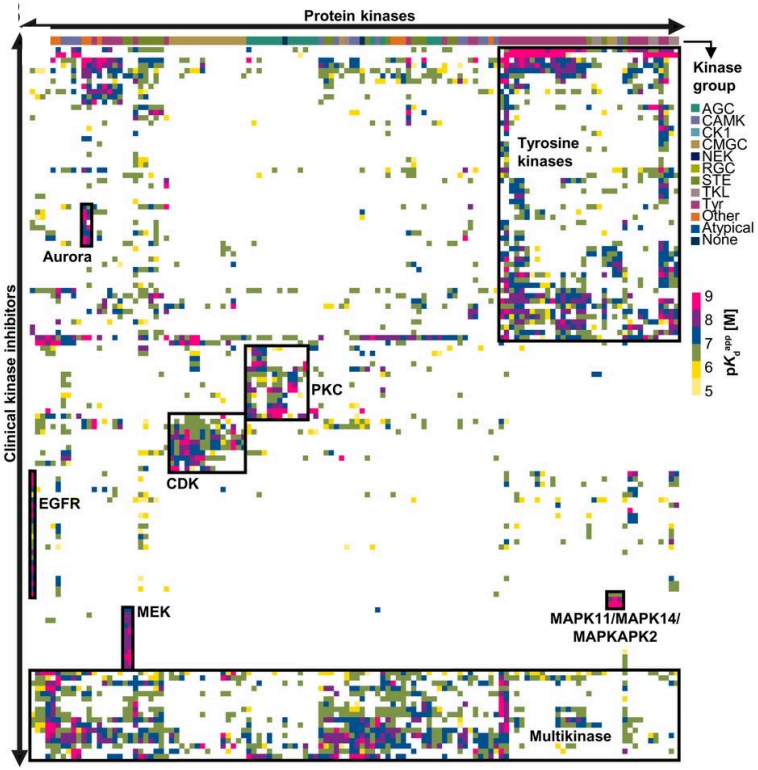
- Label-free
- Natively expressed kinases
- Cellular context

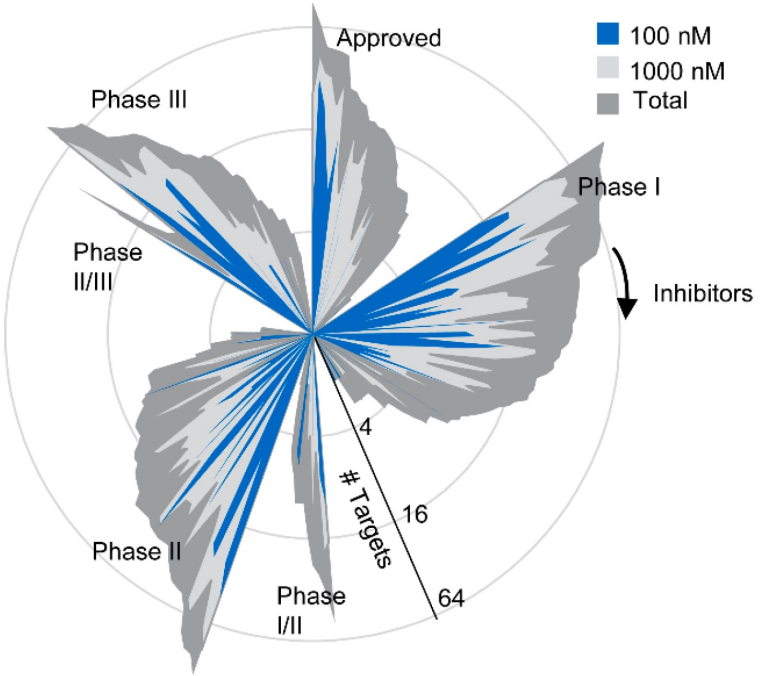


Medard et. al. JPR 2015

Nicolas Goldbach

Selectivity Profiling with Kinobeads

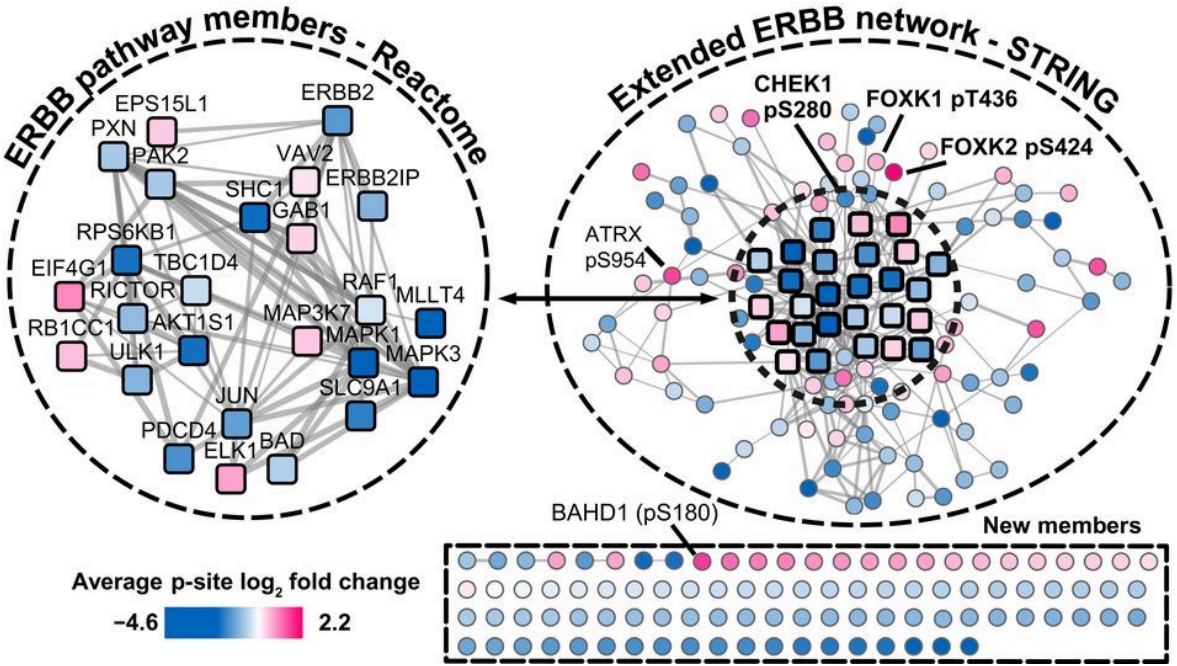




Science, **2017**, 358, 6367

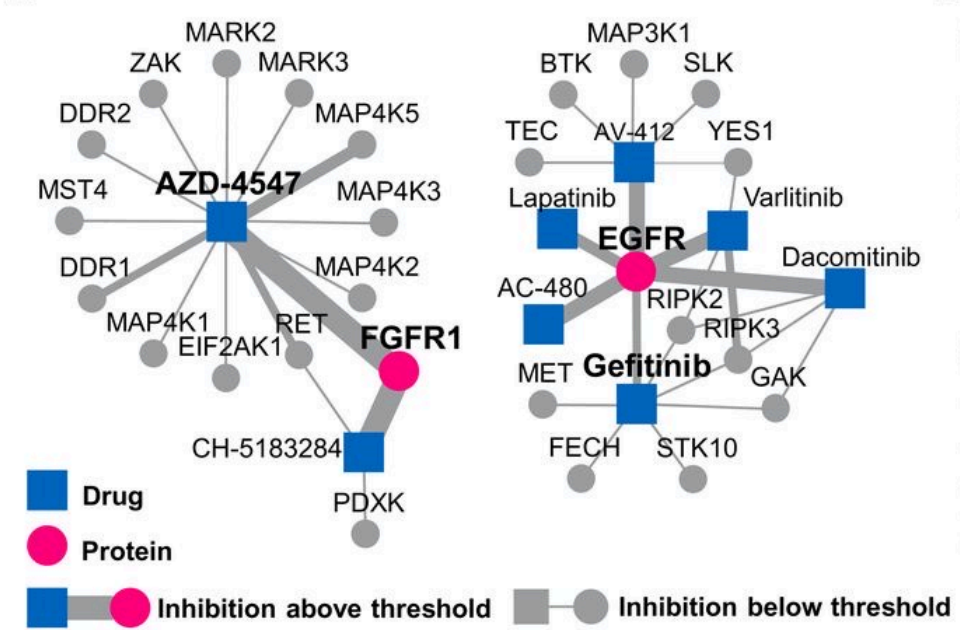
Nicolas Goldbach

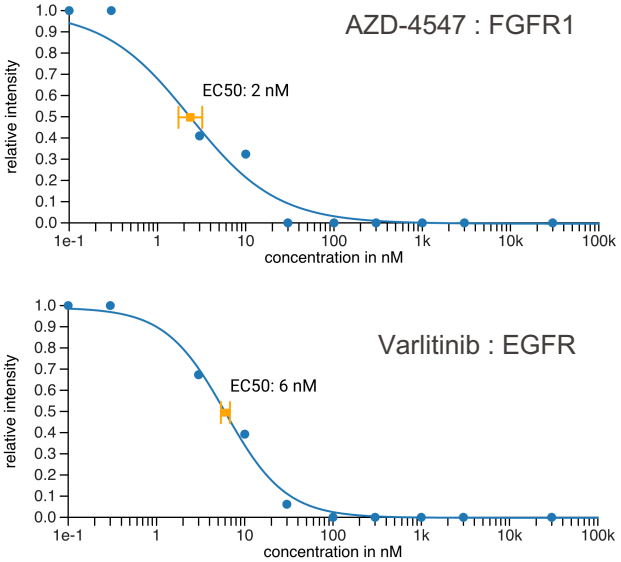
Unravelling signaling pathways: Phospho-Proteomics



Science, **2017**, 358, 6367

Data Accessability: ProteomicsDB





Science, **2017**, 358, 6367 | www.proteomicsdb.org