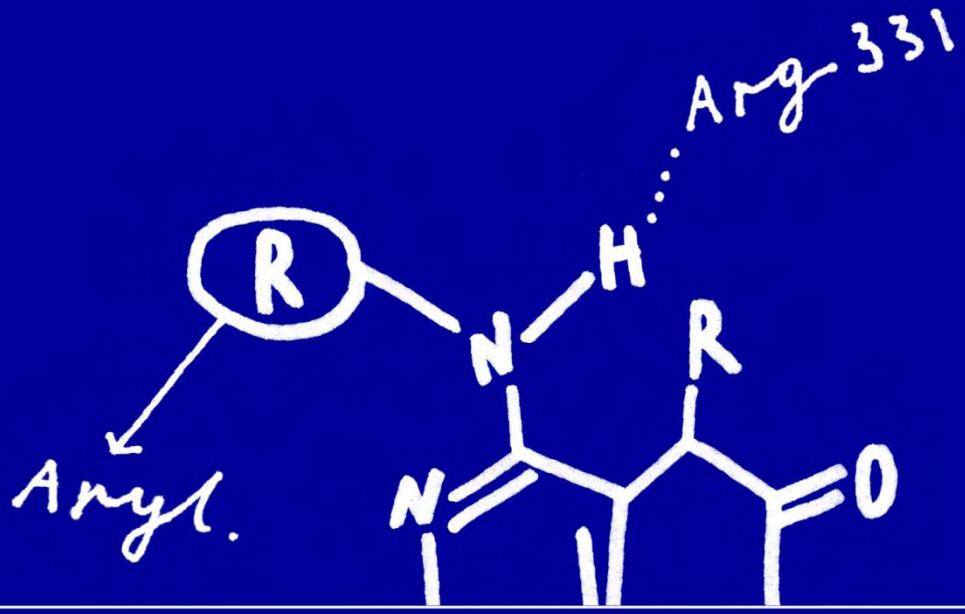
evotec

'RESEARCH NEVER STOPS'
Building innovative drug discovery alliances

EVOTEC MUNICH

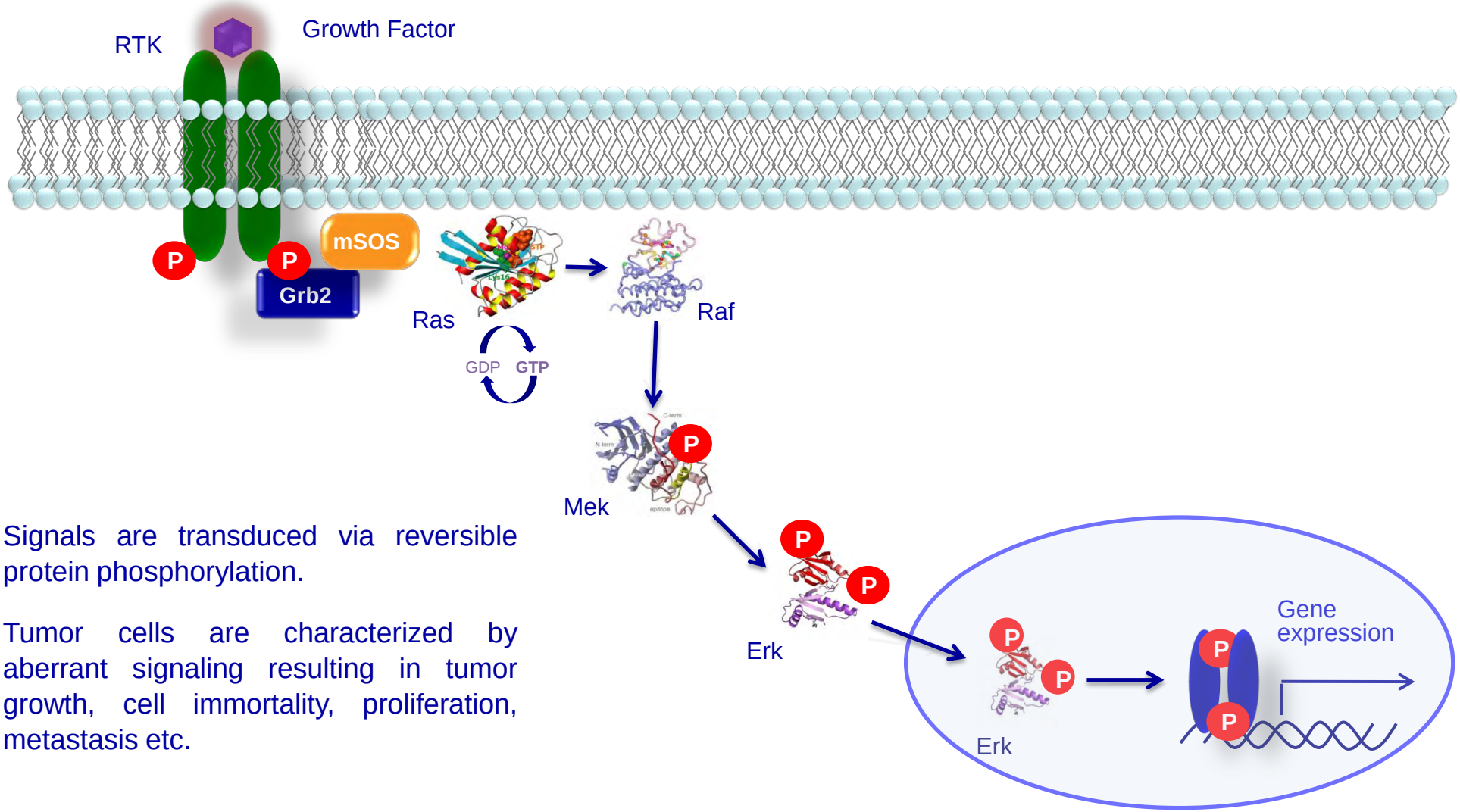
Chemical proteomics and quantitative phosphoproteomics to discover novel mechanisms of action of the approved targeted drug Sorafenib



Evotec AG, May 21st 2012

Evotec Munich

Targeted Drugs Interfere with Signal Transduction



Signals are transduced via reversible protein phosphorylation.

Tumor cells are characterized by aberrant signaling resulting in tumor growth, cell immortality, proliferation, metastasis etc.

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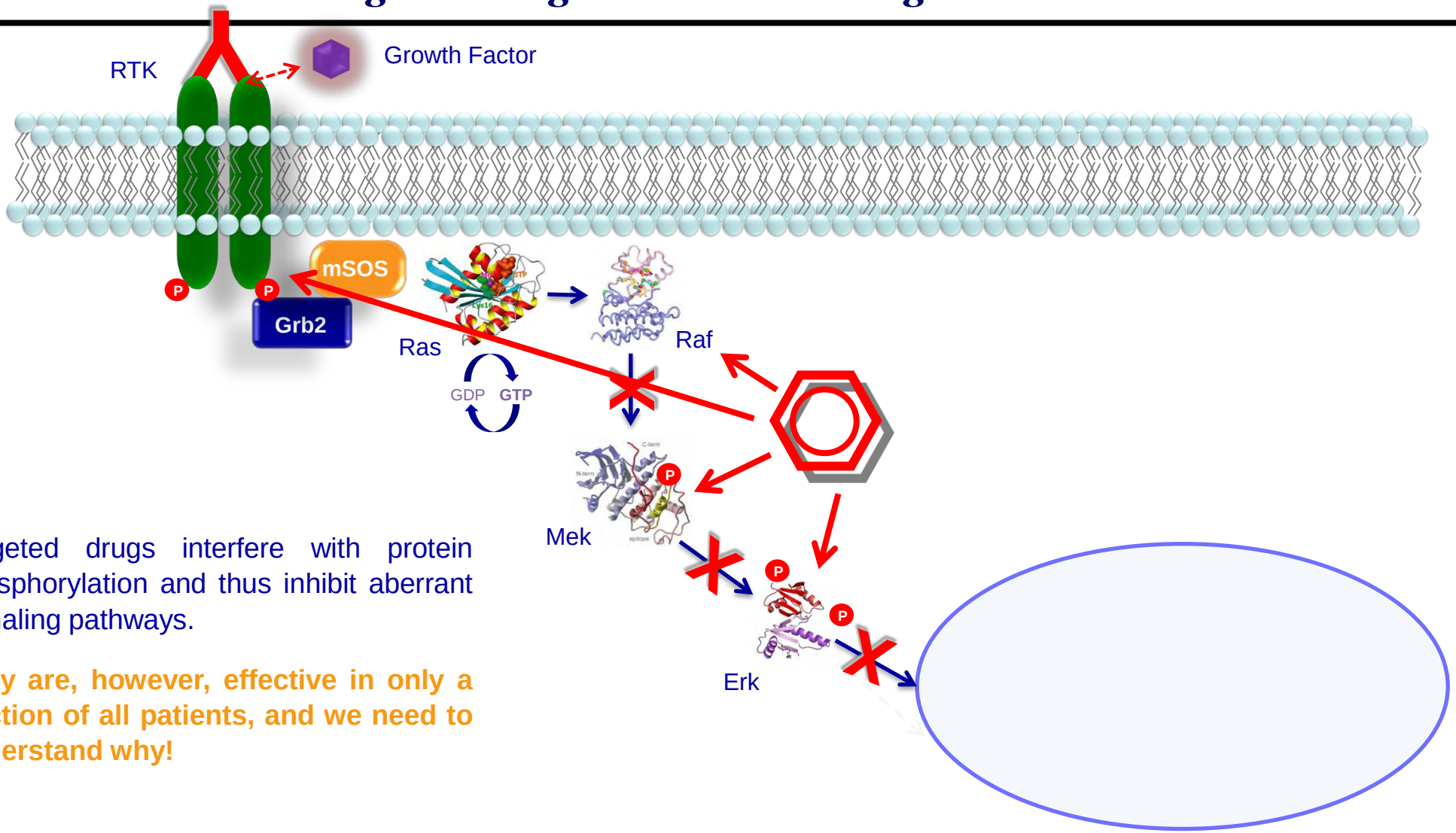
Targeted Drugs Interfere with Signal Transduction

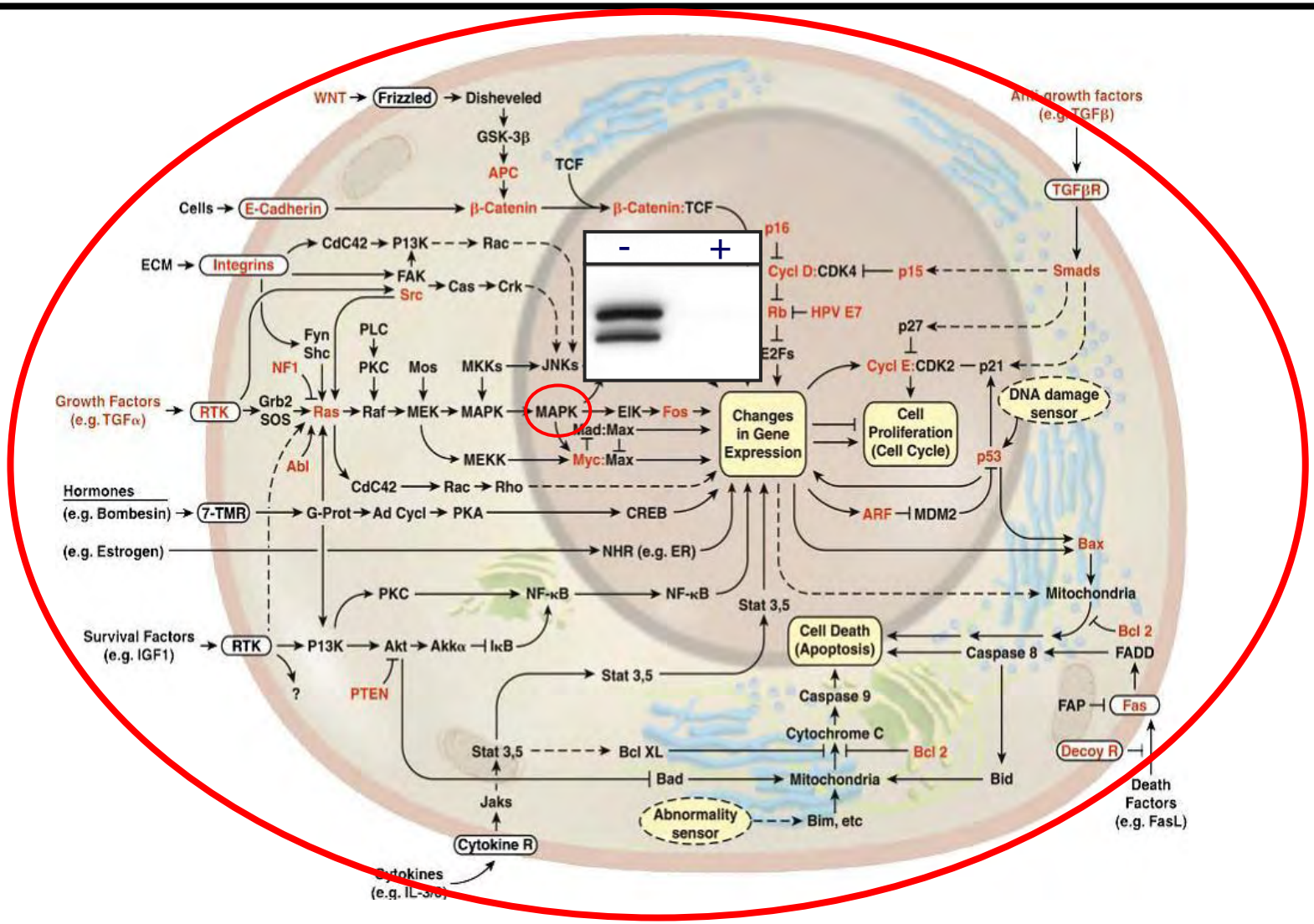


Targeted drugs interfere with protein phosphorylation and thus inhibit aberrant signaling pathways.

They are, however, effective in only a fraction of all patients, and we need to understand why!

A Global Understanding of Biological Systems

… by applying mass spectrometry based proteomics



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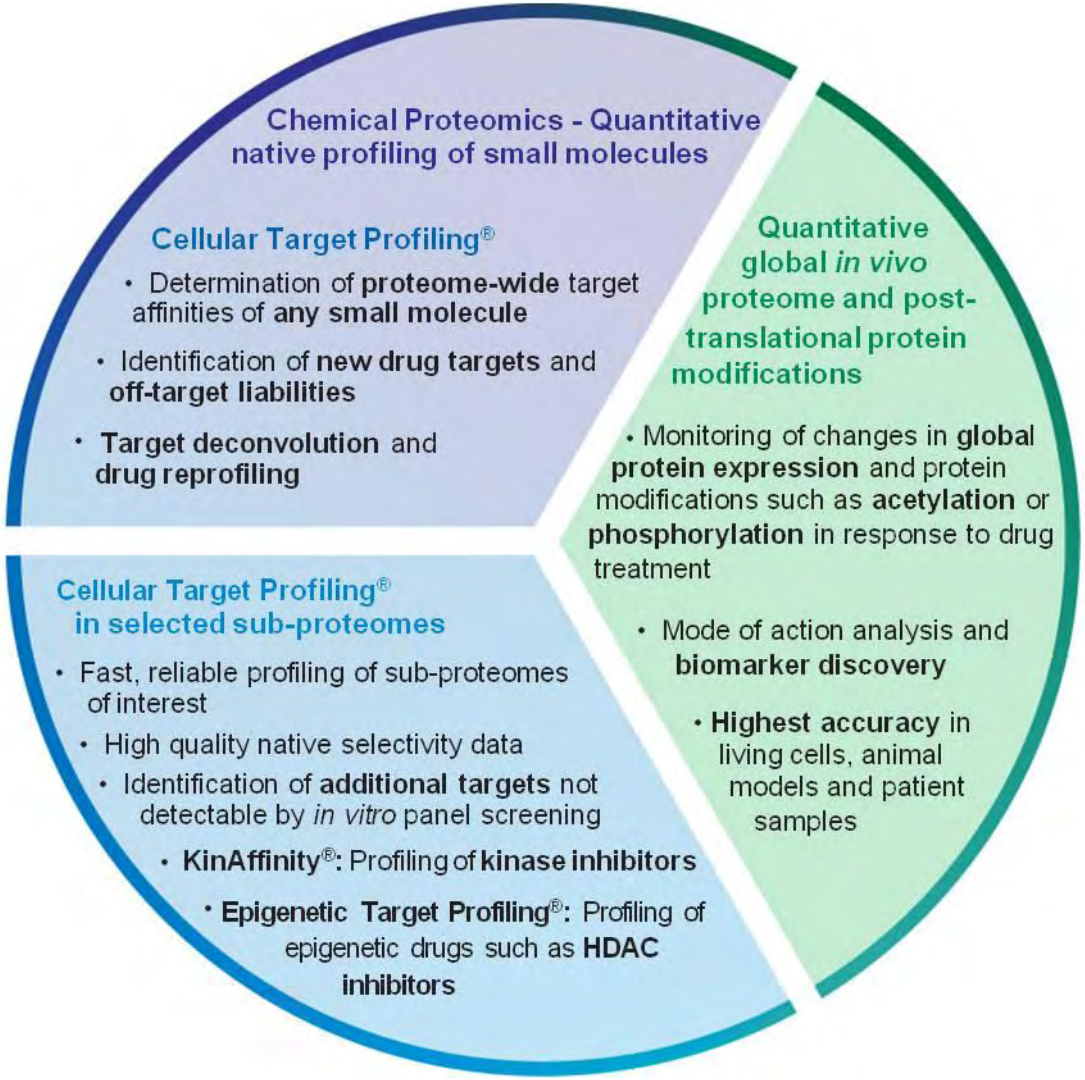
Technology Offering



About Evotec Munich

A leader in chemical proteomics and phosphoproteomics

Evotec Munich

Prof. Dr. Axel Ullrich, Max-Planck Director

- **Evotec's Center of Excellence for Proteomics and Oncology**
- Emerged from Kinaxo Biotechnologies, a Max Planck spin-off founded by the renowned cancer researcher Prof. Axel Ullrich
- Combines highest service quality standards with powerful technological innovation
- Collaborates with leading academic research laboratories including the Matthias Mann lab at the Max Planck Institute
- Has worked with numerous global pharma and biotechnology companies such as

Johnson & Johnson, Daiichi-Sankyo, Roche, Bristol-Myers Squibb, UCB, AstraZeneca, Eisai, Takeda Pharma, Boehringer Ingelheim, Bayer HealthCare

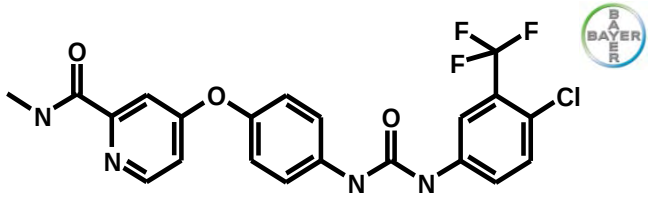
Overview

- Identification of the Target Profile of Sorafenib using Cellular Target Profiling
- Phosphoproteomics applied to cultured cell lines (Sorafenib Case Study)
- Outlook

Case Study Sorafenib

Rationale

Sorafenib (Nexavar®)



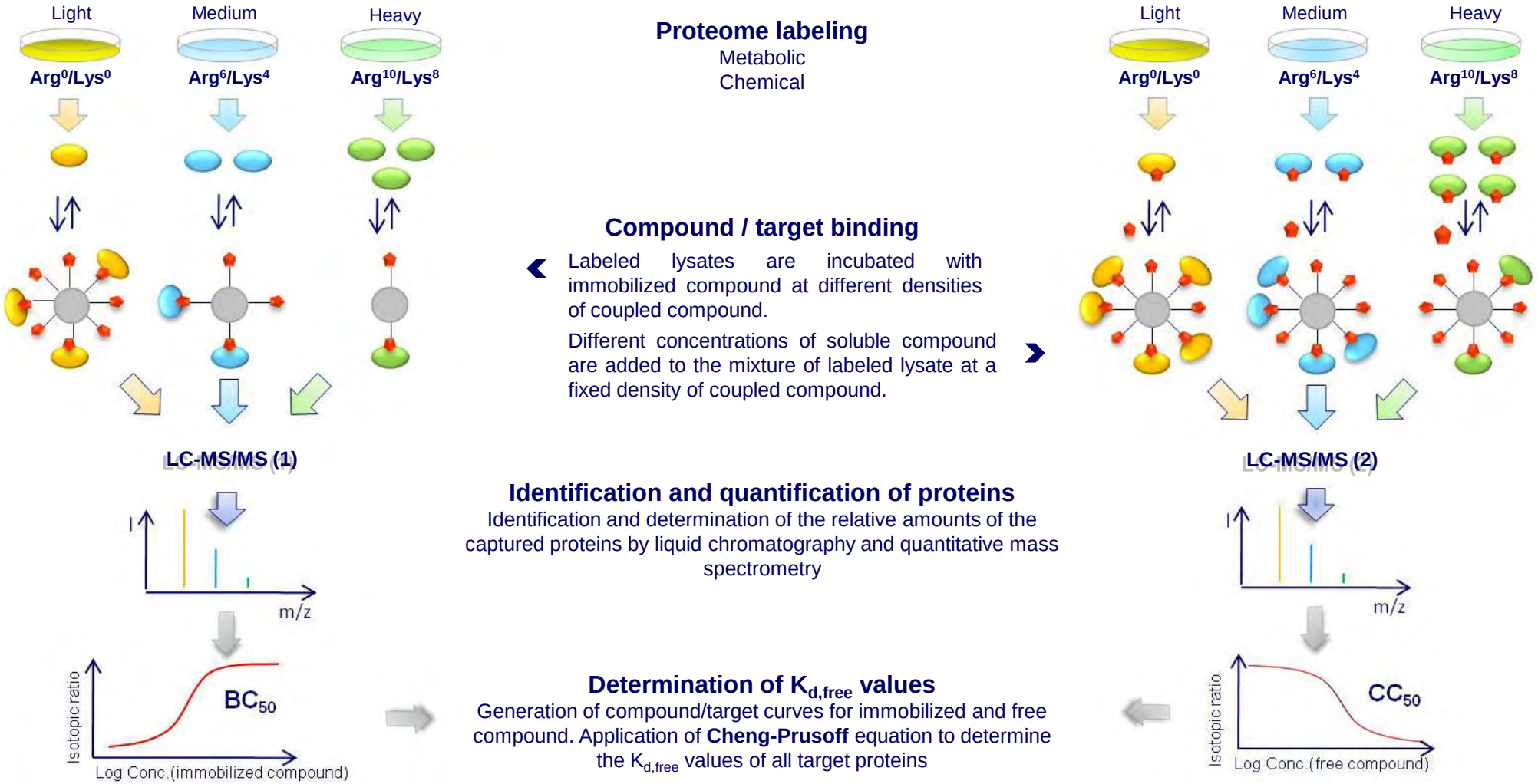
- Multi-kinase inhibitor (known targets are bRAF, VEGFR, RET, FLT3)
- Strong anti-proliferative and anti-angiogenic effects
- Approved for treatment of renal-cell carcinoma and hepatocellular carcinoma; shows promising activity in several different cancer types
- Human prostate cancer cells (PC3) are sensitive to sorafenib treatment, even though this effect cannot be explained by inhibition of the reported main targets

↓

Sorafenib's mode-of-action remains unclear in PC-3 cells

Cellular Target Profiling®

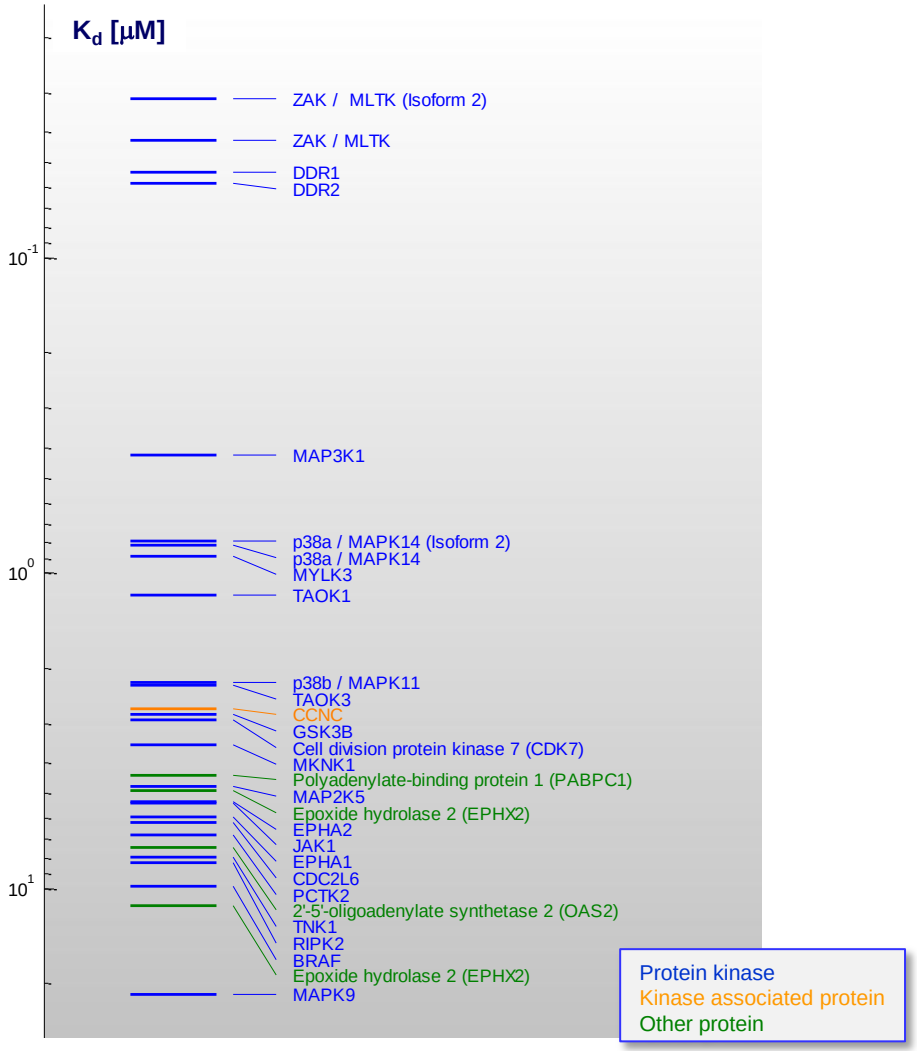
Workflow



Sharma et al., Nat Methods. 2009 Oct;6(10): 741-4
Patent Protection EP2045332B1

Cellular Target Profiling®

Target Profile of Sorafenib in PC3 cells

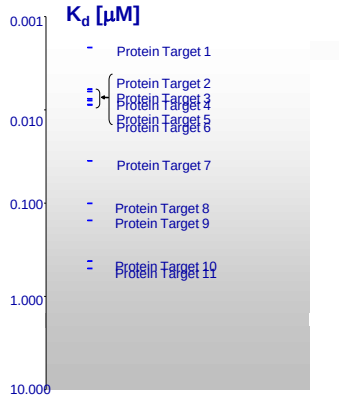


- ZAK, DDR1, DDR2, MAP3K1, MAPK14/p38α and MYLK3 bind Sorafenib with affinities better than 1µM in PC3 cells
- These kinases modulate a wide range of cellular responses such as **apoptosis, cell migration** or **cell proliferation** and might therefore contribute to the drug's effects in PC3 cells

Cellular Target Profiling - Phosphoscout

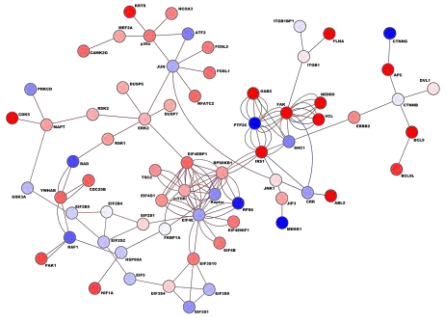
From Target Profile to the Mode-of-Action

- **Cellular Target Profiling** → Target Profile



Impact on Signaling Pathways?

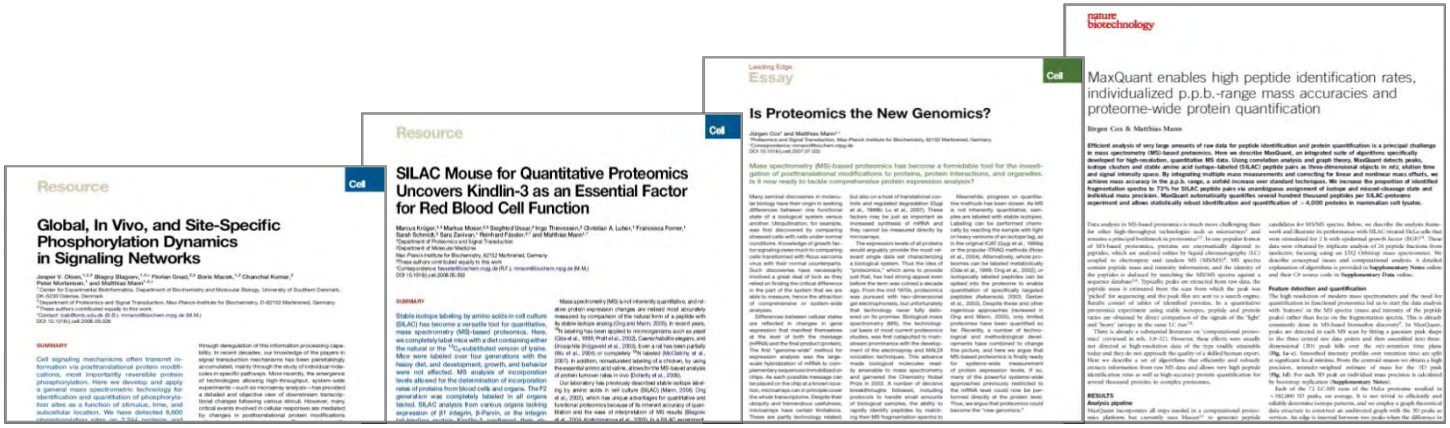
- **PhosphoScout** → Mode-of-Action analysis



PhosphoScout®

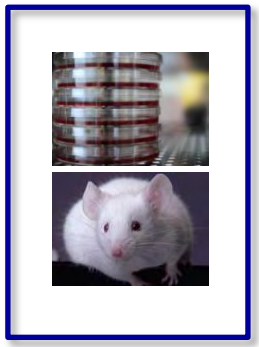
Global quantitative phosphoproteomics

- Global, unbiased and quantitative method to monitor dynamic phosphorylation events for systematic understanding of cellular behavior
- Reproducible quantification of >15.000 phosphorylation events in a single experiment
- Identification and quantification of phosphorylation sites in living cells, animal models and patient samples
- **Mode of action analysis** of targeted cancer drugs and **biomarker discovery**

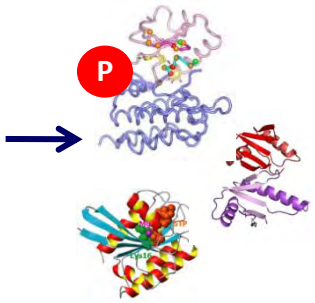


PhosphoScout®

Global Quantitative Phosphoproteomics Workflow

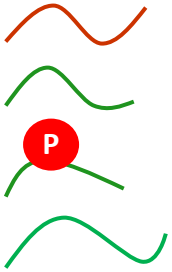


Isobaric labeling
(SILAC)

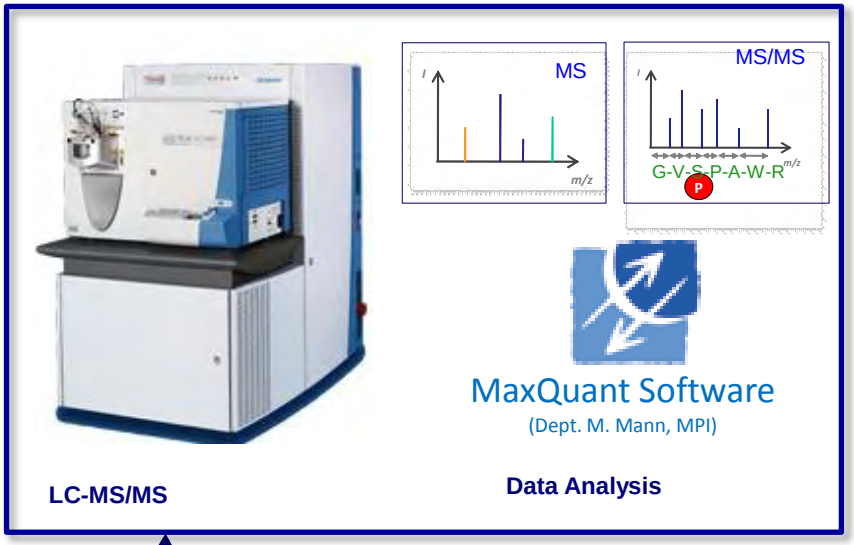


Proteins

enzymetic cleavage



Peptides



LC-MS/MS

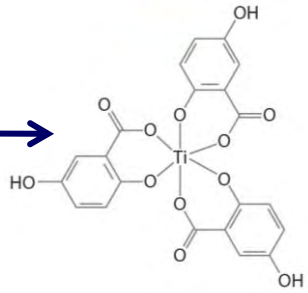
Data Analysis

Unbiased, global quantitative phosphoproteome analysis

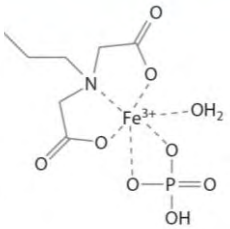
Global phosphoproteome enrichment

SCX

TiO2–DHB complex

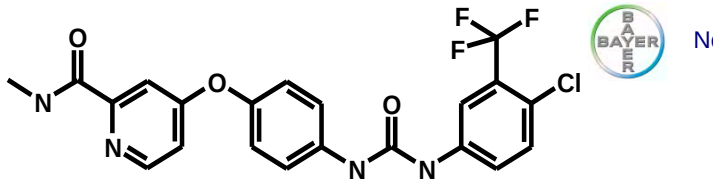


IMAC-Fe3+ phosphate complex



Case Study Sorafenib

Quantitative Phosphoproteomics Workflow

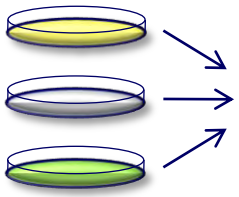


Nexavar®

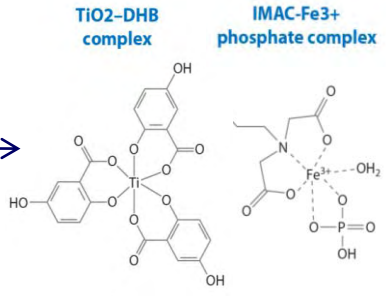
Control | *Treated (30 min)* | *Treated (90 min)*

Arg^0/Lys^0 | Arg^6/Lys^4 | Arg^{10}/Lys^8

SILAC labeled PC-3 cells

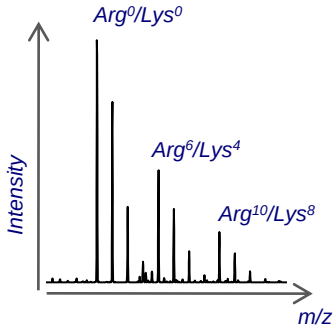


Protein extraction and subsequent enzymatic cleavage



Global phosphopeptide enrichment using inorganic affinity chromatography materials

Unbiased quantitative phosphoproteome analysis by LC-MS/MS



Mass spectrometry- based quantification

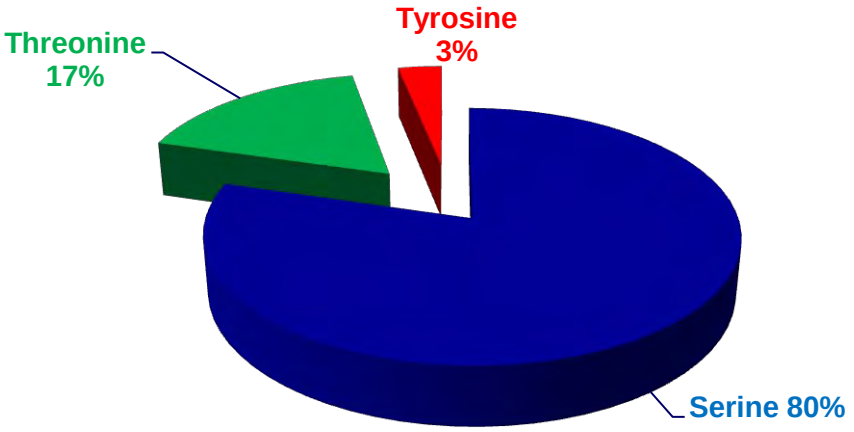
Case Study Sorafenib

Identification of Regulated Phosphorylation Sites

	All P-sites	Kinases
No. of detected phosphorylation sites	15,825	961
No. of detected proteins with phosphorylation sites	3,931	228
No. of regulated sites	1,012	68
No. of proteins with regulated phosphorylation sites	605	40

- Only phosphorylation sites that could be localized within the peptide sequence with high confidence are considered in our analysis
- Identification of differentially regulated phosphorylation sites at a false discovery rate of 5% based on a global rank test

Zhou *et al.*, Bioinformatics 23 (2007) 2073



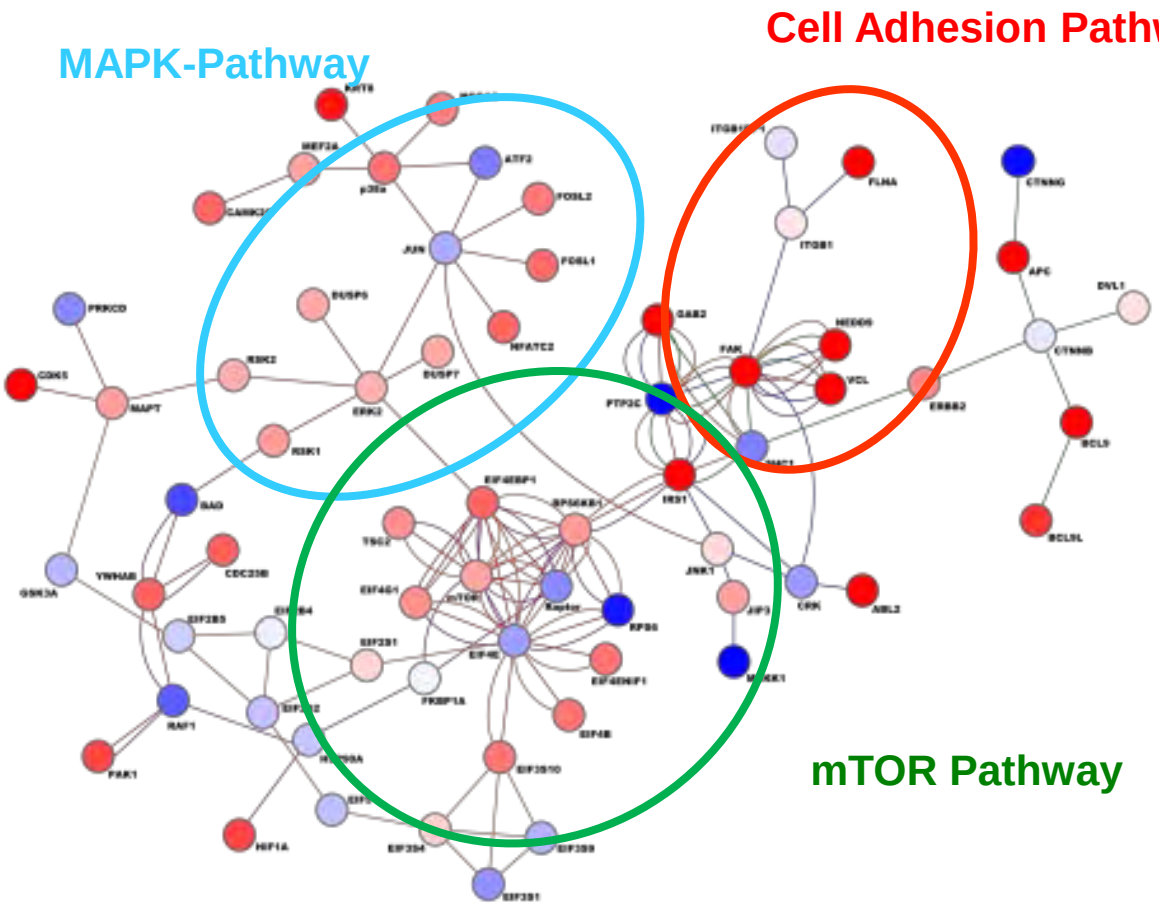
Case Study Sorafenib

Affected Signaling Pathways

KEGG Pathway	Proteins with detected P-sites	Proteins with regulated P-sites
Insulin signaling pathway	52	19
MAPK signaling pathway	74	21
mTOR signaling pathway	22	9
ErbB signaling pathway	40	13
Axon guidance	34	10
Prostate cancer	21	7
Non-small cell lung cancer	17	6

Case Study Sorafenib

Integrating data with protein-protein networks



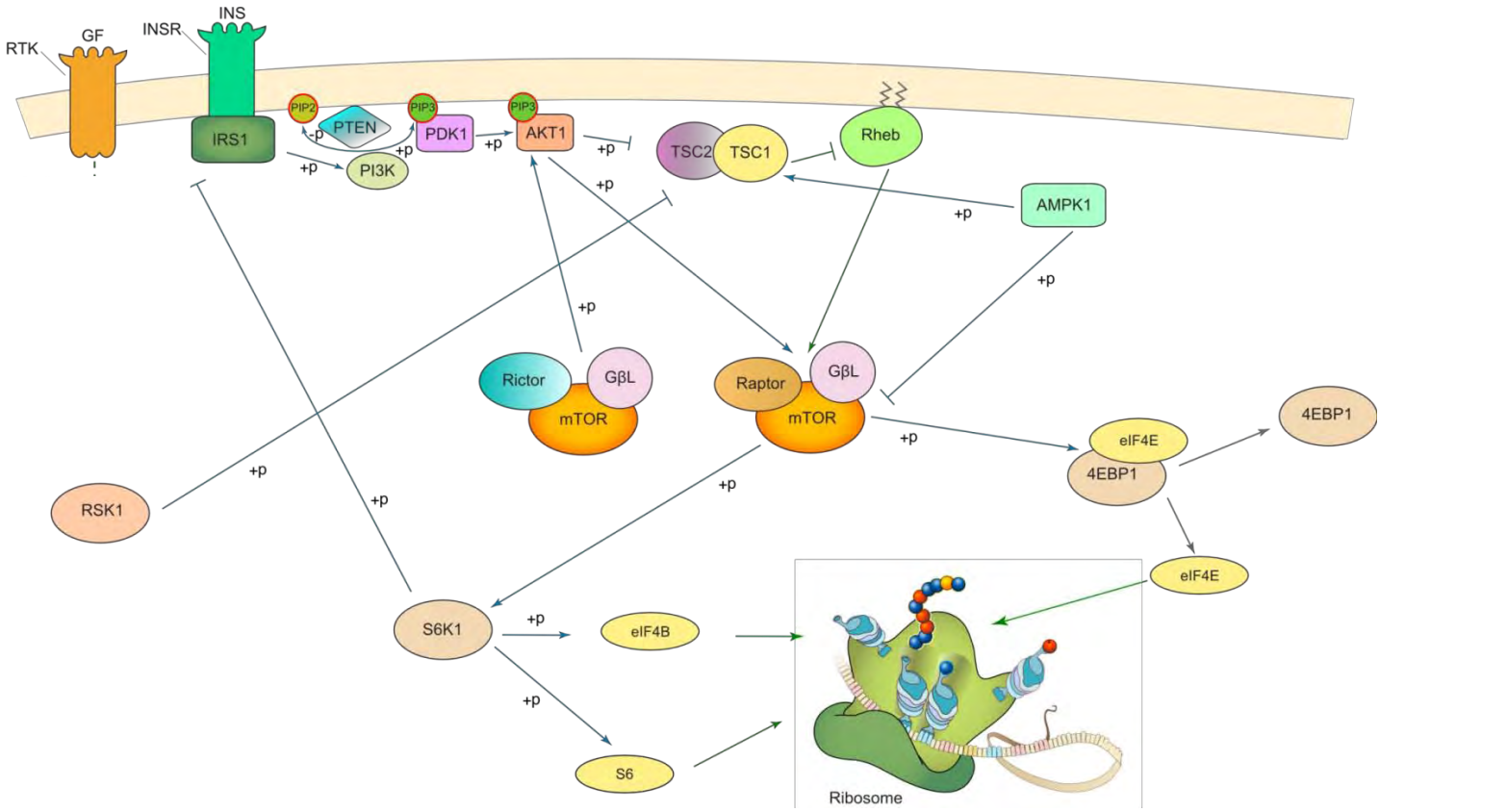
- The „SubExtractor“ algorithm combines phosphoproteomic data with information from STRING
- Identification of differentially regulated subnetworks and individual proteins in a biological context

Klammer *et al*., BMC Bioinformatics, 2010

Case Study Sorafenib

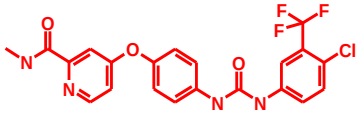
Detailed visualization of regulated phosphosites

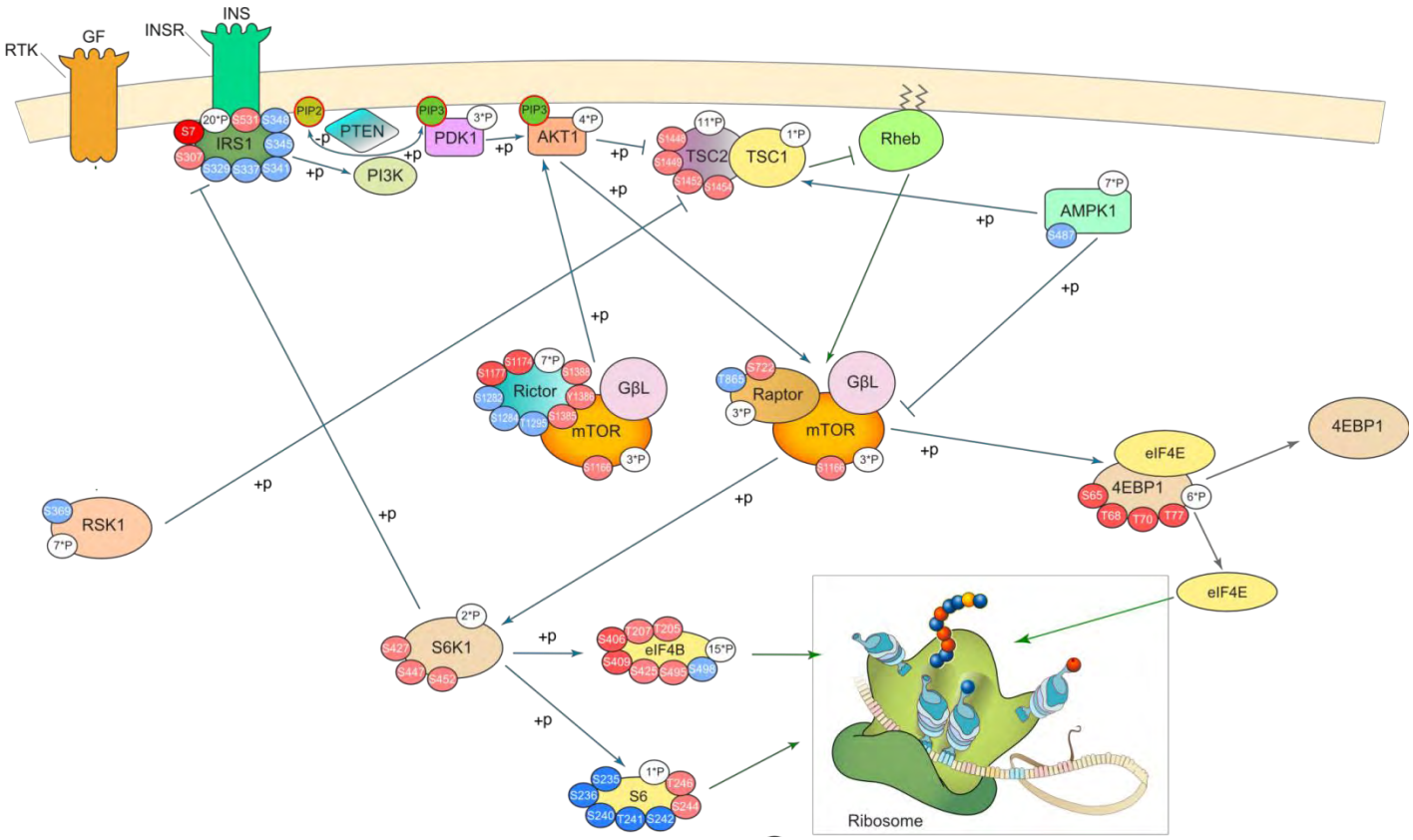
PC3 cells with PTEN mutation → activated PI3K/Akt/mTOR-pathway

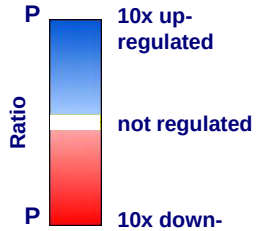


Case Study Sorafenib

Detailed visualization of regulated phosphosites



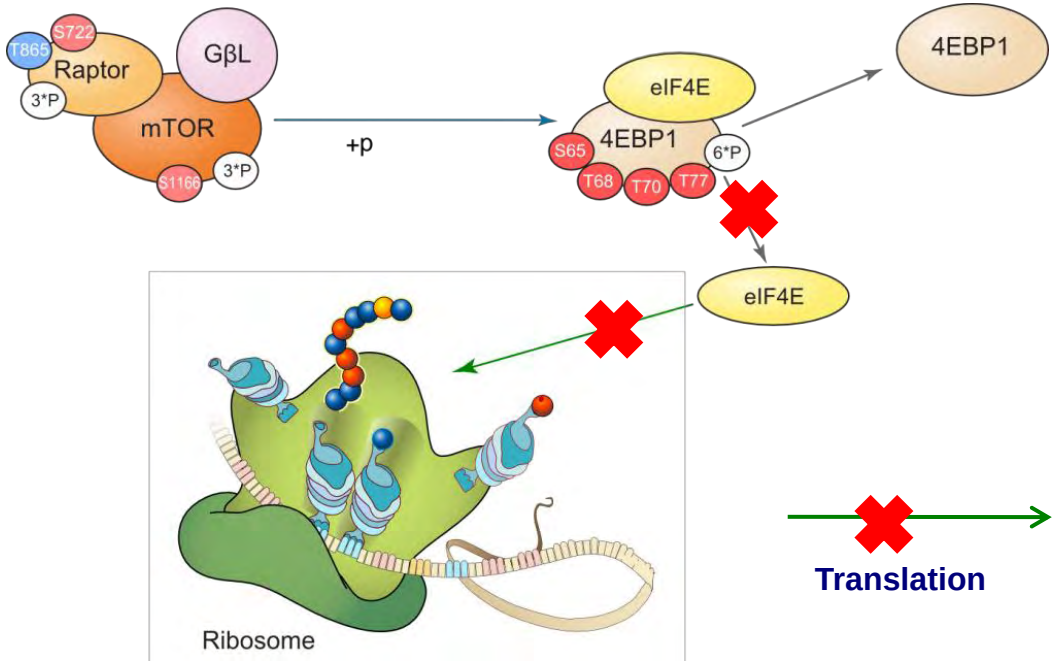




Translation

Case Study Sorafenib

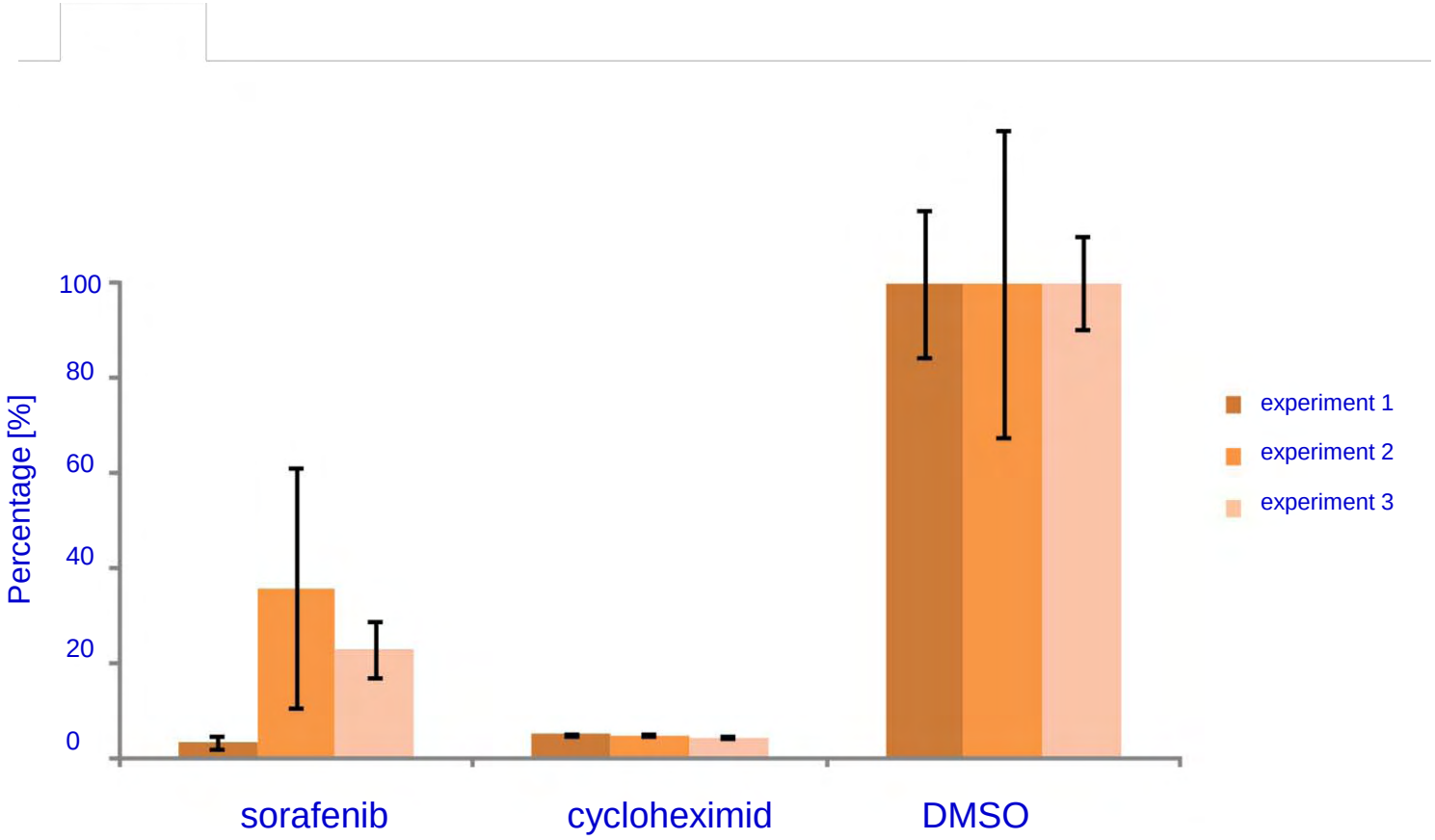
Detailed visualization of regulated phosphosites

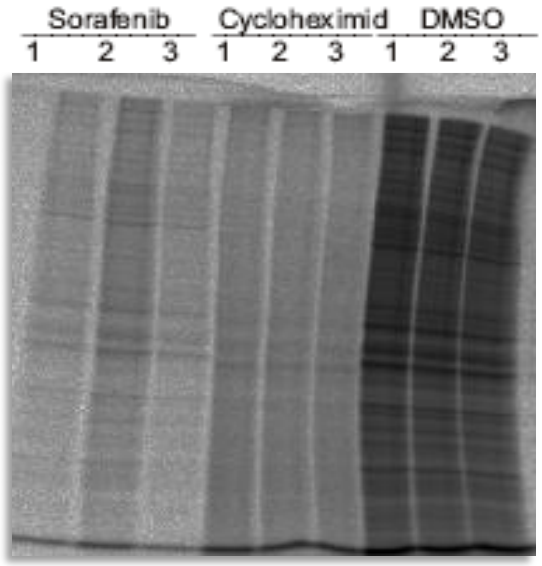


Case Study Sorafenib

Validation of sorafenib's effect on translation

PC3 - 30min S^{35} pulse after treatment with sorafenib, cycloheximid or DMSO for 90min





Case Study Sorafenib

Summary

Phosphoproteomics analysis of Sorafenib

- Statistically validated and reproducible information about the drug's mode of action
- Revealed previously unknown inhibition of the mTOR pathway
- Might lead to additional therapeutic applications with a better understanding drug efficacy, resistance mechanisms etc.

Biomarker discovery for sorafenib

KINAXO Biotechnologies and Bayer Vital GmbH Collaborate in Phosphoproteomics Biomarker Identification

Martinsried, Leverkusen, Germany, October 15, 2009. KINAXO Biotechnologies GmbH and Bayer Vital GmbH announced today that they will enter into collaboration. KINAXO will apply its quantitative phosphoproteomics technology PhosphoScout® for the identification of novel biomarkers in a clinical trial conducted by Bayer Vital.

KINAXO's phosphoproteomics platform allows annotation and quantification of regulated phosphorylation sites. Since the majority of targeted compounds used as anti-cancer drugs influence cellular signal transduction pathways, analysis of phosphorylation patterns in relation to drug administration reveals a compound's molecular mode of action. Characteristic phosphorylation sites predicting response to treatment, resistance mechanism or synergistic effects can hereby be identified as biomarkers which allow for personalized treatment plans.

Biomarkers

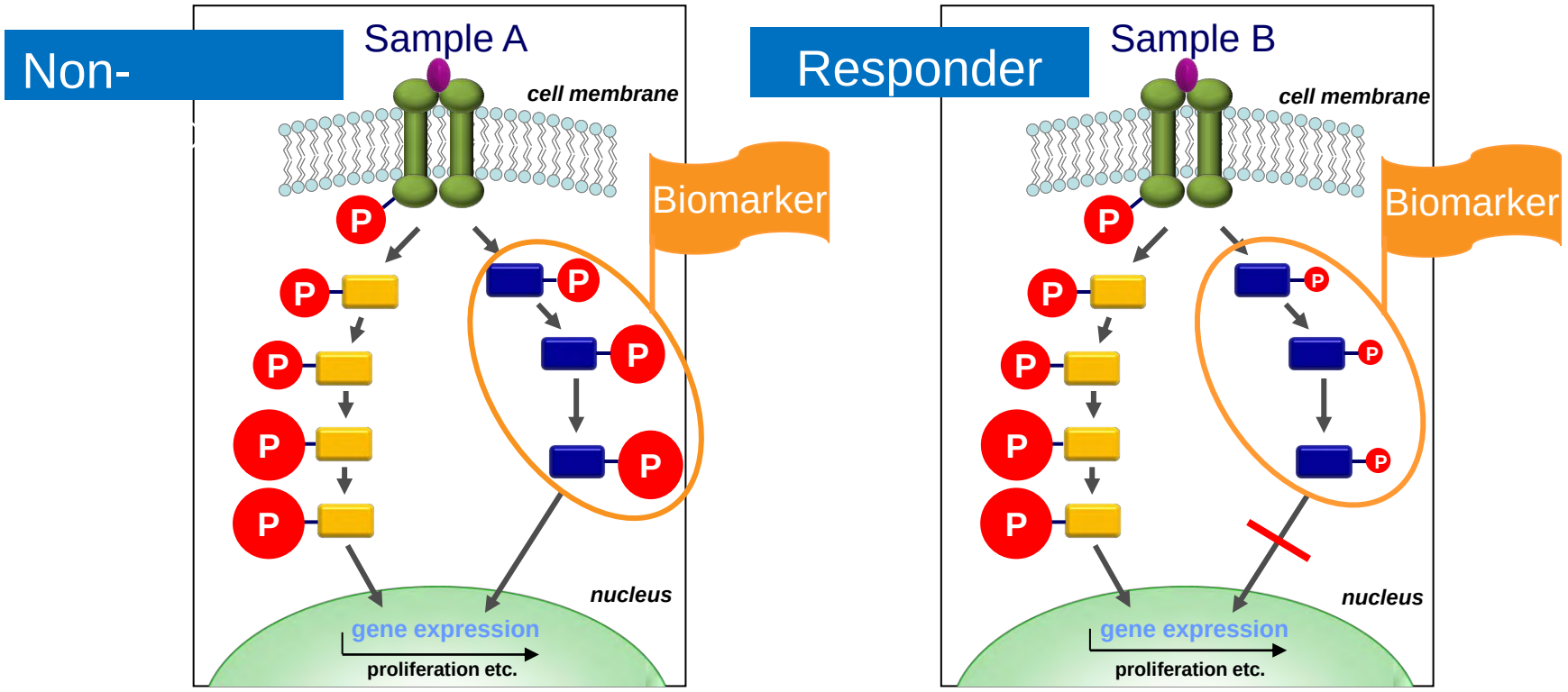
Definition Biomarker

"A biomarker is a characteristic that is objectively measured and evaluated as an indicator of **normal biologic processes**, **pathogenic processes**, or **pharmacologic responses** to a therapeutic intervention." (NIH)

- **Diagnostic** marker (e.g. PSA level for the diagnosis of prostata carcinoma)
- **Prognostic** marker (e.g. mRNA levels predicting the risk of forming metastasis for breast cancer patients)
- **Drug response** marker (e.g. FDG-PET for monitoring tumor size)
- **Stratification marker** (e.g. HER2 overexpression to stratify patients that are likely to benefit from Herceptin therapy)

Biomarkers for Sorafenib

Rationale for Biomarker Discovery



Hypothesis: Patient samples A and B are distinguishable due to their individual phosphorylation patterns that correlate with the drug's effects. These 'phosphosignatures' represent biomarkers for patient stratification.

In-vivo Phosphoproteomics

Cell culture on plastic dishes might not always recapitulate all aspects of *in vivo* tumor physiology in particular cancer cell growth in a three-dimensional environment in contact to other cells of non-tumor origin.

Extending global phosphoproteomics on animal models such as mouse xenograft models.

Mode of Action Analysis in Xenograft Models

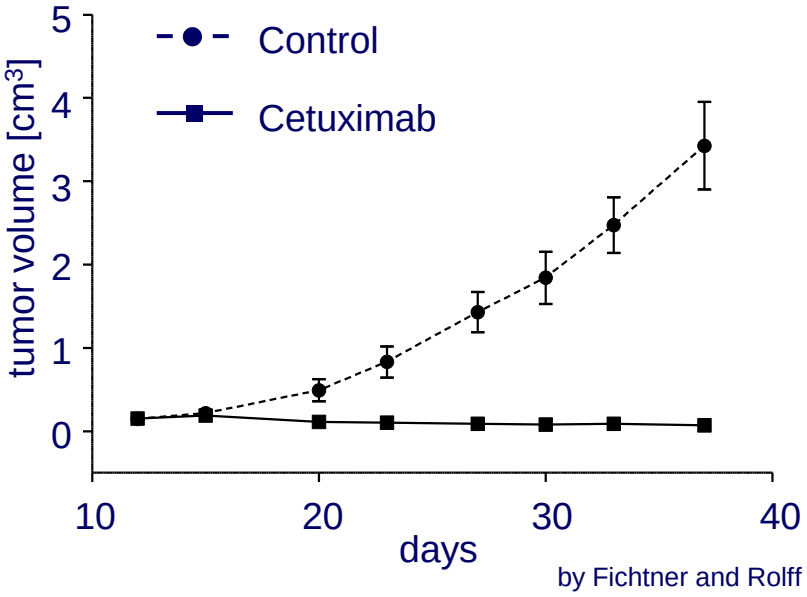
... responsive to cetuximab

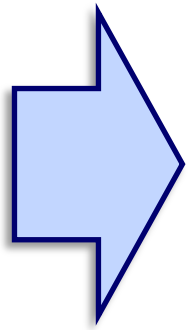
Cetuximab: monoclonal antibody directed against EGFR given for the treatment of metastatic colorectal and head/neck cancer

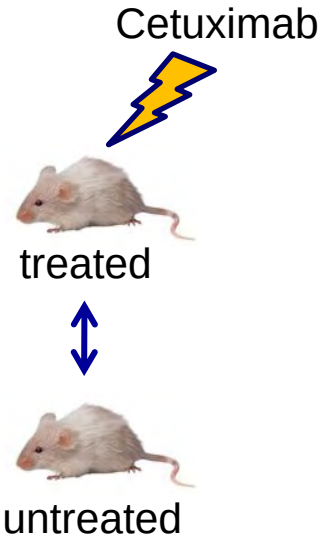
ID	Age	Sex	Smoker	Tumor-stage	Prior treatment	*EGFR*	*K-ras*	*p53*	Cetuximab response
ADC7466 (NSCLC)	57	M	s	pT2 pN1 cM0 G3 R0	No	wt	wt	R196P	++++

adapted from Fichtner et al., *Clin Cancer Res* 2008;14(20)

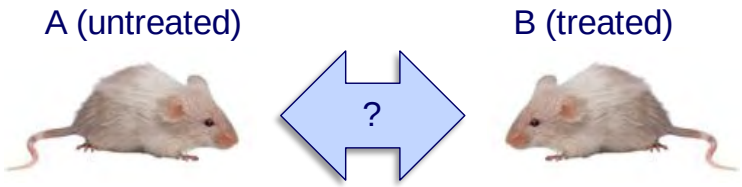
ADC7466 xenografts



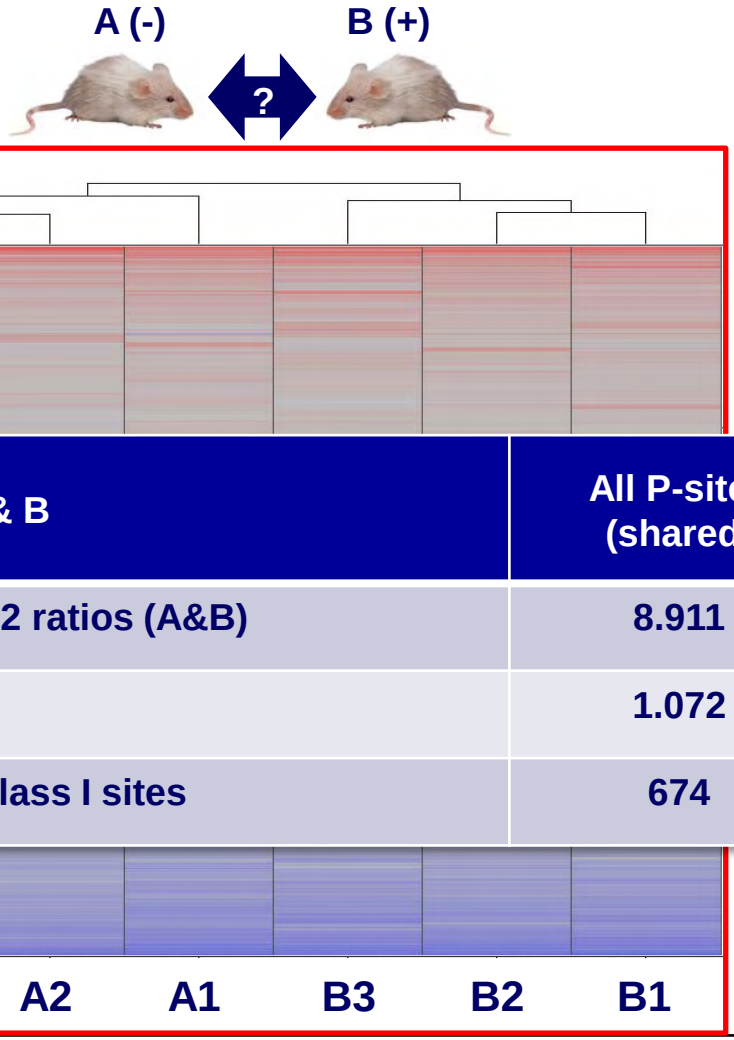




Case Study Cetuximab

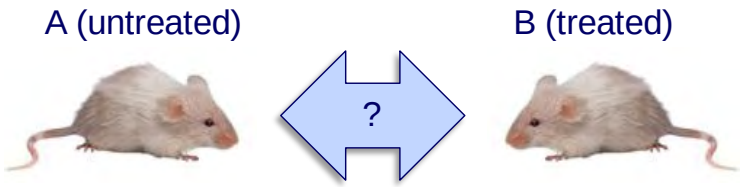


Treated vs. untreated



Found at least in 2 rep. from A & B	All P-sites (shared)	Unique Human
No. of class I sites with at least 2 ratios (A&B)	8.911	5.529
No. of regulated sites	1.072	755
No. of proteins with regulated class I sites	674	484

Case Study Cetuximab



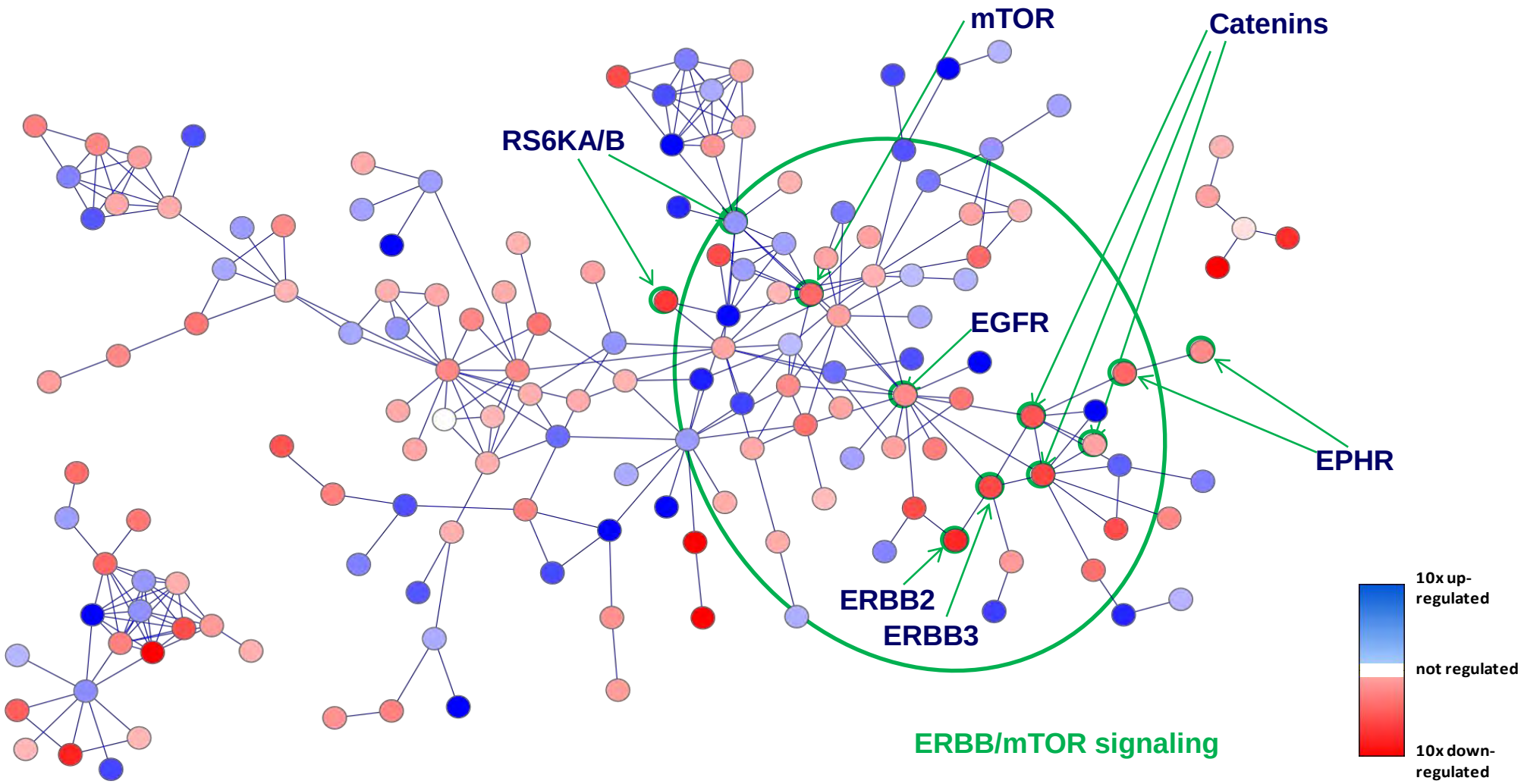
Overall results

KEGG Pathway	Proteins with detected p-sites	Proteins with regulated p-sites
Cell junctions	23	11
Focal adhesion	82	17
ErbB signaling pathway	51	12
Regulation of actin cytoskeleton	82	22

GO term	Proteins with detected p-sites	Proteins with regulated p-sites
cytoskeleton organization	351	62
GTPase regulator activity	456	65
regulation of kinase activity	219	35
regulation of cell cycle	310	41
regulation of signal transduction	465	62

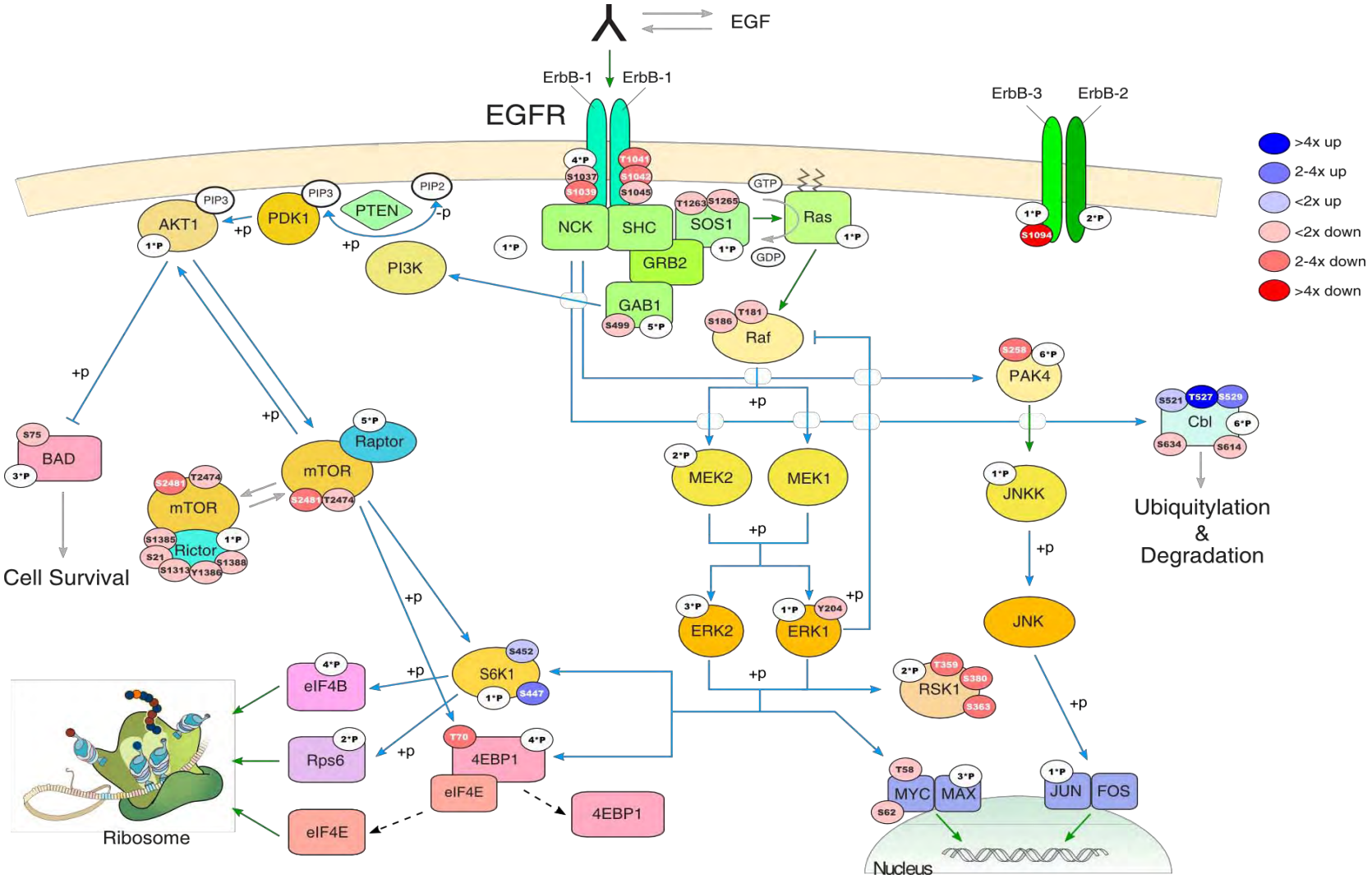
Case Study Cetuxumab

Integration with protein-protein interaction networks



Case Study Cetuximab

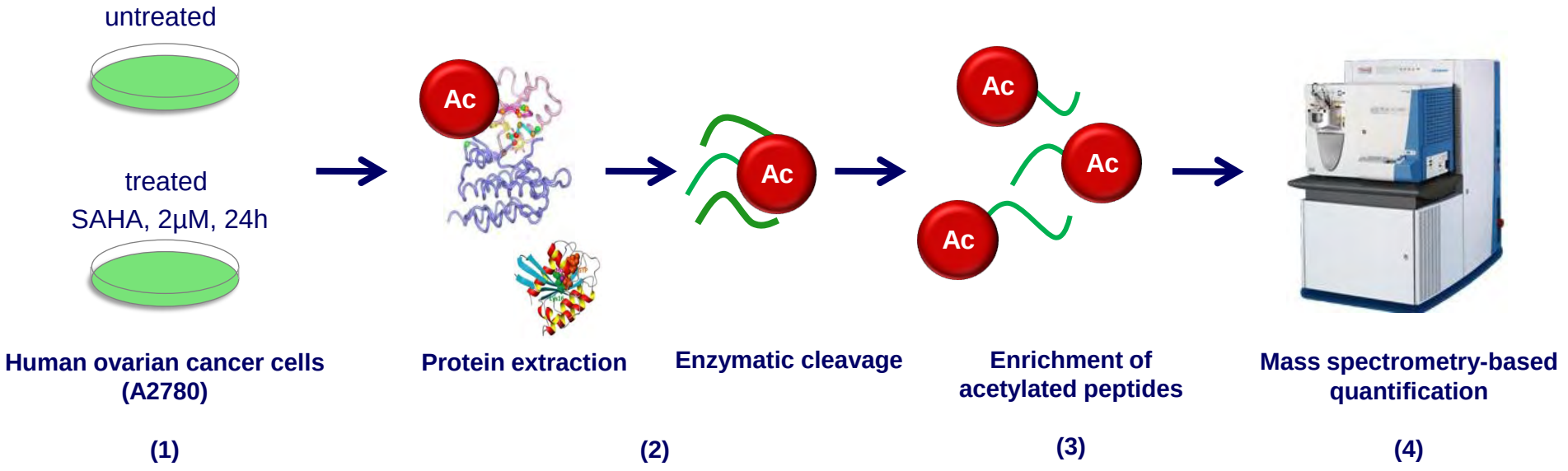
Mapping of phosphorylation sites



Outlook

Extending global analysis on other PTM‘s

Acetylomics:



Acknowledgements

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- Stefan Müller
- Jutta Fritz
- Andreas Tebbe
- Henrik Daub
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Thank you for your attention!

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