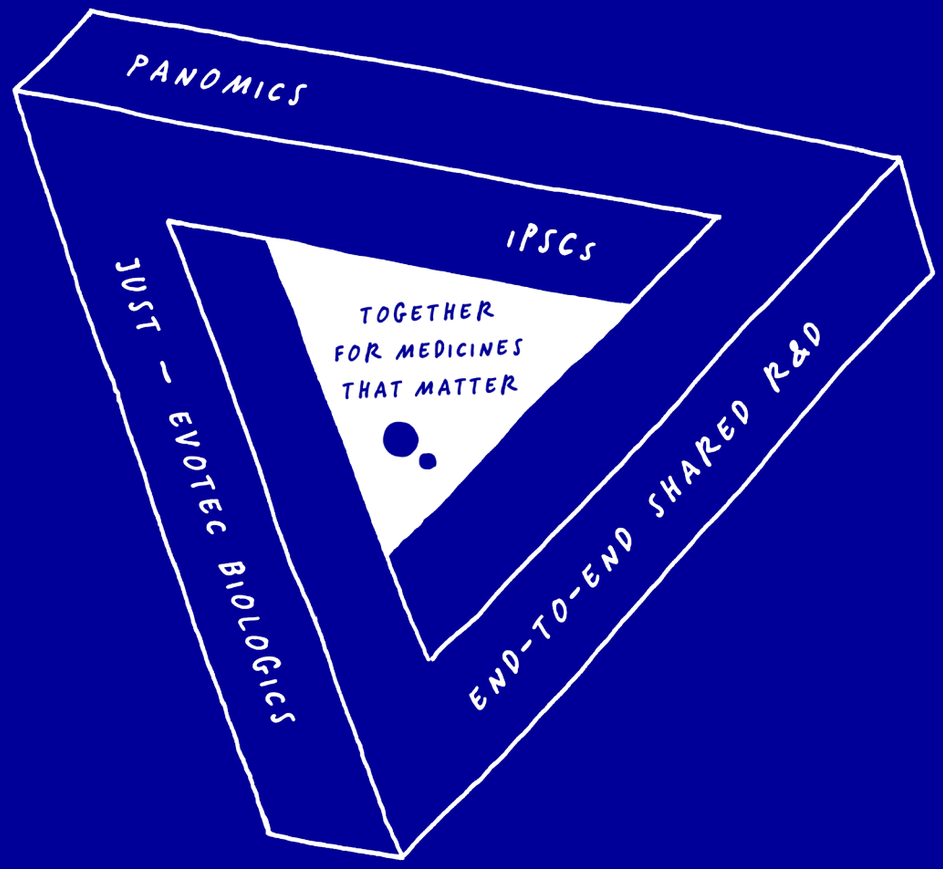
evotec

#RESEARCHNEVERSTOPS

Proteomics & Metabolomics

High-Throughput, Quantitative Mass Spectrometry Platforms to Support Drug Discovery



Global Leadership in Cutting-Edge Proteomics

Evotec's High Performance Proteomics and Metabolomics Platforms

> 50
MS instruments

> 100
Employees MUC & TLS

> 1.5 B
Datapoints generated p.a.

- Evotec operates two of the **largest proteomics & metabolomics** facilities world-wide
- Evotec has more than **20 years of experience** in mass spectrometry, chemical proteomics, metabolomics, and bioinformatics and their applications in **drug and biomarker discovery**
- Evotec drives the **paradigm shift** towards high-throughput and **high-coverage proteomics screening**
- Evotec is a proven partner of **global pharma, biotechnology, and crop science** companies as well as academic institutions and private foundations

Boehringer Ingelheim · Bristol-Myers Squibb · Takeda Pharma · Janssen · MSD · Bayer CropScience · The Mark Foundation for Cancer Research · CHDI Foundation · Bayer HealthCare

Quantitative MS Supporting Drug Discovery

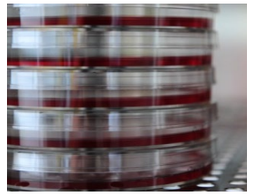
Chemical proteomics, global proteomics and metabolomics

Global Proteomics Platforms

- High-end quantitative mass spectrometry to monitor protein expression, phosphorylation, glycosylation, acetylation, ubiquitination or arginine methylation
- Targeted mass spectrometry assay development and deployment

1 *In vivo* mode-of-action analysis in cells, tissues or patients

2 Discovery and verification of biomarker candidates



Chemical proteomics

- Evotec Cellular Target Profiling™ technology to both identify and quantify interactions with cellular compound targets
- Drug photoaffinity labelling and activity-based protein profiling for covalent target capture

3 Cellular compound selectivity analysis in a native context

4 Target de-convolution of hit compounds from phenotypic screens

Metabolomics

- *In vitro* and *in vivo* quantification of metabolites in complex sample using SPE-LC–MS/MS

5 Targeted analysis in cells, tissues, body fluids or awake animals

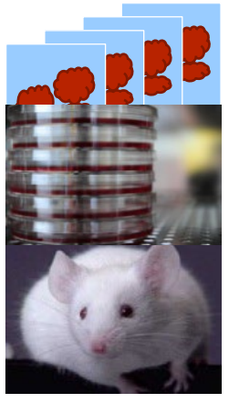
Target candidates	Target deconvolution	Biological selectivity	Mode-of-action	Target engagement	Drug candidate prioritization	Biomarker candidate ID	Biomarker verification
Target ID	**Screening**	**Hit-to-lead**	**Lead Opt**	**Pre-clinic**	**Phase I/II**		

Industry-leading Capabilities Supporting Target and Biomarker Discovery

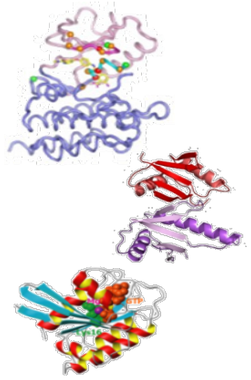
Quantitative approach to accurately measure protein abundance levels *in vivo* on a global scale

- Data independent acquisition (DIA) allows detection of up to 10,000 proteins in single-shot measurements
- Isotope labelling (e.g. SILAC) available for specific applications
- Samples incl. cultured and primary cells, fresh and FFPE tissues, plasma & CSF from animals and humans
- Typical applications: *in vivo* mode-of-action analysis in cells, tissues or patients, discovery and verification of biomarker candidates

Isotopic labelling or Label-free



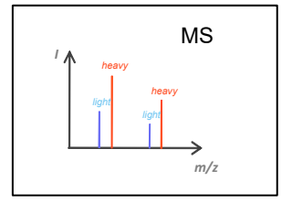
Extraction of Proteins

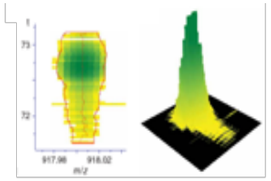


Enzymatic Cleavage

Single-shot Proteome Profiling (DIA)

LC-MS/MS

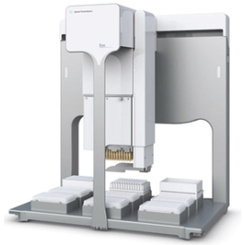




Data Analysis

ScreenPep™ – Scaling throughput in real screening scenarios

Highly sensitive detection of target candidate regulation for diverse compounds

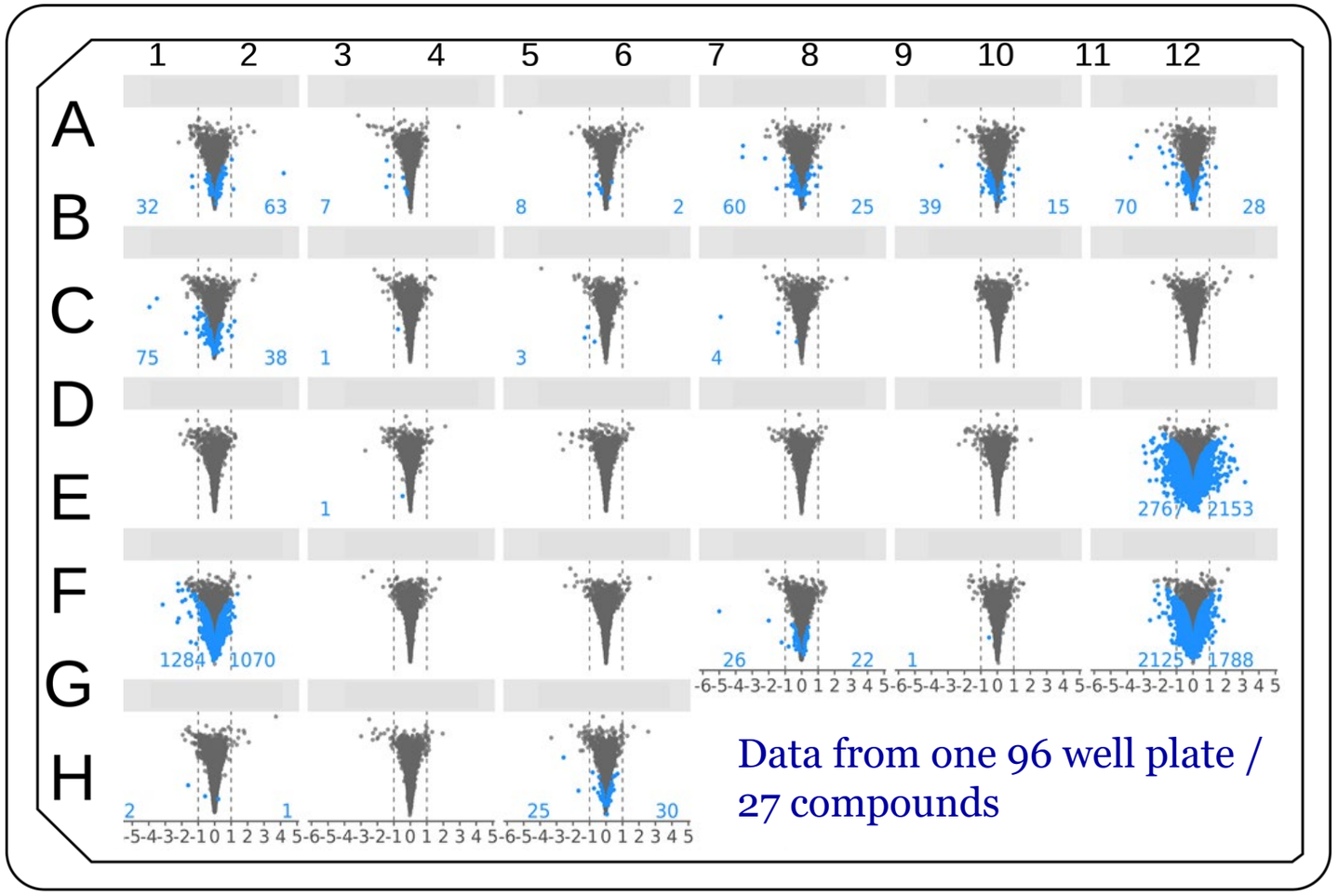


Sample prep

LC-MS/MS

DIA-NN

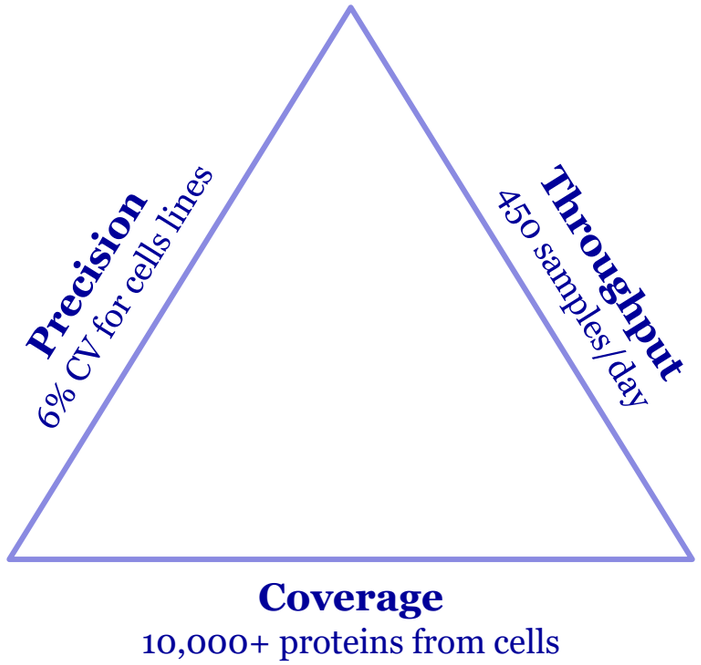
Data analysis



- Ultra-deep coverage of up to **>11,000 proteins** with median protein **CVs of ~6%** by **single-shot DIA MS**
- **Systematic scoring** and evaluation process to select target candidates for hit confirmation and **proteomic validation**

ScreenPep™ – Proteomics without compromise

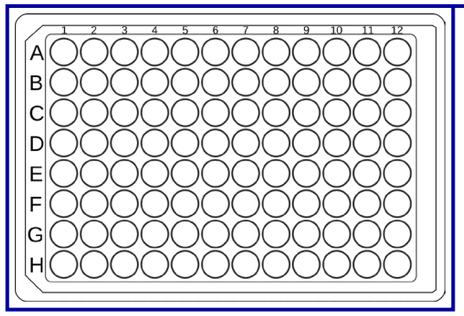
Deep proteomics at industrial scale



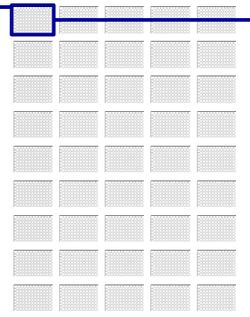
Driven by proprietary processes and workflows

- High-end mass spectrometers embedded in proprietary workflows
- Fully automated sample preparation processes
- Highly optimized, single-shot mass spectrometry
- Dedicated bioinformatics pipeline and IT infrastructure

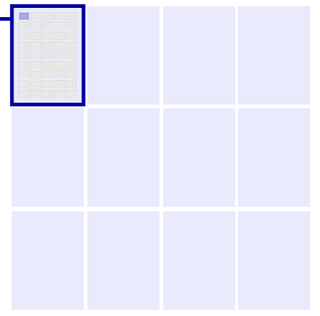
Biological samples → Automated sample preparation → Deep proteome single-shot MS analysis → Dedicated bioinformatics pipeline → Activity profiles Targets Biomarker



27 compounds per 96-well



~1,250 compounds → 45 x 96 WPs
(1 instrument per year)



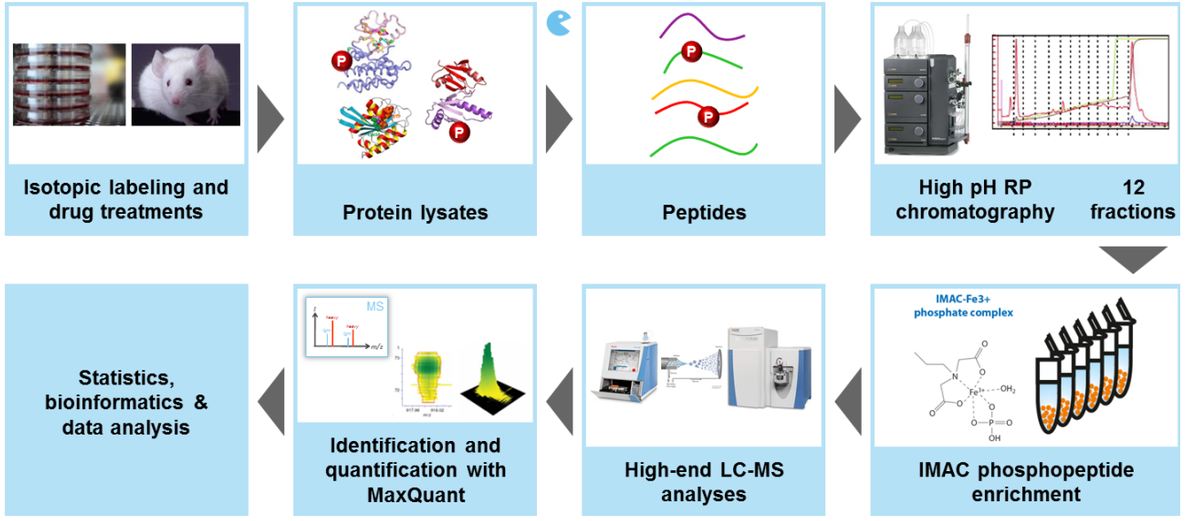
~40,000 compounds per year

In vivo Mode-of-Action Analysis of Kinase Inhibitors

Quantitative Phosphoproteomics

Quantitative phosphoproteomics for kinase inhibitor research

- Reliable measurement of more than 20,000 phosphorylation sites (pSer, pThr and pTyr) in biological replicate experiments
- Mode-of-action analysis by comprehensive investigation of signalling pathways and their response to drugs
- Related workflows enabling global measurement of **lysine acetylation, lysine ubiquitination, or protein methylation sites** to support discovery of epigenetic drugs and drugs targeting the UPS system

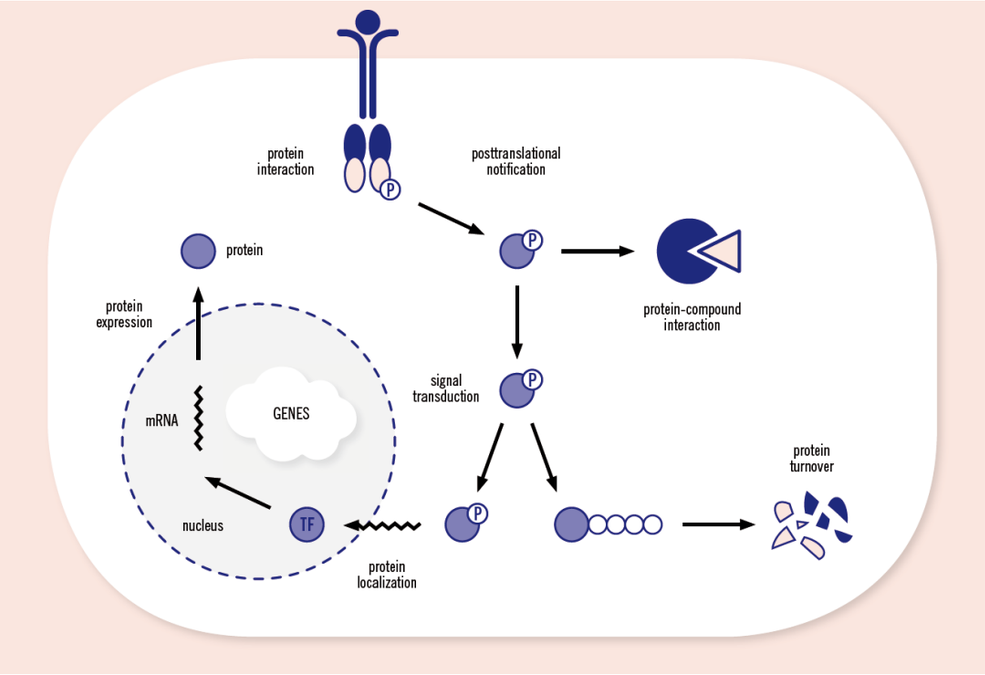


Typical project applications

- Identification of target engagement and pharmacodynamic markers
- Selection of drug candidates with maximal on- and minimal off-target activity in cellular conditions
- Explore mechanisms for differential biological activity of kinase inhibitors

Bringing Proteomic Expertise to Drug Discovery

Proteomes in time and space

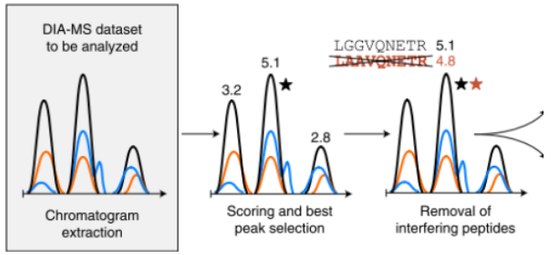


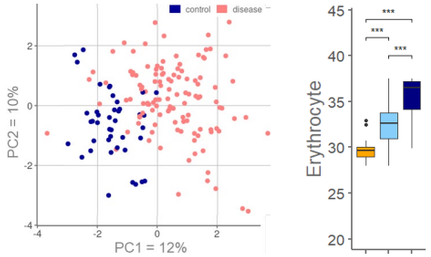
- Quantitative, deep analysis of protein expression
- Protein-protein and protein-ligand interactions
- Posttranslational modifications
- Protein localization, dynamics and turnover

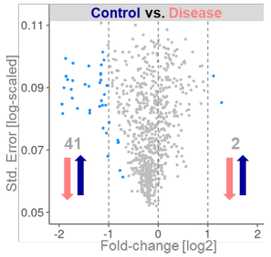
Proteome & Subcellular Proteome Profiling	**Global Proteomics**
PhosphoScout®	
Acetylomics, Methylomics, Ubiquitinomics	
Protein turnover	
Protein-Protein Interactions (AP-MS)	
Proximity Labelling	
MHC Peptidomics	
Evotec Cellular Target Profiling™ (CTP)	**Target Deconvolution**
Photoaffinity Labelling (PALMS)	
KinAffinity®	
Activity-based protein profiling	
Multiple & parallel reaction monitoring	**Targeted**

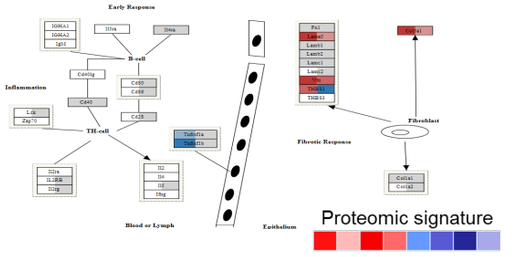
From MS Raw Data to Biological Insights

Biostatistics and Bioinformatics Pipeline for Data Preprocessing, In-Depth Analysis & Interpretation









LC-MS/MS raw data processing

- Peptide identification
- Generation of individual predicted spectral libraries from and for all sample types
- Global identification and relative quantification of peptides and proteins by spectral library comparison (“match between runs”)

Preprocessing and quality control

- Protein group inference, quantification & normalization
- Assessment of protein identifications
- Outlier detection
- Blood contamination marker analysis
- Global exploratory analyses (PCA, confounder analysis)

Differential abundance analysis

- Differential abundance analysis e.g. comparing disease and control groups
- Analysis adapted to experimental setup
- Linear models (limma)
- In-house developed HTS test for screening studies

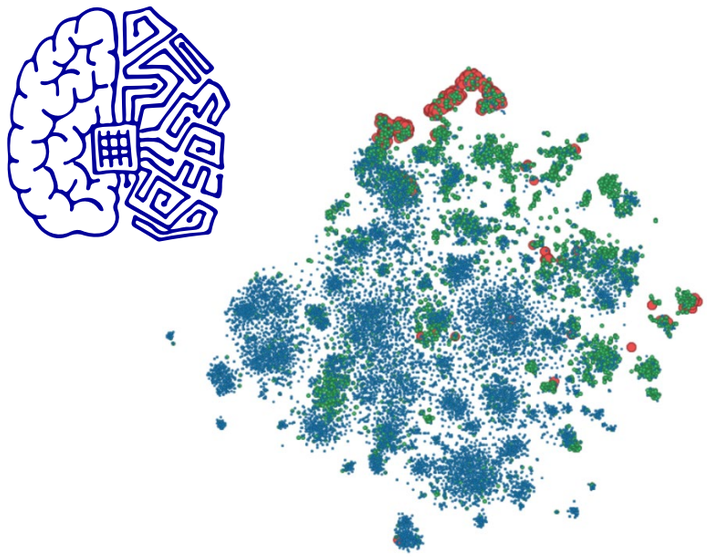
In-depth analysis & data interpretation

- Functional analyses
  - GO enrichment
  - Pathway/network analysis
  - Kinase analyses
- Dedicated analyses for specific applications
- Machine Learning (ML)
  - Target ID
  - Biomarker discovery
  - Patient stratification & diagnosis
  - Predictive drug safety

Integration of informatics and AI to further increase success

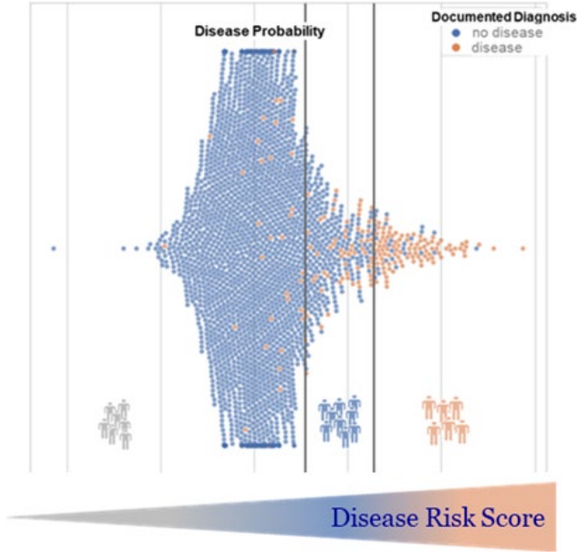
Biomarkers link all discovery and development work to patients

Biomarker identification



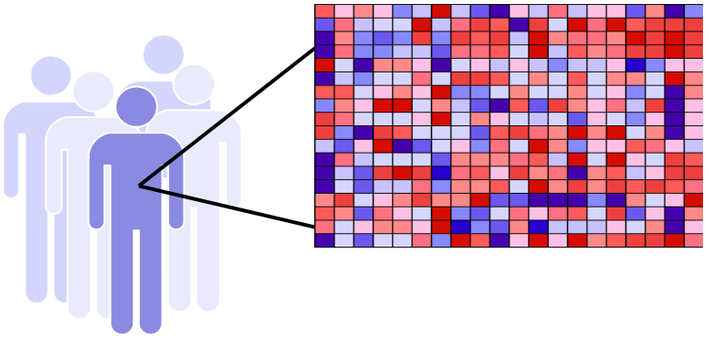
- Big Data analysis platform
- In-house quality data sets
- Data curation

Biomarker validation & optimization



- Hypothesis testing and cross-validation on new cohorts
- Multi-variate signatures

Translation of biomarkers & companion diagnostics

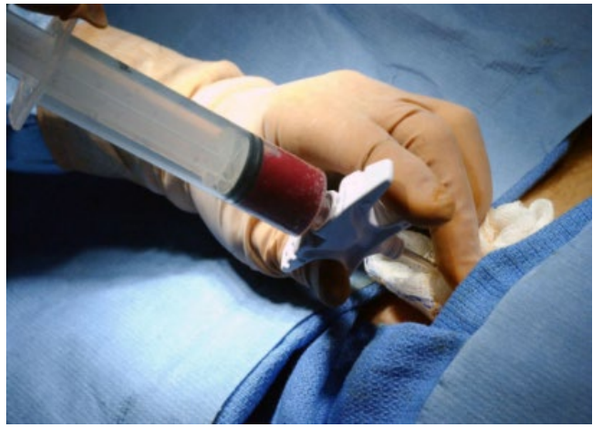


- Integration of clinical results
- Retroactive refinement of predictivity and safety

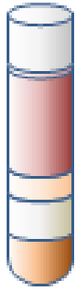
Use Case: Clinical Response Prediction in AML patients

Identification of proteomic markers for Flt3 inhibitor sensitivity[1]

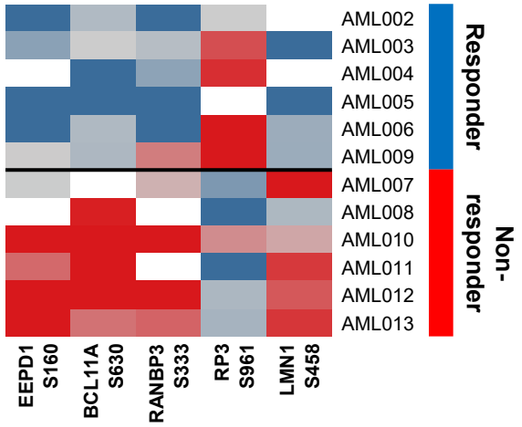
Bone marrow aspirates of AML patients



AML cell purification

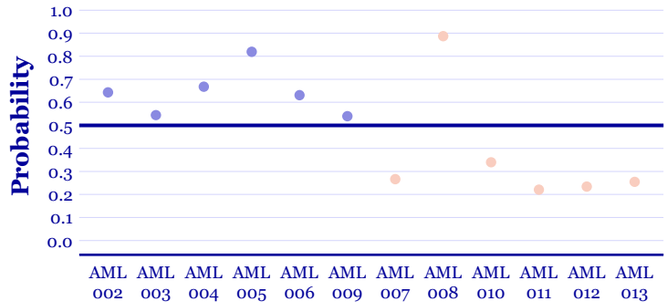


Patient AML cell phospho-proteomics and biomarker candidate identification



- Clinical collaborators provided acute myeloid leukaemia patient samples from Flt3 inhibitor (quizartinib) phase II trials, together with patient data about therapy outcome
- Quantification of patient AML cell phosphoproteomes followed by bioinformatics analysis identified predictive phosphorylation sites, which retained predictivity in an independent set of patients
- Results demonstrate potential of clinical proteomic biomarkers for efficient patient stratification

Phosphosite signature predicting Flt3 inhibitor response with high accuracy



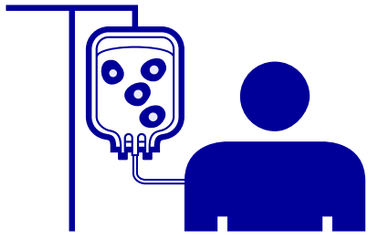
1 Schaab *et al.* (2014) *Leukaemia* **28**, 716-719

Strategic Alliance with Seer for Proteograph™ technology

Adaptation of ScreenPepTM platform for measurement of biofluid samples using Proteograph™

Clinical samples

Body fluids (plasma, serum, urine, cerebrospinal fluid) or secretome samples



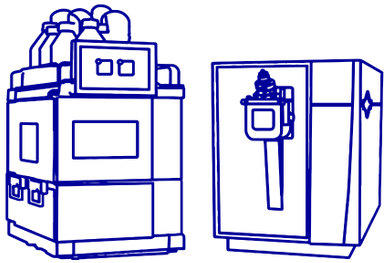
Proteograph platform

Proteograph

Incubation of beads with sample: tryptic digest & peptide clean-up

LC-MS/MS acquisition

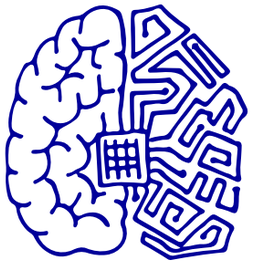
High Resolution DIA acquisition & on-the-fly data QC



- Orbitrap MS
- timsTOF MS

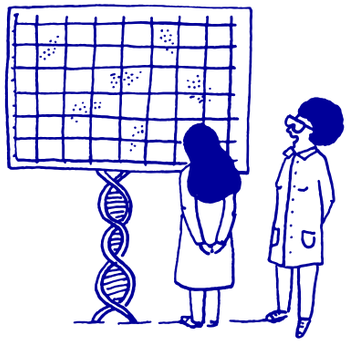
Raw data processing

Spectral library generation from single shot data



Peptide and protein identification and quantification

Bioinformatics analysis

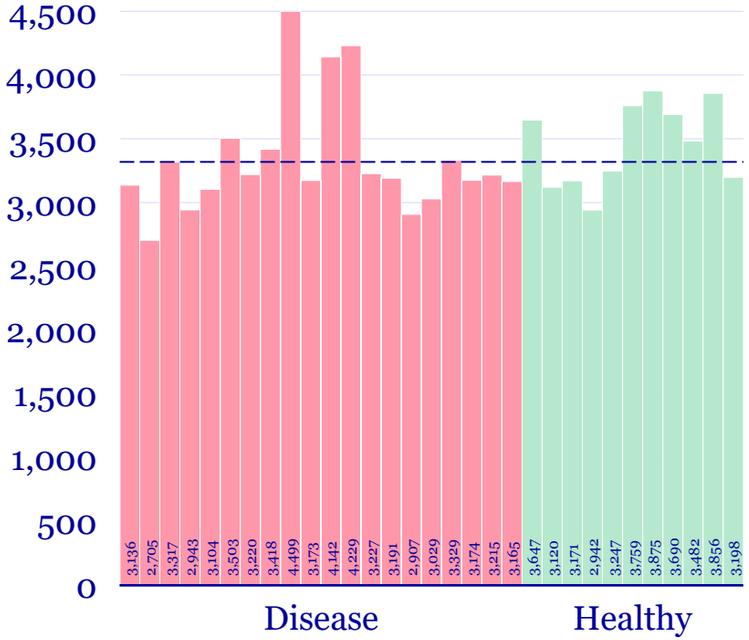


- QC & Contamination analysis
- Global exploratory analysis
- Functional analysis
- Multi-omics data integration

DIA: Data-independent acquisition

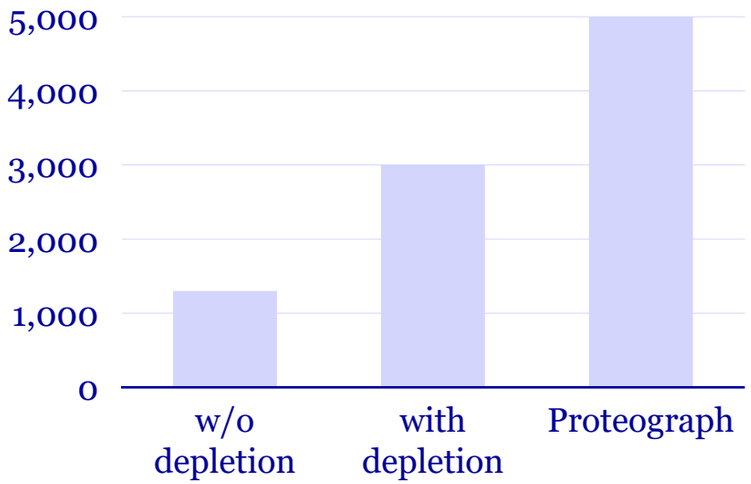
Strategic Alliance with Seer for Proteograph™ technology

Adaptation of ScreenPepTM platform for measurement of biofluid samples using Proteograph™

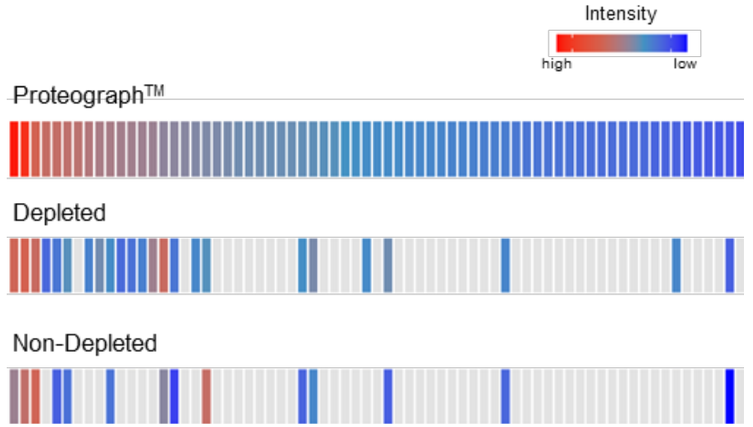


Proteome depth applying Evotec's plasma proteomics workflows

Number of detected protein groups



Detected Cytokines (GO-Term 0005125)

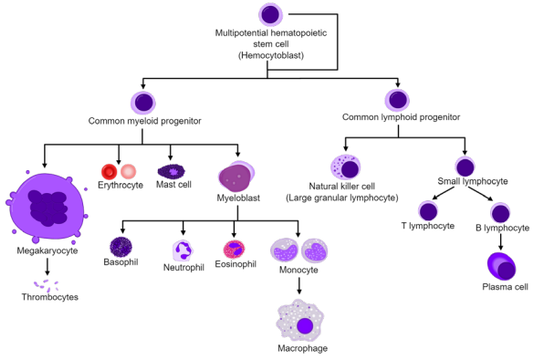


- Detection of up to **5,000 protein groups** in human serum/plasma samples by combining nanoparticles
- Significant increase of dynamic range of detected proteins → **Access to low abundant proteins such as cytokines**
- **Similar boost in coverage and sensitivity for other body fluids (e.g. CSF, urine) and secretome analysis**

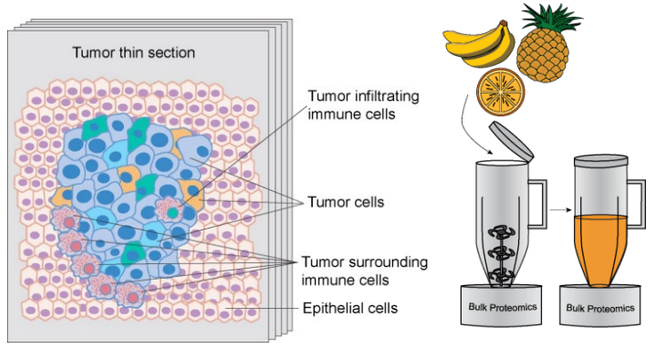
High Sensitivity Proteomics

Cell type , spatially resolved and single cell proteomics

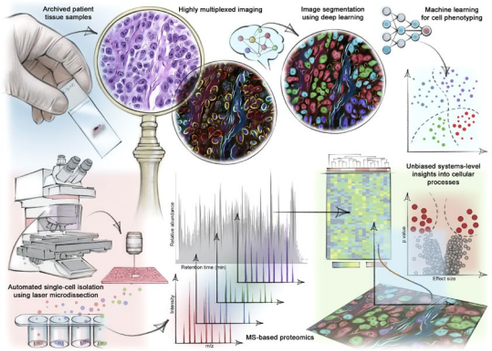
Sorted rare cell/cell populations



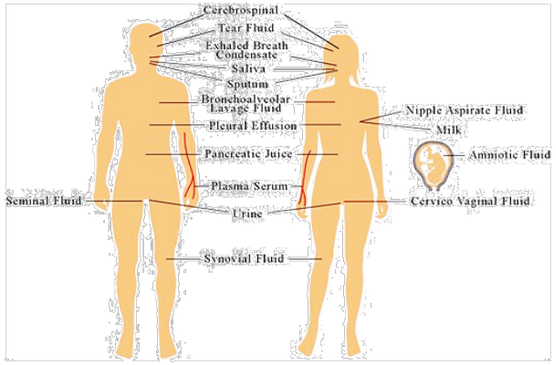
Cell type resolved



Spatial proteomics



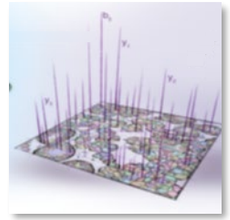
Low amount body fluids

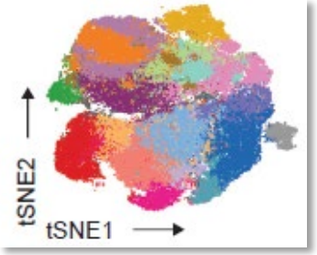


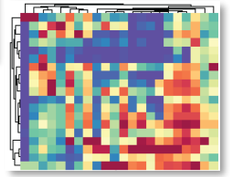
Cell sorting and cell dispensing instruments

Ultra high sensitivity LC-MS instrumentation

Dedicated bioinformatics data analysis tools

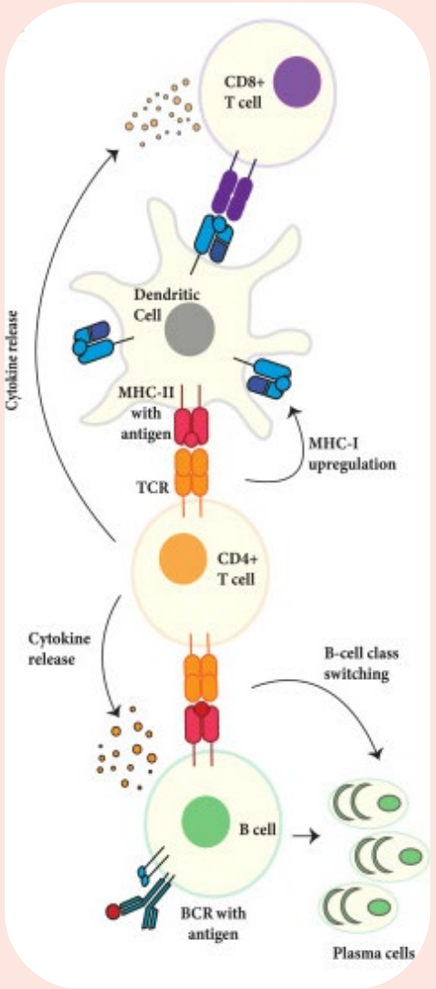






Recognition of Tumor Antigens

- Immunopeptides are antigen peptides extracellularly presented by major histocompatibility complex (MHC) class I and class II
- These peptides represent a snapshot of the extra- and intracellular proteome
- Immunosurveillance by T cells is the primary means by which the immune system protects against cancer
- The mechanism behind this is that T cells interact closely with MHC/peptide complexes of other cells via their T cell receptor (TCRs)
- If “normal” cells accrue tumorigenic genetic and molecular alterations the immune system can often recognize these cells as non-self and eliminates them
- Cytotoxic $CD8^+$ T cells recognize peptide antigens (9-12 aa) stemming from intracellular proteins presented on MHC class I molecules
- $CD4^+$ T helper cells interact with MHC class II molecules presenting peptides (10-25 aa) originating from extracellular sources or cellular proteins degraded via the endosomal pathway



Taylor, Hannah B., et al. "MS-Based HLA-II Peptidomics Combined With Multiomics Will Aid the Development of Future Immunotherapies." *Molecular & Cellular Proteomics* 20 (2021).

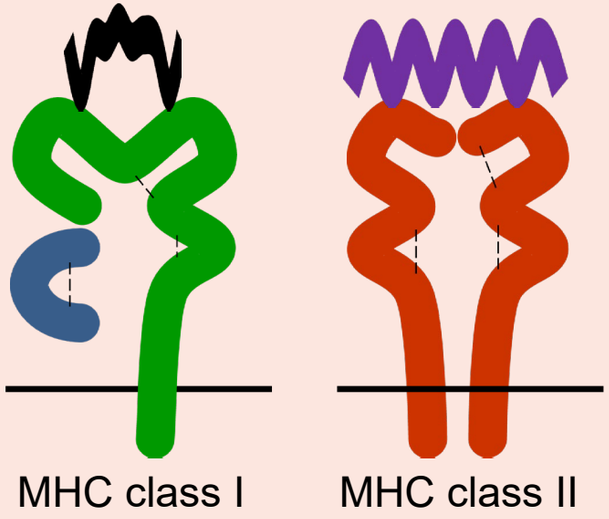
Recognition of Tumor Antigens

- While MHC I is present in all nucleated cells, MHC class II is typically expressed by specialized antigen-presenting cells and to some extent by tumor cells
- Activation of $CD4^+$ T cells via cytokines and chemokines results in stimulation of other immune cell types such as cytotoxic $CD8^+$ T cells to destroy their targets and B cells initiate antibody production

➢ The highly specific and effective modus operandi of the immune system bears huge potential for different immunotherapeutic platforms

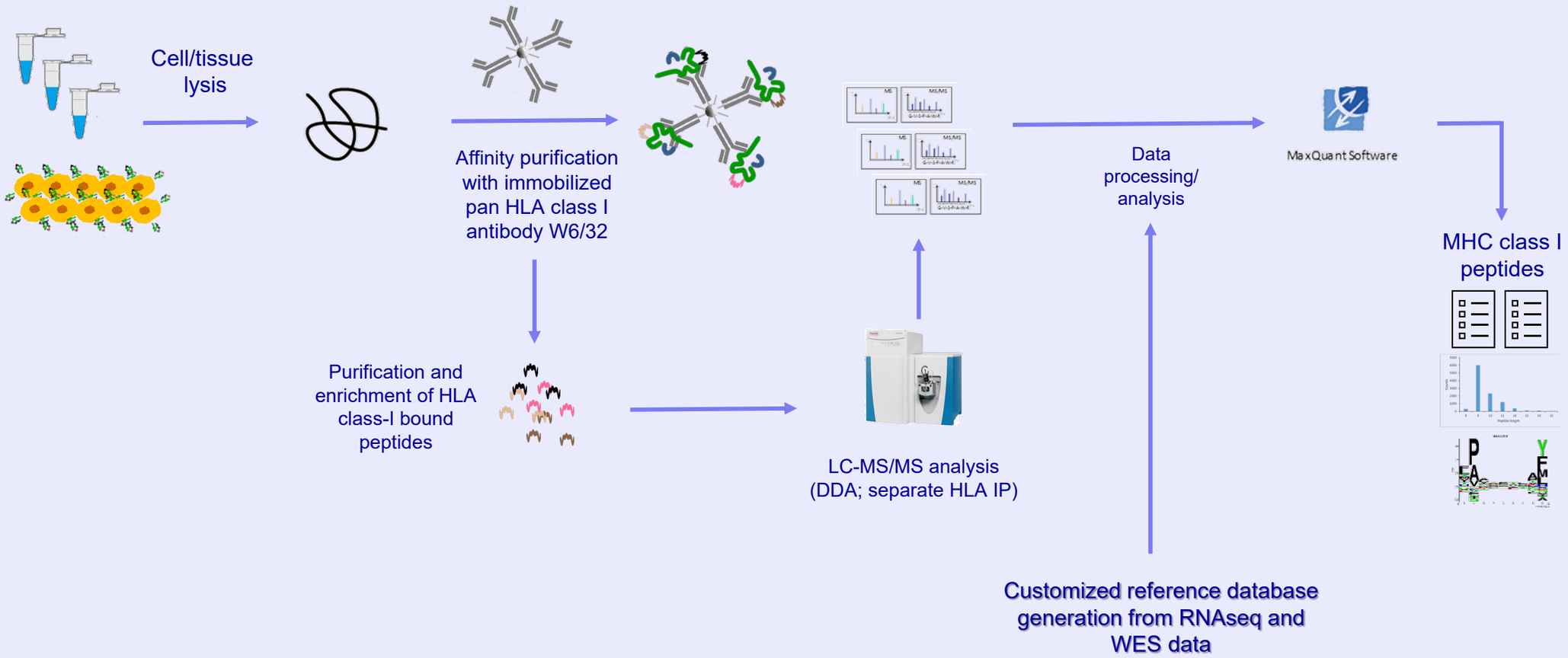
➢ Knowing tumor-specific and -associated antigens expand therapeutic opportunities

➢ Knowing both types of antigens is crucial as improved clinical response has been observed when $CD4^+$ T cells are targeted in combination with $CD8^+$ T cells



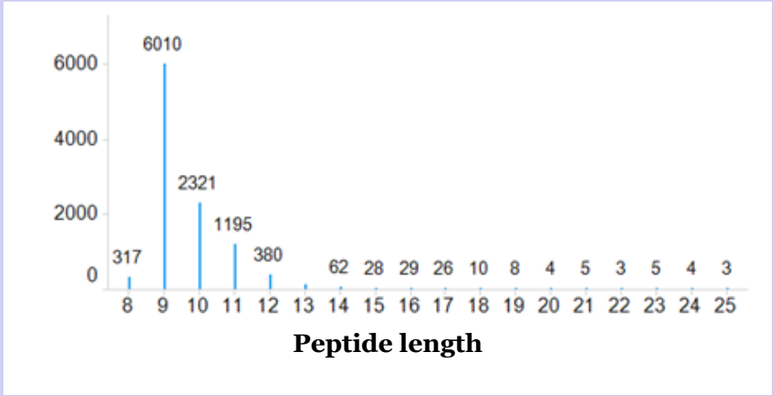
Immunopeptidomics Analysis at Evotec

Immunopeptidomics workflow (MHC class I)

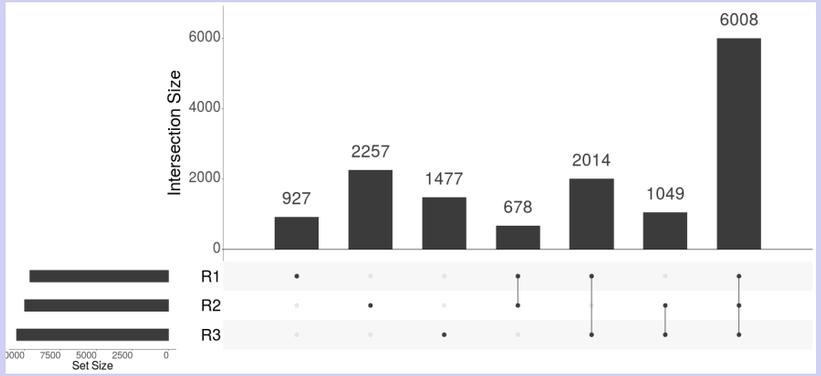


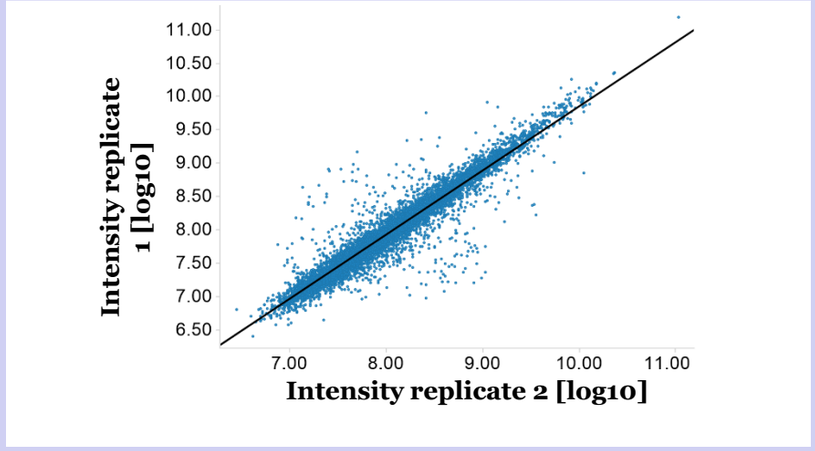
Immunopeptidomics Analysis at Evotec

Example cell line data

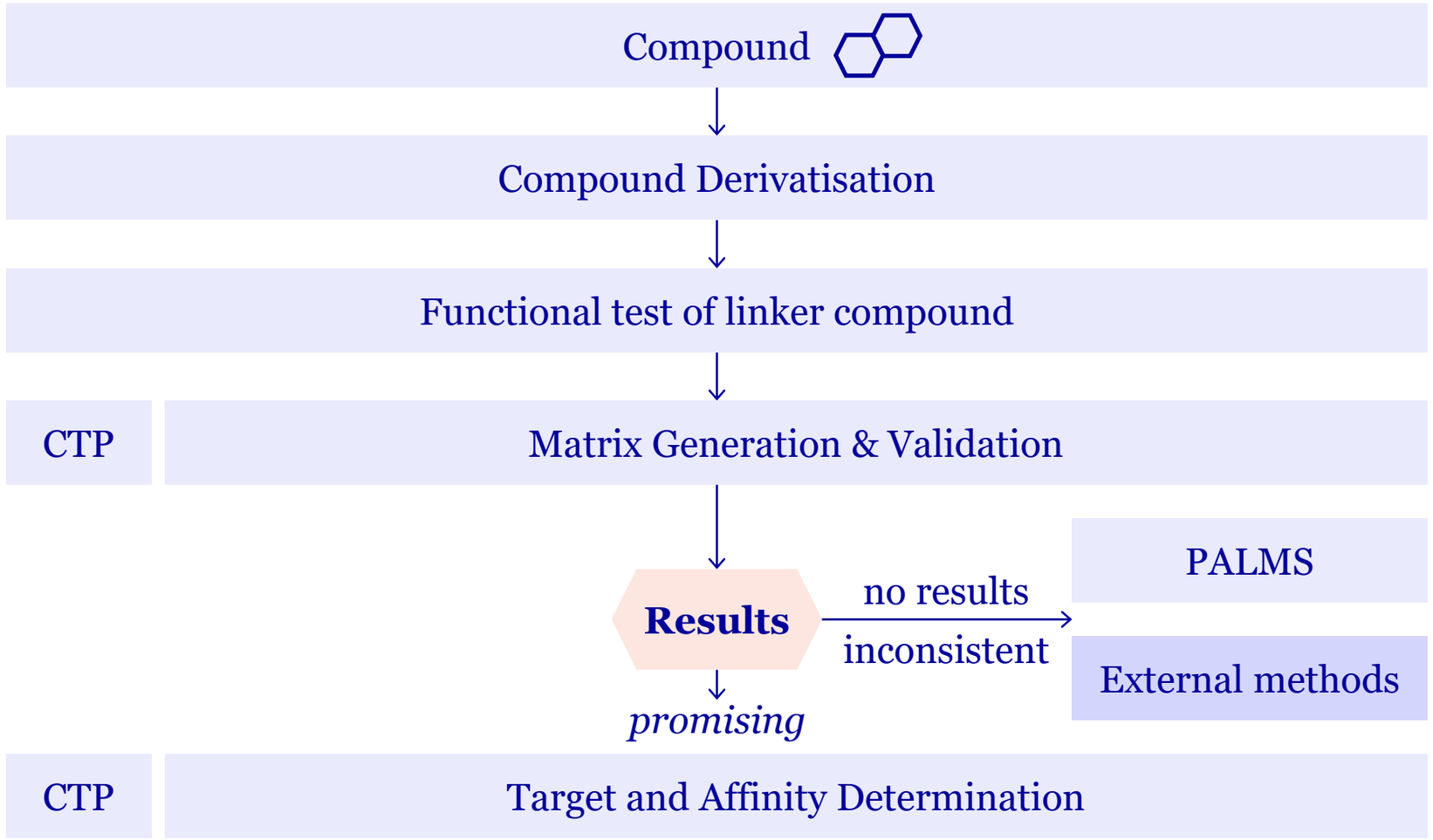


- $3x10^8$ HT-29 cells served to discover MHC class I presented peptides in triplicate analyses
- Up to 10,000 peptides were identified with 1% FDR in one replicate
- Peptides exhibited a length distribution typical for MHC class I peptides
- Replicates showed a large proportion of overlap of IDs
- And a good quantitative correlation (R^2) was observed for peptides overlapping amongst replicate samples.





Default strategy for proteomic target ID



Evotec will provide tailored solution for customer needs based on project specifics

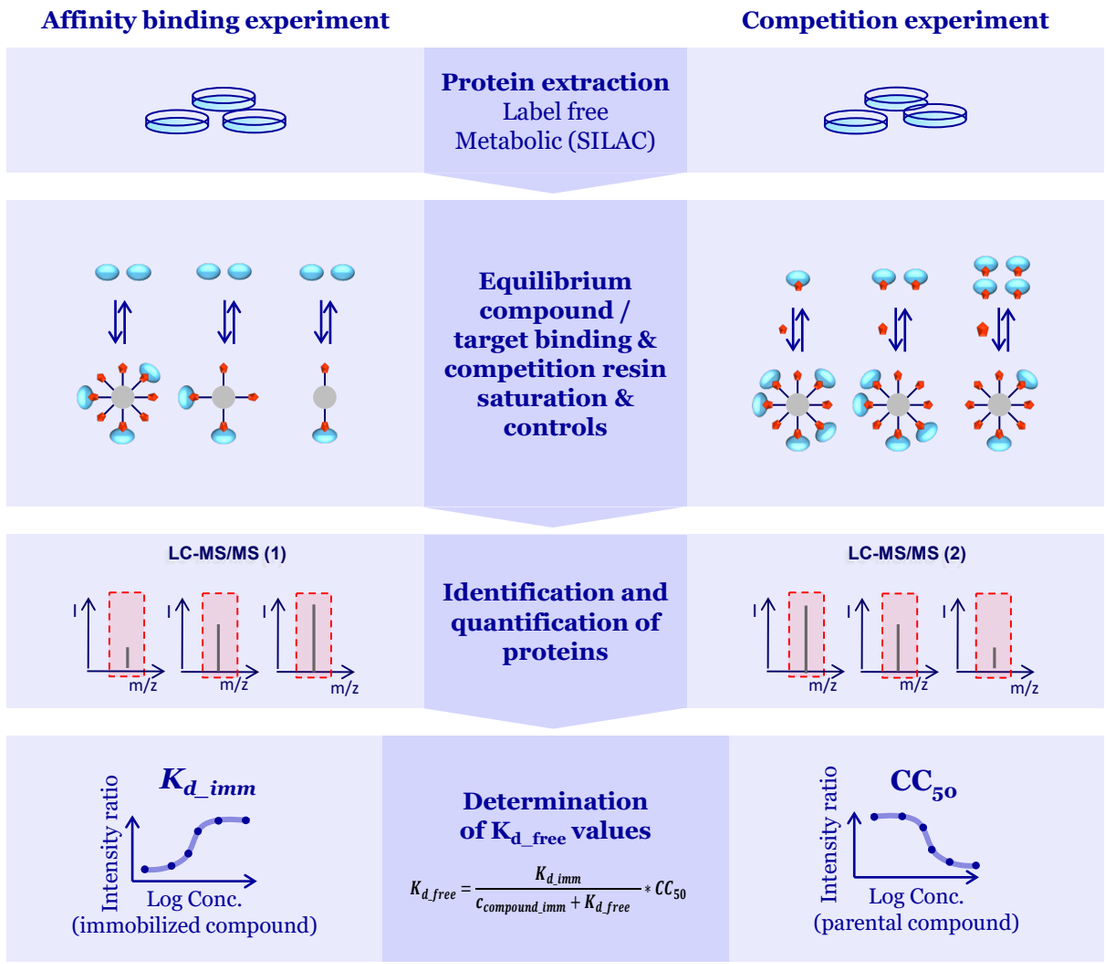
Supporting information

- Chemical structure
- SAR information
- Compound potency
- Compound metabolic stability
- Biological / cell system
- Assumed target class
- …

Evotec Cellular Target Profiling™ (CTP)

Workflow and K_d determination

- Identification of small molecule targets in any type of cell or tissue of choice
- Determination of a compound's proteome wide binding affinities (K_d values)
- Profiling of a compound against native, endogenously expressed, post-translationally modified full length proteins in the presence of cellular cofactors and native complex partners
- CTP requires a linker derivative of the active compound for target enrichment by affinity chromatography
- Synthesis of linker compounds with terminal NH2, COOH, or alkyne, azide moiety for functional immobilization
- Competition with active parental and inactive analogous compound
- CTP as mature "workhorse" approach for target ID with extensive, non-target class restricted track record in target deconvolution & profiling of various small molecules

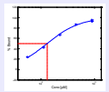
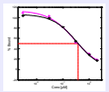
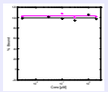
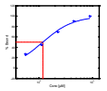
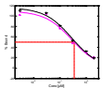
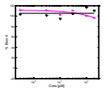
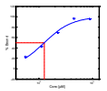
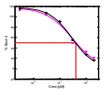
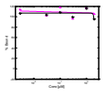
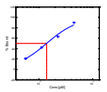
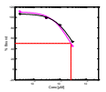
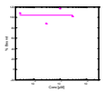
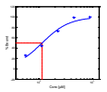
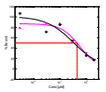
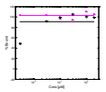


Sharma K, Weber C, Bairlein M, Greff Z, Kéri G, Cox J, Olsen JV, Daub H., Nat Methods. 2009 Oct;6(10): 741-4
Patent Protection EP2045332B1

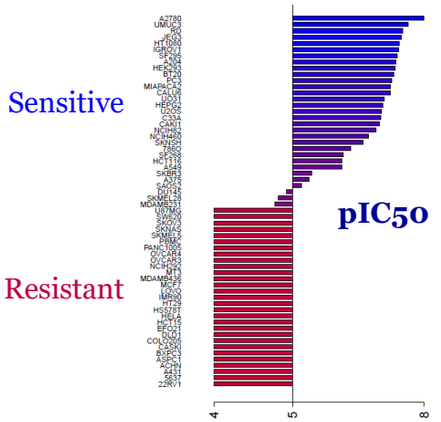
Evotec Cellular Target Profiling™ for novel target ID

Target deconvolution of hit compound from phenotypic screen

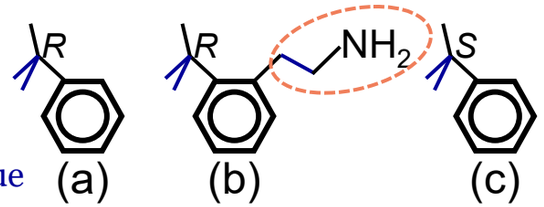
- Compound with differential effect on a subset of cancer cells
- Amine linker derivative of active compound served for generation of affinity resin
- A distomer served as negative control
- **Nicotinamide phosphoribosyltransferase (NAMPT)** was successfully identified as main target, K_d ~50 nM

Protein Name	Sequence Coverage [%]	Binding Linker Cpd	Competition Active Cpd	Competition Inactive Cpd	$K_d$free [nM]
NAMPT	**80.7**				**49.7**
EIF4A3	1.9				44.7
SIRT3	3.5				51.0
DNM1	2.0				40.8
TTN	0.1				47.8

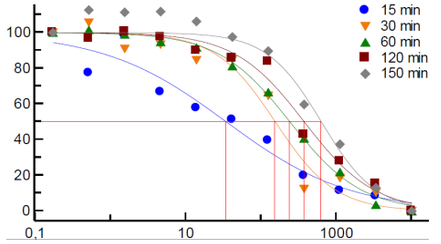
Panel of 82 cell lines



a) active parental
b) active linker derivative
c) inactive analogue

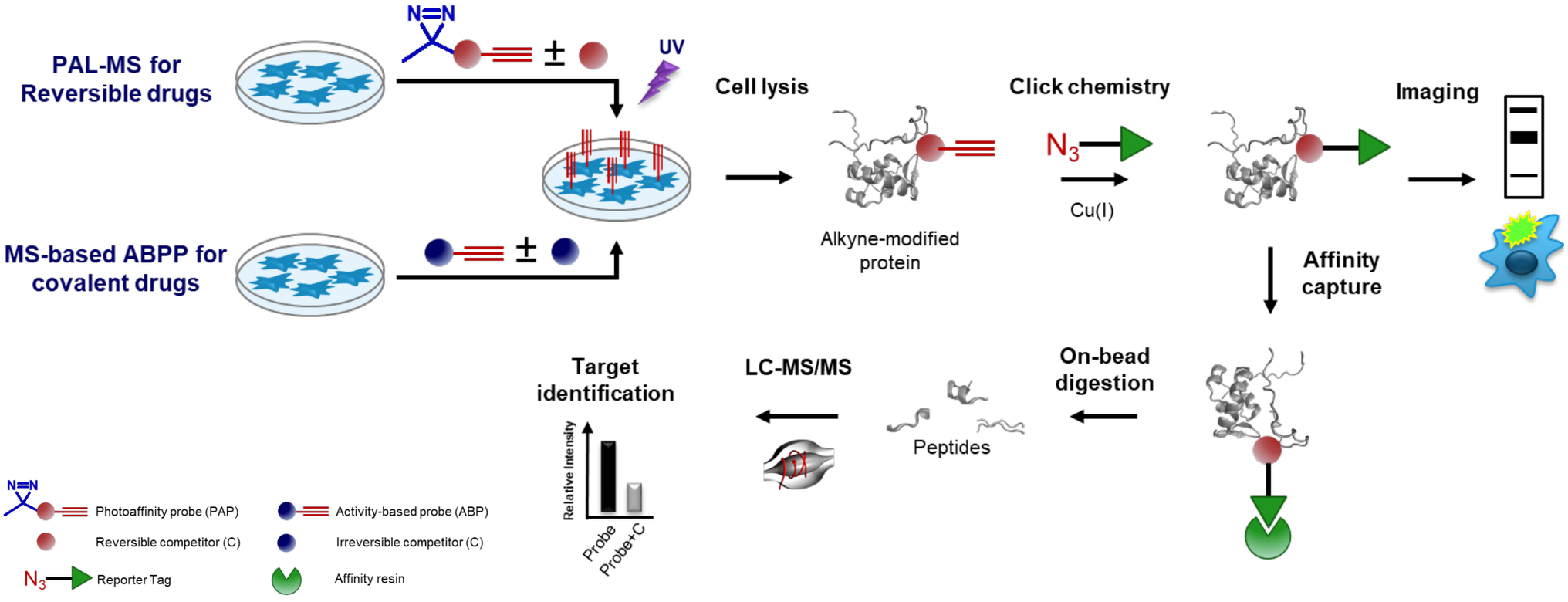


Biophysical confirmation of NAMPT binding using FCCS



Photoaffinity Labeling (PALMS) & Activity based protein profiling (ABPP)

Unbiased target deconvolution in live systems



- Identification of direct binding targets (and off-targets) of reversible or covalent drugs in live cells
- Cellular localization of the drug-target complex using fluorescence imaging microscopy

The Untargeted Metabolomics & Lipidomics Platform

Our Offer

- **Metabolites**
  400 – 2000 monitored features
- **Complex Lipids**
  300 – 1500 monitored features

Optimized and Standardized Lab Workflows

In-House Developed Processing Tools

Rich Library of Standards

High Quality Data

Biomarker Identification

- Pharmacodynamics
- Disease Mechanism
- Drug Development
- ‘Omics Integration
- Trait Prediction
- Precision Medicine

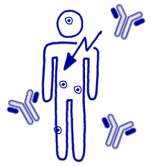
Samples

Neurological Disorders

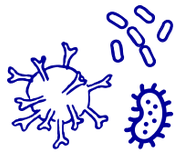
Oncology

Cardiovascular Diseases

Diabetes



Autoimmune Diseases



Infectious Diseases

Aging

The Untargeted Metabolomics & Lipidomics Platform

Our Offer

Set up project

- Support planning and designing experiment
- Advice sample collection

Level I

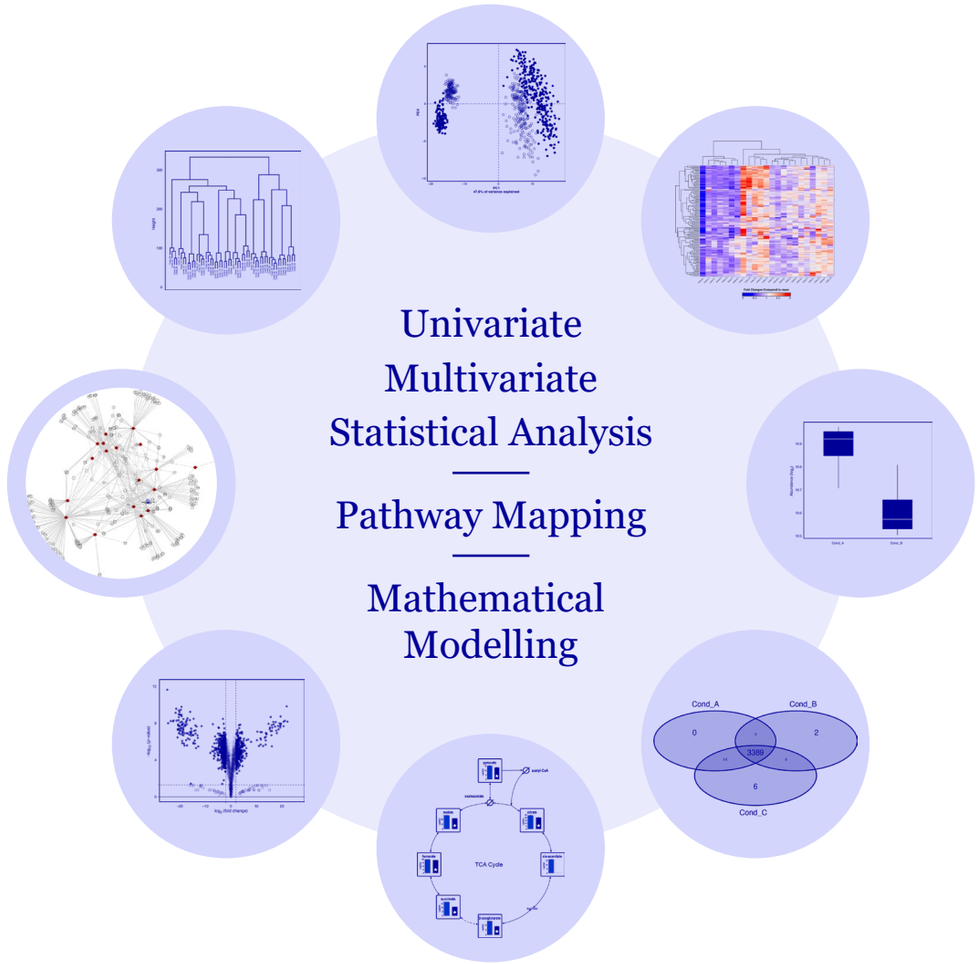
- Annotation of the content
- Exploratory statistical analysis

Level II

- Level I deliveries +
- In dept data analysis

Level III

- Level II deliveries +
- Data Modelling / Data Integration



The Untargeted Metabolomics & Lipidomics Platform

Our Offer

Biomarker Identification

- Diagnostic
  (acute / predictive)

‘Omics Integration

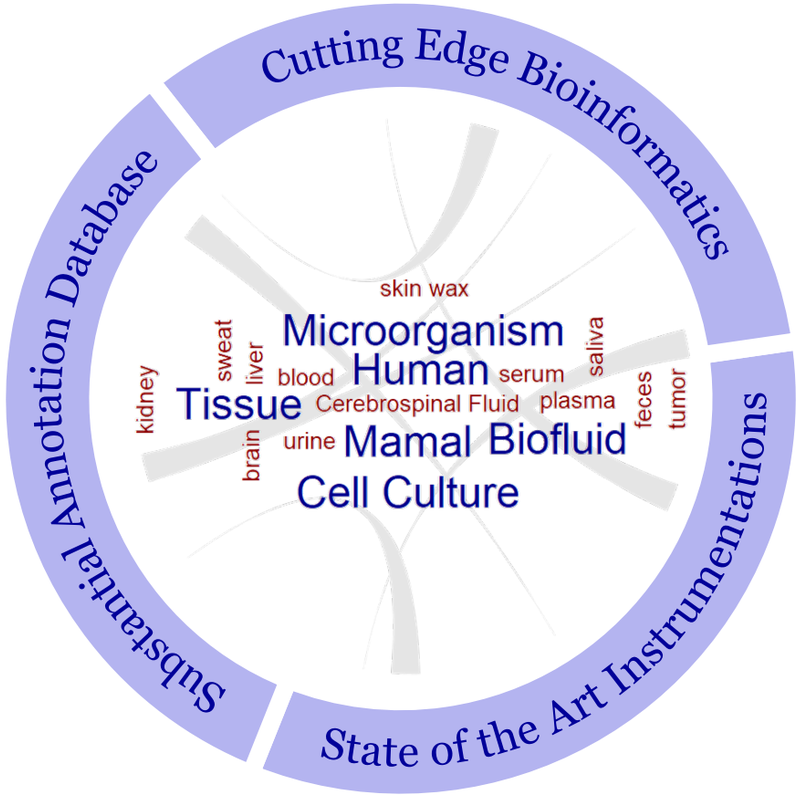
- Molecular partner identification
- Gene Identification

Trait Prediction

- Toxicity classification

Pharmacodynamics

- Quantify drug activity on a pathway or a profile



Disease Mechanism

- Better characterisation of diseases and their stages
- Validation of animal models

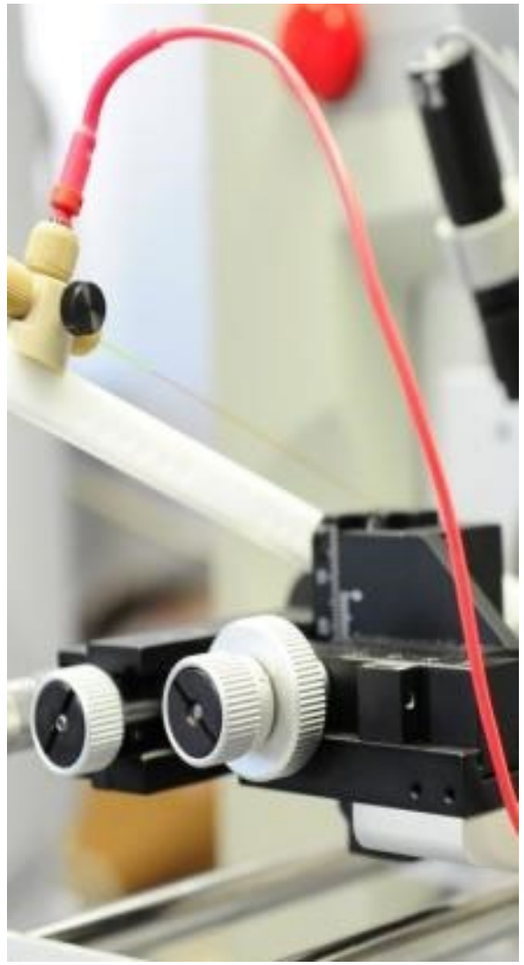
Drug Development

- Target identification
- Mode of action uncovering
- Explore mechanism of toxicity

Precision Medicine

- Patient categorisation
- Tailor drug treatments to individual's profile

Evotec Proteomic & Metabolomic Solutions



Fit for Purpose	Highly optimized experimental strategies tailored to different applications and project needs
High-End	Industry-leading capabilities in high-end quantitative mass spectrometry
High-Throughput	Industrialized, QMS supported process enables analysis of 1,000s of samples with constant high quality
Data Infrastructure	Experience and infrastructure to analyse the enormous amounts of data generated in large-scale studies
Advanced Bioinformatics	Advanced statistics and bioinformatics for systems-wide data analysis and in-depth data interpretation
Quality and Reporting	Extensive track record to deliver high quality and actionable results within agreed timelines

evotec

#RESEARCHNEVERSTOPS

Dr. Christoph Schaab
SVP Head of Proteomics & Metabolomics

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