BIOGNOSYS

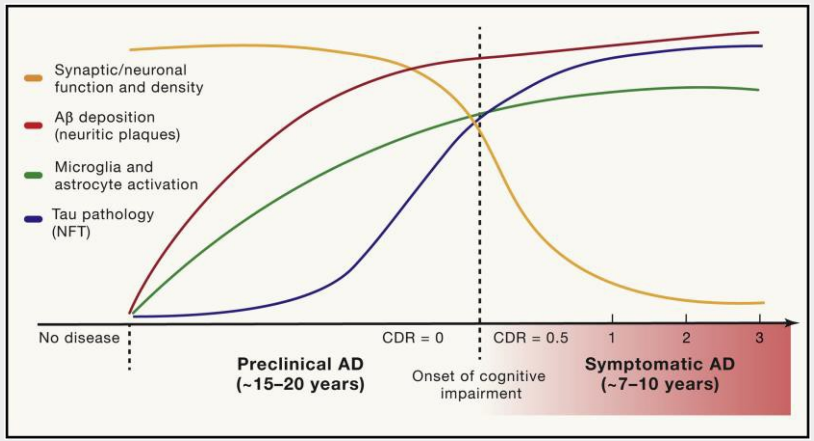
DEEP PROTEOMIC PROFILING OF CSF FROM SUBJECTS WITH ALZHEIMER'S DISEASE

CSF Biomarker Discovery Platform

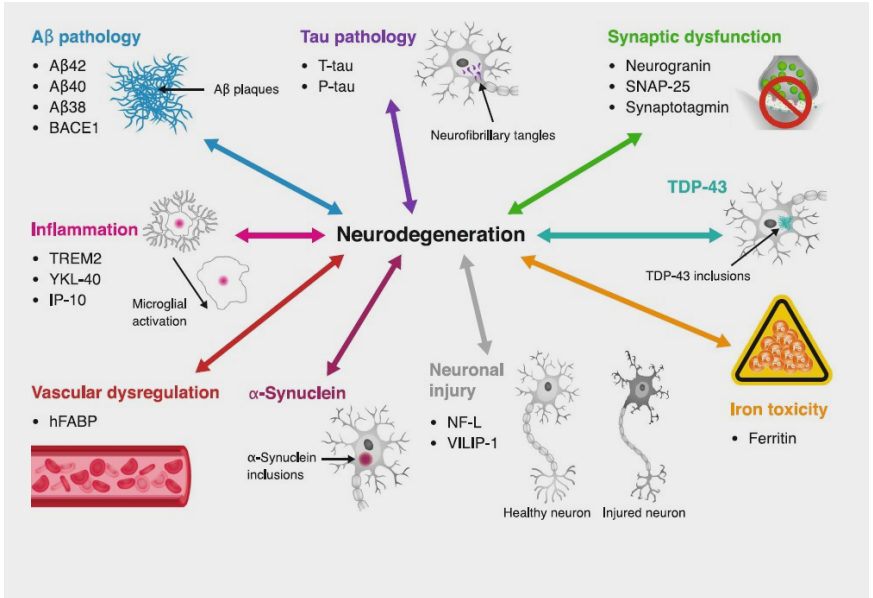
Contact us at
services@biognosys.com
www.biognosys.com

Multiple Molecular Mechanisms Describe Complex Pathobiology of Alzheimer's Disease

Long Pre-symptomaticTime



Multiple Mechanisms Involved

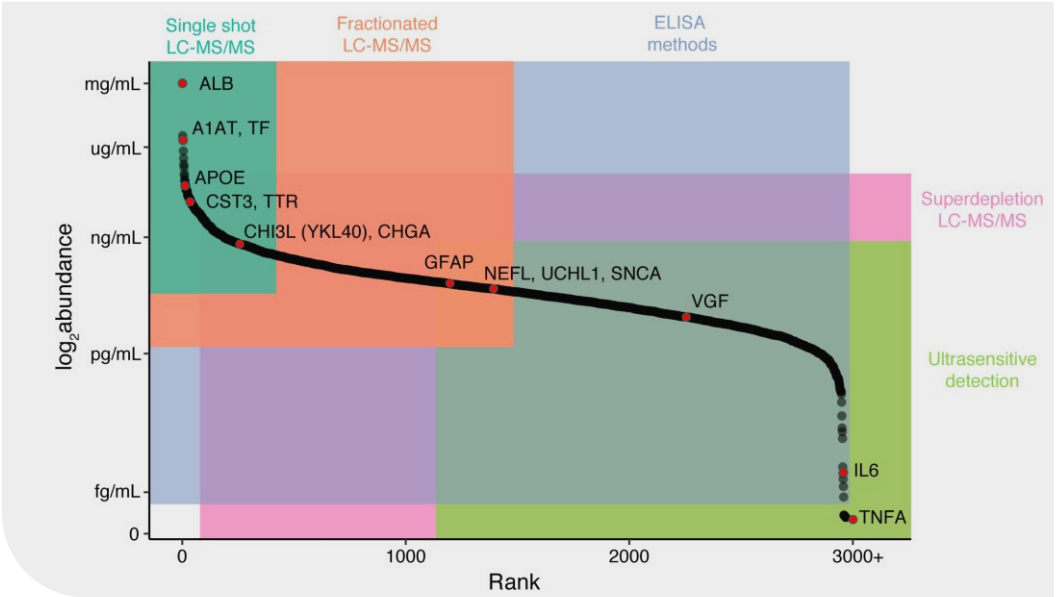


Cell (2019) 179, 2:821–853

Acta Neuropathologica (2018) 136:821–853

Multiplexed Proteomic Approaches for CSF Phenotypic Characterization

Trade-off between Sensitivity and Multiplexing



Proteomes 2018, 6, 32; doi:10.3390/proteomes6030032

- Unbiased analysis shows linkages of biomarkers to more completely characterize a disease state
- Each experimental approach has different benefits
- Single shot LC-MS/MS enables simultaneous measurement of multiple analytes
  - Ideal use of limited samples
  - Scalable to 100's -1'000's of samples
- Focus: DIA/HRM discovery proteomics
  - Validated in other sample matrices
  - Increased depth of characterization and number of analytes quantified
  - Goal – increase information content gained from single sample analysis

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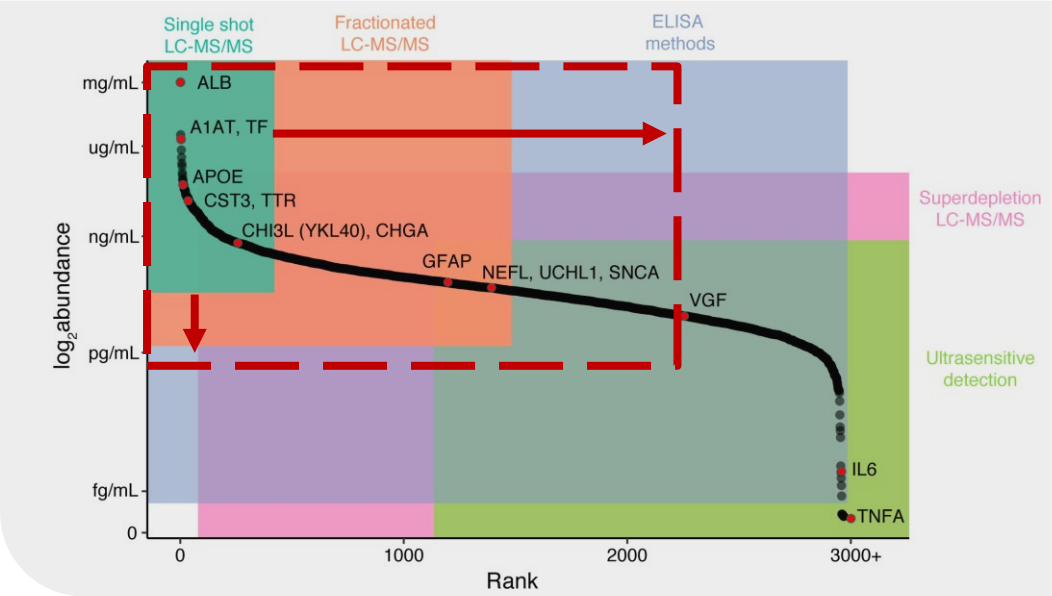

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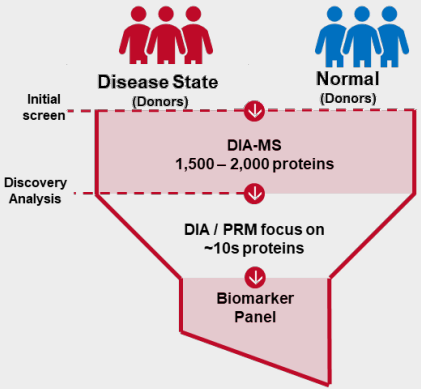
Experimental Strategy and Study Cohort

Study Design



Sporadic Alzheimer's Disease
MMSE >= 14 to <=18
compared to
age-matched normal controls

Cohort Information

Diagnosis	Normal Controls	Alzheimer's Disease
N (%)	8 (33%)	16 (67%)
Median MMSE (0-30; 95% CI)	30 (28-30)	16 (15-17)
Median Age (yrs; 95% CI)	66 (55-74)	63 (53-74)

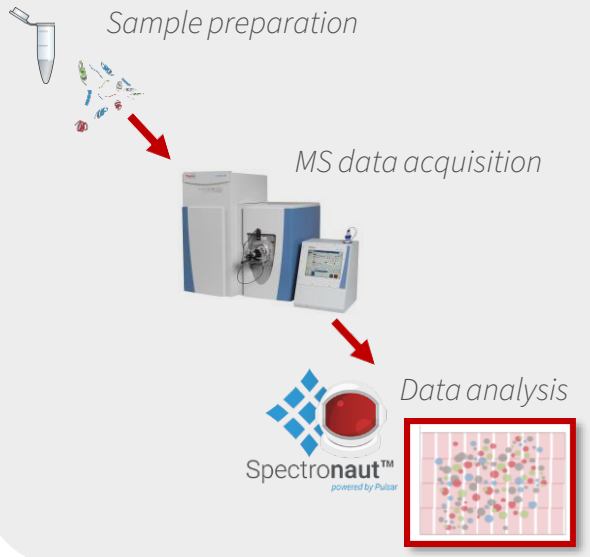
Study Focus

- Select a case-controls cohort to highlight dysregulated proteins and pathways associated with Alzheimer's Disease
- Subjects in Alzheimer's cohort have severe dementia so this cohort will be reflecting late stage biology

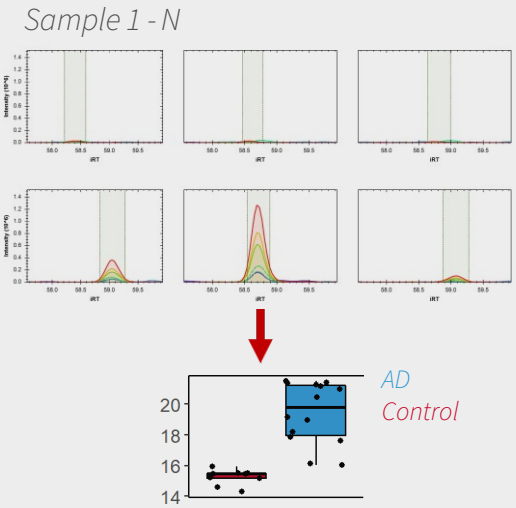
Unbiased Global Protein Quantification

HRM/DIA-MS Platform

Robust Experimental Workflow



Protein Quantification

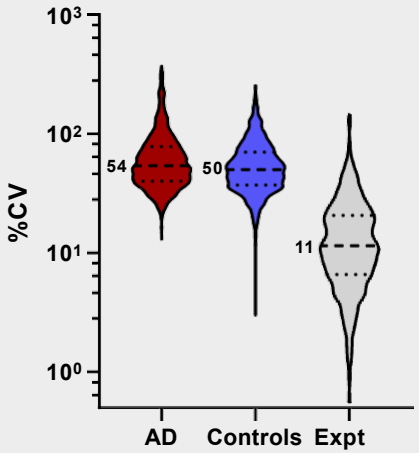


CSF Proteome Coverage

- 1’535 proteins quantified on average in each sample
- 1’924 proteins quantified across the sample cohort
- 1’719 proteins included for statistical testing

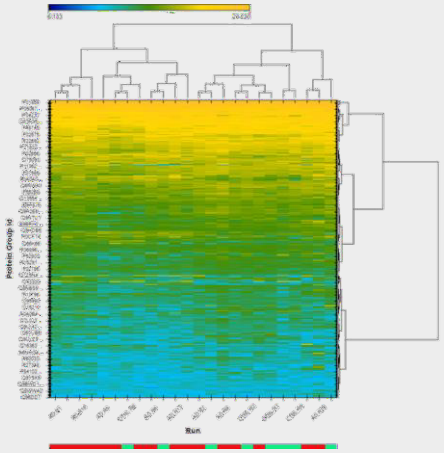
Exploratory Analysis of Global Proteomes and Proteins Associated with AD Phenotype

Inter-subject Heterogeneity



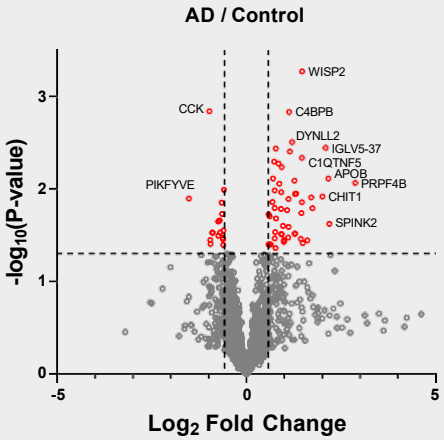
Biological variance is much greater than the experimental precision

Subtle Global Changes



Global clustering shows partial separation of groups [7] Subtle changes in AD CSF proteome

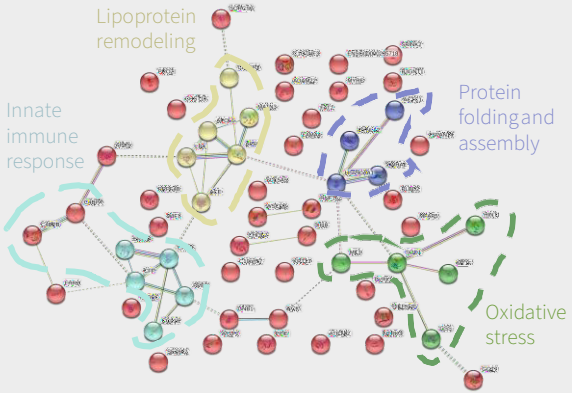
Group-wise Statistical Testing



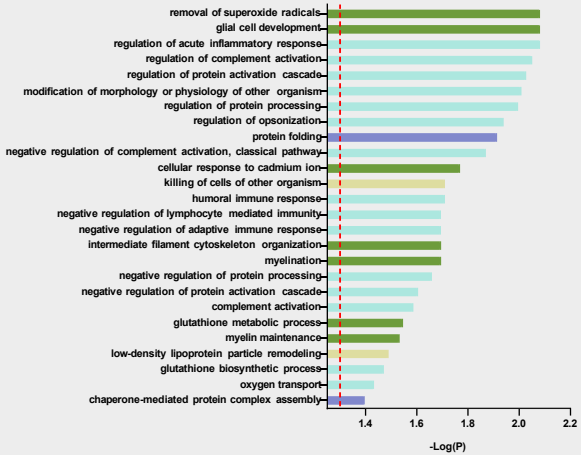
73 proteins differentially expressed including: WISP2, C1QTNF5, C4BPA/B, ApoB, CHIT1

Functional Analysis of Protein Biomarker Candidates

Functional Groupings



Biological Process(GO)

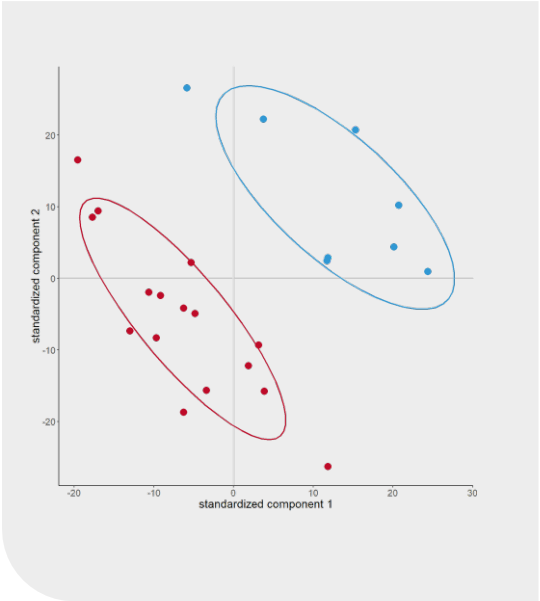


Coverage of Selected Known Markers

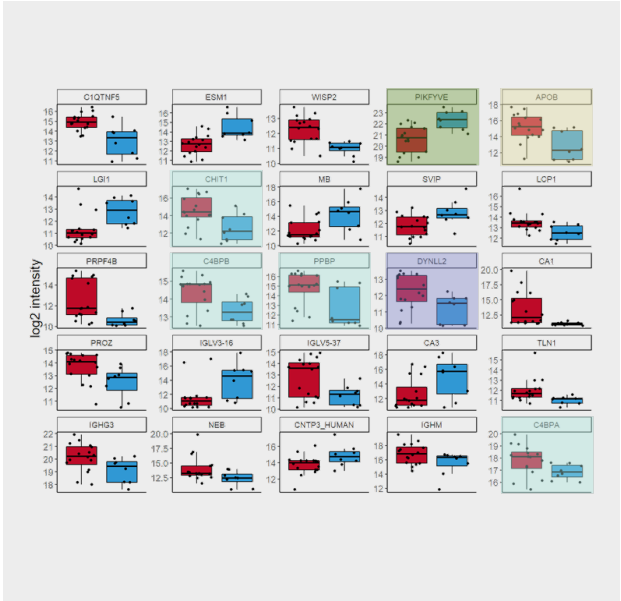
Aβ pathology	APP
	BACE1
Tau pathology	Tau
Synaptic function	SNAP25
	NEUG
	SYT7
Inflammation	IP10
	TREM2
	CHI3L1
Synapse loss	CLSTN1
	GLUR4
	NRXN2

Identification of Driver Protein Biomarker Candidates

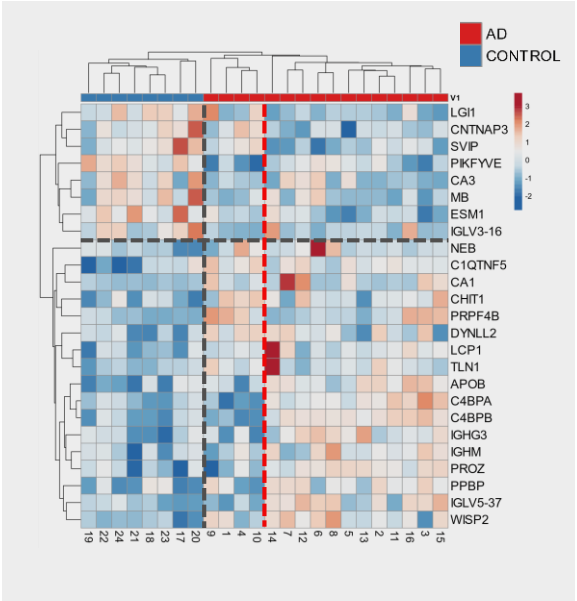
Multivariate PLS-DA Analysis



Quantification of Top 25 Candidates

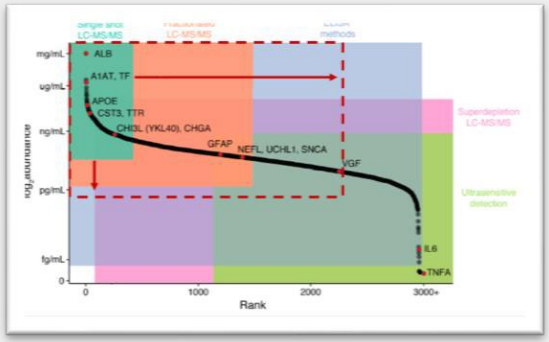


Protein Signatures Revealed Subpopulation

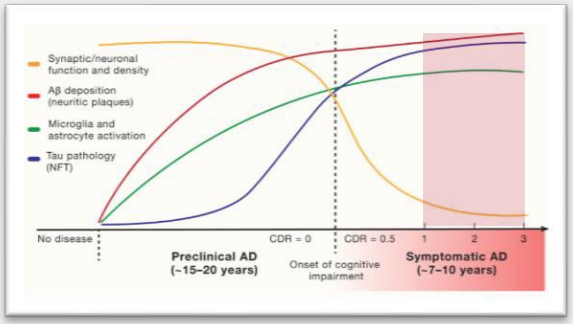


Summary and Potential for CSF Proteomics in Drug Development for Neurological Disorders

HRM™-MS Expands Access to CSF Biology



Insights into Disease and Drug Mechanisms



Potential for CSF Proteomics

- Robust quantification of 1’500-1’900 CSF proteins
- Scalable workflow to profile 100s-1’000s of samples
  - Longitudinal study
- Panel(s) of protein signature can be deployed for patient stratification and correlation with observations from MRI, PET

BIOGNOSYS

ACCELERATING BIOPHARMA R&D WITH NEXT GENERATION PROTEOMICS

Biognosys Proteomics Platform

Greg Moore
Director of Business Development
greg.moore@@biognosys.com
www.biognosys.com

SUPPORTING BIOMARKER RESEARCH IN DRUG DEVELOPMENT

Biomarker Discovery
HRM-MS™

Protein Quantification
HRM-MS™ & MRM/PRM

Target Deconvolution
LIMITED PROTEOLYSIS

Capabilities

- Optimized reproducible workflows
- Novel Biomarker Discovery
- Novel Target Identification
- Understanding Drug Resistance
- Determine Mechanism of Action
- Small Molecule Target Validation and Deconvolution

Experience

- 100+ Projects per year
- 54+ Publications
- 250+ Pharma, biotech & academic partners

Roche, Genentech, Bayer, Pliant, Nestlé, Moderna, Epizyme, Max-Planck-Gesellschaft, Freenome …

FROM PIONEER TO LEADER IN NEXT GENERATION PROTEOMICS

2008

Company Foundation

Dr. Ruedi Aebersold leading proteomics pioneer

Spinoff ETH zürich

2012

DIA/SWATH/HRM™ MassSpectrometry

Proprietary platform

Discovery Proteomics HRM

2013

HRM™ and Biognosys Software

Next-generation quantitative proteomics workflow with dedicated software packages

Spectronaut™ powered by Pulsar

SpectroDive™ powered by Pulsar

SpectroMine™ powered by Pulsar

QuiC™ powered by Pulsar

2018

Drug Target Deconvolution Platform

Novel target deconvolution technology – exclusive license

Global Presence

100+ Projects per year
250+ Pharma, biotech & academic partners

Roche, Genentech, Bayer, Pliant, Nestlé, Moderna, Epizyme, Max-Planck-Gesellschaft, Freenome, ...

2021

Implementation of GCP Laboratory

ENABLING RESEARCH ACROSS THE DRUG DISCOVERY AND DEVELOPMENT PIPELINE

Discovery Biology & Discovery

- Target Deconvolution
- Mechanism of Action
- Target Validation, Novel Target Discovery
- Disease Biology Profiling

Preclinical

- Animal Model Profiling
- Translational Biomarker Discovery
- Validation of Protein Expression
- Toxicity Profiling

Early & Late Clinical

- Tissue
- Blood
- CSF
- Urine
- …

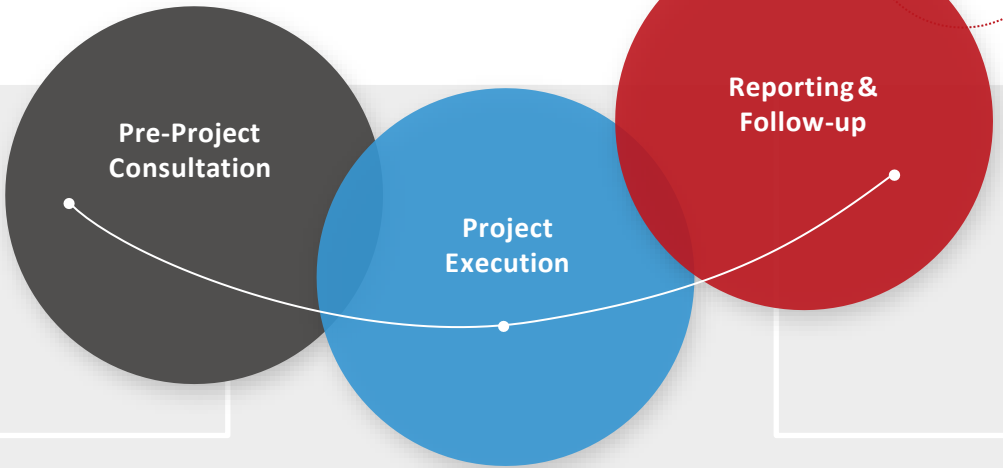
WORKING WITH US: TYPICAL SAMPLE REQUIREMENT

Biological Sample	Cell Culture	Plasma/Serum	Tissue	Urine	CSF
Sample amount required	10^5 – 10^6 cells*	10 µl / 100µl if depletion	1 – 5 mg*	500 µl	50 – 100 µl
Recommended HRM™ workflow	ID+, HighD	SpeeD, ID	ID+, HighD	ID+	ID+
Approx. Number of proteins quantified	3'000 – 9'000	300 – 600 (non-depleted) 400 – 1'400 (depleted)	3'000 – 9'000	600 – 2'400	500 – 1'400
Sample storage conditions	fresh frozen freeze-dried	fresh frozen	fresh frozen freeze-dried FFPE	fresh frozen	fresh frozen

* for phospho-proteomics minimum of 10^7 cells and 10 mg tissue

WORKING WITH US, AN EXAMPLE: 50 HUMAN PLASMA SAMPLES

Turnaround: 5 weeks



Initial contact

Project discussion

Research plan and quotation

Project commitment

Protein quantification tables

Full report

Raw data

Live report presentation

Project Initiation

Samples arrival at Biognosys

Sample Processing

1.5 weeks

Sample preparation for MS analysis

Spectral library generation

Project Quantification with HRM-ID Workflow

2.5 weeks

Sample measurement

Customized Data Analysis

1 week

In-depth statistics

Pathway analytics

Data interpretation