Expanded View Figures

A

Workflow

Metabolic labeling of glycoproteins (GP) with ManNAz

1 Million cells

40 Million cells

Shedding

Secretion

Lectin based GP enrichment

Click reaction of azide-functionalized GP

GP bound to magnetic bead (MB)

GP modified with biotin

Streptavidin pull down

Washing to eliminate contaminations

Washing to eliminate contaminations

1% SDS
8M Urea
20% ACN

1% SDS

SDS-PAGE fractionation (14x)

Albumin excluded

Tryptic digestion

On-bead

In-gel

Tryptic peptides

nLC-MS/MS mass spectrometry analysis

Label-free quantification

DDA vs DIA

DDA

B

10% of input

ConA Eluate

Albumin

C

MS1 scan

MS2 scan

Cycle time (out of scale)

m/z

D

# sources:

Total=484

E

Zielinska et al. (396)

Joshi et al. (235)

Fang et al. (407)

Liu et al. (231)

Uniprot (400)

Albumin excluded

Tryptic peptides

F

BR 1

BR 2

BR 3

BR 4

SPECS

DDA

DDA

DDA

DDA

BR 1

BR 2

BR 3

BR 4

hiSPECS

DDA

DDA

DDA

DDA

Figure EV1.
Figure EV1. Benchmarking hiSPECS against SPECS.

A Comparison of the novel hiSPECS (green) and the previous SPECS (blue) protocol (Kuhn et al, 2012). hiSPECS uses lectin-based glycoprotein enrichment followed by covalent binding to magnetic beads which improves sample processing. On-bead tryptic digestion is followed by mass spectrometry analysis and label-free quantification (LFQ) using data-independent or data-dependent acquisition (DIA vs DDA). In contrast, SPECS depends on biotinylation of the azide-functionalized glycoproteins, followed by streptavidin pull-down, SDS-gel-based fractionation into 14 gel slices, and DDA analysis only.

B Coomassie-stained gel showing the prominent reduction in albumin as a result of glycoprotein enrichment with ConA. Left lane: secretome (conditioned medium) of primary neurons before glycoprotein enrichment. 10% of the volume was loaded that was used for the ConA enrichment. Right lane: eluate after glycoprotein enrichment using ConA beads. The albumin band is highlighted with a red arrow.

C A representative peptide density plot of a neuronal secretome using the hiSPECS method and analyzed in DDA mode. The m/z ratio is plotted against the retention time. One MS1 scan (purple) is followed by 20 MS2 scans covering a range of 300–1,400 m/z with an overlap of 1 m/z between adjoining m/z windows. The m/z windows were adjusted to achieve equal numbers of peptides.

D Identified glycoproteins in the secretome of primary neurons using the DIA hiSPECS protocol according to UniProt, (Fang et al, 2016), (Joshi et al, 2018), (Liu et al, 2017), or (Zielinska et al, 2010).

E Venn diagram illustrating in which sources the proteins of (D) were found to contain a glycosylation site. As expected for the glyco-secretome, more than 85% of the quantified secretome proteins were annotated as glycoproteins.

F Representative Pearson correlations of log₂-transformed protein LFQ intensities of four biological replicates (BR) processed with either the SPECS or hiSPECS method. In the previous SPECS studies, sample pairs were separated on the same gel to achieve high reproducibility, whereas the correlation of biological replicates run on different gels was rather low. Thus, protein LFQ ratios of the individual replicates were used for statistical evaluation. The blue squares indicate samples run on the same gel during sample preparation [SPECS data obtained from (Kuhn et al, 2012)].
Figure EV2.
Figure EV2. Quality control of cell type-resolved mouse brain secretome resource.

A Data transformation of the hiSPECS DIA secretome analysis of the brain cell types. Log$_2$ transformation of the intensities ($N = 1$). The central band indicates the median, the boxes indicate the lower or upper quartile, the whiskers indicate minimum or maximum, and dots indicate outliers.

B Functional annotation clustering with DAVID 6.8 (da Huang et al, 2009a; da Huang et al, 2009b) for gene ontology term cellular component (FAT) of the 995 hiSPECS proteins identified using the mouse proteome as background. The dot sizes indicate the log$_2$ enrichment score.

C Fold change in cell type-specific proteins in the brain cells. Log$_2$ ratio of the average abundance in the specific cell type to the median abundance in the other cell types in the hiSPECS secretome or lysate analysis (Sharma et al, 2015) is shown. Known cell type-specific marker proteins are highlighted for each cell type which reveals a strong enrichment in the lysate and secretome of the primary brain cells verifying the quality and comparability of the primary cultures. For example, the ectodomain of the membrane protein NCAM2 (with an y-axis value of 3 in the log$_2$ scale) is secreted about eightfold more from oligodendrocytes compared with the median of the other three cell types and also expressed at a higher level in this cell type compared with the median of the other three cell types. Horizontal lines indicate the mean.

D Principal component analysis (PCA). The secretomes of the four cell types segregated based on the two major components of all 995 proteins identified in at least 5 biological replicates in one cell type, which accounted for 44.9% and 19% of the variability, respectively.
Figure EV3.
Figure EV3. Top 50 cell type-specifically enriched proteins in the secretome resource.

A Heat map of the top 50 differentially secreted proteins (Bonferroni’s P adj < 0.05 using the R package Limma (Ritchie et al, 2015)) across the four cell types from hierarchical clustering. For the missing protein quantification data, an imputation approach was undertaken using data missing at random within a left-shifted Gaussian distribution by 1.8 standard deviation. The rows represent the differentially secreted proteins, and the columns represent the cell types with their replicates. The colors represent log-scaled protein levels with blue indicating the lowest, white indicating intermediate, and red indicating the highest protein levels.

B Functional annotation clustering with DAVID 6.8 (da Huang et al, 2009a; da Huang et al, 2009b) for gene ontology term biological process (FAT) of the cell type-specific secretome proteins (Table EV4). All proteins detected in the hiSPECS brain secretome study have been chosen as the background. The dot sizes indicate the enrichment score.
Specifically secreted from astrocytes

Specifically secreted from microglia

Specifically secreted from neurons

Specifically secreted from oligodendrocytes

Figure EV4. Protein levels in the brain cell secretome vs lysate proteome.

Comparison of the iSPECS secretome resource and lysate data by Sharma et al (2015). The % enrichment is indicated normalized to the average of the most abundant cell type. For example, APLP1 is similarly abundant in lysates of neurons and oligodendrocytes, but only secreted to a relevant extent from neurons. Thus, APLP1 is classified as a cell type-specifically secreted protein. Horizontal lines indicate the mean.
Figure EV5. Substrate candidate identification of the Alzheimer’s protease BACE1 as proof of principle of the hiSPECS method.

A The same experiment as in Fig 5A and B, but using DDA instead of DIA for mass spectrometric data acquisition. The volcano plot shows changes in protein levels in the secretome of primary cultured neurons (hip + ctx) upon BACE1 inhibitor C3 treatment using the hiSPECS DDA method. The negative log_{10}-transformed P-value (two-sample t-test) of each protein is plotted against its log2 fold change comparing inhibitor-treated and control condition (N = 11). The gray hyperbolic curves depict a permutation-based false discovery rate estimation (P = 0.05; s0 = 0.1). Significantly regulated proteins (P < 0.05) are indicated with a dark blue dot, known BACE1 substrates are indicated with blue letters, and unknown ones are indicated with black letters. The two newly validated BACE1 substrates CD200 and ADAM22 are indicated in red. SEZ6 and IL6ST are not depicted in the volcano plot because they were only identified in the control condition.

B Correlation of the hiSPECS DDA to DIA method. Plotted are the log2 fold changes between C3 treatment and DMSO control samples (N = 11). The two newly validated BACE1 substrates CD200 and ADAM22 are indicated in red, known BACE1 substrates in blue, and unknown ones in black.

C The volcano plot (details described in (A)) shows changes in protein levels in the secretome of primary cultured neurons isolated either from the hippocampus (hip) or from cerebral cortex (ctx) using the hiSPECS DIA method. Proteins exclusively detected in one group are indicated.

D Correlation analysis of the average log2 LFQ values of the secretome proteins of (C).

E Venn diagram indicating the overlap of quantified proteins in the secretome of hippocampal vs cortical neurons. Proteins were considered robustly quantified if detected with at least 5 of 6 biological replicates.

F The same experiment as (A), however, using only hippocampal neurons and hiSPECS DIA (N = 8). Proteins were considered if quantified in at least 6 of 8 biological replicates in one group.

G Correlation of the hiSPECS DIA method comparing hippocampal to mainly cortical (mixed ctx and hip, as described in legend to panel A neuronal secretome; Fig 5B). Plotted are the log2 fold changes between C3 treatment and DMSO control samples. The newly validated BACE1 substrate CD200 is indicated in red, known BACE1 substrates in blue, and unknown ones in black.
Only detected in control samples: SEZ6, IL6ST

Only detected in hip samples: ADGRL2, EPHA4, GALNT18, HS6ST3, ICAM5, ISLR, LRRTM1, MEGEB, P10404, SLC30A1, SLIT3, SMOC1, TMEM108

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Figure EVS.