Simple targeted assays for metabolic pathways and signaling: a powerful tool for targeted proteomics

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Current content of STAMPS

Table S1: Current data content of STAMPS

| Registered organisms | Mouse |
|----------------------|-------|
| Registered tissues   | Brain, Blood, Eye, Fat, Gut, Heart, Kidney, Liver, Lung, Spleen |
| Number of metabolic pathways | 56 |
| Number of signaling pathways | 49 |
| Number of proteins     | 16,810 |
| Number of unique peptides | 116,873 |
| Number of spectra       | 152,000 |
| Number of proteins in pathways | 3,505 |
| Number of PRM / SRM validated Peptides | 670 |

Information about present pathways and the pathway structure including the protein and metabolite node location and connection were initially extracted from KEGG database\(^1\), UniProt\(^2\) and NCBI\(^3\).

Table S2: Distribution of identified peptides / spectra among the different tissues. Take into account that there are overlaps of peptides / spectra between the tissues.

| tissue | peptides | spectra | tissue | peptides | spectra |
|--------|----------|---------|--------|----------|---------|
| Blood  | 22058    | 25933   | Heart  | 13432    | 16557   |
| Brain  | 79128    | 98698   | Kidney | 41385    | 52447   |
| Eye    | 14145    | 16662   | Liver  | 23043    | 28791   |
| Fat    | 62454    | 76789   | Lung   | 30925    | 38341   |
| Gut    | 7476     | 8186    | Spleen | 35144    | 41565   |
### Current curators of STAMPS

Table S3: current pathway curators of STAMPS

| Curated pathway                                      | Curator                                           | Institute                        |
|------------------------------------------------------|---------------------------------------------------|----------------------------------|
| Adrenergic signaling in cardiomyocytes               | Prof. Kristina Lorenz                             | ISAS Dortmund                    |
| Arachidonic acid metabolism                          | Prof. Dr. Nils Helge Schebb; Nicole M. Hartung, PhD | University of Wuppertal          |
| Glycerolipid metabolism                              | Douglas Mashek, PhD                               | University of Minnesota          |
| Glycerophospholipid metabolism                       | Prof. Christer S. Ejsing                         | University of southern Denmark   |
| Glycosphingolipid biosynthesis – ganglio series       | Federico Torta, PhD; Bo Burla, PhD                | National University of Singapore |
| Glycosphingolipid biosynthesis – globo and isoglobo series | Federico Torta, PhD; Bo Burla, PhD                | National University of Singapore |
| Glycosphingolipid biosynthesis – lacto and neolacto series | Federico Torta, PhD; Bo Burla, PhD                | National University of Singapore |
| Immunological pathways                               | Dr. Olga Shevchuk                                 | ISAS Dortmund                    |
| PPAR signaling pathway                               | Dr. Robert Ahrends                                | ISAS Dortmund                    |
| Sphingolipid metabolism                              | Prof. Thorsten Hornemann                          | University Hospital Zurich       |
| Steroid biosynthesis                                 | Dr. Hans-Frieder Schött                           | ISAS Dortmund                    |
| Steroid hormone synthesis                            | Dr. Hans-Frieder Schött                           | ISAS Dortmund                    |
| UPR signaling                                        | Dr. Jan Medenbach                                 | University of Regensburg          |
Overview of assay development time for insulin signaling (including 140 proteins)

Table S4: time comparison of assay development time between STAMPS and other databases. All time specifications are expressed as hh:mm:ss.

| Tested proteomics pipeline | Single steps                                    | Time for each step | Complete time needed |
|----------------------------|------------------------------------------------|--------------------|----------------------|
| STAMPS                     | download assay                                 | 00:00:32           | 00:00:32             |
| PeptideAtlas               | download FASTA file\(^a\)                      | 01:18:06           | 01:18:59             |
|                            | download of protein library                    | 00:00:53           |                      |
| ProteomicsDB               | download FASTA file\(^a\)                      | 01:18:06           | 01:18:06 + 00:40:30 - n |
|                            | create spectral library\(^b\)                   | 00:40:30 - n       |                      |
| Picky                      | download FASTA file\(^a\)                      | 01:18:06           | 01:19:29             |
|                            | download assay                                 | 00:01:23           |                      |
| NIST library               | download FASTA file\(^a\)                      | 01:18:06           | 01:19:45             |
|                            | download of protein library                    | 00:01:39           |                      |

\(^a\) Downloading enzyme list from KEGG (00:03:02), converting KEGG ID to Uniprot accession numbers (01:13:53) and downloading Fasta file from Uniprot (00:01:11)

\(^b\) Including downloading the raw file (00:01:38 - n), converting (00:20:57 - n), peptide identification with SearchGUI (00:11:05 - n), protein inference with PeptideShaker (00:05:02 - n) and spectral library creation with Skyline (00:01:48 - n), where n is the number of files taken to create the spectral library

The time evaluation is based on a domain expert using all mentioned tools and frameworks to create a fasta file and a spectral library containing all necessary protein information of the insulin signaling pathway. Note, that for PeptideAtlas & NIST library only complete spectral libraries for mouse could be downloaded as well as a complete library was created when using raw files from ProteomicsDB. In Picky, only ≤ 100 IDs can be used in one assay. When only human experiments were available in those databases, we took human instead of mouse data.
## Feature comparison

Table S5: Feature comparison between STAMPS and other databases offering spectral data

| Feature                                      | STAMPS | PeptideAtlas\(^1\) | ProteomicsDB\(^2\) | Picky\(^3\) | NIST Library\(^4\) |
|----------------------------------------------|--------|---------------------|---------------------|-------------|---------------------|
| Human curation of spectra                    | X      | n/a                 | n/a                 | n/a         | n/a                 |
| Pathway curation by domain experts           | X      |                     |                     |             |                     |
| Statistical support for method dev.          | X      |                     |                     | X           |                     |
| Web-editor for editing pathways              | X      |                     |                     |             |                     |
| High-resolution spectra                      | X      |                     |                     | X           |                     |
| Downloadable spectra                         | X      | X                   |                     | X           | X                   |
| Protein selection                            | X      |                     |                     |             |                     |
| Downloadable protein sequence                | X      | X                   | X                   | X           |                     |
| Peptide selection                            | X      |                     |                     |             |                     |
| Peptides from real data                      | X      |                     |                     | X           |                     |
| Spectra selection                            | X      |                     |                     |             |                     |
| Spectrum view                                | X      |                     |                     | X           | X                   |
| Mouse data                                   | X      | X                   |                     | X           | X                   |
| Human data                                   | (X)    | X                   | X                   | X           | X                   |
| Proteins tissue resolved                     | X      |                     |                     |             |                     |
| Proteins chromosome resolved                 | X      |                     | X                   |             |                     |
| Proteins pathway resolved                    | X      |                     |                     |             |                     |
| Visual protein selection                     | X      |                     |                     |             |                     |
| Protein batch selection                      | X      |                     |                     |             | X                   |
| Proteins enzymatically resolved              | X      |                     |                     |             | X                   |
| Proteins subcellular resolved                | X      |                     |                     |             | X                   |
| Assay development                            | X      |                     |                     |             | X                   |
| Integration with cons. analysis tool\(^*\)   | X      |                     |                     |             | X                   |

\(^{(X)}\) = available in the next version  
\(n/a\) = information not available  
\(^{*}\)e.g. Skyline

\(^1\) [http://www.peptideatlas.org/](http://www.peptideatlas.org/)  
\(^2\) [https://www.proteomicsdb.org/](https://www.proteomicsdb.org/)  
\(^3\) [https://picky.mdc-berlin.de/](https://picky.mdc-berlin.de/)  
\(^4\) [https://peptide.nist.gov/](https://peptide.nist.gov/)
Application – Methods

Cell culture

Preadipocytes were cultivated in minimum essential medium (Mem-α) supplemented with 20% fetal bovine serum (FBS) and 1% penicillin/streptomycin (PSG) at 37 °C and 5% CO₂. Medium change was done every two days followed by propagation with trypsin if cells were at 80% confluence. The experiment consisted out of 4 different conditions: control, induced insulin sensitivity, induced insulin resistance and both together. For each of the 4 conditions, cells were grown until 80% of confluence to start the treatment. For inducing insulin sensitivity, cells were treated with 1 µM of rosiglitazone for 48 h. To induce insulin resistance, the treatment was done with 10 ng/ml TNFα for 24 h. The last condition was prepared by adding first 1 µM rosiglitazone. After the incubation time of 48 h, cells were washed and 10 ng/ml TNFα was added for additional 24 h. The control condition comprised preadipocytes grown to 80% confluence and treated with just 0.01% Dimethylsulfoxid (DMSO). Harvested cells were washed with PBS, centrifuged and snap frozen. See Figure S1 for a summarizing flow chart.

Sample preparation

Cell pellets were lysed with a 1% SDS lysis buffer. Frozen mouse organs were homogenized by grinding in liquid nitrogen, one at a time. To achieve this, each organ were placed in a liquid nitrogen-cooled ceramic mortar separately. The tissues were manually grinded with a pestle to obtain a fine powder (sterile conditions). The powder was collected in Eppendorf reaction tubes and 50 µg of each prepared organ were lysed using a 1% SDS lysis buffer. To remove interfering DNA, the samples were treated with Benzonase® for 30 min at 37 °C, followed by protein precipitation using 3 volumes of ice cold acetone overnight at -20 °C. Precipitated proteins were harvested by centrifugation at 20,000 g for 20 min at 4 °C and dissolved in 100 µl 8 M Urea for 3 h at room temperature (RT). Protein amount was checked by conducting a BCA according to the manufacturer’s instructions. Samples were reduced by adding 10 mM tris(2-carboxyethyl)phosphine (TCEP) for 30 min at 37 °C and alkylated by adding 15 mM of iodoacetamide (IAA) for 30 min at 21 °C (dark). Hydrolysis of proteins was done overnight at 37 °C using a trypsin to protein ratio of 1:100. The reaction was stopped by adding 2 µl of formic acid (FA, 99%). The samples were desalted using solid phase extraction with C18 filter cartridges, washed with 0.1% trifluoroacetic acid (TFA) and eluted with 80%
acetonitrile (ACN). Cleaned samples were dried by using a vacuum concentrator. Concentration was adjusted to 1 µg/µl with 0.1% TFA. Proteolytic digests were checked for complete digestion after desalting by using monolithic column separation (PepSwift monolithic PS-DVB PL-CAP200-PM, Dionex) on an inert Ultimate 3000 HPLC (Dionex, Germering, Germany) by direct injection of 1 µg sample. A binary gradient (solvent A: 0.1% TFA, solvent B: 0.08% TFA, 84% ACN) ranging from 5-12% B in 5 min and then from 12-50% B in 15 min at a flow rate of 2.2 µL/min and at 60 °C, was applied. UV traces were acquired at 214 nm.

For pH8 fractionation each of the digested and desalted samples was first dried using a vacuum concentrator. Subsequently, the peptides were brought back into solution in a buffer consisting of 10 mM ammonium acetate and 0.4 mM formiate (pH 8.0) and separated on a C18 RP column (50µg). The separation and fractionation was performed with the following gradient by solvent B (84% acetonitrile in 10 mM ammonium acetate, 0.4 mM formiate, pH 8.0): 3-10% B for 10 min, 10-25% B for 35 min, 25-40% for 20 min, 40-95% for 10 min., 95% for 5 min. and 20 min. equilibration at 3% B. The individual fractions were collected in a time interval of 60 s, each sample being divided into 16 fractions. The fractions were collected in concatenated manner. After fractionation, the individual samples were dried in a vacuum concentrator and recorded in 0.1% TFA (1 µg/µl) for the subsequent nano LC-MS/MS analysis.

**Method development and targeted LC-MS analysis**

To develop the method for the targeted LC-MS/MS analysis, we used STAMPS to select proteins and peptides for the tricarboxylic acid (TCA) cycle and insulin signaling pathway according to the pathway browser and the built spectral library. After importing the method into Skyline, three transitions were chosen for each peptide that showed the best signal to noise ratio. In addition, synthetic peptides were synthesized for each chosen proteotypic peptide to avoid false positive peak annotation and to confirm the correct retention time. Synthetic peptides were labeled with [13C6] R and [13C6] K. After creating a scheduled SRM method with 5 minute windows, we were able to scan for the proteins of one whole pathway per measurement by keeping the maximum concurrent occurring transitions always below 100.
Chromatographic separation of peptides was achieved on an UltiMate 3000 RSLC nano System (Dionex) using a 75 μm × 2 cm C18 pre column coupled to a 75 μm × 50 cm C18 reversed phase main column (Acclaim Pepmap, Thermo Scientific). A 30 min gradient (0.1% FA, 2 – 35% ACN) was applied with a flow speed of 250 nl/min. Peptides were ionized by using a nano spray ESI-source and analyzed on a TSQ Vantage mass spectrometer.

**Figure S1 | Summarizing flow chart of the different steps for sample preparation.** 1: Control condition comprising only normal grown preadipocytes for a period of 72 h containing 0.01% of DMSO. 2: Steps to prepare insulin-resistant cells by treating them with 10 ng/ml TNFα. 3: Steps to prepare insulin sensitive cells by treating them with 1 μM of rosiglitazone for 48 h. 4: Combination of treatments to check if TNFα is able to revert the effect of rosiglitazone.

**Application – Results**

To determine the practicability of the workflow, we compared STAMPS with state-of-the-art targeted proteomics tools (Table S4). We measured the time to create a targeted assay for insulin-signaling. Here, a time reduction of $\geq 150$-fold was achieved compared to the second fastest tool. A list of all features in comparison to other state-of-the-art tools is shown in Table S5.

Next, to confirm the applicability of STAMPS, we selected insulin-signaling and the tricarboxylic acid cycle (TCA), two pathways important for the overall metabolism of higher
eukaryotes. Key proteins of these pathways were analyzed after inducing insulin resistance and sensitivity (Figure 2, S1 - S3).

Figure S2 | Overview of all measured proteins part of the TCA. In total 24 proteins of the TCA were measured. Colors represent the two unique peptides used per protein for the targeted analysis. Standard deviation was calculated with 3 biological replicates of each condition (n=3). Numbers represent the different treatments: (1) DMSO control, (2) 10 ng/ml TNFα, (3) 1 µM rosiglitazone, (4) initial 48 h rosiglitazone plus 24 h TNFα afterwards. Asterisks were used to show significant changes in the peptide abundance compared to the control or by comparing the last 2 conditions. For better visualization, a dashed black line was added to show the trend of the respective treatment.
Figure S3 | Overview of all measured proteins part of the insulin signaling pathway. In total 30 proteins of the insulin signaling pathway were measured, representing the key players. Colors represent the two unique peptides used per protein for the targeted analysis. Standard deviation was calculated with 3 biological replicates of each condition (n=3). Numbers represent the different treatments: (1) DMSO control, (2) 10 ng/ml TNFα, (3) 1 µM rosiglitazone, (4) initial 48h rosiglitazone plus 24h TNFα afterwards. Asterisks were used to show significant changes in the peptide abundance compared to the control or by comparing the last 2 conditions. For better visualization, a dashed black line was added to show the trend of the respective treatment.
Tutorials

Creating an assay using the pathway browser

One of the major tasks in targeted proteomics is building up methods to analyze proteins of one or multiple pathways to uncover their behavior upon different treatments revealing coherencies and patterns, for instance in the metabolism. To facilitate those studies and gain results as shown in Figure 2, STAMPS provides a multitude of intuitive interfaces for fast protein selection, spectra reviewing, assay building and is interlinked with Skyline.

1) Select pathway browser

The first page of the STAMPS\(^5\) website is leading to all main protein selection options as visible in Figure S4. Clicking on “Pathway Browser” in the lower panel will lead to the interactive main pathway browser window (Figure S5). The view can be shifted (right click) and the zoom can be changed (mouse wheel or magnifier icons on bottom right position). The metabolic pathways are plotted in a graph-based fashion. Metabolites are presented as circles; proteins which act in the same metabolic reaction are grouped in protein boxes. By directly clicking on metabolite or protein labels, additional information is displayed. Links to other Pathways are indicated in round-cornered boxes. By clicking on them, the according pathway will be opened.

\(^5\)https://stamps.isas.de
2) Select pathway(s)

In the upper panel, the pathway of interest can be chosen. For a better overview, pathways are grouped into metabolic and signaling pathways. Clicking on one of those options will open a drop down menu. Former protein selections are preserved when switching back to a pathway. Additional functions are (i) changing the organism (in the current version, only mouse data is available), (ii) various filters for viewing proteins and (iii) a case insensitive search function for key words like a protein/metabolite name, description, accession number or even peptide sequence. When selecting a protein searched this way, the view is automatically centered and zoomed on the hit in the pathway map.

3) Select proteins

Proteins of choice can be (de)selected by clicking on their checkboxes. Double-clicking on a protein group box will (de)select all proteins of the according box. By clicking and holding down the left mouse button on a free space and moving the mouse, a protein selection area will be spanned. When releasing the button, all proteins within this selection window will be (de)selected.

4) Pathway statistical overview

Figure S5 | The interactive pathway browser is the main protein selection window.
The user has the possibility to get a statistical overview for each pathway by clicking on the arrows in the upper right corner. A window will appear giving information of the total protein number of the selected pathway, protein coverage, number of selected proteins and additional information regarding the number of peptides, spectra and validation (see Figure S6).

![Figure S6](image)

**Figure S6** | Statistics box displaying all relevant information for a certain pathway.

5) **Check the protein selection and review spectra**

Having selected all proteins for an assay, the user can review the selection by clicking on “Check spectra” in the upper panel. A new window opens as shown in Figure S7. On the left-hand side, a table is showing all selected proteins. The user has the possibility to expand each protein to show its corresponding peptides that are stored with an according spectrum in the database. By clicking on the precursor mass, the corresponding spectrum pops up showing y and b ions. All proteins, peptides or spectra can be (de)selected on this screen for the final assay by checking or unchecking the blue boxes. To refine the assay even more, specific filter criteria can be applied. For instance, the user can filter for peptide length and precursor charge as well as choose between variable and fixed modifications of the oxidation of methionine and/or the carbamidomethylation of cysteine. In addition, there is the possibility to filter for specific tissues. The user has to take into consideration that only proteins, peptides and precursors will be displayed,
which were identified and stored in our database and in addition satisfy the adjusted filter criteria.

6) Download the assay

Once the selection is done, the user can proceed to download the assay by clicking on “Go to download” on the bottom right side of the dialog. A spectral library in .blib format, a fasta file containing all selected protein sequences and a Skyline project file are being created on the fly and packed as a .zip file offered for download. After downloading and unzipping, the Skyline project file can easily be opened in Skyline\(^6\) with the complete assay ready to start the targeted analyses.

![Figure S7](https://skyline.ms)

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**Figure S7 | Review dialog for (de)selecting proteins, peptides or single spectra in the assay.**

Creating an assay using the accession list

1) Open the protein selection window via accession IDs

For this step, a list of UniProt\(^7\) accession IDs is required. On the main page (consider Figure S4) one has to click on “Accession Search” in the lower panel.

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\(^6\) [https://skyline.ms](https://skyline.ms)

\(^7\) [https://www.uniprot.org](https://www.uniprot.org)
2) Paste your protein accession IDs

A window for inserting the accession IDs is being opened (as seen in Figure S8). The drop down menu is used to select the desired species. The user can type in the accession IDs into the text field. If there is a longer list, it is possible to copy and paste it into the text field. Click on “Next” to proceed.

3) Check the protein selection and review the spectra

The review dialog appears as known from the previous instructions (Figure S7), proceeding with step 5 of the pathway browser tutorial.

Creating an assay using the chromosome browser

1) Open protein selection window via chromosome browser

Another way of building up assays for targeted LC-MS/MS analysis is by selecting proteins of interest according to their gene location on the chromosomes. On the main page (consider Figure S4) click on “Chromosome Search” in the lower panel.

2) Paste your protein accession IDs

A window for selecting genes within the chromosomes appears (as illustrated in Figure S9). Here, the desired species can be chosen in the upper panel. The user can select different regions on each chromosome by clicking on a specific spot and adjusting the size of the selection box by using the mouse wheel. Alternatively it is possible to drag and drop
the box along the chromosome or by entering a specific chromosome location in the upper panel.

On the right-hand side, a gene table lists all registered genes available in the particular chromosome region including gene name and accession number. Genes can be (de)selected with the checkboxes to the right. When the selection is complete, one can proceed by clicking on “Next”. Only proteins and peptides according to the selected genes that were identified and stored in the background library will be displayed.

3) Check your protein selection and review spectra

The review dialog appears as known from the previous instructions (Figure S7), proceeding with step 5 of the pathway browser tutorial.

Figure S9 | Chromosome browser for selecting proteins within regions of chromosomes.

Using the pathway editor to create / update / delete pathways

To offer a simple way for the pathway curators to create, update, or delete pathways, an access-restricted pathway editor was developed. Within this editor it is possible to insert, update, or delete pathway, protein and metabolite information and the according attributes (e.g. pathway name, protein accession number, metabolite EC number etc.). An intuitive drag and drop concept was used to facilitate the pathway editing.
1) Open the pathway editor

Open the editor\(^8\) and type in the credentials. The editor is split into a top menu strip, a tool box with all functions such as buttons on the left hand side and the current view (canvas) on the pathway on the right hand side (consider Figure S10).

![Figure S10 | Basic view on the pathway editor.](https://stamps.isas.de/admin)

The toolbox itself is split into two types of functions i) for creating visual entities (protein nodes, metabolite nodes, edges, etc.) and ii) for alternating the entities (highlight labels, move entities, etc.). The pathway to be edited can be selected via the buttons “Metabolite Pathways” or “Signaling Pathways” as well as species, respectively.

2) Creating a new pathway

To create a new pathway which is not yet registered in the database, click on “Manage entries”. In the opening window (consider Figure S11), the user can choose to work on pathway group, single pathway, protein or metabolite entries. Pathway entries contain three attributes, i) the pathway name, ii) the membership to a pathway group and iii) a flag if the pathway is a signaling pathway or not. When clicking at the bottom on “New pathway”, a dialog is opening requesting those attributes. For instance, one can type in: pathway name = “UPR Signaling”, pathway group = “Other pathway”, signaling = checked. After adding a pathway, its attributes can be furthermore edited in the “Manage entries” window (Figure S11).

\(^8\) [https://stamps.isas.de/admin](https://stamps.isas.de/admin)
3) Inserting entities into the pathway

Be aware that a recently created pathway is (obviously) empty. Therefore, nothing is visible when selecting the pathway via “Metabolite Pathways” or “Signaling Pathways”. Furthermore, in the regular STAMPS pathway browser (Figure S5), empty pathways are not selectable. When clicking on “Protein” in the “Create entity” section of the toolbox, the protein node creation mode is active. Moving over the canvas, an empty protein node is being displayed. When clicking on an arbitrary position on the workspace, a protein node is being spotted on this position and the user can continue to create another protein node. The mode can be deactivated by either clicking once again on “Protein” in the “Create entity” section or selecting another function. Creating pathway nodes, metabolite nodes, labels, or membranes works the same way (Figure S12).

In the next step, the nodes can be filled with entries from the database. Active modes have to be deactivated before. Now, the user can simply click on the nodes and an according pathway, protein, or metabolite selection window appears. Note, that pathway and metabolite nodes are single nodes whereas multiple proteins can be assigned to one protein node. After assigning entries to the node entities, one can rotate the metabolite.
labels by activating the “Rotate metabolite label” mode and clicking on the according metabolite.

**Figure S12 | View on the pathway editor canvas after inserting certain functional nodes.**

**Figure S13 | View on the pathway editor canvas after inserting entries into the according entities.**

4) **Drawing edges and alter entities**

To draw edges, “Edges” mode in the “Create entity” section must be activated first. Subsequently, anchor points appear for all visual nodes in the canvas. By clicking on one anchor point, holding the mouse button, moving the mouse and releasing over another anchor point, an edge will be drawn and routed automatically. It is allowed to draw edges from each anchor point to each other anchor point, except connecting two anchors of the same node.
By default, the edges are solid and both their endings are not decorated. When clicking on “Change edge type” and clicking on a certain edge the appearance of the edge is changing with each click. In this way one can create solid and dashed edges as well as add arrows and other symbols on the end of each edge. Figure S14 shows an example. Additional functions are moving node entities and highlighting either metabolite or standalone labels. The last function offers deleting either edges or nodes. When deleting a node, all connected edges will be deleted as well.
Statistics

We added several statistical overviews on different levels comprising spectral, protein, and pathway level. Overviews can be accessed over the main page of STAMPS and are dynamically adjustable. The data is taken directly from the protein and spectral database and the figures are always generated on the fly. Figure S15 illustrates all statistical overviews. Shown are e.g. normalized distributions of protein abundances over pathways or over tissues, absolute number of proteins, the normalized spectrum abundance factor, protein isoelectric point versus the protein masses as well as the mass residual distribution between the theoretical spectra and the actually measured precursors.
Figure S15 | Statistical overviews. All figures are interactive allowing the user to change parameters.

(1) Kanehisa, M.; Goto, S. Nucleic Acids Research 2000, 28, 27-30.
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(3) Sherry, S. T.; Ward, M.-H.; Kholodov, M.; Baker, J.; Phan, L.; Smigielski, E. M.; Sirotkin, K. Nucleic Acids Research 2001, 29, 308-311.