Supporting Information

Proteomic sample preparation through extraction by unspecific adsorption on silica beads for ArgC-like digestion

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For supporting tables S1-S10, please consult the respective Excel file.

Peptide lists are provided separately as XLSX files, archived in one ZIP file to minimize file size.

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Protein extraction

S1 Extraction performance of silica beads analyzed with SDS PAGE

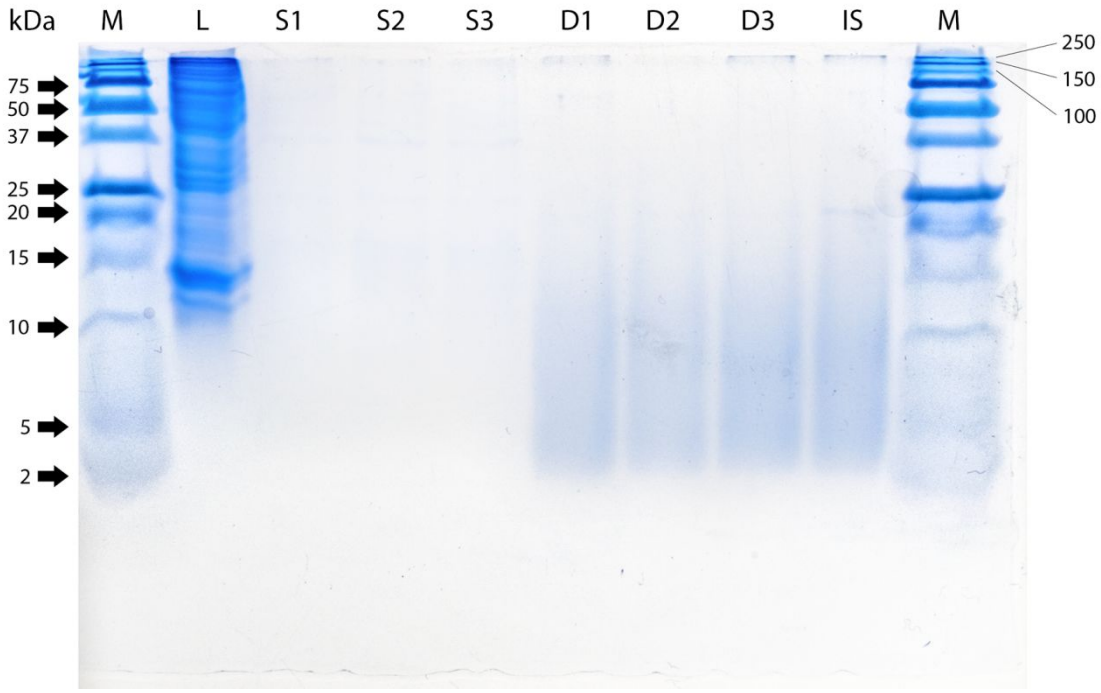

Figure S1

Cells were harvested and lysed with urea solution (sup. table S1) as described in materials and methods. Triplicates of lysates were incubated on magnetic silica beads and the supernatant was saved for analysis (S1-S3). The protein on the beads was digested with trypsin (overnight, 37°C) and the supernatant/peptide mixture collected (D1-D3). Lysate (L) and supernatant (S1-3; D1-3) volume equivalent to 20µg protein was applied to the gel electrophoresis.

Tryptic digestion on silica beads of HEK 293T cell lysate. SDS PAGE with 12% polyacrylamide (PAA) gel stained with Coomassie Blue. Peptides from the supernatant after digestion are seen in D1-D3 and in IS (in-solution digest, control) at 15-2 kDa. A faint trypsin band can be seen for IS (~23 kDa), but not for D1-D3, suggesting that trypsin remains on the silica surface.

Comparison ABP, FASP and AP-IS

S2 Comparison of distributions regarding GRAVY score and isoelectric points

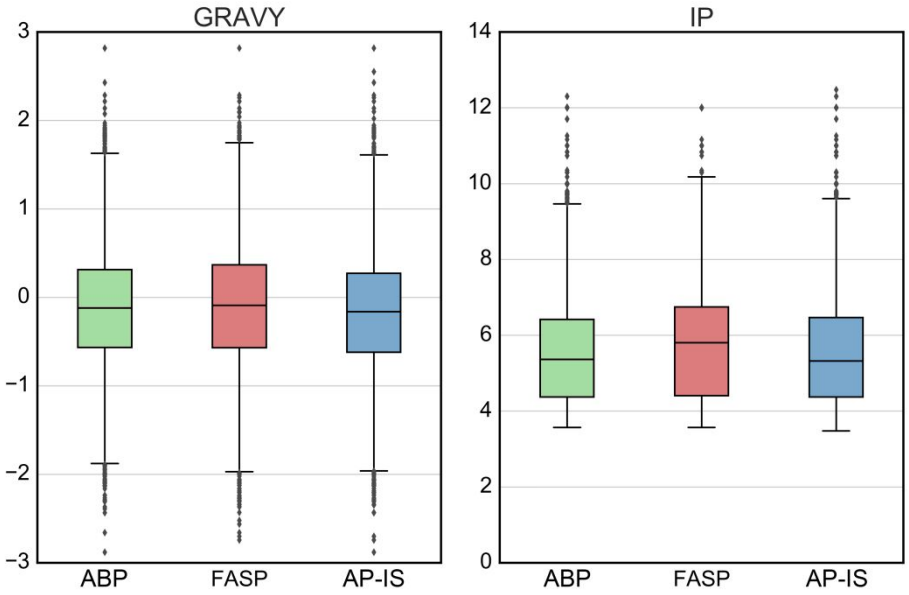

Figure S2 – Comparisons of Gravy scores and isoelectric points between proteins identified after FASP, ABP and AP/IS

S3 Distribution of peptide lengths of identified proteins

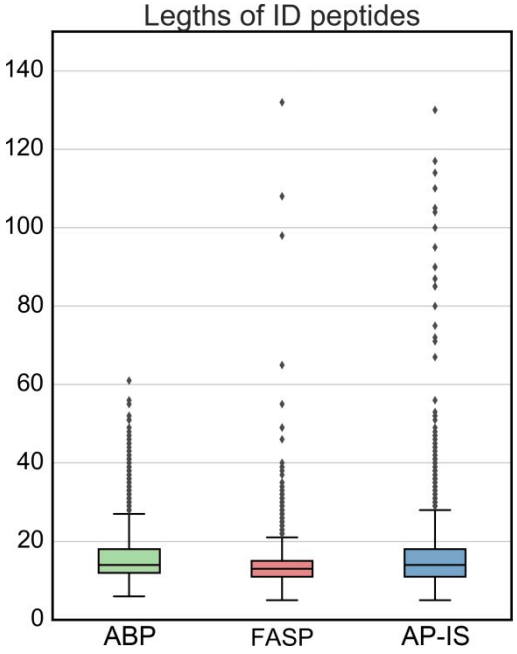

Figure S3 – Peptide lengths were calculated in numbers of amino acids

S4 Oxidation rates of methionine

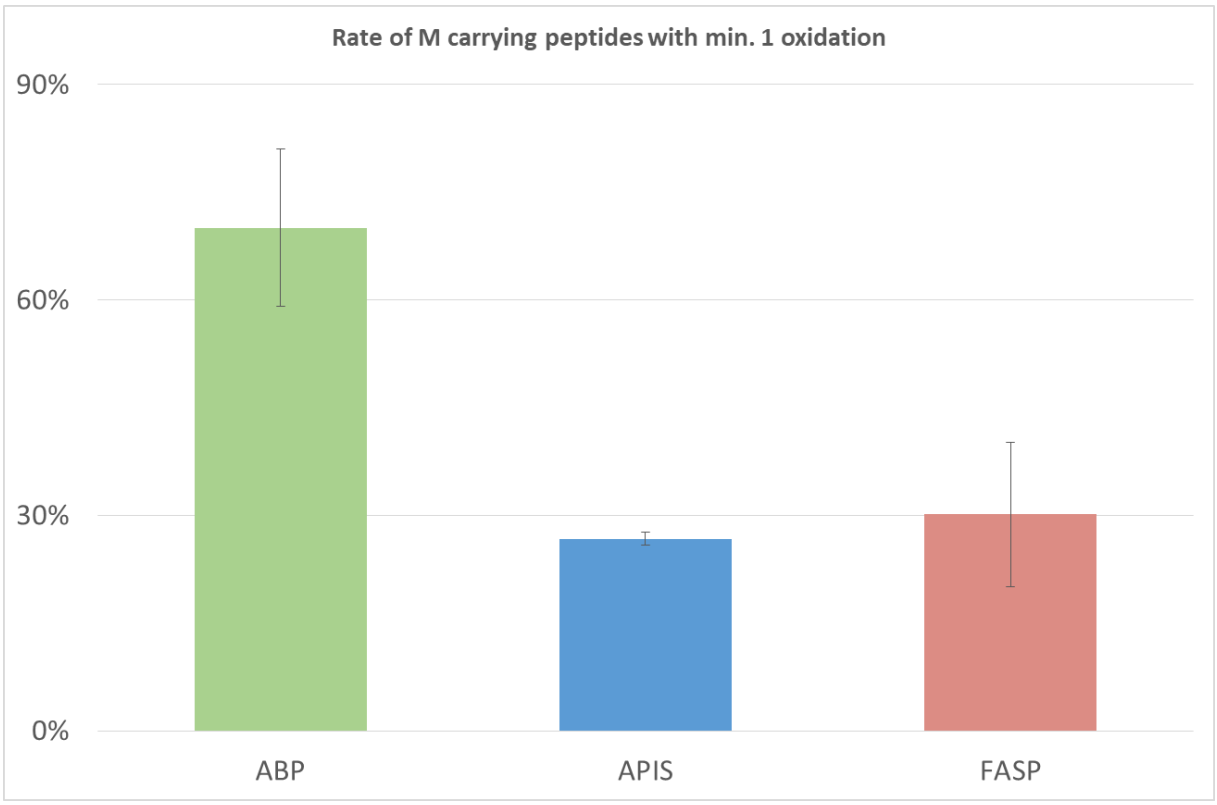

Figure S4: Oxidation rate of methionines. The rate of peptides carrying methionines (M) of which at least one oxidized was calculated in comparison to FASP and AP-IS.

S5 Physicochemical qualities of exclusive peptides

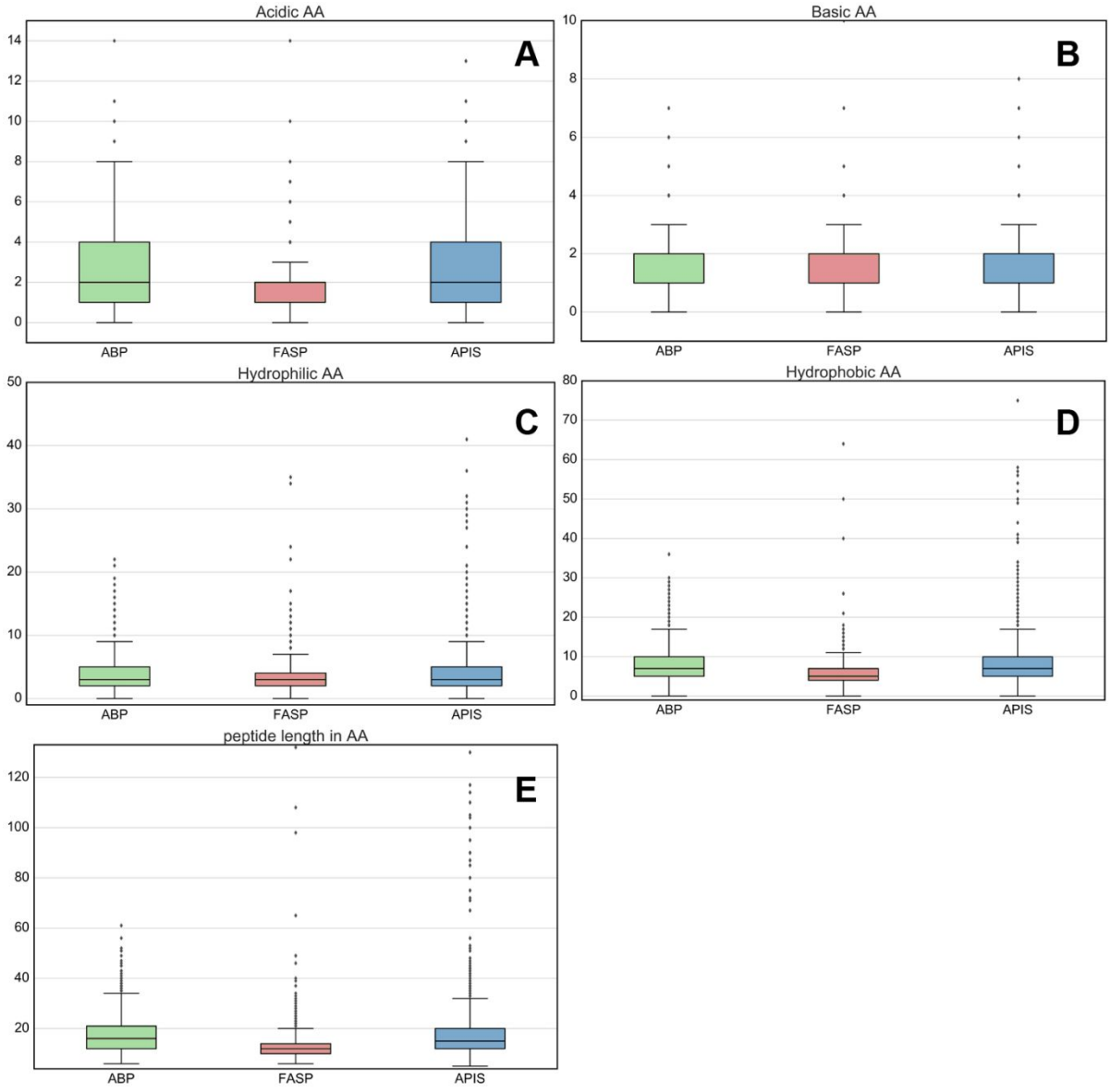

Figure S5: Physicochemical qualities of exclusive peptides. Peptides exclusive to each approach have been analyzed for their amino acid (AA) composition and therefore physicochemical attributes. Total amount of acidic (A) and basic (B) amino acids. Hydrophilic (C) or hydrophobic (D) tendencies as well as the overall peptide length (E) were compared.

Performance of ArgC-like digestion

S6 Overlaps of identified proteins

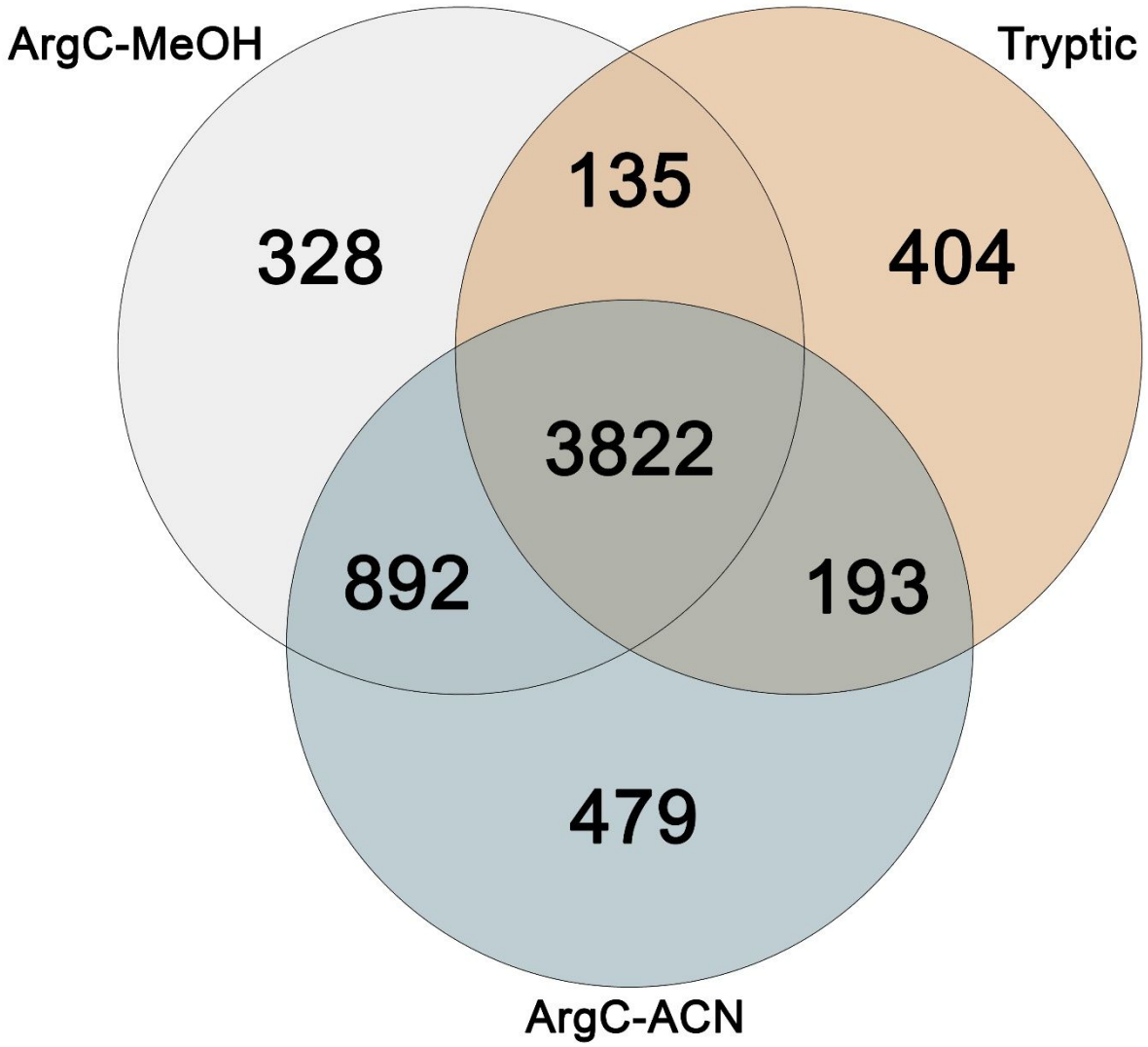

Figure S6 - Venn diagram of all identified proteins for each protocol. Intersection of proteins identified in total among all three approaches (Tryptic, ArgC-like in methanol and ArgC-like in acetonitrile)

S7 Comparison of GRAVY scores and IPs

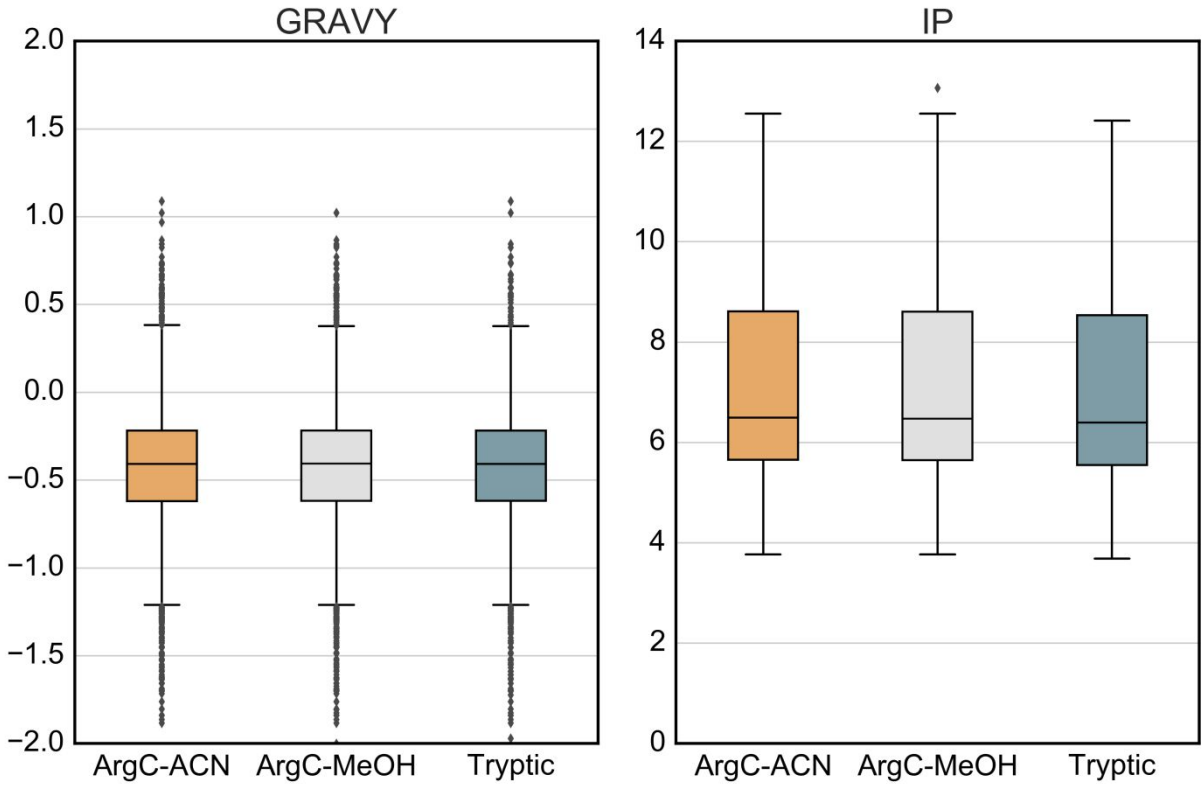

Figure S7 - Analysis of potential differences in identified proteins regarding GRAVY score and isoelectric points (IP) when comparing ArgC-like approaches with tryptic digestion.

S8 Physicochemical qualities of tryptic and ArgC-like peptides

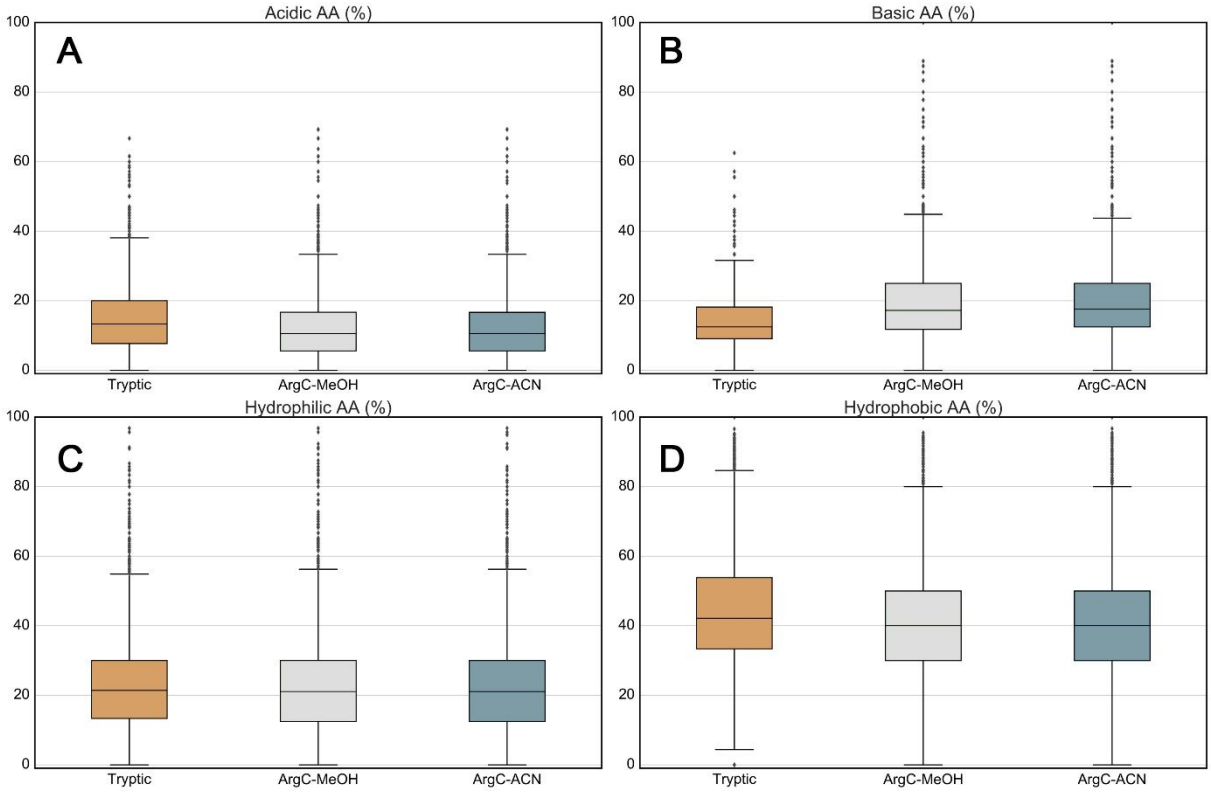

Figure S8 - Peptide composition by amino acid (AA) characteristics:

Percentage of Acidic Amino Acids (A), Basic AA (B), Hydrophillic AA (C) and Hydrophobic AA (D)

Data Analysis

S9 Database search (KNIME workflow)

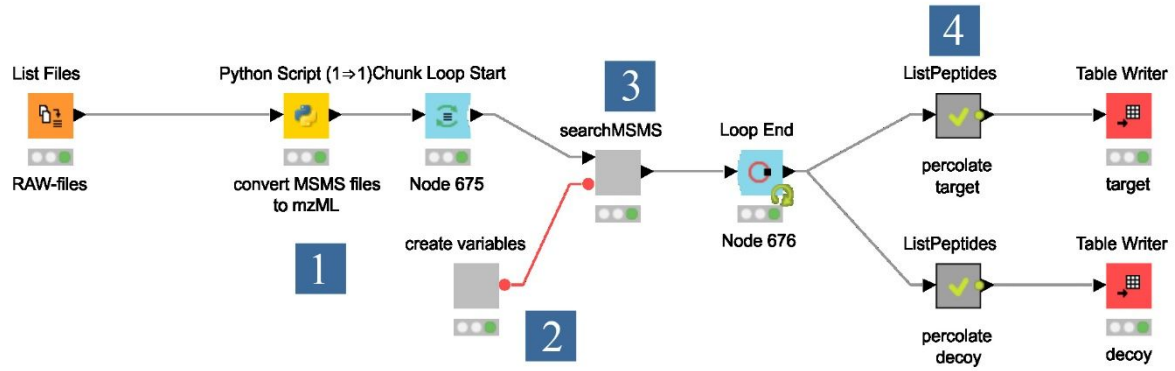

Figure S9: Mascot database search and percolation as streamlined in KNIME

1. RAW file were converted to open source file format mzML via MSConvert (Mascot)
2. Search parameters of table S1 were applied to the database search
3. Mascot search was performed successively for each file
4. Peptide lists were percolated and saved as tables (Supp. Lists: /Peplists)

S10 Determination of subcellular location and histone count (KNIME WORKFLOW)

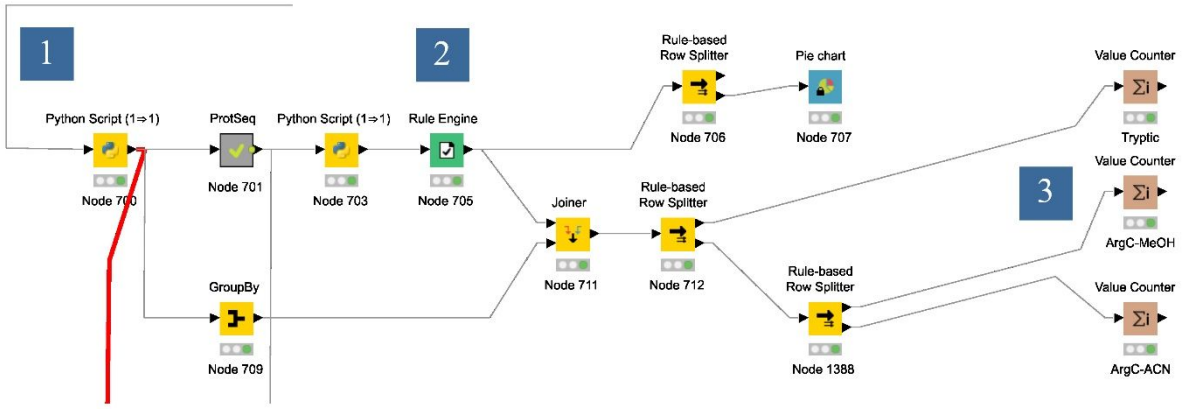

Figure S10: KNIME workflow associating identified proteins with subcellular tags

1. Combined Peptide list (all protocols) joined with dat (Mascot) file
2. Subcellular location entry extracted from dat-file and assigned as tag

3. Tag count for each protocol separately, produced lists in Supp. Lists: /Origins

An equivalent procedure was performed for histone tags

Protocols provided by manufacturers

Pierce™ BCA Protein Assay Kit (Thermo Scientific)

Dilution scheme for BSA Standard

Dilution Scheme for Standard Test Tube Protocol and Microplate Procedure (Working Range = 20-2,000µg/mL)

Vial	Volume of Diluent (µL)	Volume and Source of BSA (µL)	Final BSA Concentration (µg/mL)
A	0	300 of Stock	2000
B	125	375 of Stock	1500
C	325	325 of Stock	1000
D	175	175 of vial B dilution	750
E	325	325 of vial C dilution	500
F	325	325 of vial E dilution	250
G	325	325 of vial F dilution	125
H	400	100 of vial G dilution	25
I	400	0	0 = Blank

- Preparation of working reagent (WR) by mixing 50 parts of BCA Reagent A with 1 part of BCA Reagent B
- Pipette 10µL of each standard or unknown sample replicate into a microplate well (working range = 125-2000µg/mL)
- Add 200µL of the WR to each well and mix plate thoroughly on a plate shaker for 30 seconds.
- Cover plate and incubate at 37°C for 30 minutes.
- Cool plate to RT.
- Measure the absorbance at or near 562nm on a plate reader (TECAN, see materials and methods)

Gel staining solutions

Coomassie Blue

1. After electrophoresis, place the gel into a clean container with 100 mL of fix solution (50% methanol, 7% acetic acid) and agitate on an orbital shaker for 30 minutes. Repeat once more with fresh fix solution. Pour off the used fix solution.
2. Stain gel with Coomassie Blue solution (See sup. table S1) overnight
3. Destaining with (40% methanol, 10% acetic acid) until bands are clearly visible, replenish when necessary
4. Transfer to a new container and store in 5% acetic acid

SYPRO Ruby

1. After electrophoresis, place the gel into a clean container with 100 mL of fix solution (50% methanol, 7% acetic acid) and agitate on an orbital shaker for 30 minutes. Repeat once more with fresh fix solution. Pour off the used fix solution.
2. Add 60 mL of SYPRO Ruby gel stain. Agitate on an orbital shaker overnight
3. Transfer the gel to a clean container and wash in 100 mL of wash solution (10% methanol, 7% acetic acid) for 30 minutes.

FASP Protein Digestion Kit (Expedeon)

1. Mix up to 30 µL (0.4 mg) of a protein extract with 200 µL of Urea Sample Solution (provided in the kit) in the Spin Filter and centrifuge at 14,000 x g for 15 min.
2. Add 200 µL of Urea Sample Solution to the Spin Filter and centrifuge at 14,000 x g for 15 min.
3. Discard the flowthrough from the collection tube
4. Add 10 µL 10X Iodoacetamide Solution (provided in the Kit) and 90 µL Urea Sample Solution to the Spin Filter and vortex for 1 min; incubate without mixing for 20 min in the dark.
5. Centrifuge the Spin Filter at 14,000 x g for 10 min.
6. Add 100 µL of Urea Sample Solution to the Spin Filter and centrifuge at 14,000 x g for 15 min.
7. Repeat this step twice. Discard the flowthrough from the collection tube.
8. Add 100 µL of 50 mM Ammonium Bicarbonate Solution provided with the FASP Kit to the Spin Filter and centrifuge at 14,000 x g for 10 min. Repeat this step twice.
9. Add 75 µL Digestion Solution (enzyme to protein ratio 1:100) and vortex for 1 min. Wrap the tops of the tubes with Parafilm to minimize the effects from evaporation.
    - Digestion has been carried out with the same trypsin solution and the same volume as AP-IS and ABP (trypsin:protein 1:100)
10. Incubate the Spin Filter in an incubator at 37°C for 4 –18 h.
11. Transfer the Spin Filter to a new collection tube.
12. Add 40 µL of 50 mM Ammonium Bicarbonate Solution. Centrifuge the Spin Filter at 14,000 x g for 10 min. Repeat this step once.
13. Add 50µL 0.5 M Sodium Chloride Solution provided with the FASP Kit and centrifuge the Spin Filter at 14,000 x g for 10 min.
14. Filtrate contains digested proteins. Acidify the filtrate with TFA to the desired pH and desalt.

Magnetic Beads

NHS-Beads (Bioclone) – Protein immobilization

Blocking of reactive surface groups was done as described in steps 5-6

16. Combine the protein solution and magnetic beads. Resuspend the magnetic beads and mix very well by pipetting or vortexing. Incubate the reaction with continuous rotation at room temperature for 4-6 hours or overnight.
17. Place the tube on the magnetic separator for 1-3 minutes. Remove the supernatant while the tube remains on the separator.
18. Remove the tube from the separator and resuspend the beads with 1 ml wash buffer by vortex for 30 seconds. Place the tube on the magnetic separator for 1-3 minutes. Remove the supernatant while the tube remains on the separator.
19. Wash beads 3-4 times with 1 ml wash buffer (or 1 M NaCl) as described above.
20. Add 0.5-1ml blocking buffer to the beads and incubate at room temperature for 1 hour or at 4 °C overnight.
21. Beads can be resuspended in any buffer after removing the supernatant

Aldehyde-Beads (Bioclone)

Blocking of reactive surface groups was done as described in steps 4-6

1. In a fume hood, combine protein solution, 10µL NaCNBH3 solution (10µL per milliliter of total volume, final concentration 50 mM) and magnetic beads.
2. Resuspend the magnetic beads and mix very well by pipeting. Incubate the reaction with continuous rotation at room temperature or 4°C overnight.
3. Wash beads 3 times with 1 ml Coupling buffer (0.1 M sodium phosphate, pH 7.0)
4. Add 0.5-1ml blocking buffer (1 M Tris•HCl, 0.05% NaN3, pH 7.4) and 5-10µL NaCNBH3 solution (10ul per milliliter of total volume, final concentration 50 mM) to the beads and incubate at room for 1 hour or at 4°C overnight.
5. Wash beads 4-6 times with 1 ml Washing buffer (1 M NaCl, 0.05% NaN3)
6. Beads can be resuspended in any buffer after removing the supernatant

Silica beads (Bioclone)

Silica beads were used as described in the manuscript after optimization experiments.

Further supporting content

The listed supporting tables are provided in the respective Excel file

Supporting tables (SuppTables.xlsx):

Table S1	Reagents, buffers and solutions prepared in-house
Table S2	Mascot Search parameters
Table S3	Missed cleavages and False discovery rates (comparison ABP, AP/IS, FASP)
	S3a IDs and FDRs
	S3b MCs (all peptides)
	S3b MCs (exclusive peptides)
	S3d Protein ID overlaps
Table S4	Methionine oxidation rate
Table S5	Carbamylation rate of lysines
Table S6	Missed cleavages and False discovery rates (comparison Tryptic and ArgC-like)
	S6a MCs
	S6b IDs and FDRs
Table S7	Protein ID overlaps
Table S8	Protein Origins
	S8a Histones
	S8b Cellular substructures
Table S9	Hits above identity threshold
Table S10	Precursor mass ranges

Peptide lists (Peplists.zip):

PEPLIST_ABP_FASP_APIS.xlsx	Peptides used for identification comparing ABP, FASP and AP/IS
PEPLIST_ArgClike.xlsx	Peptides used for identification comparing ArgC-like and tryptic digestion
PEPLIST_Carbamylation.xlsx	Peptides used for identification of competing variable modifications on lysine.