Hydrazide method from Bernd Wollscheid

Hofmann 2015, [Proteomics Clin Appl.](http://www.ncbi.nlm.nih.gov/pubmed/26076441)

For the Cys-Glyco-CSC/ Glyco-CSC technology, glycans of cell surface proteins were oxidized with sodium meta-periodate and then biotinylated with biocytin hydrazide. The Lys-CSC technology applied cleavable, amine reactive sulfosuccinimidyl-2-(biotinamido)-ethyl-1, 3’-dithiopropionate (sulfo-NHS-SS-biotin; Pierce, Rockford, IL, USA) to biotinylate cell surface proteins. Cells were homogenized and iodoacetamide was added to the lysis buffer in order to preserve disulfide bridges of cell surface proteins. Afterwards, cell debris and nuclei were removed by differential centrifugation and membrane pellets were generated by ultracentrifugation. Membrane pellets were solubilized by indirect sonication and proteins were digested overnight with trypsin. Biotinylated peptides were bound to streptavidin beads and unspecific, not biotinylated peptides were removed by stringent washing. Cysteine containing ‘piggyback’ peptides and lysine containing peptides were eluted from streptavidin beads by chemical reduction. Glycopeptides were eluted enzymatically from the streptavidin beads with PNGase F. Produced free thiols of cysteine containing peptides were alkylated with iodoacetamide and peptides were desalted and dried in a SpeedVac concentrator. Finally, peptides were resolubilized and analyzed individually by LC-MS.

**LC-MS analysis**

The Cys-Glyco-CSC/ Glyco-CSC and the Lys-CSC technology were applied twice for each cell line, which resulted in 6 peptide samples for each cell line. Each peptide sample was analyzed twice on an Eksigent Nano LC system (Eksigent Technologies, Dublin, CA, USA) connected to a hybrid linear ion trap LTQ Orbitrap (Thermo Scientific, Waltham, MA, USA), which was equipped with a nanoelectrospray ion source (Thermo Scientific). Peptide separation was carried out on a RP-HPLC column (75 µm inner diameter and 10 cm length) packed in-house with C18 resin (Magic C18 AQ 3 µm; Michrom Bioresources, Auburn, CA, USA) using linear gradients from 95% solvent A (water, 0.2% formic acid, and 1% acetonitrile) and 5% solvent B (water, 0.2% formic acid, and 80% acetonitrile) over 3 min to 90% solvent A and 10% solvent B and then to 65% solvent A and 35% solvent B over 39 min at a flow rate of 0.2 µl/ min. The data acquisition method was set to acquire one high resolution MS scan in the Orbitrap followed by three or five collision induced dissociation MS/MS scans in the linear ion trap. For a high resolution MS scan, 2x106 or 5x105 ions were accumulated over a maximum time of 400 ms and the FWHM resolution was set to 60 000 (at m/z 400). Only MS signals exceeding 500 ion counts triggered a MS/MS attempt and 104 ions were acquired for a MS/MS scan over a maximum time of 200 ms or 250 ms. The normalized collision energy was set to 35% and one or three microscans were acquired for each MS/MS spectrum. Singly charged ions were excluded from triggering MS/MS scans.

Boheler 2014, stem cell report

Cell Surface Capturing (CSC-Technology): Cells were washed with PBS and oxidized by treatment with 1 mM sodium meta-periodate (Pierce, Rockford, IL) in PBS pH 7.6 for 15 min at 4°C followed by 2.5 mg/ml biocytin hydrazide (Biotium, Hayward, CA) in PBS pH 6.5 for 1 hour at 4°C. Cells were then collected from the plate and homogenized in 10mM Tris pH 7.5, 0.5 mM MgCl2 and the resulting cell lysate was centrifuged at 2500 x g for 10 min at 4°C. The supernatant was centrifuged at 210,000 x g for 16 hours at 4°C to collect the membranes. The supernatant was removed and the membrane protein pellet was washed with 25 mM Na2CO3 to disrupt peripheral protein interactions. The resulting membrane pellet was resuspended in 300µl 100 mM NH4HCO3, 5 mM Tris(2-carboxyethyl) phosphine (Sigma, St. Louis, MO), and 0.1% (v/v) Rapigest (Waters, Milford, MA) with continuous vortexing and proteins were allowed to reduce for 10 min at 25°C followed by alklylation with 10 mM iodoacetamide for 30 min. The sample was incubated with 1 µg Lys-C and 20 µg proteomics grade trypsin (Promega, Madison, WI) at 37°C for 16 hrs. Samples were centrifuged at 14,000 rpm for 10 min to remove particulates. The resulting peptide mixture was incubated with 450 µl bead slurry of UltraLink Immobilized Streptavidin PLUS (Pierce, Rockford, IL) for 1 hour at 25°C. Beads were sequentially washed with 10mL each of 0.05% Triton X-100 in 100 mM NH4HCO3, 5M NaCl, 100 mM NH4HCO3, 100 mM Na2CO3, and 80% isopropanol to remove non-specific peptides and lipids. Beads were resuspended in 100 mM NH4HCO3 and 500 units glycerol-free endoproteinase PNGaseF (New England Biolabs, Ipswich, MA) and incubated at 37°C for 16 hrs with end-over-end rotation to release the peptides from the beads. Collected peptides were desalted and concentrated using a C18 MicroSpin™ column (Harvard Apparatus, Holliston, MA) according to manufacturer’s instructions.

Zeng 2009 nature methods

**Optimization of periodate oxidation with** **1-Acid Glycoprotein (****1-AGP).** Conditions for periodate oxidation were optimized using 1-AGP protein (200 μg/ml in PBS, pH 7.4). Asialo 1-AGP was prepared by mild acid hydrolysis (pH 1.6, 80 ̊C, 1 hour) followed by extensive dialysis. For optimizing NaIO4 concentration, 1-AGP was oxidized with 1 to 20 mM NaIO4 at 4 ̊C for 5 minutes. For optimizing NaIO4 reaction time, 1-AGP was oxidized with 1 mM NaIO4 at 4 ̊C for 5 to 60 minutes. Excess periodate and formaldehyde (generated during periodate oxidation) were removed by gel filtration (spin columns from Pierce Net). Periodate oxidation of 1-AGP was followed by reaction with 100 μM aminooxy-biotin (Invitrogen Corporation) and 10 mM aniline (Sigma-Aldrich) at pH 6.7, RT for 60 minutes. The protein was then resolved by gel electrophoresis and the extent of biotinylation visualized by western analysis with streptavidin- HRP (1:10000, Jackson Immunoresearch Laboratories, Inc.). Equivalent loading of protein was confirmed by staining a duplicate gel with EZ-Blue (Sigma-Aldrich) or silver stain (Invitrogen Corporation).

**Periodate oxidation of sialic acids on cells.** BJA-B K20 or K88 were washed with Dulbecco's phosphate buffered saline, pH 7.4 (Invitrogen; PBS) and suspended to 1106 cells/mL in PBS containing 1 mM sodium periodate (Sigma-Aldrich). CHO monolayers cultured on coverslips in 6-well plates were immersed in 1 mL PBS containing 1 mM sodium periodate. Unless otherwise indicated, the periodate treatment was carried out for 30 minutes at 4 C, then quenched with 1 mM glycerol before washing in PBS.

**Aniline catalyzed oxime ligation.** Unless otherwise indicated, the aniline catalyzed oxime ligation was conducted as follows. Periodate treated or NeuAc labeled K20 or K88 cells were washed in PBS and suspended to 1106 cells/mL in PBS/5% FBS (pH 6.7) containing 100 μM aminooxy biotin and 10 mM aniline. CHO monolayers cultured on coverslips in 6-well plates were immersed in 1 mL PBS/5% FBS (pH 6.7) containing 100 μM aminooxy biotin and 10 mM aniline. The reaction was carried out at 4 C for 90 minutes with gentle agitation on an end- over-end rotator or a rocking tray.