Description of Catalogues (July 2, 2009)

Pre-001: Preparation of mixed N-Glycans.

The starting material for this procedure can be purified protein, cell extract or tissue. Protein is solubilized and denatured with SDS/2-ME. NP-40 is added to "buffer" the SDS. PNGase F is added and the mixture incubated overnight at 37°C. The mixture is applied to a Sep-Pak C18 cartridge equilibrated in water. Proteins and the majority of detergent bind to the resin. The run-through and water washes are applied to a porous graphitized carbon (PGC) cartridge. Under aqueous conditions, oligosaccharides bind to the PGC cartridge, while salts and buffers pass through unretarded. Carbohydrates are eluted with 30% acetonitrile, 0.1% TFA in water while any residual detergent remains bound to the cartridge. The resulting desalted N-glycans are dried and ready for further analysis.

Pre-002: Preparation of Mixed O-Glycans

The starting material for this procedure can be purified protein, cell extract or tissue. The sample is incubated with 0.1 M NaOH/1M NaBH4 at 45°C overnight to release O-glycans by way of alkaline induced β-elimination. The sample is passed through a cartridge packed with Dowex 50 (H^+ form) to remove peptide and Na^+. The flow through is collected and lyophilized. Repeated addition of methanol/acetic acid to form volatile methylborates followed by drying under a stream of nitrogen yields desalted, dried, reduced O-glycans ready for further analysis.

Pre-003: Preparation of Mixed Glycolipid-derived Glycans

The starting material for this procedure can either be purified glycolipids or crude lipid extracts, e.g., Pre-004. Lipid mixtures are treated with endoglycoceramidase to release the glycan from the lipid. The mixture is passed over a Sep-Pak C18 cartridge to remove enzymes and lipids and the run-through is applied to a PGC cartridge. Under aqueous conditions, oligosaccharides bind to the PGC cartridge, while salts and buffers pass through unretarded. Carbohydrates are eluted with

30% acetonitrile, 0.1% TFA in water while any residual detergent remains bound to the cartridge. The resulting desalted glycolipid-derived glycans are dried and ready for further analysis.

Pre-004: Preparation of Total Lipid Extracts

The starting material for this procedure is typically tissues. Tissues are sequentially extracted with chloroform: methanol, twice with each of the following ratios 2:1, 1:1 and 1:2. The pooled extracts are dried and resuspended in chloroform: methanol 2:1.

Pre-005: Preparation of Cellular Metabolites-TCA Extraction

The starting material for this procedure is typically tissue or cell pellets. Freshly isolated material is preferable. Samples are treated with TCA (5-10% w/v final). The precipitated macromolecules are removed by centrifugation. The supernatant is treated with an equal volume of Freon: trictylamine (3:1) to extract the TCA and the aqueous phase is stored frozen or lyophilized.

Pre-006: Preparation of Glycosaminoglycans

The starting material for this procedure is typically tissue or cell pellets. Hard tissues are grinded on liquid nitrogen or soft tissues are homogenized on ice bath on protease treatment buffer. Samples are digested overnight with Pronase/ Protease at 37°C and centrifuged at 14000rpm for 20 minutes. The supernatant is passed through a DEAE column and the bound glycosaminoglycan (GAG) is eluted with 2M NaCl. The GAG is then desalted on PD10-size exclusion column and purified GAG is lyophilized and used for further analysis.

Pre-007: Preparation of Glycopeptides

Protein samples are treated with Trypsin, at 37°C for 16-18 hours, followed by destruction of the enzyme by immersing the sample in 100°C for 5 minutes. The sample is then passed through Sep-Pac C18 column and the glycoprotein fraction is eluted at 15-30% Acetonitrile fraction.

Pre-008: Analysis of Sulfate and Phosphate

Oligosaccharides and glycoconjugates may contain covalently attached non-carbohydrate groups; among those, sulfate and phosphate are quite common. The presence and quantity of those groups is determined by subjecting the sample to pyrolysis, followed by autosampler injection and separation of the anions on an ion-exchange (Dionex IonPac) column and detected by suppressed conductivity detector. Samples are hydrolyzed using 0.3N HCl at 150°C for 30 minutes. Hydrolyzed samples are dried on speed vac and pyrolsed for 15 seconds, cooled, dissolved in water and injected on the Dionex IonPac column.

Pre-009: Extraction of Semi-Rough or Smooth LPS (Hot Phenol water extraction)

Bacterial cells are washed by PBS once and then twice with distilled water to remove the adhered media. The cells are suspended in water (7mL water per gm or bacterial cell) and sonicated for 1 minute to make the cell-suspension. The cell suspension is stirred on oil/or water bath maintained at 65°C for 10 minutes using a magnetic stirrer. 7mL of 90% phenol is pre-heated to 65°C and added to the aqueous bacterial suspension. The mixture is stirred for 30 minutes at 65°C and immediately cooled below 10°C on an ice-water bath. The reaction mixture is centrifuged at 4000rpm for 40 minutes at 10°C. The top layer containing Smooth LPS is removed carefully and dialyzed against distilled water for 3-4 days with changing of water each day until the smell of phenol is undetectable. The extracted LPS are treated with DNase/RNase and Proteinase K to remove contaminating nucleic acid and proteins respectively. Finally the LPS are precipitated by ultracentrifugation at 120,000g for 6 hours at 5°C.

Pre-010: Extraction of Rough LPS (Phenol-Chloroform-Petroleum Ether extraction method)

Bacterial cells are washed with deionized water (once), 90% ethanol (twice), acetone (twice) and di-ethyl ether (once), and dried in vacuum desiccators. The

dried cells are grinded to fine powder and reaction mixture in the ratio of 2:5:8 of 90% phenol: chloroform: petroleum-ether (40-60°C fractions) is added (200 mL of reaction mixture for 50 gm of dried cell or equivalent). This cell-solvent mixture is stirred at high speed maintaining the temperature near 10°C for 30 minutes. The extracted material is centrifuged at 3500 rpm for 10 minutes at 10°C. Supernatant is collected and chloroform and petroleum ether is removed from the mixture by rotary evaporation. Rough LPS is precipitated from the phenol layer by careful addition of water dropwise. Precipitated LPS is centrifuged and repeatedly washed with 5:1 Di-ethyl ether: acetone mixture and dried by Nitrogen flush.

Pre-011: Capsular polysaccharide extraction

Cells are shaken with PBS or 1% aqueous phenol for 3 hours at room temperature and centrifuged at 7000 rpm for 20 minutes at 5°C. Crude CPS is isolated from supernatant by cold absolute ethanol precipitation. CPS is removed by centrifugation at 14000 rpm for 20 minutes at 5°C. The precipitate is treated with Nuclease and Proteinase K and dialyzed against water. Enzyme treated CPS is further purified by size exclusion chromatography or weak anion exchange chromatography.

Pre-012: Bacterial Lipid Extraction

Bacterial lipids are extracted from peletted cells using Bligh-Dyers extraction protocol. Pellet cells approximately $1x10^7$ cells are suspended in 3mL $CHCl_3$: CH_3OH (3:1) mixture and vortexed thoroughly. 0.8mL of water is added again vortexed and the solution is allowed to sit at room temperature for at least 30 minutes. Spinning at 3000 rpm for 5 minutes pellets the cellular debris. The solvents are transferred to a new tube without transferring any of the pelleted debris. 1mL of $CHCl_3$ and 1mL water are added to each sample (upon addition of $CHCl_3$ a biphasic solution should result). The samples are vortexed and spun at 3000 rpm for 5 minutes. The upper aqueous/ methanolic phase is removed and

the lower chloroform layer is transferred to a glass tube and dried down using dry nitrogen flush. The lipid residues are stored at -80°C for future analysis.

Pre-013: Sugar nucleotide extraction

Cells are pelleted by centrifugation washed once in ice-cold phosphate buffered saline and then lysed by sonication or lysis buffer. The lysed cells are centrifuged at 16000 rpm for 10 minutes at 4°C to remove insoluble material and the supernatant is dried under nitrogen. The samples are dissolved in 200ul of 9% butanol and extracated three times with 400ul of 90% butan-1-ol to remove lipids. The resulting aqueous phase is dried under nitrogen and resuspended in 5mM ammonium bicarbonate and sugar nucleotides are extracted using EnviCarb graphitized carbon columns (Supelco). Columns are prepared by washing with 3mL of 80% acetonitrile, 0.1% triflouroacetic acid followed by 2mL water. The samples are loaded in 5mM ammonium bicarbonate and the column is washed with 2mL water, 2mL 25% acetonitrile and 2mL 50mM TEAA buffer and finally eluted with 2mL of 25% acetonitrile, 50mM TEAA buffer. Eluate is freeze-dried and stored at -80°C prior to analysis.

Modifications

Mod 001: Alditol Acetate derivative for GC-MS (AA)

Dry glycan samples are hydrolysed by 4N TFA at 100°C for 4 hours, followed by removal of the acid by dry nitrogen flush. The acid is removed completely by coevaporation twice using 1:1 isopropanol: water mixture under dry nitrogen flush. Hydrolysed samples are reduced overnight by Sodium borohydride in 1M ammonium hydroxide solution. Excess borohydride is neutralized by 30% acetic acid and boric acid is removed as their methyl borate. Samples are finally treated with 1:1 acetic anhydride: pyridine mixture at 100°C for 1h. Pyridine and acetic anhydride is removed by nitrogen flush and alditol acetate is extracted with dichloromethane.

Mod 002: Trimethyl Silyl derivative for GC-MS (TMS)

The starting material for this procdure is typically glycoprotein, glycolipid or glycan. Buffer and salt should be minimized. Samples should be completely dried or lyophilized. For 0.1-0.25mg of sample 250μl 1M Methanolic Hydrochloric is added and methanolysed at 80°C for 16-18h. Methanolic hydrochloric acid is removed by dry nitrogen flush, and coevaporated once more with dry methanol. Samples are re-N-acetylated by 4:1:1 methanol: pyridine: acetic anhydride at 100°C for 1h. Reagents are removed by dry nitrogen flush and finally treated with Tri-Sil (Pierce) reagent at 80°C for 30 minutes. The TMS derivative of the monosaccharides is extracted with hexane and ready for injection on GC-MS.

Mod 003a: Par-O-Methylation of glycans (PM)

The starting material for this procedure should be purified and completely dried glycolipids or glycan. Samples are dissolved in dry DMSO and stirred for several hours until the sample is completely dissolved. Powdered Sodium hydroxide or Sodium hydroxide slurry in DMSO is added as base and kept for vigorous stirring for 1-2 hours. Samples are cooled on an ice bath and 200μl of methyl iodide is added and continued stirring for 1 hour. Another aliquot of 100μl of methyl iodide is added and stirred for 30 minutes. To the reaction mixture 0.5mL of chloroform is added and stirred for 10 minutes, and the reaction is stopped by adding 1mL of water. The methylated glycan is extracted in the chloroform layer, dried and used for further analysis by MALDI mass spectrometry or as PMAA derivative for linkage analysis.

Mod 003b: Partially methylated Alditol Acetate (PMAA)

Partially methylated alditol acetate derivative is done after permethylation of glycans as described in Mod 003a. The PM samples are hydrolyzed by 4N TFA at 100°C for 6hours. Followed by removal of the acid, reduction and acetylation are carried out as described in Mod-001.

Mod 004: Fatty acid methyl ester (FAME)

Dried samples are methanolysed by 1M methanolic HCl at 80°C for 18 hours. The acid is removed by nitrogen flush, half-saturated NaCl is added to the samples and fatty acid methyl ester is extracted using chloroform. The chloroform layer is back extracted with water and is dried by nitrogen flush. Fatty acid samples are dissolved in hexane and injected on GC-MS.

Mod 005: Mild acid treatment for releasing Sialic acid

The starting material for this procedure is typically isolated glycan and glycolipids, although glycopeptides and intact proteins may also be deemed appropriate depending on the nature of the sample. Sample is dissolved in a final concentration of 2 M HOAc and heated to 80°C for 3 hours. Acetic acid is removed by drying.

Mod 006: De-O-Acetylation of glycans

De-O-Acetylation of glycans is achieved by treating the samples with base 50mM of Sodium hydroxide at room temperature for 3 hours or by treatment with anhydrous hydrazine. Sodium hydroxide is neutralized by 30% acetic aicd under cold conditioning and the glycan is purified by PD10-size exclusion chromatography. Anhydrous hydrazine treated material is precipitated by cold (-80°C) acetone, the precipitate is washed once more with cold acetone, and the precipitate is dissolved in water and lyophilized.

Mod-007: Enzymatic degradation of Chondroitin Sulfate (GAG-CS)

The starting material for this procedure is dried, desalted glycosaminoglycan. Glycosaminoglycans are dissolved in buffer appropriate for the lyase(s) to be used. A cocktail mixture of Chondroitinase ABC is added and the polysaccharide is digested at 37°C for 18 hours. Following digestion, the mixture is fractionated using a 10,000 MWCO filter to remove enzyme and undigested

glycosaminoglycan chains. The supernatant is dried and used for Pro-004 or Mod-009.

Mod-008: Enzymatic degradation of Heparin Sulfate (GAG-HS)

The starting material for this procedure is dried, desalted glycosaminoglycan. Glycosaminoglycans are dissolved in buffer appropriate for the lyase(s) to be used. A mixture of Heparinase I, II and III is added and the polysaccharide is digested at 37°C for 18hours. Following digestion, the mixture is fractionated using a 10,000 MWCO filter to remove enzyme and undigested glycosaminoglycan chains. The supernatant is dried and used for Pro-005 or Mod-009.

Mod-009: Isotopic Aniline tagging for GAG disaccharide analysis by mass spectrometry (GRIL-Glycan Reductive Isotope Labeling)

1 pmol to 10 nmol of HS and CS disaccharides is transferred to 1.5-ml microcentrifuge tubes and dried down in a centrifugal evaporator. 15 µl of $^{12}C_6$ aniline or $^{13}C_6$ aniline and 15 µl of 1 M $NaCNBH_3$ (Sigma-Aldrich) freshly prepared in dimethyl sulfoxide: acetic acid (7:3, v/v) are added to each sample. Reactions are carried out at 65°C for 4 hours or, alternatively, at 37°C for 16 hours, and then dried in a centrifugal evaporator. The dried samples are resuspended in running buffer and used for LCQ-MS analysis (Pro-009).

Mod-010: 2AB labeling of glycans

For labeling with 2-Aminobenzamide (2-AB) 100 ul of the cyanoborohydride mixture (prepared by adding 6.5 mg of sodium cyanoborohydride in 200µl of 35:65 glacial acetic acid: DMSO mixture) are added to 6 mg of 2-AB reagent, vortexed and sonicated to mix thoroughly. 10 ul of 2AB reagent are added to each sample and incubated at 65°C for 2.5 hours. After 2AB labeling the samples are purified by passing through a Glycoclean S-cartridge dried on a speed vac and is ready for further analysis.

COMPOSITIONS

Com-001: Monosaccharide analysis by HPAEC-PAD

The starting material for this procedure is typically glycoprotein, glycolipid or glycan. Buffer and salt should be minimized. Samples (0.1mg) are treated with 200µl of 2 M TFA at 100°C for 4h to cleave all glycosidic linkages. Acid is evaporated by dry nitrogen flush, repeated co-evaporation with 1:1 isopropanol, with water helping to get rid of the acids completely. Glycolipid and glycoprotein samples are thus passed through SepPak C18 cartridge and evaporated to remove the acid. Finally, the samples are dissolved in water and analyzed by HPAEC-PAD using a CarboPac PA-1, PA-10 or PA-20 column. Monosaccharide standards found are treated in parallel and used for calibration of HPAEC-PAD response.

Com-002: Uronic acid analysis by HPAEC-PAD

The starting material for this procedure is typically any proteoglycan, plant polysaccharide or bacteria-derived glycan. Dried samples are treated with 2M TFA at 100°C for 6 hours to cleave all glycosidic linkages. Alternatively, methanolysis may be used to hydrolyze glycosidic bonds and concomitantly form the methyl glycoside and methyl ester derivatives. Methanolysis is followed by 0.5N TFA hydrolysis to remove these methyl groups at 100°C for 1 hour. After drying and removing the acids from the hydrolyzate, samples are dissolved in water and analyzed by HPAEC-PAD using a CarboPac PA-1 column. Common uronic acid standards (Glucuronic, Galacturonic and iduronic acids) are treated in parallel and used for calibration of HPAEC-PAD response.

Com-003: Sialic acid analysis by HPAEC-PAD

The starting material for this procedure can be glycoprotein, glycolipid or glycan. The sample is dissolved in a final concentration of 2 M HOAc and heated to 80°C for 3 hours to release sialic acids. The released sialic acids are collected by ultra-

filtration through a 3,000 MWCO filter, dried and analyzed by HPAEC-PAD using a Dionex CarboPac PA-1 column eluted with a sodium acetate gradient that separates N-acetylneuraminic acid and N-glycolylneuraminic acid. As little as 100 pmol of individual sialic acid can be detected. Known standards are run in parallel, sialic acids are identified by elution position and quantitation is done in reference to known amounts of N-acetylneuraminic acid injected in parallel. The printout of the results shows the profile and individual sialic acid content expressed in nmol present in the volume injected. Blanks (that represent background from the methodology used for sample preparation) are required for optimum quantitative results.

Com-004: Analysis of Anions by Ion Chromatography

The starting material for this procedure can be glycoprotein, glycolipid or glycan. Oligosaccharides and glycoconjugates may contain covalently attached non-carbohydrate groups; among those, sulfate and phosphate are quite common. The presence and quantity of those groups is determined by subjecting the sample to pyrolysis, followed by autosampler injection and separation of the anions on an ion-exchange (Dionex IonPac) column. Conductivity detector is used in conjunction with a suppressor to get optimum results. Blanks (that represent background from the methodology used for sample preparation) are required and it is recommended that samples be run in duplicate for optimum quantitative results.

Com-005: Reverse phase HPLC of DMB-Sialic acid and determination by Fluorescence detector

The starting material for this procedure can be glycoprotein, glycolipid or glycan. The sample is dissolved in a final concentration of 2 M HOAc and heated to 80°C for 3 hours to release sialic acids. The released sialic acids are collected by ultra-filtration through a 3,000 MWCO filter and derivatized with DMB. The fluorescent sialic acid derivatives are analyzed by reverse-phase HPLC with on-line fluorescence detection. The method can detect as low as 250 fmol of individual

sialic acid. Identification is based on known standards run in parallel, and quantitation is done in reference to known amounts of N-acetylneuraminic acid derivatized and injected in parallel.

Com-006: GC-MS of TMS derivative

Trimethylsilyl derivatives of methyl glycosides of neutral and N-Acetylated amino sugars and trimethylsilyl derivative of methyl glycoside methyl ester of uronic acids are successfully separated by GC-MS using either DB-1 or DB-5 capillary column. TMS sugars are detected from their retention time and by electron impact (EI) ionization mass spectrum. Analysis requires standards to be prepared and analyzed in parallel. The aforementioned monosaccharides are identified by elution position and fragmentation profile. Blanks (that represent background from the methodology used for sample preparation) are required for optimum quantitative results.

Com-007: GC-MS of AA derivative

Alditol acetate derivative of neutral and amino sugars are separated on DB-1 capillary column. Neutral pentoses and deoxy-hexose sugars are better resolved in SP-2330 capillary column. AA sugars are detected from their retention time and by electron impact (EI) ionization mass spectrum. Analysis requires standards to be prepared and analyzed in parallel.

Com-008: GC-MS of PMAA derivative (linkage analysis)

The starting material for this procedure should be purified glycolipid or glycan. These are methylated according to the Ciucanu-Kerek or Hakomori methods, as appropriate. Purification methods used include extraction, Sep-Pak cartridge purification, dialysis or chromatography over LH-20 depending on the sample. Permethylated products are cleaved by acetolysis/hydrolysis (when amino sugars are present) or acid hydrolysis. Partially methylated monosaccharides are reduced, and partially methylated alditols are derivatized to obtain partially methylated alditol acetates. Analysis of these products is done by GC-MS using

DB-5 or equivalent capillary column. Identification is achieved by using a combination of retention times (as compared to those of known standards analyzed under the same conditions) and mass fragmentation pattern.

Com-009: GC-MS of Fatty acids

Fatty acid methyl esters are dissolved in hexane and analyzed by GC-MS in EI or CI mode. DB-1 or DB-5 can separate the fatty acids and the mass fragmentation patterns are used to identify saturated and unsaturated fatty acids. Hydroxy-fatty acids are components of bacterial lipids and can be detected by characteristic mass fragmentation pattern. The hydroxyl fatty acids are treated with Tri-Sil, thereby forming trisilyl methyl ether of fatty acid methyl ester.

Com-010: LCQ-MS Single runs no sample prep (Per hour)

Suitable sample are dissolved in 1:1 methanol: water mixture containing 0.1% formic acid and data collected on positive mode. For negative mode ammonium hydroxide is added on the solvent mixture. The liquid chromatography is done using suitable column and solvent buffer compatible with mass spectroscopy.

Com-011: Glycan analysis by LCQ-MS

Glycan samples are separated on suitable column (reverse phase or amino-bonded column) and connected online to the mass spectroscope. Acetonitrile: water mixture containg ammonium fromate or formic acid can be used as mobile phase for amino-bonded column and Methanol: acetonitrile and water is used for reverse phase columns.

Com-012: Nucleotide sugar analysis by HPAEC-UV

The mixture of sugar nucleotide is profiled by anion exchange chromatography using a Dionex PA-1 column equilibrated in either water or 10mM NaOH. Elution is effected by an increasing gradient of NaOAc concentration. UV detection is typically performed at 260 nm. The dominant species present in this analysis is typically nucleotide phosphates. Sugar nucleotides are well resolved using a

combination of the two gradient systems described above, however, true estimates of sugar nucleotide concentrations may first require the removal of nucleotide phosphates. Detection limits of ~1 nmol/peak are typical. Control analysis consists of profiling a standard mixture of sugar nucleotides.

PROFILINGS

Pro-001: **HPAEC-PAD Profile of N-Glycans**

The starting material for this procedure is a mixture of desalted N-glycans, e.g., Pre-001. Reduced N-glycans can be similarly analyzed. The sample is dissolved in water and profiled by High pH Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD) using either a Dionex Carbo-Pac PA-1 or PA-100 column. Increasing concentrations of NaOH and NAOAc effect elution. Species elute in order of increasing negative charge. Elution time is also affected by the size of the oligosaccharide, the nature of the glycosidic linkages and the presence of fucose residues. Control analysis consists of profiling a standard mixture of fetuin for sialylated N-glycans and RNaseB for high mannose N-glycans.

Pro-002: O-Glycan profiling

The starting material for this procedure is a mixture of desalted (generally reduced) O-glycans, e.g., Pre-002. The sample is dissolved in water and profiled by High-pH Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD) using either a Dionex Carbo-Pak PA-1 or PA-100 column. Increasing concentrations of NaOH and NaOAc affect elution. Species elute in order of increasing negative charge. Elution time is also affected by the size of the oligosaccharide, the nature of the glycosidic linkages and the presence of fucose residues. Gradient conditions differ from those used for N-glycans. Control analysis consists of profiling a standard mixture of either bovine submaxillary gland O-glycans, or milk oligosaccharides.

Pro-003: 2AB-labelled glycan profiling

2-AB labeled glycans are separated by HPAEC and detected by fluorescence detection; this is a very sensitive method and can determine the glycans in picomolar levels. 2-AB labeled glycans are dissolved in water and separated on PA-1 or PA-100 column using sodium hydroxide and sodium acetate gradient. The detection is done by an online fluorescence detector.

Pro-004: Profile of Glycosaminoglycan-derived Disaccharides by Ion Pair RP-HPLC with UV and Fluorescent Detection

The starting material for this procedure is typically a mixture of disaccharides obtaining by the action of lyases. This method is prefered for chondroitin- and dermatan-derived disaccharides. Disaccharides are adsorbed to the C18 column by virtue of their ionic interactions with the hydrophobic ion pair reagent tetrabutylammonium hydrogen sulfate. Elution is affected with a gradient of increasing acetonitrile concentration. Disaccharides are detected by the absorbance at 232 nm due to the presence of the double bond resulting from the action of the lyases. UV detection has a detection limit of ~100 pmol. The effluent from the UV detector is mixed with NaOH and 2-cyanoacetamide, heated to 130°C to form a fluorescent adducts and then detected fluorometrically. The limit of detection of the fluorscence detection is ~5 pmol. Control analysis consists of a mixture of known glycosaminoglycan-derived disaccharides.

Pro-005: Profile of Glycosaminoglycan-derived Disaccharides by Anion Exchange HPLC with UV and Fluorescent Detection

The starting material for this procedure is typically a mixture of disaccharides obtaining by the action of lyases. This method is prefered for heparin/heparan sulfate-derived disaccharides. Elution is effected with a gradient of increasing NaCl concentration. Disaccharides are detected by the absorbance at 232 nm due to the presence of the double bond resulting from the action of the lyases. UV detection has a detection limit of ~100 pmol. The effluent from the UV

detector is mixed with NaOH and 2-cyanoacetamide, heated to 130°C to form a fluorescent adducts and then detected fluorometrically. The limit of detection of the fluorscence detection is ~5 pmol. Control analysis consists of a mixture of known glycosaminoglycan-derived disaccharides.

Pro-006: Single HPLC run (No sample preparation based on hourly charge)

Pro-007: MALDI-TOF profiling of glycans

Starting material must be purified glycans, permethylated glycans or glycolipids. Native glycans are dissolved in water at a concentration of 2µg/µl and mixed in 1:1 ratio with DHB as matrix and spotted on the MALDI plate. Permethylated glycans are dissolved in absolute methanol and mixed with matrix in 1:1 ratio and spotted on the MALDI plate. Glycolipid samples are dissolved in 3:1 chloroform: methanol mixture before spotting. The samples are air-dried and analyzed in positive mode, in order to do a negative mode analysis for samples that bear sialic acids or negative ions such as sulfate, and phosphate THAP is used as matrix.

Pro-008: Cellular metabolite analysis

The starting material for this procedure is typically a TCA extract from cells or tissues, e.g. Pre-005. The mixture is profiled by anion exchange chromatography using a Dionex PA-1 column equilibrated in either water or 10mM NaOH. Elution is affected by a gradient of increasing NaOAc concentration. UV detection is typically performed at 260 nm. The dominant species present in this analysis are typically nucleotide phosphates. Sugar nucleotides are well resolved using a combination of the two gradient systems described above, however, true estimates of sugar nucleotide concentrations may first require the removal of nucleotide phosphates. Detection limits of ~1 nmol/peak are typical. Control analysis consists of profiling a standard mixture of sugar nucleotides.

Pro-009: GRIL LCQ-MS for GAG disaccharide Analysis

An LCQ classic quadrupole ion trap mass spectrometer equipped with an electrospray ionization source, and a quaternary high-performance liquid chromatography pump (Thermo-Finnigan, San Jose, CA) is used for disaccharide analyses. Aniline isotopic and non-isotopic disaccharides prepared by Mod-009 are separated on a C18 reversed-phase column (0.46 cm x 25 cm, Vydac) with the ion pairing agent dibutylamine (DBA, Sigma-Aldrich). The solvent gradient used for eluting the samples are: 100% buffer A (8mM acetic acid, 5mM DBA) for 10 minutes, 17% buffer B (70% methanol, 8 mM acetic acid, 5 mM DBA) for 15 minutes; 32% buffer B for 15 minutes, 40% buffer B for 15 minutes, 60% buffer B for 15 minutes; 100% buffer B for 10 minutes; and 100% buffer A for 10 minutes. The most highly substituted disaccharides elute at 60% buffer B (42% methanol). Ions of interest are monitored in negative ion mode, and signal intensity is optimized for a representative species of disaccharide. To minimize in-source fragmentation of sulfated disaccharides, the capillary temperature and spray voltage are kept at 140 °C and 4.75 kV, respectively.

MISCELLANEOUS

Mis-001: Consultation Time (Per Hour)

It is the policy of Glycotechnology Core that each project be given a maximum of 30 minutes of discussion at no charge with the Core Director. Additional time for individualized analysis of the issue, help in selecting the best protocol for the isolation, purification and analysis of glycoconjugates and interpretation of data is available through consultation with the Core Director, depending upon available time.

Mis-002: Additional Analytical Time (Per Hour)

There may be an additional charge if custom methods are requested or if there is any analysis which is not listed on the website. This charge will not be imposed without prior consultation with the investigator.

Mis-003: Method development based on time and materials used

Glycotechnology Core is always willing to do a method development and discover new strategies for analyzing glycans. This can be done upon request; however, the customer must provide materials or columns as needed. A fee will be charge for the establishment of a new method and the subsequent transfer of the method to the customer.

Mis-004: Fraction collection by HPLC (Per Fraction)

Glycotechnology Core can collect fractions from HPLC for the customer for further analysis, and can also send them to the customer. Shipping charges and expenses are the responsibility of the customer.

Mis-005: Powerpoint slide preparation.

Many of the instruments in Glycotechnology Core utilize proprietary software that is not commonly found in most research laboratories. Glycotechnology Core can therefore provide any data profile in the form of a Microsoft PowerPoint presentation, thus eliminating the need to scan data printouts.

Mis-006: NMR analysis on the glycan samples will be done upon request.