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SUPPLEMENTARY INFORMATION

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Glycosylation in health and disease

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Supplementary information

Supplementary Table 1: Common mammalian saccharides

Monosaccharide	Abbreviation	Symbol
Arabinose	Ara	
Fructose	Fru	
Fucose	Fuc	
Galactose	Gal	
Galactosamine	GalN	
N-acetylgalactosamine	GalNAc	
Glucose	Glc	
Glucosamine	GlcN	
N-acetylglucosamine	GlcNAc	
Glucuronic acid	GlcA	
Mannose	Man	
Muramic acid	Mur	
Neuraminic acid	Neu	
Sialic acid	Sia	
Rhamnose	Rha	
Ribose	Rib	
Xylose	Xyl	

Supplementary Table 2: Methodologies and techniques for the analysis of glycosylation

Method	Data Obtained	Techniques and/or Reagents	Applications
Acid hydrolysis coupled with chromatographic separation and detection	Monosaccharide compositional analysis	HPEAC-PAG, GC-FL, GC- MS	Fungal polysaccharides enhance cell-mediated immunity[1]
HPLC profiling of glycoconjugates (released glycans, glycopeptides, GAGs, etc)	Separation of glycoconjugates, identification of glycoconjugates by comparison to reference compounds	HILIC-UPLC	Plasma *N*-glycans as biomarkers[2]
Mass spectrometry (MS, most often combined with HPLC)		LC-MS	For review see[3]
Intact glycoproteins and other glycoconjugates	Glycoform heterogeneity in context of intact glycoconjugate	Top-down MS	Characterization of biosimilars and therapeutic antibodies[4]
Glycopeptides and partially cleaved glycoconjugates	Identification, composition, relative abundance, partial quantitation, site-specific context of glycoform heterogeneity.	Glycosidases + proteases + LC-MS	HIV-1 Env multiple *N*-glycans[5]; Glycoproteins as disease markers[6]
Released glycans	Identification, composition, overall trends of glycan composition for the entire sample	PNGase F release + HILIC HPLC + MS & MS/MS	Profiling of cell-surface glycans review[7]
Tandem mass spectrometry (MS/MS)	Confirmation of glycan composition, some linkage information, identification of sites of attachment for multi-chain glycoconjugates	CID, ETD, EDD	IgA1 *O*-glycans[8]; Chondroitin sulfate GAG[9]; C1-inhibitor[10]
NMR	3D structural analysis of glycoconjugates including linkage information, dynamics of glycan interactions		Glycoprotein analysis[11]; Heparan sulfate[12]
Lectin	Selective identification/quantification of structural epitopes in cells, tissues and biological fluids	Lectin-Western Blot, Lectin-ELISA	Quantitative lectin ELISA[13]; for review see[14]
Lectin affinity chromatography	Selective separation/enrichment of glyconjugate epitopes	Jacalin, wheat-germ agglutinin, ConA	Biomarker analysis[15,16]
Glycan-specific antibodies	Detection of selective glycan epitopes in cell culture and tissues.	RL2 & CTD110 (*O*-GlcNAc); 2G12, PG9 (*N*-glycans on HIV Env)	*O*-GlcNAc[17,18]; HIV *N*-glycans[19]; for review see[20]
Exoglycosidases (Combined with various detection methods)	Sequence, anomeric configuration, partial linkage confirmation	α2,3 neuraminidase, α1,6 mannosidase	Assisted annotation of *N*- glycans[21]
Glycosidase inhibitors	Can alter glycan production pathways in the cellular environment	Mannosidase inhibitors; neuraminidase inhibitors	Kifunensine[22]; Review of neuraminidase inhibitors[23]
Chemo-enzymatic synthesis of glycans, glycopeptides, and glycoconjugates	Molecular tools for testing specific glycoforms/glycoconjugates in isolation instead of the context of complex mixtures		For review see[24,25]
Engineered glycosyltransferases	Labeling of glycoconjugates for detection or selective attachment of synthetic glycans.		Single-glycoform glycoprotein[26]; Modulating IgG effector function[27]

Aptamers	Create novel molecular inhibitors or sensors of specific glycoconjugate epitopes.	Oligonucleotides or peptides from random sequence pool	Protein-glycan inhibitors[26]; Molecular sensors[28]
Array technologies			
Glycan arrays	High throughput screening of glycan interacting biomolecules across individualized glyconjugates	Synthetic glycans and glycopeptides	Cell-based glycan arrays[29]; For review see[30]
Lectin arrays	High throughput screening for multiple specific glycoconjugate epitopes in heterogeneous or complex mixtures.		Serum biomarkers[31]; Detection of pathogenic bacteria[32]
Molecular modeling and molecular dynamics simulations	Visualization and estimations of glycoconjugates as part of larger macromolecular complex to identify intra- and intermolecular interactions that cannot be accomplished by other techniques.	PDB, NMR data, GlyCAM	For review see[33]
Genomics, transcriptomics	Cell or tissue-specific expression profiles of glycosyltransferases, gene variants, gene mutations		Regulation of glycan structures in tissues[34]
Glycomics and glycoproteomics	Global identification/quantitation of glycoconjugates in biological systems.	All tools above	For review see[35]
Bioinformatics	Combining glycoconjugate profiling with omics-based data to find consensus of biological function and outcomes.	Glycomics, genomics, proteomics	IgG glycosylation[36]; Cell surface analysis of B-cell lymphoma[37]
Medical bioinformatics	Combining glycan analysis and omics technologies with patient clinical data	Pattern recognition, phenomics, PubMed + omics data	For review see[38,39]

Abbreviations: HPAEC-PAD, high performance anion-exchange chromatography-pulsed amperometric detection; GC-FL, gas chromatography fluorescence; GC-MS, gas chromatography-mass spectrometry; HILIC-UPLC, hydrophilic interaction liquid chromatography-ultra-performance liquid chromatography; LC-MS, liquid chromatography-mass spectrometry; PNGase F, Peptide:*N*- glycosidase F; MS/MS, tandem mass spectrometry; CID, collision-induced dissociation; ETD, electron-transfer dissociation; EDD, electron-detachment dissociation; ELISA, enzyme-linked immunosorbent assay; PDB, protein data bank.

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