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Glycosidases as an analytical tool in glycomics assays

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Abbreviations

2'FL	2-Fucosyllactose
αGal-LeX	α(1–3)Gal-Lewis X
BBSRC	Biotechnology and Biological Sciences Research Council
CAZymes	Carbohydrate-Active enZymes
CBM	Carbohydrate binding module
CE	Capillary electrophoresis
CFG	Consortium for Functional Glycomics
CGE	Capillary gel electrophoresis
CID	Collision induced dissociation
CQA	Critical quality attribute
CZE	Capillary zone electrophoresis
ECD	Electron capture dissociation
EDC	1-Ethyl-3-(3-(dimethylamino)-propyl)carbodiimide hydrochloride
ELISA	Enzyme linked immune sorbent assay
ESI	Electrospray ionisation
FLD	Fluorescence detection
FRET	Förster resonance energy transfer
FT-ICR	Fourier transform-ion cyclotron resonance
Fuc	Fucose
Gal	Galactose
GalNac	<i>N</i> -acetylgalactosamine
GH	Glycoside hydrolase
GiRP	Girard's Reagent P
Glc	Glucose
GlcNac	<i>N</i> -acetylglucosamine
HILIC	Hydrophilic interaction liquid chromatography
HMOs	Human milk oligosaccharides
HOBt	1-hydroxybenzotriazole
HRP	Horseradish peroxidase
IBD	Inflammatory bowel disease
ICR	Ion cyclotron resonance
IMAC	Immobilized metal affinity chromatography
IMS	Ion mobility mass spectrometry
IPTG	Isopropyl B-D-thiogalactopyranoside
ITC	Isothermal titration calorimetry
LacNAc	<i>N</i> -acetyl-D-lactosamine
LC	Liquid chromatography
LeA	Lewis A
LeB	Lewis B
LED	Light emitting diodes
LeX	Lewis X
LeY	Lewis Y
LIF	Laser induced fluorescence
MALDI	Matrix assisted laser desorption ionisation
Man	Mannose
MD	Molecular dynamics

MS	Mass spectrometry
NAD⁺	Nicotinamide adenine dinucleotide
NADP⁺	Nicotinamide adenine dinucleotide phosphate
Neu5Ac	5- <i>N</i> -acetylneuraminic acid
NHAc	Acetamido group
NMR	Nuclear magnetic resonance
NP-40	Nonidet P-40
PAD	Pulsed amperometric detection
PBS	Phosphate buffered saline solution
PNGase F	Peptide <i>N</i> -glycosidase F
pNP-Fuc	4-nitrophenyl α -L-fucopyranoside
QA	Quality assurance
QC	Quality control
qPCR	Quantitative polymerase chain reaction
RMSD	Root mean square deviation
RP	Reverse phase
RSD	Relative standard deviations
S/N	Signal to noise
SAD	Single anomalous diffraction
SDS	Sodium dodecyl sulfate
Sia	Sialic acid
sLeX	Sialyl Lewis X
SPBS	Sodium phosphate buffer solution
SPE	Solid-phase extraction
SPR	Surface plasmon resonance
SSN	Sequence similarity networks
STD	Saturation transfer difference
TOF	Time of flight
TPNG	Total plasma <i>N</i> -glycome
TSNG	Total serum <i>N</i> -glycome

Curriculum vitae

Osmond D. Rebello was born on the 21th of July, 1992 in Dubai, U.A.E. In 2013, he obtained a bachelor's degree in Chemistry and Industrial microbiology from Pune University, India. and in 2016, he obtained a master's degree in Biochemistry and Molecular Biology from Bremen University, Germany. During his master's studies, Osmond worked as an assistant laboratory technician at the Nano-SIMS facility of the Max Planck Institute, Bremen. During this period, Osmond gained an immense interest in analytical chemistry and instrumentation and so focused his master internships and thesis towards an analytical chemistry track. For his master's thesis, Osmond developed assays for quantifying the binding affinity of lectin domains from trans-sialidases in the laboratory of Prof. dr. Sorge Kelm, Bremen University.

In 2017, Osmond obtained a three-year Marie Curie fellowship as an early-stage researcher for the GLYSIGN consortium. During this project, Osmond was primary based at Ludger Ltd., United Kingdom, as a R&D scientist developing glycomics techniques. In collaboration with Prof. dr. Nathalie Juge of Quadram Institute, Dr. Lucy Crouch of University of Birmingham and Dr. David Bolam of Newcastle University, Osmond was involved in the identification of several novel exoglycosidases by LC-MSⁿ techniques. Additionally, Osmond also developed a plate-based spectrophotometric glycomics technique. Osmond spent a portion of this period working on research projects at the Center for Proteomics and Metabolomics (CPM) in Leiden University Medical Center (LUMC) where he developed mass spectrometry-based and capillary electrophoresis-based glycomics assays and techniques under the guidance of Dr. David Falck and Prof. dr. Manfred Wührer. Osmond also contributed to automating a IgG capture protocol onto a Hamilton liquid handling robotic platform. His contribution to the GLYSIGN consortium is compiled into this PhD. thesis.

Since 2020, Osmond is working as a R&D scientist / mass spectrometry engineer at Spectrometry Vision B.V. (MSVision), Netherlands. In this role, Osmond pursues a career in mass spectrometry and other analytical instrumentation development.

List of publications

This thesis

1. **A novel glycosidase plate-based assay for the quantification of galactosylation and sialylation on human IgG**
Osmond D. Rebello, Richard A. Gardner, Paulina A. Urbanowicz, David N. Bolam, Lucy I. Crouch, David Falck, Daniel I. R. Spencer.
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2. **A Matrix-Assisted Laser Desorption/Ionization—Mass Spectrometry Assay for the Relative Quantitation of Antennary Fucosylated N-Glycans in Human Plasma**
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3. **Fucosidases from the human gut symbiont Ruminococcus gnavus**
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Other

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6. **GLP-2 Is Locally Produced From Human Islets and Balances Inflammation Through an Inter-Islet-Immune Cell Crosstalk**
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7. TLR4 triggered complex inflammation in human pancreatic islets

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Jessica Nüße, Ursula Mirastschijski, Mario Waespy, Janina Oetjen, Nadine Brandes, **Osmond Rebello**, Federico Paroni, Sørge Kelm and Frank Dietz

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