Glycosidases as an analytical tool in glycomics assays

Rebello, O.D.

Acknowledgements

Firstly, I want to thank my promoter Prof. dr. Manfred Wuhrer for offering me this PhD position, mentoring me in my scientific research and making me believe in my capabilities.

I would like to thank my co-promotor Dr. David Falck for mentoring me in my scientific research, indulging my scientific curiosity and instilling upon me an analytical mindset. I will greatly miss our long fury filled scientific discussions / debates.

I would also like to acknowledge and thank Dr. Daniel Spencer for recruiting me into Ludger Ltd. which made obtaining this PhD possible. I am glad to be mentored under him because I learnt something which academia would never teach me i.e. professionalism and work attitude in a commercial and industrial scientific organisation.

Collaborations with other institutes and research groups were a vital part of my thesis, and so I want to acknowledge and thank my collaborators Dr. Lucy Crouch, Dr. Arnaud Basil, Dr. Haiyang Wu, Prof. dr. Nathalie Juge and Dr. David Bolam. These collaborations introduced me into such an exciting world of interdisciplinary research that tickled my interest, and which made my thesis possible.

Of course, I would like to acknowledge my colleagues at the CPM, Steffen, Katarina, Di, Sander, Tamas, Alan and Iwona for their friendship and creating a great work environment for my time at the LUMC.

Importantly vital, I acknowledge the financial grant organisations that made my PhD possible, 1) The European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement GlySign No 722095 and 2) The Innovate UK Biocatalyst grant Glycoenzymes for Bioindustries (BB/M029042/).

Lastly but most importantly, I want to acknowledge and thank all my colleagues at Spectrometry Vision B.V. (MSVision) for their love and encouragement during the completion of my PhD.

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	*N*-acetylgalactosamine
GH	Glycoside hydrolase
GiRP	Girard's Reagent P
Glc	Glucose
GlcNac	*N*-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	*N*-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-*N*-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 *N*-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 *N*-glycome
TSNG	Total serum *N*-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-MS^n 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 Wuhrer. 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.
   Glycoconjugate Journal 37, 691–702, 2020

2. **A Matrix-Assisted Laser Desorption/Ionization—Mass Spectrometry Assay for the Relative Quantitation of Antennary Fucosylated N-Glycans in Human Plasma**
   Osmond D. Rebello, Simone Nicolardi, Guinevere S. M. Lageveen-Kammeijer, Jan Nouta, Richard A. Gardner, Wilma E. Mesker, Rob A. E. M. Tollenaar, Daniel I. R. Spencer, Manfred Wuhrer and David Falck
   Frontiers in Chemistry 8, 138, 2020

3. **Fucosidases from the human gut symbiont Ruminococcus gnavus**
   Haiyang Wu**, Osmond Rebello**, Emmanuelle H. Crost, C. David Owen, Samuel Walpole, Chloe Bennati Granier, Didier Ndeh, Serena Monaco, Thomas Hicks, Anna Colvile, Paulina A. Urbanowicz, Martin A. Walsh, Jesus Angulo, Daniel I. R. Spencer, Nathalie Juge
   Cellular and Molecular Life Sciences, 78:675–693, 2021

Other

4. **Complex *N*-glycan breakdown by gut Bacteroides involves an extensive enzymatic apparatus encoded by multiple co-regulated genetic loci**
   Justina Briliūtė, Paulina A. Urbanowicz, Ana S. Luis, Arnaud Baslé, Neil Paterson, **Osmond Rebello**, Jenifer Hendel, Didier A. Ndeh, Elisabeth C. Lowe, Eric C. Martens, Daniel I. R. Spencer, David N. Bolam & Lucy I. Crouch
   Nature Microbiology 4, 1571–1581, 2019

5. **Carbon recycling efficiency and phosphate turnover by marine nitrifying archaea**
   Travis B. Meador, Niels Schoffelen, Timothy G. Ferdelman, **Osmond Rebello**, Alexander Khachikyan, Martin Könneke
   Science Advances, 6(19):eaba1799, 2020

6. **GLP-2 Is Locally Produced From Human Islets and Balances Inflammation Through an Inter-Islet-Immune Cell Crosstalk**
   Wei He, **Osmond D. Rebello**, Antonia Henne, Fabian Nikolka, Thomas Klein and Kathrin Maedler
   Frontiers in Endocrinology, 12:69712005, 2021

7. **TLR4 triggered complex inflammation in human pancreatic islets**
   Wei He, **Osmond Rebello**, Rocco Savino, Rosa Terracciano, Carole Schuster-Klein, Beatrice Guardiola, Kathrin Maedler
   Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease 1865(1), 86-97, 2018

8. **Two new isoforms of the human hepatoma-derived growth factor interact with components of the cytoskeleton**
   Jessica Nüße, Ursula Mirastschijski, Mario Waespy, Janina Oetjen, Nadine Brandes**,** **Osmond Rebello**, Federico Paroni, Sørge Kelm and Frank Dietz
   Biological Chemistry 397(5), 417-436, 2016