Rapid and sensitive methods for the analysis and identification of O-glycans from glycoproteins

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Abbreviations

2-AA	2-aminobenzoic acid
2-AB	2-aminobenzamide
AI-ECD	activated ion-electron capture dissociation
AP	2-aminopyridine
APP	amyloid precursor protein
APTS	1-aminopyrene-3,6,8-trisulfonic acid
BAP	biotin pentylamine
BSM	bovine submaxillary glands mucin
BSSL	bile salt-stimulated lipase
CDG	congenital disorder of glycosylation
CID	collision induced fragmentation
DTT	dithiothreitol
ECD	electron capture dissociation
EDTA	ethylenediaminetetraacetic acid
EMEA	European Medicines Evaluation Agency
ESI	electrospray ionization
ETD	electron transfer dissociation
Fab	fragment antigen binding

Fc	fragment cristallizable
FDA	US Food and Drug Administration
FLR	fluorescence
FTICR	fourier transform ion cyclotron resonance
Fuc	fucose
Gal	galactose
GalNAc	N-acetylgalactosamine
GalNAc	N-acetylgalactosamine
GC	gas chromatography
GCQAs	Glycosylation Critical Quality Attributes
Glc	glucose
GlcNAc	N-acetylglucosamine
GlcNAc	N-acetylglucosamine
Hex	hexose
HexNAc	N-acetylhexosamine
HILIC	hydrophilic interaction liquid chromatography
HPAEC-PAD	high pH anion exchange chromatography with pulsed amperometric detection
HPLC	high-performance liquid chromatography
ICH	International Conference on Harmonization

IgA	immunoglobulin A
IgG	immunoglobulin G
IgM	immunoglobulin M
IRMPD	infrared multiphoton dissociation
LC	liquid chromatography
LIF	laser-induced fluorescence
m/z	mass over charge ratio
mAb	monoclonal antibody
MALDI	matrix assisted laser desorption
Man	mannose
MS	mass spectrometry
NeuAc	N-acetylneuraminic acid
NMR	nuclear magnetic resonance
PGC	porous graphitized carbon chromatography
PMP	1-phenyl-3methyl-5pyrazolone
PSD	post source decay
PTM	post-translational modifications
QbD	Quality by Design
QC	quality control

QTOF	quadrupole time-of-flight
RP	reversed-phase chromatography
sIgA	secretory immunoglobulin A
TFA	trifluoroacetic acid
TOF	time of flight
UDP-GalNAc	N-acetyl galactosaminyltransferase
UHPLC	ultra high performance liquid chromatography
UTI	urinary tract infection
WAX	weak anion exchange chromatography

Curriculum Vitae

Radoslaw Pawel Kozak was born on November 5th 1983, in Wloclawek, Poland. He attended high school in Plock, Poland from which he graduated in 2002. He then started his studies at University of Warsaw, Poland where he earned his M.Sc. degree in Organic Chemistry in June 2007. The research for his Masters thesis was performed in the Laboratory of Stereocontrolled Organic Synthesis under the supervision of Prof. Dr. R.R. Sicinski. The project focused on the synthesis of hydrindane precursors of vitamin D with modified side chains.

In 2008 he moved to Oxford, UK and in December 2008 he joined Ludger Ltd as a Scientist, in the Development and Glycoprofiling groups. Within those groups he has been involved in the development of new techniques for glycan release and derivitization for MS and HPLC/UPLC, and also glycan profiling of biopharmaceuticals. In June 2010 he began his PhD entitled: “Rapid and sensitive methods for the analysis and identification of O-glycans from glycoproteins” in collaboration with Department of Parasitology of the Leiden University Medical Center. In January 2016 he was promoted to a Senior Scientist at Ludger Ltd.

List of publications

[1] **R.P. Kozak**, L. Royle, R.A. Gardner, D.L. Fernandes, M. Wuhrer, Suppression of peeling during the release of O-glycans by hydrazinolysis. Anal.Biochem. 423 (2012) 119-128.

[2] G. Zauner, **R.P. Kozak**, R.A. Gardner, D.L. Fernandes, A.M. Deelder, M. Wuhrer, Protein O-glycosylation analysis. Biol.Chem. 393 (2012) 687-708.

[3] C. Gao, Y. Liu, H. Zhang, Y. Zhang, M.N. Fukuda, A.S. Palma, **R.P. Kozak**, R.A. Childs, M. Nonaka, Z. Li, D.L. Siegel, P. Hanfland, D.M. Peehl, W. Chai, M.I. Greene, T. Feizi, Carbohydrate sequence of the prostate cancer-associated antigen F77 assigned by a mucin O-glycome designer array. J.Biol.Chem. 289 (2014) 16462-16477.

[4] G.V. Harlalka, A. Lehman, B. Chioza, E.L. Baple, R. Maroofian, H. Cross, A. Sreekantan-Nair, D.A. Priestman, S. Al-Turki, M.E. McEntagart, C. Proukakis, L. Royle, **R.P. Kozak**, L. Bastaki, M. Patton, K. Wagner, R. Coblentz, J. Price, M. Mezei, K. Schlade-Bartusiak, F.M. Platt, M.E. Hurles, A.H. Crosby, Mutations in B4GALNT1 (GM2 synthase) underlie a new disorder of ganglioside biosynthesis. Brain 136 (2013) 3618-3624.

[5] **R.P. Kozak**, L. Royle, R.A. Gardner, A. Bondt, D.L. Fernandes, M. Wuhrer, Improved nonreductive O-glycan release by hydrazinolysis with ethylenediaminetetraacetic acid addition. Anal.Biochem. 453 (2014) 29-37.

[6] A. van Diepen, A.J. van der Plas, **R.P. Kozak**, L. Royle, D.W. Dunne, C.H. Hokke, Development of a Schistosoma mansoni shotgun O-glycan microarray and

application to the discovery of new antigenic schistosome glycan motifs. Int.J.Parasitol. 45 (2015) 465-475.

[7] **R.P. Kozak**, C.B. Tortosa, D.L. Fernandes, D.I. Spencer, Comparison of procainamide and 2-aminobenzamide labeling for profiling and identification of glycans by liquid chromatography with fluorescence detection coupled to electrospray ionization-mass spectrometry. Anal.Biochem. 486 (2015) 38-40.

[8] V. Dotz, R. Haselberg, A. Shubhakar, **R.P. Kozak**, D, Flack, Y. Rombouts, D. Reusch, G.W. Somsen, D.L. Fernandes, M. Wuhrer, Mass spectrometry for glycosylation analysis of biopharmaceuticals. Trends in Analytical Chemistry. 73 (2015) 1-9.

[9] C. Phansopa, **R.P. Kozak**, L.P. Liew, A.M. Frey, T. Farmilo, J.L. parker, D.J. Kelly, R.J. Emery, R.I. Thompson, L. Royle, R.A. Gardner, D.I. Spencer, G.P. Stafford,Characterization of a sialate O-acetyl esterase (NanS) from the oral pathogen Tannerella forsythia that enhances sialic acid release by NanH its cognate sialidase. Biochemical Journal. 472 (2015) 157-167.

[10] A. Bondt, S. Nicolardi, B.P. Jansen, K. Stavenhagen, D. Blank, G.S. Kammeijer, **R.P. Kozak**, D.L. Fernandes, P.J. Hensbergen, J.M. Hazes, Y.E. van der Burgt, R.J. Dolhain, M. Wuhrer, Longitudinal monitoring of immunoglobulin A glycosylation during pregnancy by simultaneous MALDI-FTICR-MS analysis of N- and O-glycopeptides. Scientific Reports. 6 (2016) 1-12.

[11] A. Shubhakar, **R.P. Kozak**, K.R. Reiding, L. Royle, D.I. Spencer, D.L. Fernandes, M. Wuhrer, Automated High-Throughput Permethylation for

Glycosylation Analysis of Biologics Using MALDI-TOF-MS. Anal Chem. 17 (2016) 8562-9.

[12] **R.P. Kozak**, P.A. Urbanowicz, C. Punyadeera, K.R. Reiding, B.C. Jansen, L. Royle, D.I. Spencer, D.L. Fernandes, M. Wuhrer, Variation of human salivary O-glycome. PLoS One. 9 (2016) 1-15.

Acknowledgements

I would like to express my appreciation and thanks to my PhD advisor and CEO of Ludger Ltd. Dr Daryl Fernandes, for encouraging my research and for allowing me to start my PhD journey and grow as a glyco-scientist.

Special mention goes to my enthusiastic supervisor Professor Manfred Wuhrer for his patience and guidance. His knowledge of glycosylation and experience in the development of glycoanalytical techniques especially for mass spectrometry is of great value to PhD student. My entire PhD progressed with many constructive discussions with him on data analysis, writing manuscripts and my thesis.

Dozens of people have helped and taught me immensely at Ludger. Dr Richard Gardner welcomed me to the lab and introduced the hydrazinolysis procedure. Dr Daniel Spencer introduced mass spectrometry, shared his knowledge and time to explain the importance of mass spectrometry in the glyco-world.

I would like to take this opportunity also to thank Dr Louise Royle from Ludger. She is a good teacher and has offered great help and guidance during my PhD. She is very well organised and she always thinks ahead of time. I have received tremendously valuable advice from her on how to perform good experiments and how to organise my tasks in the most efficient way. She shared with me her great experience in experimental procedures, especially in O-glycan release, exoglycosidase sequencing and O-glycan analysis. I appreciate her comments and suggestions.

I would also like to thank all of my friends and colleagues from Ludger and LUMC who supported me in the lab, in writing, and encouraged me to strive towards my goal.

Lastly, I would like to thank my supportive, encouraging and patient wife Marta whose support during different stages of this PhD has been so appreciated. Thank you.