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9-28-2017

Increased trans-glycosylation activity of hexosaminidases for synthesis of human milk oligosaccharides

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Recommended Citation

Jan Muschiol; Jesper Holck; Peter K. Busk; Lene Lange; Jørn D. Mikkelsen; Anne S. Meyer; and Shariza B. Jamek,, "Increased trans-glycosylation activity of hexosaminidases for synthesis of human milk oligosaccharides" in "Enzyme Engineering XXIV", Pierre Monsan, Toulouse White Biotechnology, France Magali Remaud-Simeon, LISBP-INSA, University of Toulouse, France Eds, ECI Symposium Series, (2017). http://dc.engconfintl.org/enzyme_xxiv/116

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Increased *trans*-Glycosylation Activity of Hexosaminidases for Synthesis of Human Milk Oligosaccharides

Enzyme Engineering XXIV

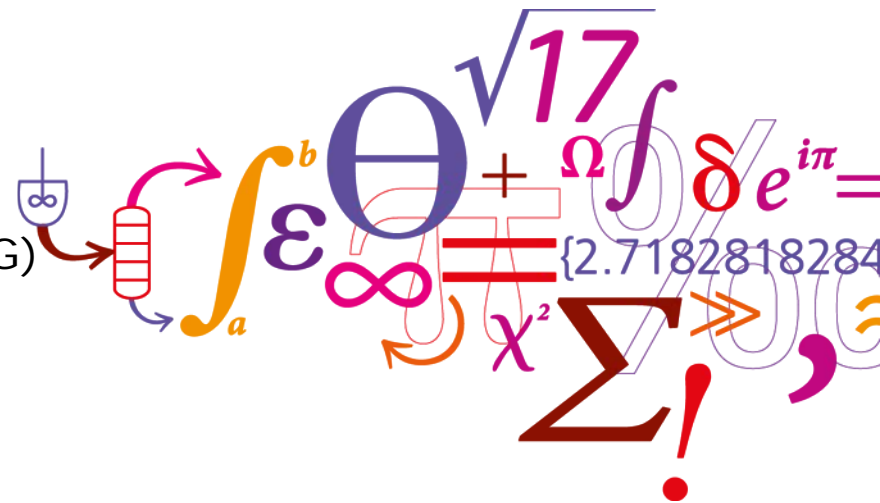
28/09-2017

Jan Muschiol

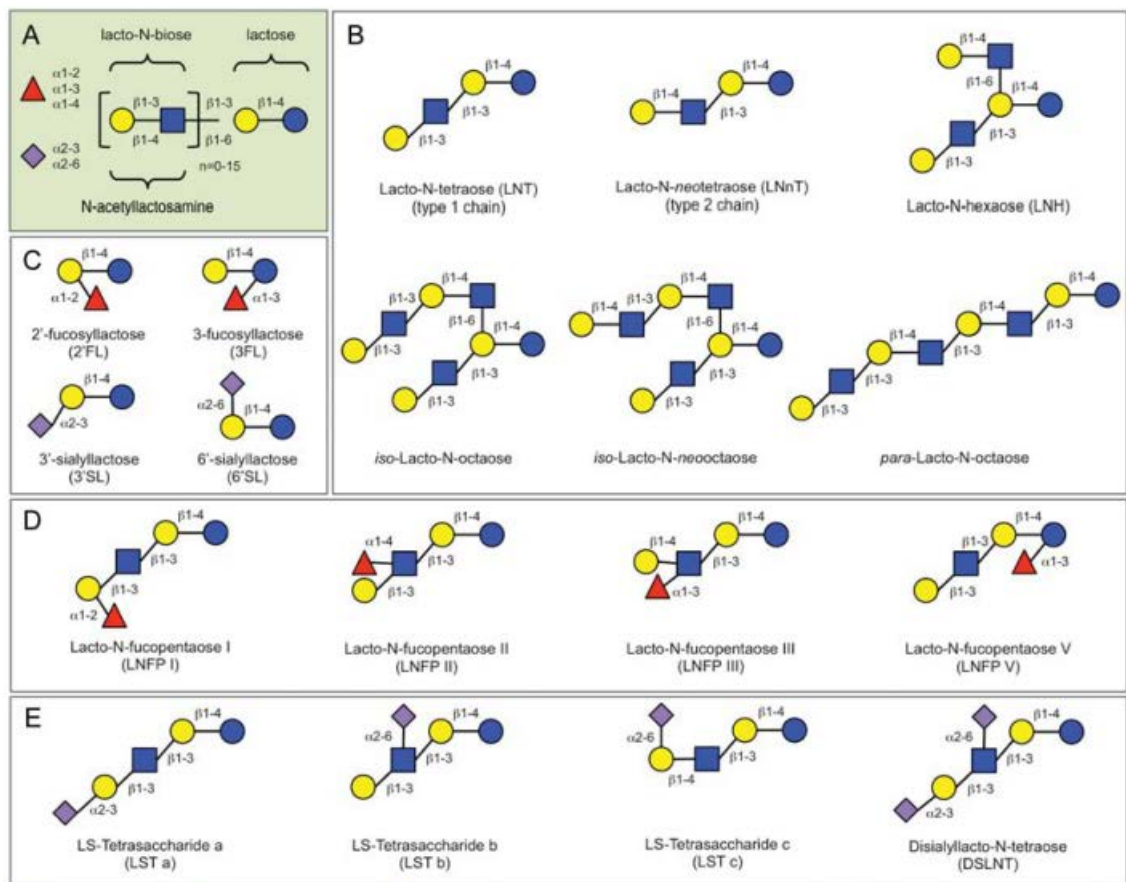
Center for Bioprocess Engineering (BIOENG)

DTU Chemical Engineering

Technical University of Denmark



Human Milk Oligosaccharides (HMOs)



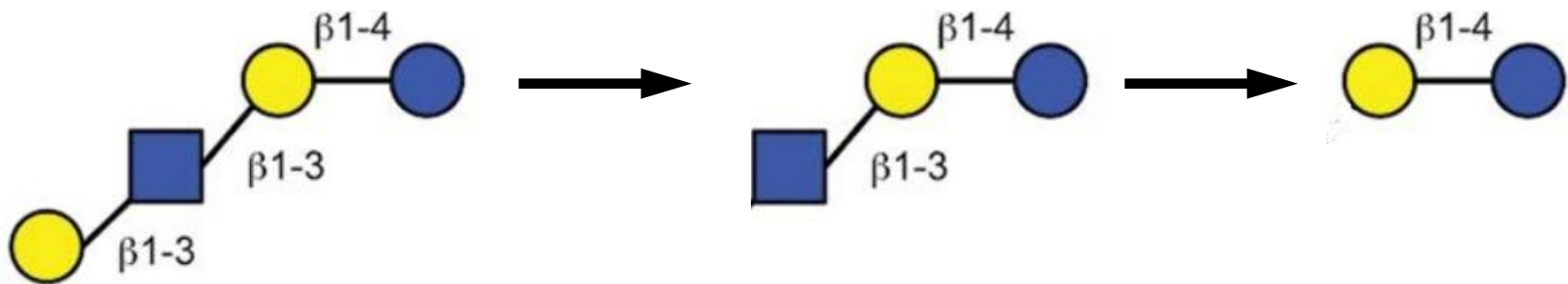
- Glucose (Glc)
- Galactose (Gal)
- ▲ Fucose (Fuc)
- N-Acetylglucosamine (GlcNAc)
- ◆ N-Acetylneuraminic acid (NeuNAc)

Postulated beneficial effects:

- Prebiotic
- Antiadhesive antimicrobial
- Modulatory on intestinal epithelial cell responses
- Immune modulatory
- Protective against necrotizing enterocolitis
- Nutritional for brain development

Bode (2012), *Glycobiology* 22:1147–1162.

Human Milk Oligosaccharides - Retrosynthetic Approach -



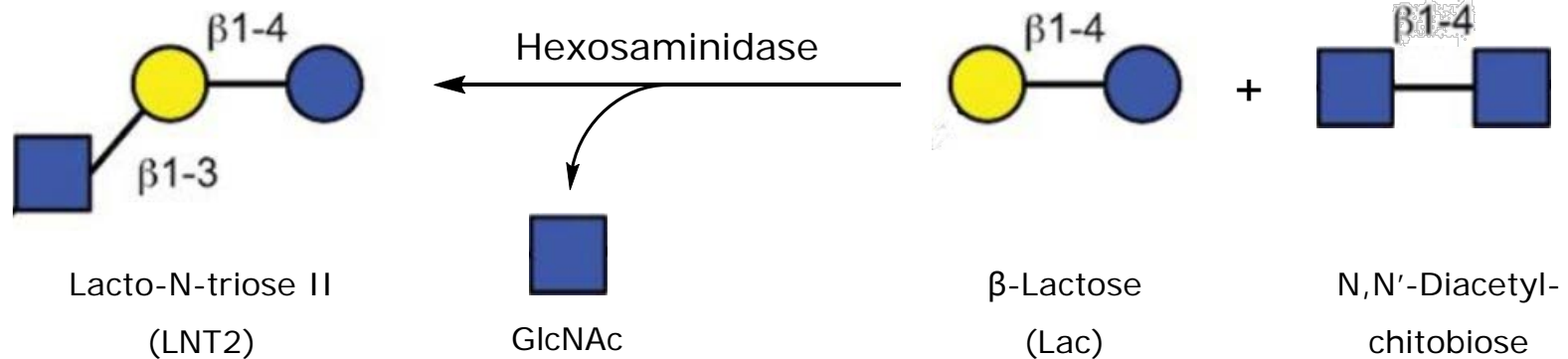
Lacto-N-tetraose
(LNT)

Lacto-N-triose II
(LNT2)

β-Lactose
(Lac)

- Glucose (Glc)
- Galactose (Gal)
- N-Acetylglucosamine (GlcNAc)

Human Milk Oligosaccharides - Retrosynthetic Approach -



Yield after 3 h

HEX1: 4.0%

HEX2: 5.5%

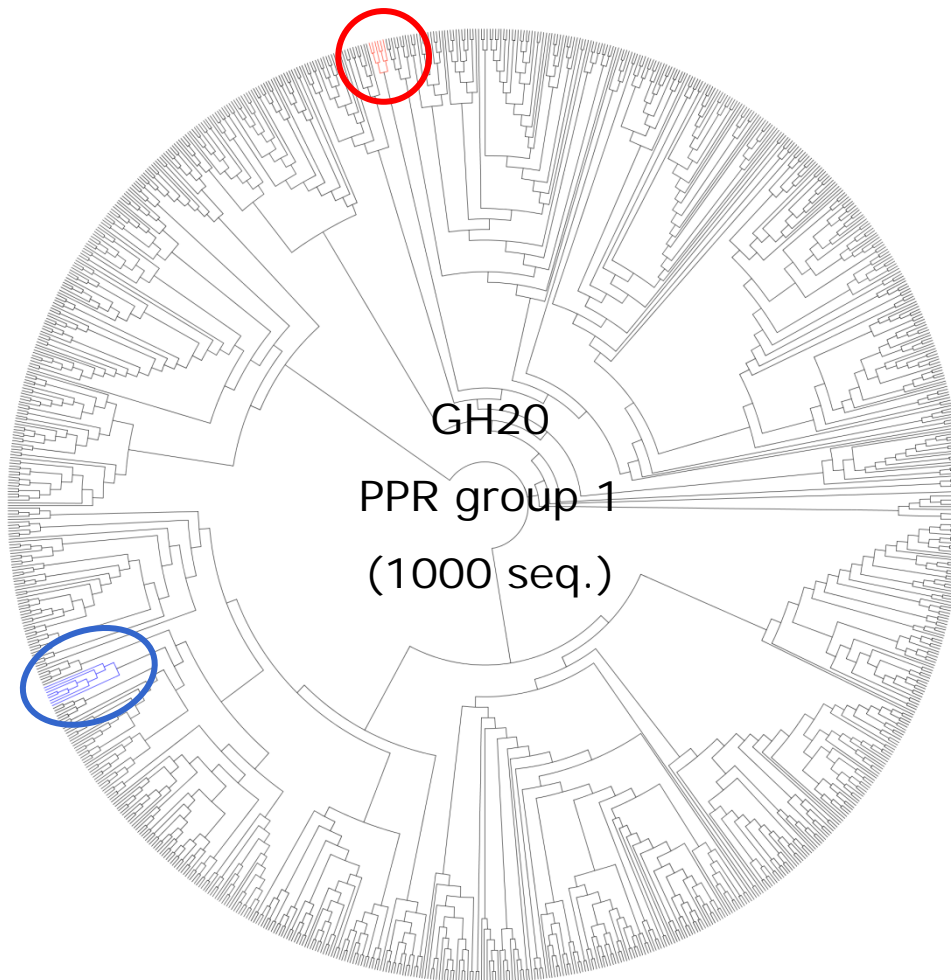
(based on donor,

incl. all detected isomers)

- Glucose (Glc)
- Galactose (Gal)
- N-Acetylglucosamine (GlcNAc)

Nyffenegger, et al. (2015) Appl Microbiol Biotechnol 99: 7997–8009.

Peptide Pattern Recognition (PPR)



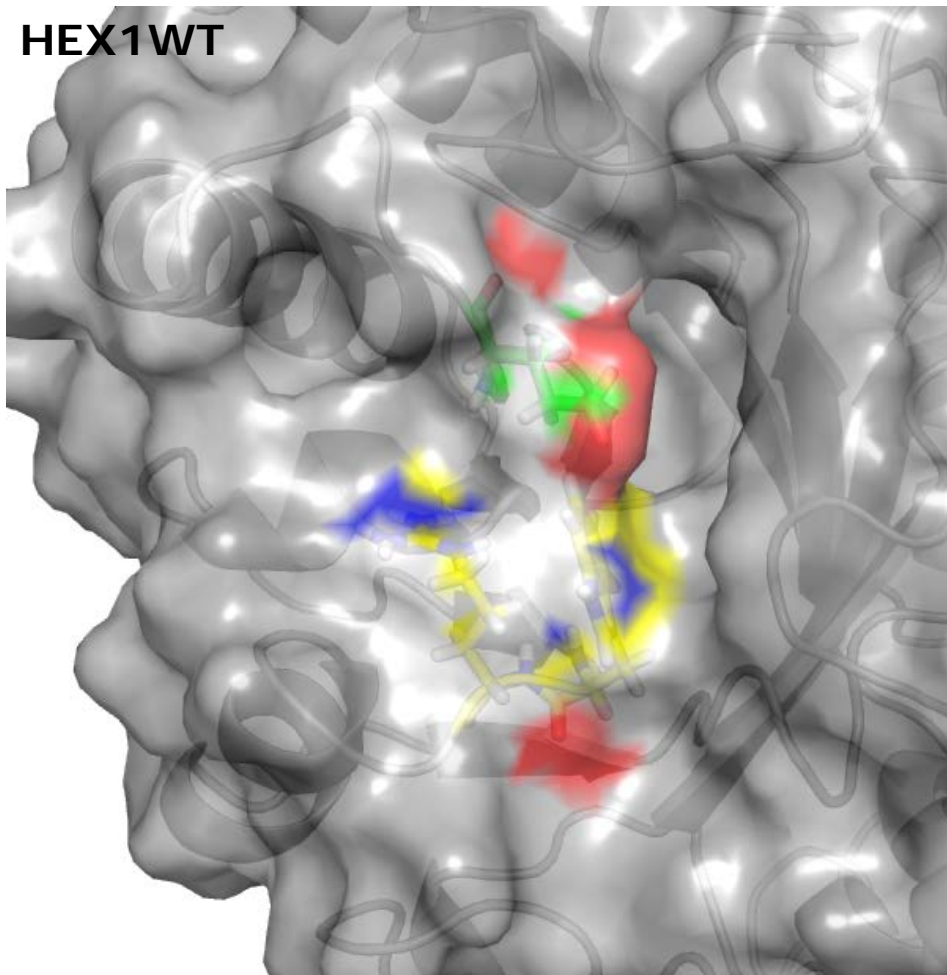
- Peptide Pattern Recognition in GH family 20 (3000 seq.) by Peter K. Busk
- HEX1 and HEX2 both in group 1 (1000 seq.)

Busk & Lange (2013) Appl Environ Microbiol 79: 3380–3391.

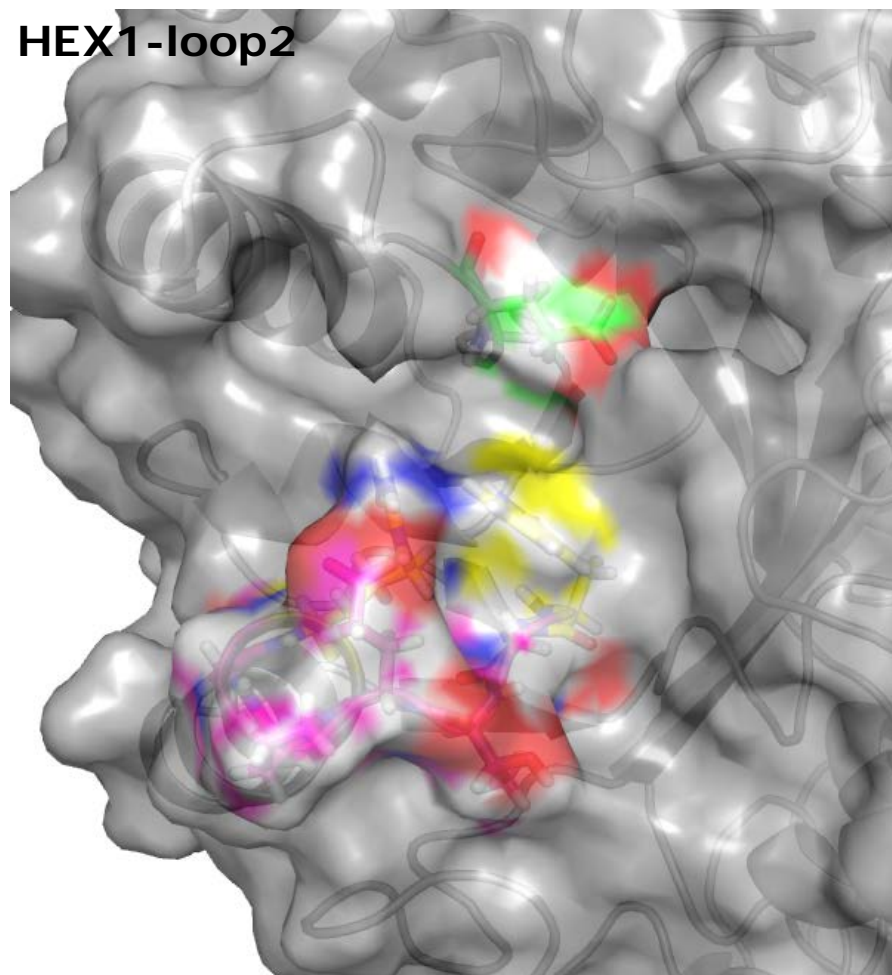
Busk, et al. (2017) BMC Bioinformatics 18: 214.

Homology modeling of loop engineered hexosaminidases

HEX1WT



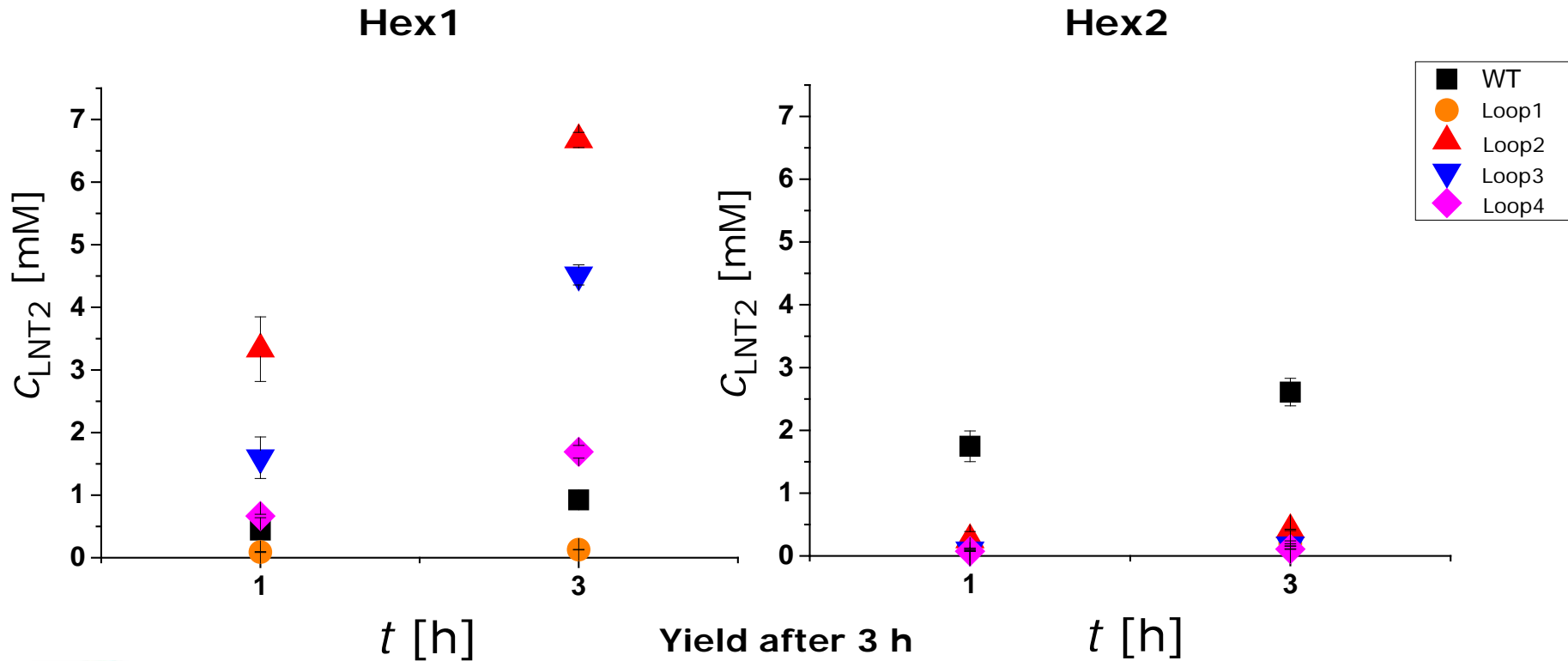
HEX1-loop2



Modeling templates: 4C7D, 1JAK, 4C7G, 1M03, 1M04

Jamek, et al., manuscript submitted

LNT2 synthesis by loop engineered hexosaminidases



HEX1-loop2: 17.0%
(based on donor,
incl. all detected isomers)



Shariza B. Jamek

Jamek, et al., manuscript submitted

Acknowledgement

Shariza B. Jamek
Jesper Holck
Birgitte Zeuner
Peter K. Busk
Jørn D. Mikkelsen
Anne S. Meyer



BIOENG in front of the Hasselbalch house (Snekkersten, DK)

