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

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

Enzyme Engineering XXIV

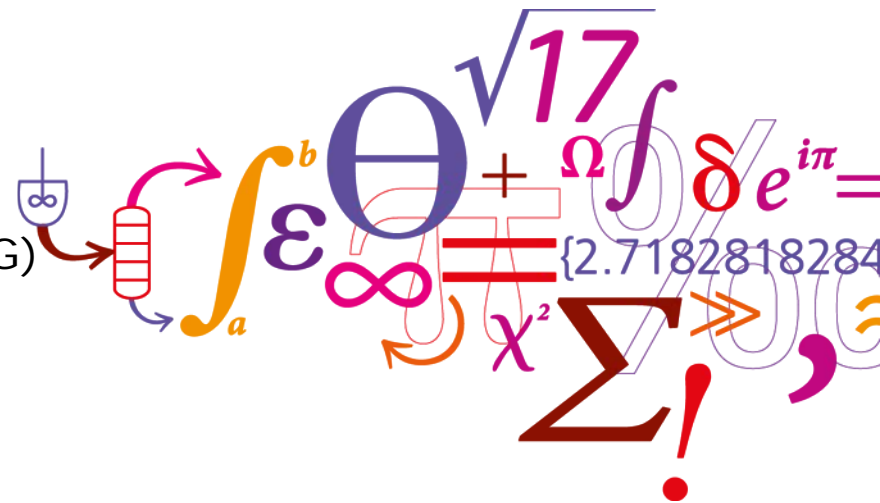
28/09-2017

Jan Muschiol

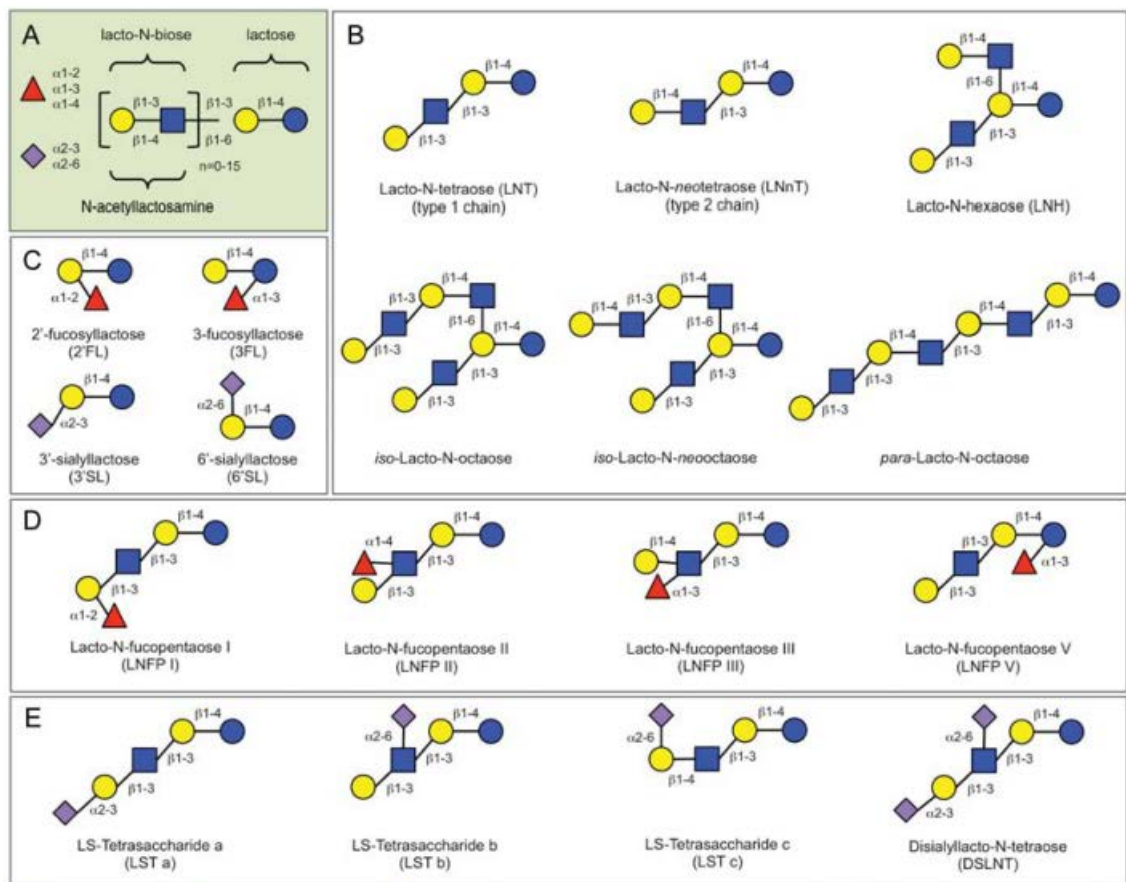
Center for Bioprocess Engineering (BIOENG)

DTU Chemical Engineering

Technical University of Denmark



Human Milk Oligosaccharides (HMOs)



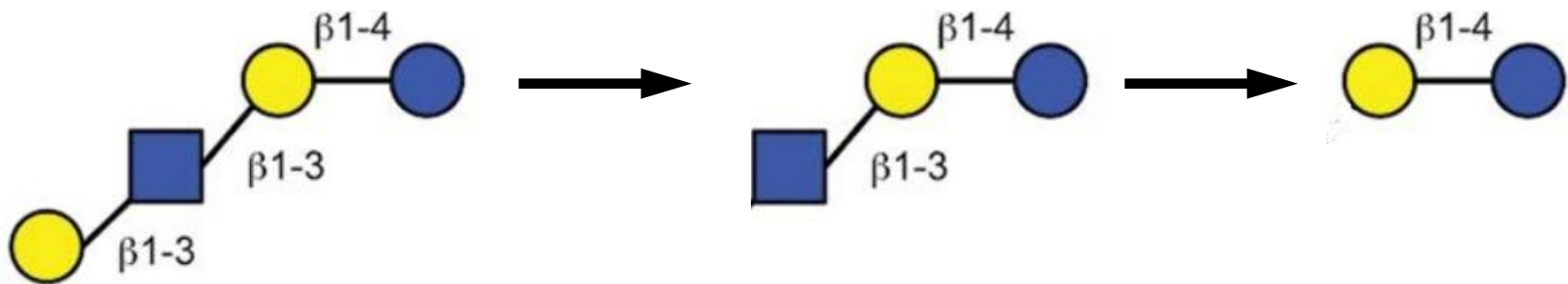
- Glucose (Glc)
- Galactose (Gal)
- ▲ Fucose (Fuc)
- N-Acetylglucosamine (GlcNAc)
- ◆ N-Acetylneuraminic acid (NeuNAc)

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 -



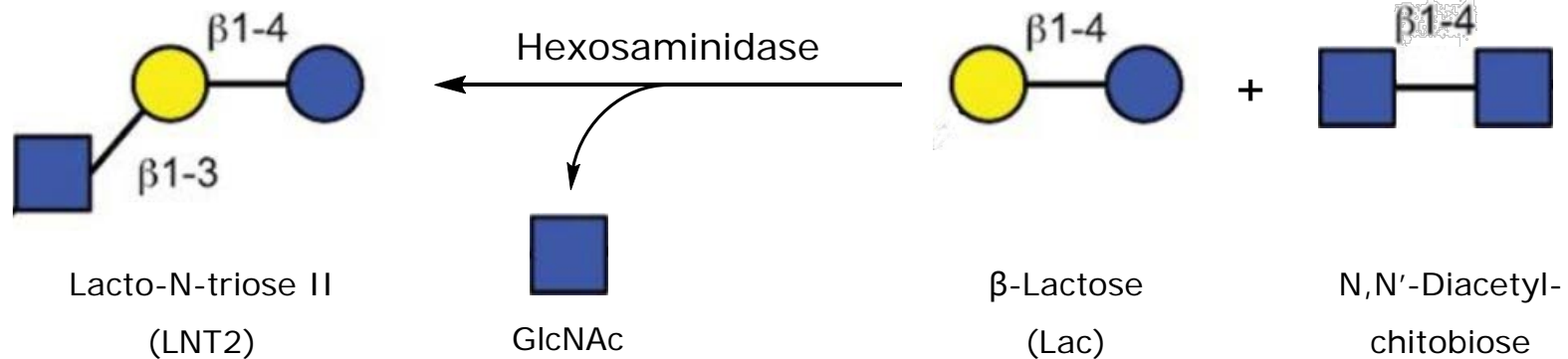
Lacto-N-tetraose
(LNT)

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%

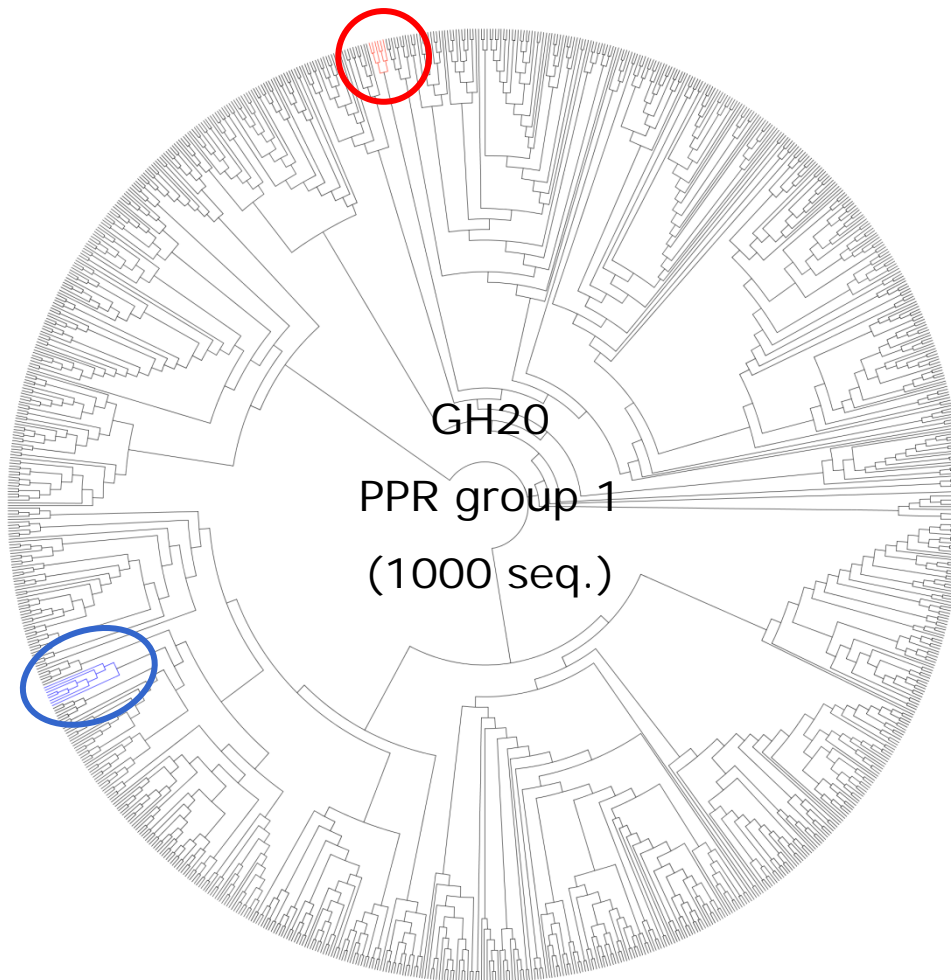
(based on donor,

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)



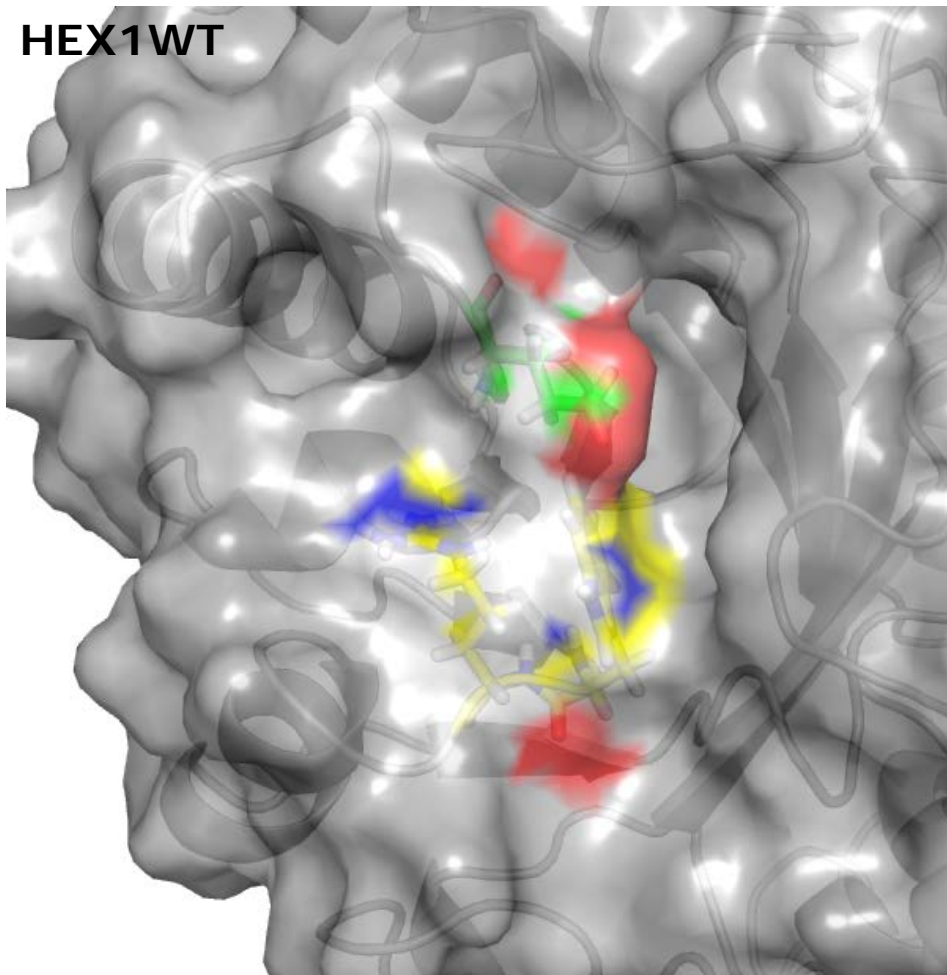
- Peptide Pattern Recognition in GH family 20 (3000 seq.) by Peter K. Busk
- HEX1 and HEX2 both in group 1 (1000 seq.)

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

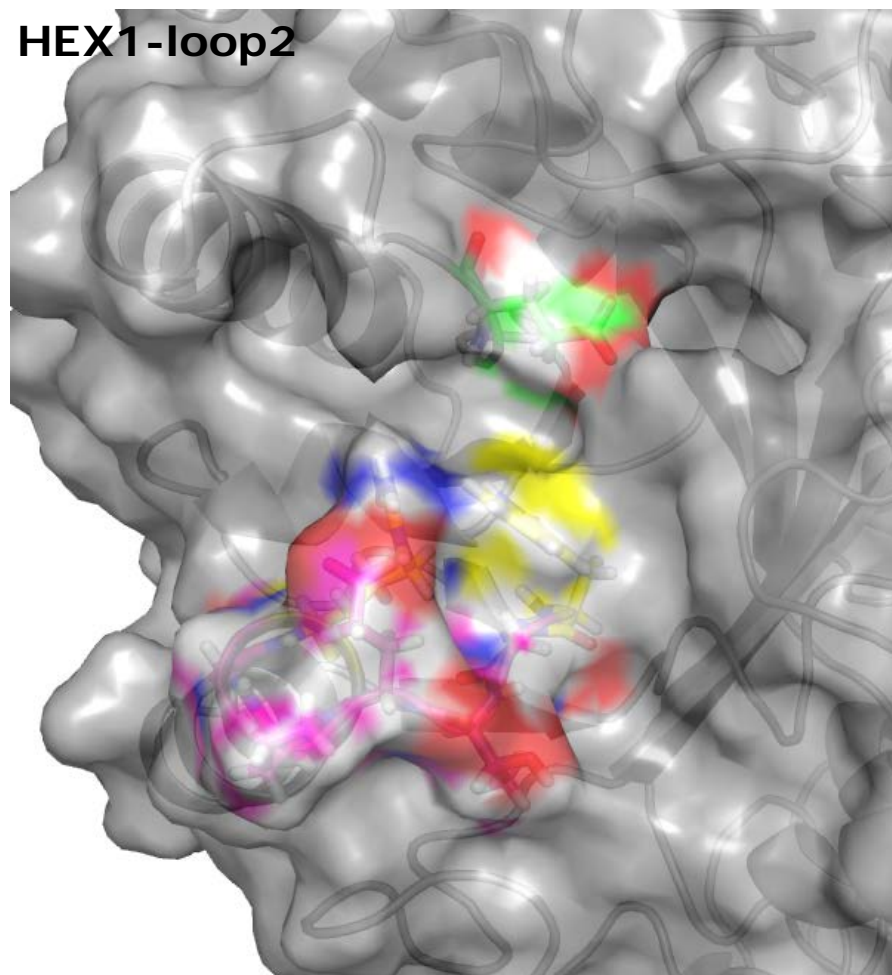
Busk, et al. (2017) BMC Bioinformatics 18: 214.

Homology modeling of loop engineered hexosaminidases

HEX1WT



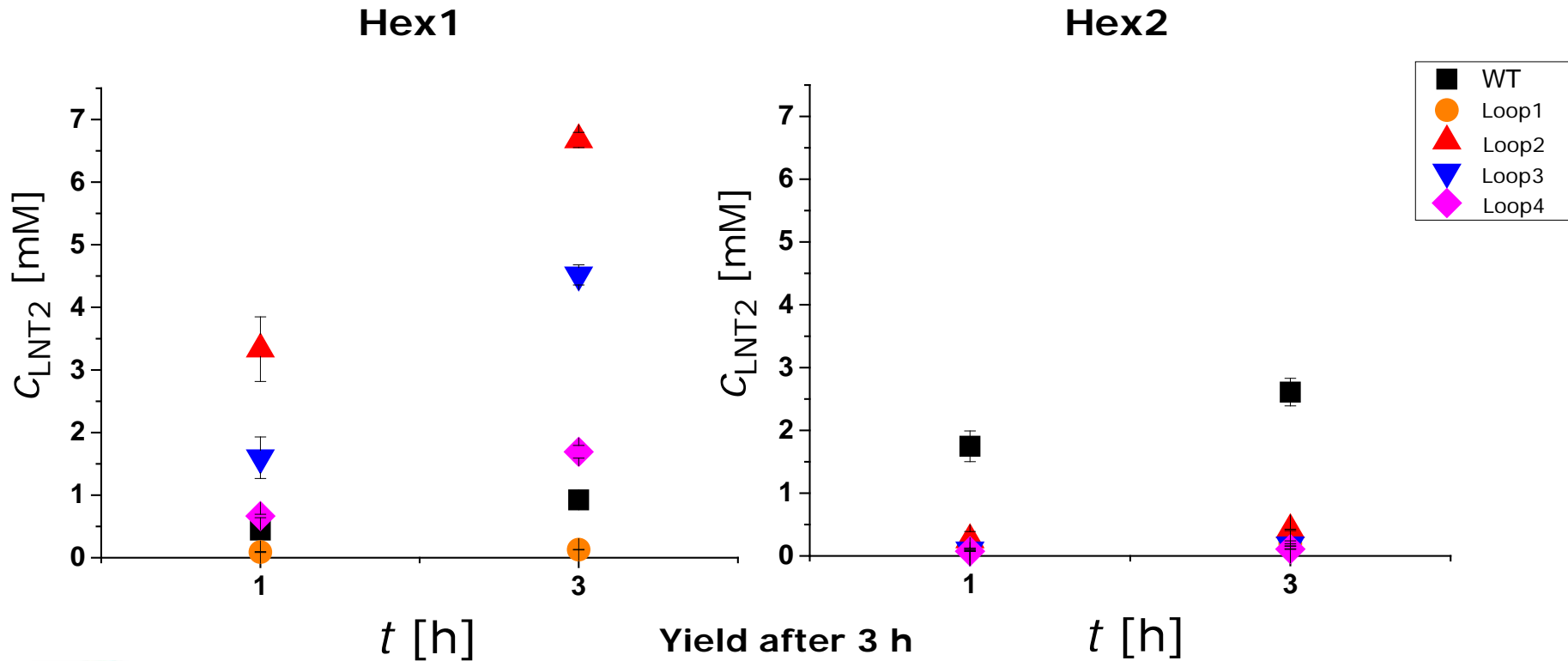
HEX1-loop2



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

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Anne S. Meyer



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

