Danmarks Tekniske Universitet

DTU

A Rational Approach to Identification of Wild Type Trans-sialidases for the Production of Human Milk Oligosaccharides

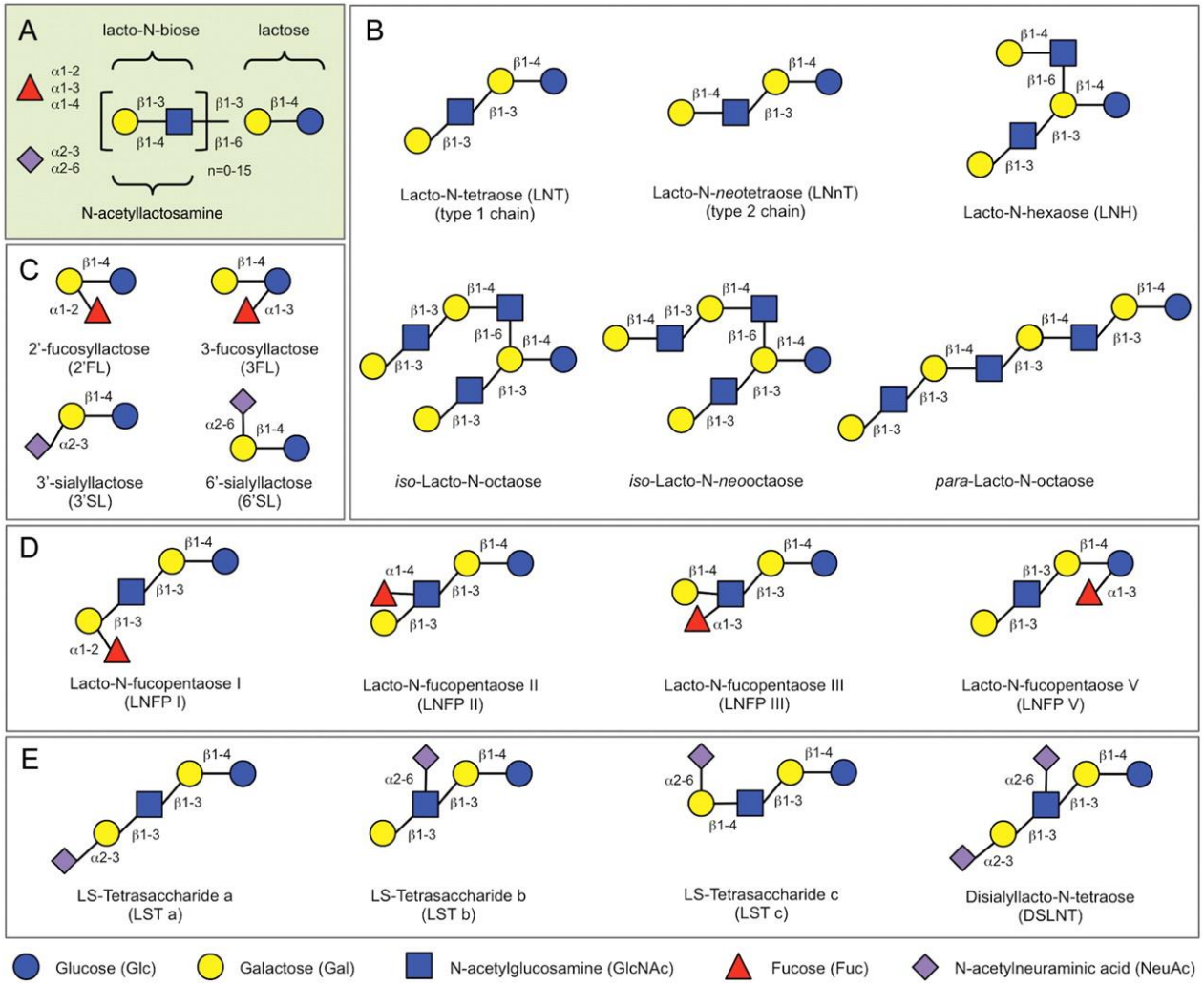
By Rune Thorbjørn Nordvang
BioEng
Institute for Chemical and Biochemical Engineering
Technical University of Denmark

Glycobiology World Congress
August 11th 2015
Philadelphia

Outline

- Human Oligo Saccharides - structures and properties
- The BioEng HMO production setup (for sialyllated HMOs)
- In silico Trans-sialidase discovery
  - Strategy development
  - In silico results
- In vitro verification
  - Experimental
  - In vitro results
- Conclusions & Perspectives

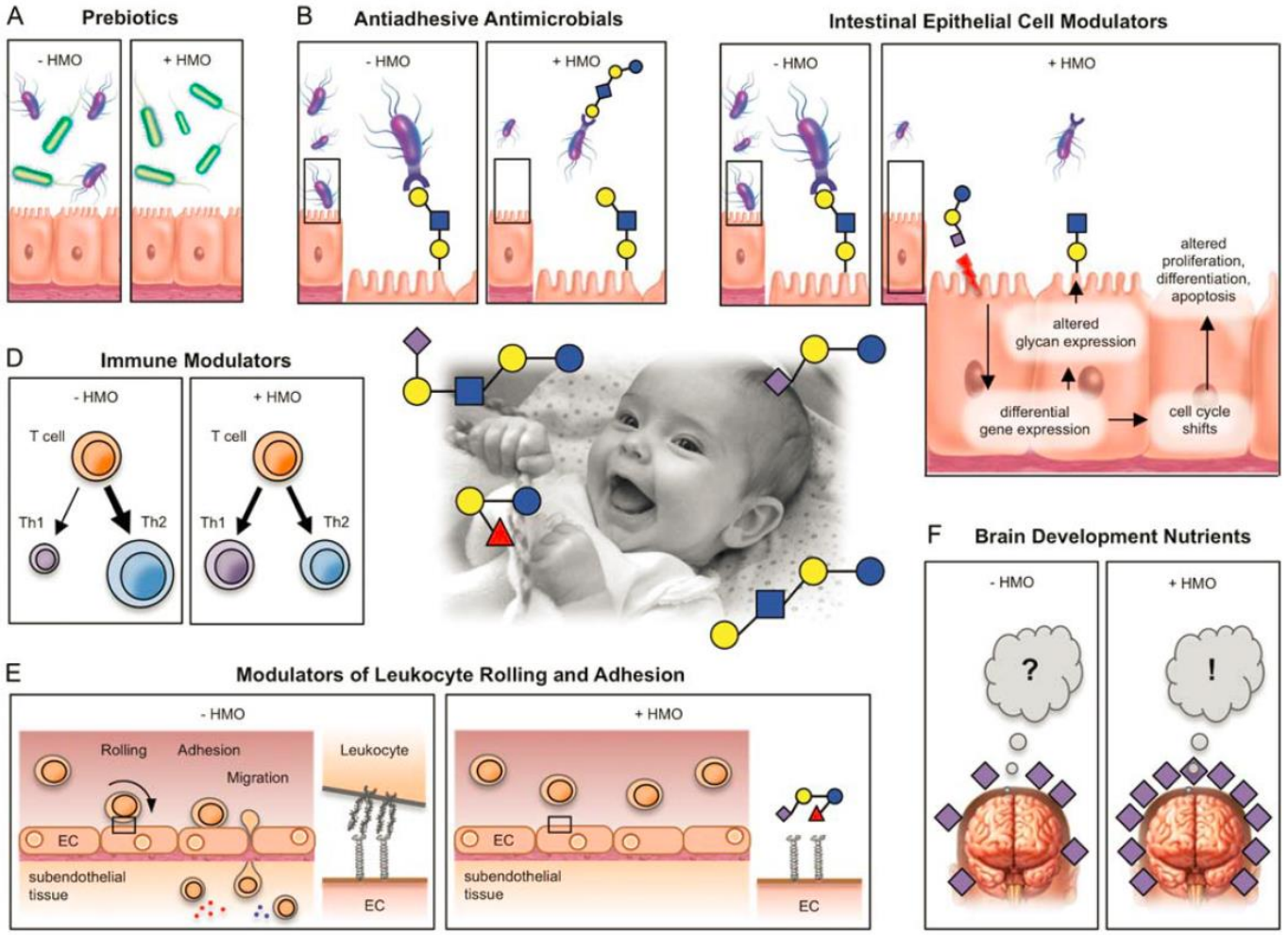
HMO blueprint and selected HMO structures



Bode L Glycobiology 2012;22:1147-1162

GLYCOBIOLOGY

Enzyme-catalysed synthetic HMO production setup



Bode L Glycobiology 2012;22:1147-1162

GLYCOBIOLOGY

Enzyme-catalysed synthetic HMO production setup

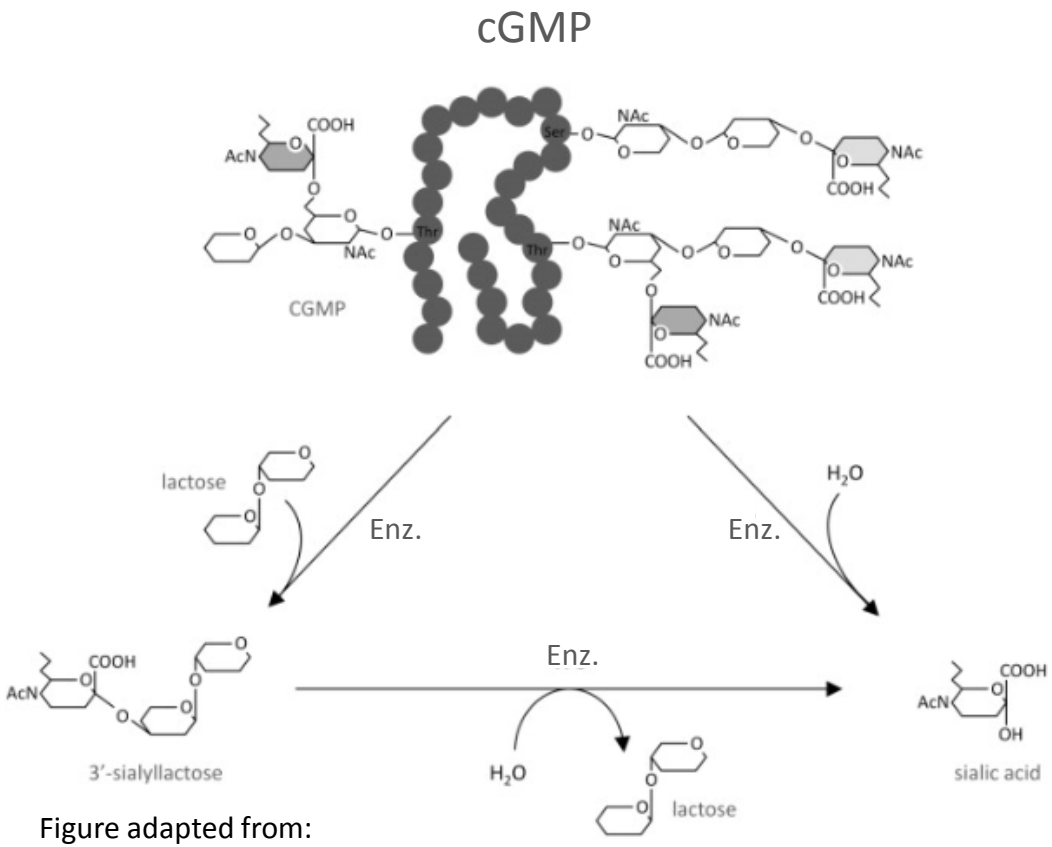
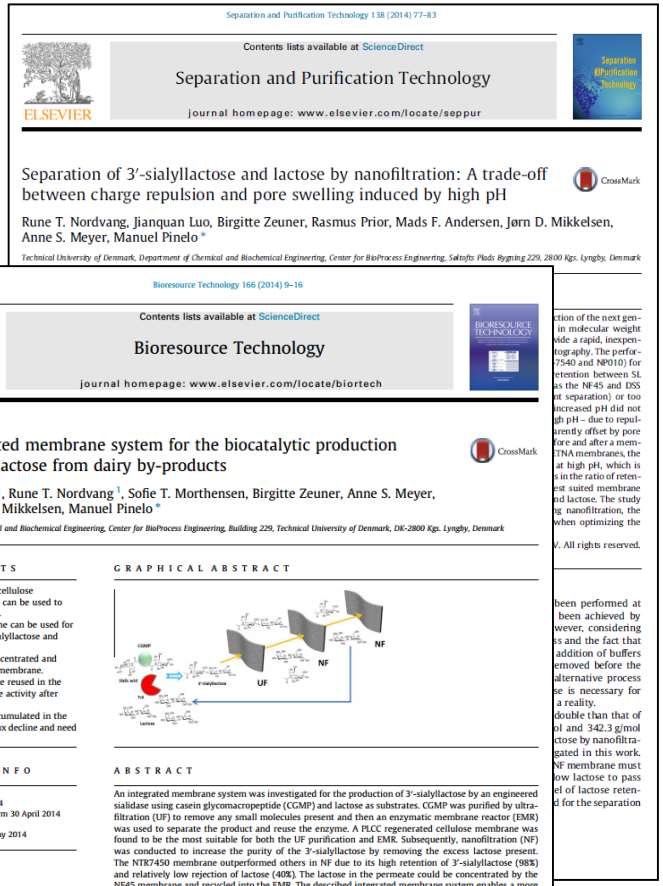


Figure adapted from:
Zeuner B, *et al.* 2014 Enzyme and Microbial Technology 55:85-93



Separation and Purification Technology 138 (2014) 77–83

Contents lists available at ScienceDirect

Separation and Purification Technology

journal homepage: www.elsevier.com/locate/seppur

Separation of 3′-sialyllactose and lactose by nanofiltration: A trade-off between charge repulsion and pore swelling induced by high pH

Rune T. Nordvang, Jianquan Luo, Birgitte Zeuner, Rasmus Prior, Mads F. Andersen, Jørn D. Mikkelsen, Anne S. Meyer, Manuel Pinelo *

Technical University of Denmark, Department of Chemical and Biochemical Engineering, Center for BioProcess Engineering, Søltofts Plads Bygning 229, 2800 Kgs. Lyngby, Denmark

Bioresource Technology 166 (2014) 9–16

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

An integrated membrane system for the biocatalytic production of 3′-sialyllactose from dairy by-products

Jianquan Luo [1], Rune T. Nordvang [1], Sofie T. Morthensen, Birgitte Zeuner, Anne S. Meyer, Jørn Dalgaard Mikkelsen, Manuel Pinelo *

Department of Chemical and Biochemical Engineering, Center for BioProcess Engineering, Building 229, Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark

HIGHLIGHTS

- PLCC regenerated cellulose membrane (5 kDa) can be used to configure the EMR.
- NTR7450 membrane can be used for separation of 3′-sialyllactose and lactose.
- Lactose can be concentrated and recycled by NF45 membrane.
- Tr6 sialidase can be reused in the EMR and retain the activity after centrifugation.
- CGMP residues accumulated in the EMR results in a flux decline and need to be removed.

GRAPHICAL ABSTRACT

ARTICLE INFO

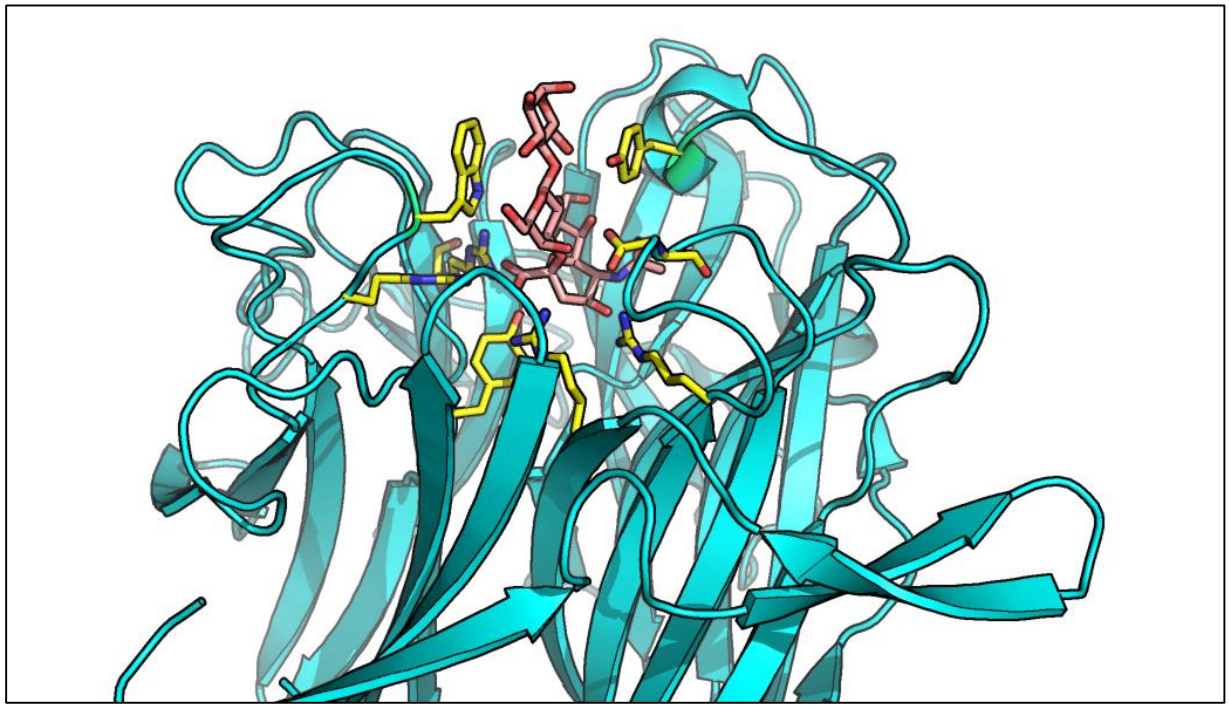
Article history:
Received 4 March 2014
Received in revised form 30 April 2014
Accepted 2 May 2014
Available online 10 May 2014

Keywords:
Ultrafiltration
Nanofiltration
Enzyme
EMR
Human milk oligosaccharides

ABSTRACT

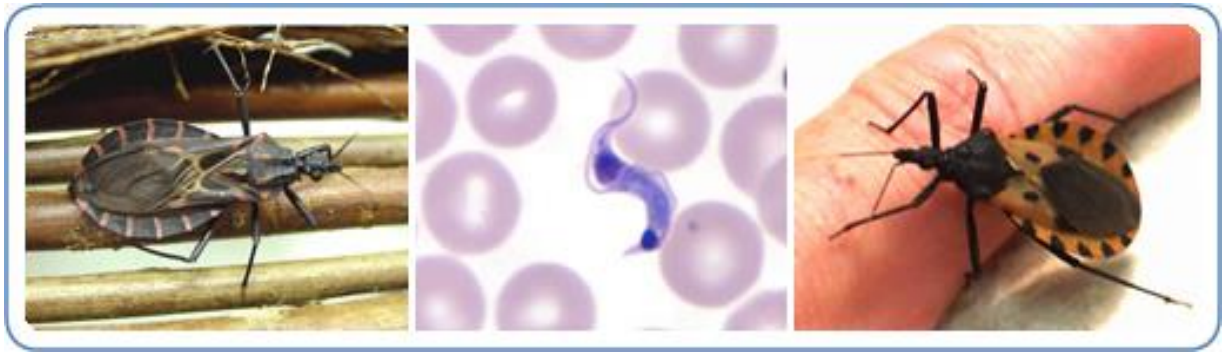
An integrated membrane system was investigated for the production of 3′-sialyllactose by an engineered sialidase using casein glycomacropeptide (CGMP) and lactose as substrates. CGMP was purified by ultrafiltration (UF) to remove any small molecules present and then an enzymatic membrane reactor (EMR) was used to separate the product and reuse the enzyme. A PLCC regenerated cellulose membrane was found to be the most suitable for both the UF purification and EMR. Subsequently, nanofiltration (NF) was conducted to increase the purity of the 3′-sialyllactose by removing the excess lactose present. The NTR7450 membrane outperformed others in NF due to its high retention of 3′-sialyllactose (98%) and relatively low rejection of lactose (40%). The lactose in the permeate could be concentrated by the NF45 membrane and recycled into the EMR. The described integrated membrane system enables a more economic and efficient enzymatic production of 3′-sialyllactose.

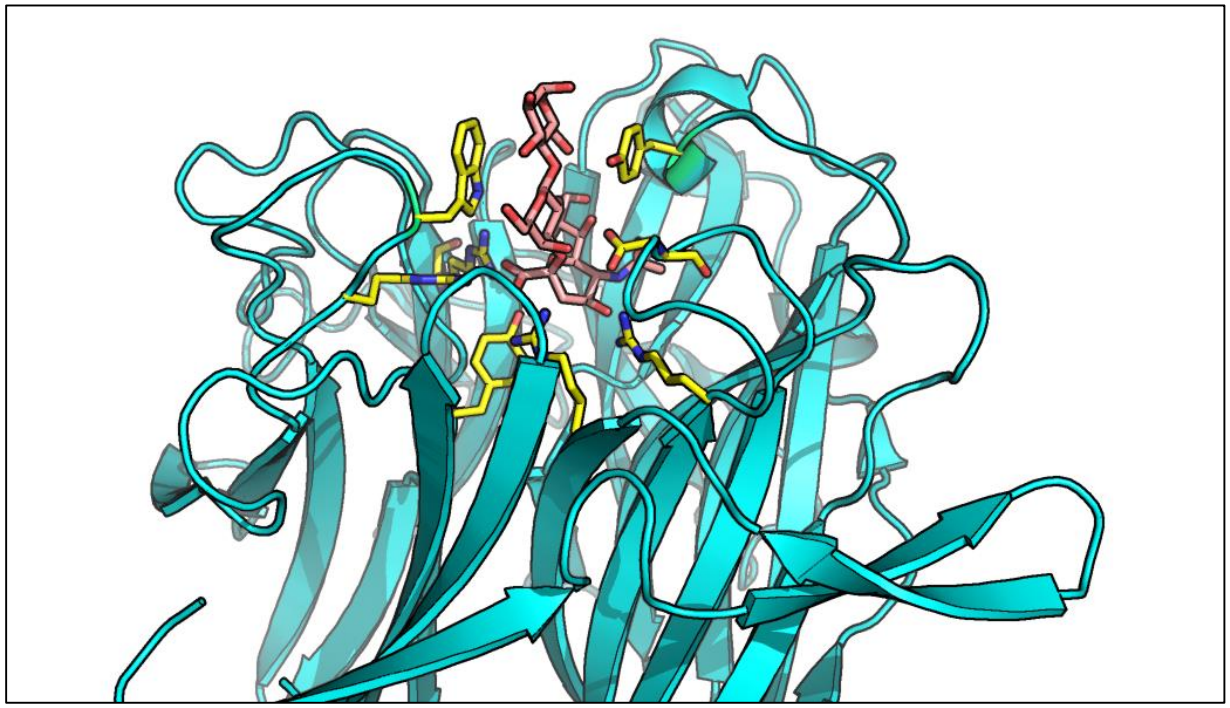
One native trans-sialidase is known



TcTS model based on Amaya M.F., et.al. (2004) Structure 12: 775–784

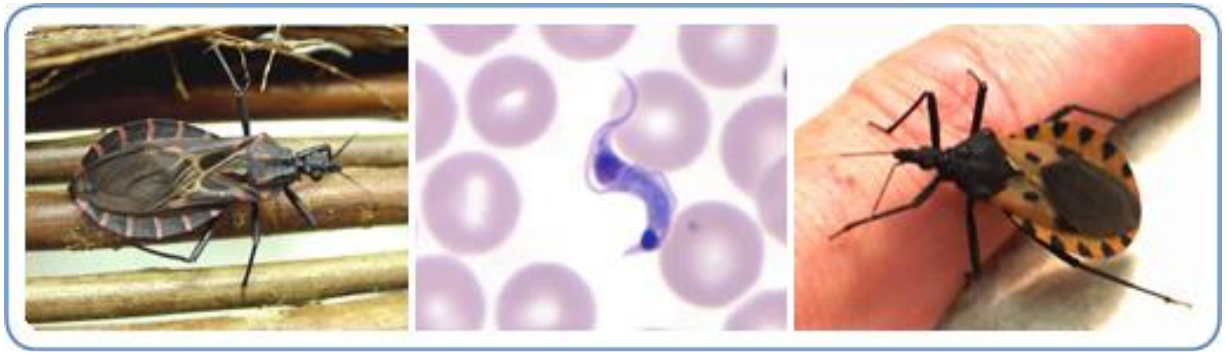
The trans-sialidase of Trypanosomas cruzi





TcTS model based on Amaya M.F., et.al. (2004) Structure 12: 775–784

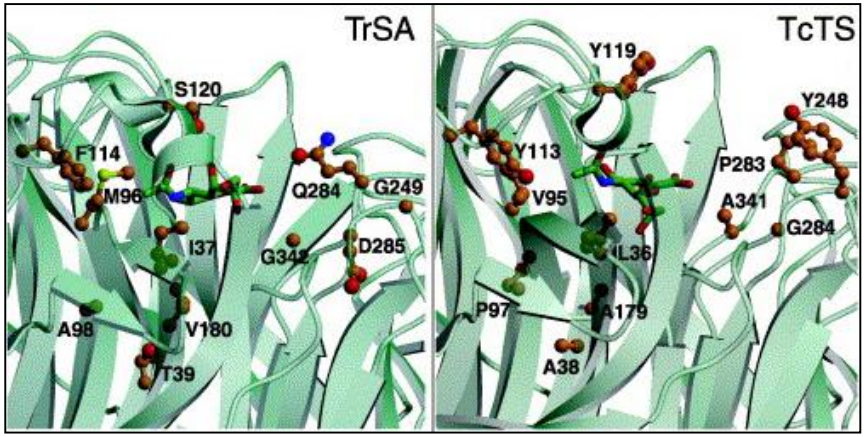
The trans-sialidase of Trypanosomas cruzi



- Not a sialyl-transferase
- Virulence factor in a human pathogen

TcTS model based on Amaya M.F., et.al. (2004) Structure 12: 775–784

A quintuple mutant of it's close cousin from the non-pathogenic T. *rangeli*



doi:10.1016/j.jmb.2004.09.031 *J. Mol. Biol.* (2005) **345**, 923–934

JMB

Available online at www.sciencedirect.com

SCIENCE DIRECT®

ELSEVIER

A Sialidase Mutant Displaying *trans*-Sialidase Activity

Gastón Paris[1]*, Laura Ratier[1], María Fernanda Amaya[2], Tong Nguyen[2] Pedro M. Alzari[2] and Alberto Carlos C. Frasch[1]

[1]*Instituto de Investigaciones Biotecnológicas-Instituto Tecnológico de Chascomús CONICET-UNSAM, CC30 1650, San Martín, Buenos Aires Argentina*

[2]*Unité de Biochimie Structurale CNRS URA 2185, Institut Pasteur, 25 rue du Dr Roux 75724 Paris, France*

**Corresponding author*

Trypanosoma cruzi, the agent of Chagas disease, expresses a modified sialidase, the *trans*-sialidase, which transfers sialic acid from host glycoconjugates to β-galactose present in parasite mucins. Another American trypanosome, *Trypanosoma rangeli*, expresses a homologous protein that has sialidase activity but is devoid of transglycosidase activity. Based on the recently determined structures of *T. rangeli* sialidase (TrSA) and *T. cruzi trans*-sialidase (TcTS), we have now constructed mutants of TrSA with the aim of studing the relevant residues in transfer activity. Five mutations, Met96-Val, Ala98-Pro, Ser120-Tyr, Gly249-Tyr and Gln284-Pro, were enough to obtain a sialidase mutant ($TrSA_{5mut}$) with *trans*-sialidase activity; and a sixth mutation increased the activity to about 10% that of wild-type TcTS. The crystal structure of $TrSA_{5mut}$ revealed the formation of a *trans*-sialidase-like binding site for the acceptor galactose, primarily defined by the phenol group of Tyr120 and the indole ring of Trp313, which adopts a new conformation, similar to that in TcTS, induced by the Gln284-Pro mutation. The transition state analogue 2,3-didehydro-2-deoxy-*N*-acetylneuraminic acid (DANA), which inhibits sialidases but is a poor inhibitor of *trans*-sialidase, was used to probe the active site conformation of mutant enzymes. The results show that the presence of a sugar acceptor binding-site, the fine-tuning of protein–substrate interactions and the flexibility of crucial active site residues are all important to achieve transglycosidase activity from the TrSA sialidase scaffold.

Keywords: *trans*-sialidase; sialidase; *Trypanosoma cruzi*; *Trypanosoma rangeli*; protein engineering

Introduction

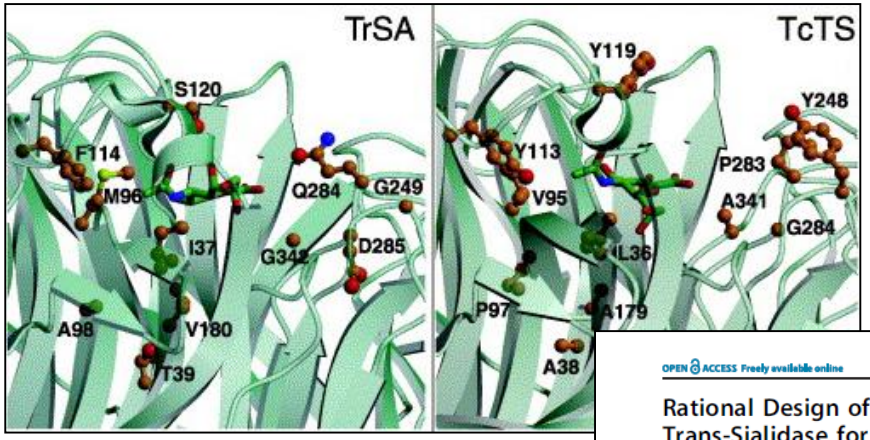
Sialic acids are nine-carbon monosaccharides and comprise a family of about 40 members found in the outermost position of oligosaccharide chains of glycoproteins and glycolipids.[1] Advances in fields as different as cell adhesion and morphogenesis[2,3] lymphocyte homing and inflammation,[4] viral, bacterial, and, protozoarian pathogenesis and nutrition[5], and regulation of the immune system[6,7] stress the biological importance of sialic acids[4,8–10] and of the enzymes, sialidases and sialyltransferases, that regulate their presence and cell surface distribution.

Trypanosoma cruzi, the agent of Chagas disease and *Trypanosoma brucei*, the agent of the disease known as sleeping sickness in humans (*T. brucei* spp. *gambiense* and *T. brucei* spp. *rhodesiense*) and ngana in domestic animals (*T. brucei brucei*), are unable to synthesize sialic acid. Instead, these parasites express a glycosylphosphatidylinositol (GPI)-anchored surface *trans*-sialidase (TS) that scavenges sialic acid from host glycoconjugates.[11,12] The TS from *T. cruzi* (TcTS) has been extensively studied. TcTS was suggested to be involved in relevant processes such as parasite survival from the complement-mediated host immune response,[13] host cell invasion[14–16] and *T. cruzi* pathogenesis.[17,18] The involvement of TcTS in these processes is likely due to its capacity of sialylating the mucin molecules that cover the parasite surface with a dense protective layer.[19] TcTS has a globular catalytic domain and a C-terminal extension of tandemly repeated 12 amino

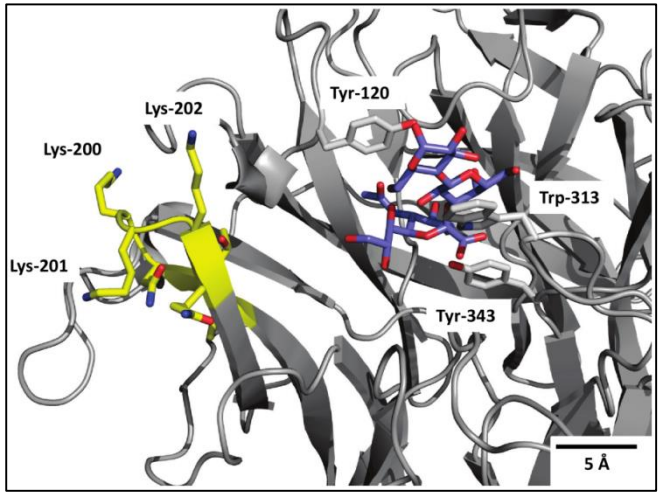
Abbreviations used: TS, *trans*-sialidase; TcTS, *Trypanosoma cruzi trans*-sialidase; TbTS, *Trypanosoma brucei trans*-sialidase; TrSA, *Trypanosoma rangeli* sialidase; GPI, glycosylphosphatidylinositol; MuNANA, 2′-(4-methylumbelliferyl)-α-D-*N*-acetylneuraminic acid; DANA, 2, 3-didehydro-2-deoxy-*N*-acetylneuraminic acid; wt, wild-type.

E-mail address of the corresponding author: gparis@iib.unsam.edu.ar

Directed evolution



Tr13



doi:10.1016/j.jmb.2004.09.031 J. Mol. Biol. (2005) 345, 923–934

JMB Available online at www.sciencedirect.com SCIENCE DIRECT® ELSEVIER

A Sialidase Mutant Displaying *trans*-Sialidase Activity

Gastón Paris[1]*, Laura Ratier[1], María Fernanda Amaya[2], Tong Nguyen[2] Pedro M. Alzari[2] and Alberto Carlos C. Frasch[1]

hagas disease, expresses a modified
hich transfers sialic acid from host
present in parasite mucins. Another
oma rangeli, expresses a homologous
t is devoid of transglycosidase activity.
structures of *T. rangeli* sialidase (TrSA)
we have now constructed mutants of
levant residues in transfer activity. Five
er120-Tyr, Gly249-Tyr and Gln284-Pro,
mutant ($TrSA_{5mut}$) with *trans*-sialidase
ased the activity to about 10% that of
e of $TrSA_{5mut}$ revealed the formation of
for the acceptor galactose, primarily
20 and the indole ring of Trp313, which
to that in TcTS, induced by the Gln284-
e analogue 2,3-didehydro-2-deoxy-*N*-
hich inhibits sialidases but is a poor
d to probe the active site conformation
w that the presence of a sugar acceptor
rotein–substrate interactions and the
esidues are all important to achieve
TrSA sialidase scaffold.

Trypanosoma cruzi; *Trypanosoma rangeli*;

rases, that regulate their presence and
distribution.
na cruzi, the agent of Chagas disease
soma brucei, the agent of the disease
leeping sickness in humans (*T. brucei*
se and *T. brucei* spp. *rhodesiense*) and
omestic animals (*T. brucei brucei*), are
ynthesize sialic acid. Instead, these
press a glycosylphosphatidylinositol
ed surface *trans*-sialidase (TS) that
sialic acid from host glycoconju-
he TS from *T. cruzi* (TcTS) has been
studied. TcTS was suggested to be
relevant processes such as parasite
om the complement-mediated host
sponse,[13] host cell invasion[14–16] and
ogenesis.[17,18] The involvement of TcTS
cesses is likely due to its capacity of
he mucin molecules that cover the
face with a dense protective layer.[19]
globular catalytic domain and a C-
ension of tandemly repeated 12 amino

OPEN ACCESS Freely available online PLOS ONE

Rational Design of a New *Trypanosoma rangeli* Trans-Sialidase for Efficient Sialylation of Glycans

Carsten Jers[1], Malwina Michalak[1], Dorte M. Larsen[1], Kasper P. Kepp[2], Haiying Li[3], Yao Guo[1], Finn Kirpekar[3], Anne S. Meyer[1], Jørn D. Mikkelsen[3]*

1 Center for BioProcess Engineering, Department of Chemical and Biochemical Engineering, Technical University of Denmark, Lyngby, Denmark, 2 Department of Chemistry, Technical University of Denmark, Lyngby, Denmark, 3 Department of Biochemistry and Molecular Biology, Southern University of Denmark, Odense, Denmark

Abstract

This paper reports rational engineering of *Trypanosoma rangeli* sialidase to develop an effective enzyme for a potentially important type of reactivity: production of sialylated prebiotic glycans. The *Trypanosoma cruzi* trans-sialidase and the homologous *T. rangeli* sialidase has previously been used to investigate the structural requirements for trans-sialidase activity. We observed that the *T. cruzi* trans-sialidase has a seven-amino-acid motif (197–203) at the border of the substrate binding cleft. The motif differs substantially in chemical properties and substitution probability from the homologous sialidase, and we hypothesised that this motif is important for trans-sialidase activity. The 197–203 motif is strongly positively charged with a marked change in hydrogen bond donor capacity as compared to the sialidase. To investigate the role of this motif, we expressed and characterised a *T. rangeli* sialidase mutant, Tr13. Conditions for efficient trans-sialylation were determined, and Tr13's acceptor specificity demonstrated promiscuity with respect to the acceptor molecule enabling sialylation of glycans containing terminal galactose and glucose and even monomers of glucose and fucose. Sialic acid is important in association with human milk oligosaccharides, and Tr13 was shown to sialylate a number of established and potential prebiotics. Initial evaluation of prebiotic potential using pure cultures demonstrated, albeit not selectively, growth of Bifidobacteria. Since the 197–203 motif stands out in the native trans-sialidase, is markedly different from the wild-type sialidase compared to previous mutants, and is shown here to confer efficient and broad trans-sialidase activity, we suggest that this motif can serve as a framework for future optimization of trans-sialylation towards prebiotic production.

Citation: Jers C, Michalak M, Larsen DM, Kepp KP, Li H, et al. (2014) Rational Design of a New *Trypanosoma rangeli* Trans-Sialidase for Efficient Sialylation of Glycans. PLoS ONE 9(1): e83902. doi:10.1371/journal.pone.0083902

Editor: Patrick C. Cirino, University of Houston, United States of America

Received April 29, 2013; **Accepted** November 18, 2013; **Published** January 3, 2014

Funding: This work was supported by the Strategic Research Council in Denmark, http://fsr.dk; The project number is 09-067134. The title is "Enzymatic production of human milk oligosaccharides". The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have filed a patent application ("A mutant sialidase having trans-sialidase activity for use in production of sialylated glycans"; European Patent Application No. 13163551.8). This does not alter the authors' adherence to all the PLOS ONE policies on sharing data and materials, as detailed online in our guide for authors. All material relating to the patent is available in connection with this submission and the present article was used as the framework to design the patent application.

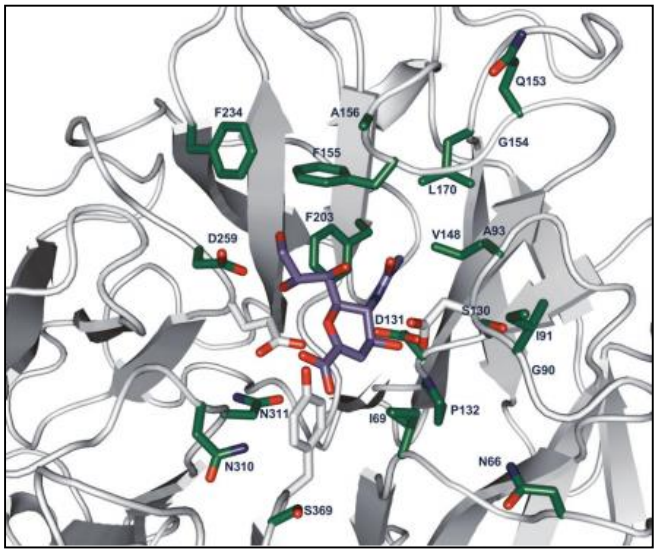
* E-mail: jdm@kt.dtu.dk

Introduction

For production of human milk oligosaccharides (HMOs), glycan sialylation can be achieved chemically as well as enzymatically [1]. To achieve enzymatic synthesis, a trans-sialidase (TcTS) derived from *T. cruzi*, the causative agent of Chagas disease, has previously proven useful by transferring sialic acid from a donor to an acceptor glycan [2]. However, for industrial production of food-grade HMOs, it is a drawback that the enzyme constitutes an important virulence factor within *T. cruzi* [3]. Redesigning mutants of the non-pathogenic *T. rangeli* sialidase (TrSA) that possesses relatively low trans-sialidase activity [4] provides an attractive alternative for application in bioconversion processes. TrSA is

TrSA, W313 (corresponds to W312 in TcTS) is found in a different conformation due to a Q284P substitution, while the Y120 (corresponds to Y119 in TcTS) is replaced by serine [8]. In addition to these differences in the acceptor binding site, a conserved D97 hydrogen bonds differently to sialic acid in the two enzymes, possibly due to the substitutions V96M and P98A. Correction of both the acceptor-binding site (S120Y, G249Y, and Q284P) and the sialic acid binding pocket (M96V, and A98P) is required to confer trans-sialidase activity (1% of TcTS activity) to TrSA, and the additional single mutations I37L (in this study named Tr6) and G342A further increase activity to 10% of the TcTS activity [9,4]. Kinetic data, however, indicate that the

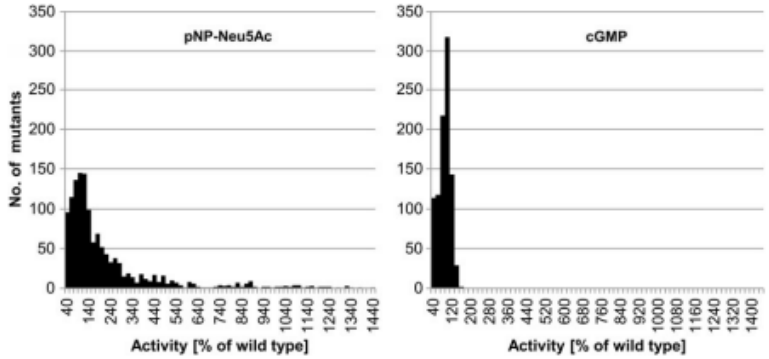
Compared to M. viridifaciens



Jers C, et al. Oxford jour. doi: 10.1093/protein/gzu054

No trans-sialidase activity observed…

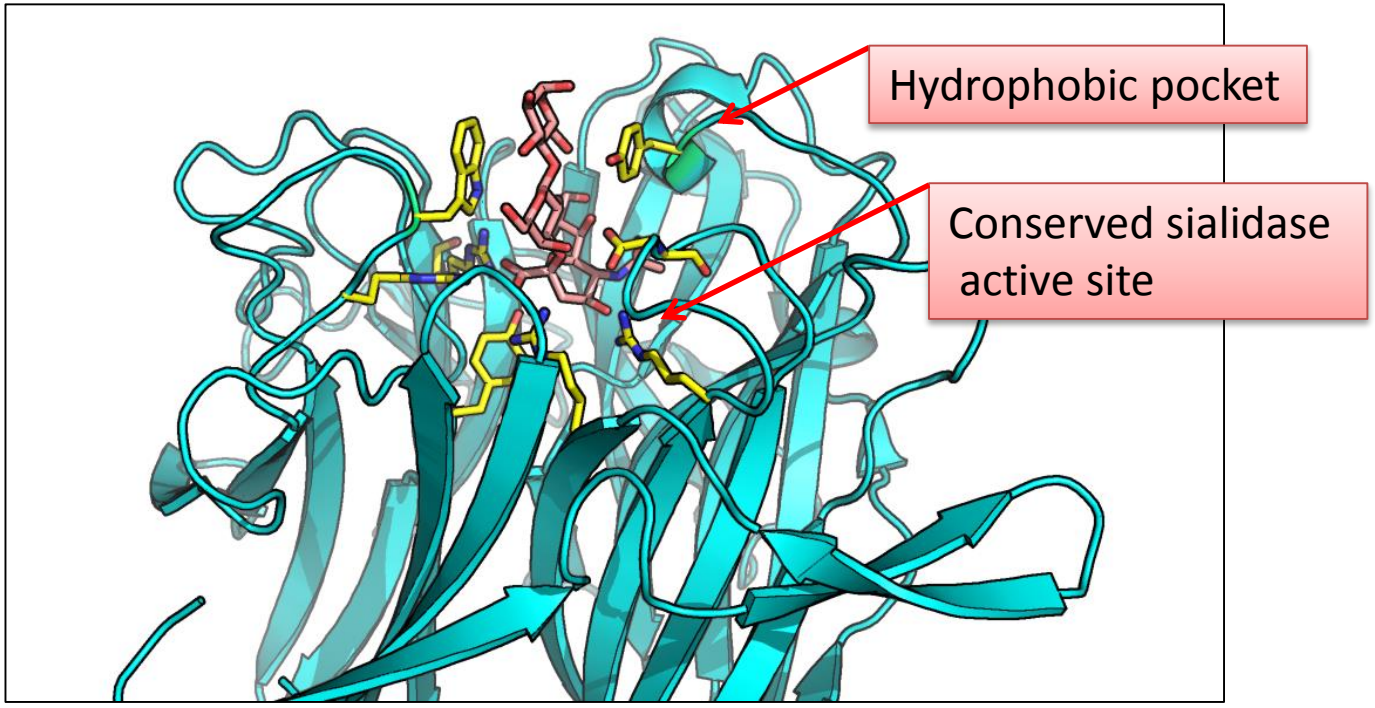
…From a pupulation of more than 200 constructed mutants



All avancements based on TcTS

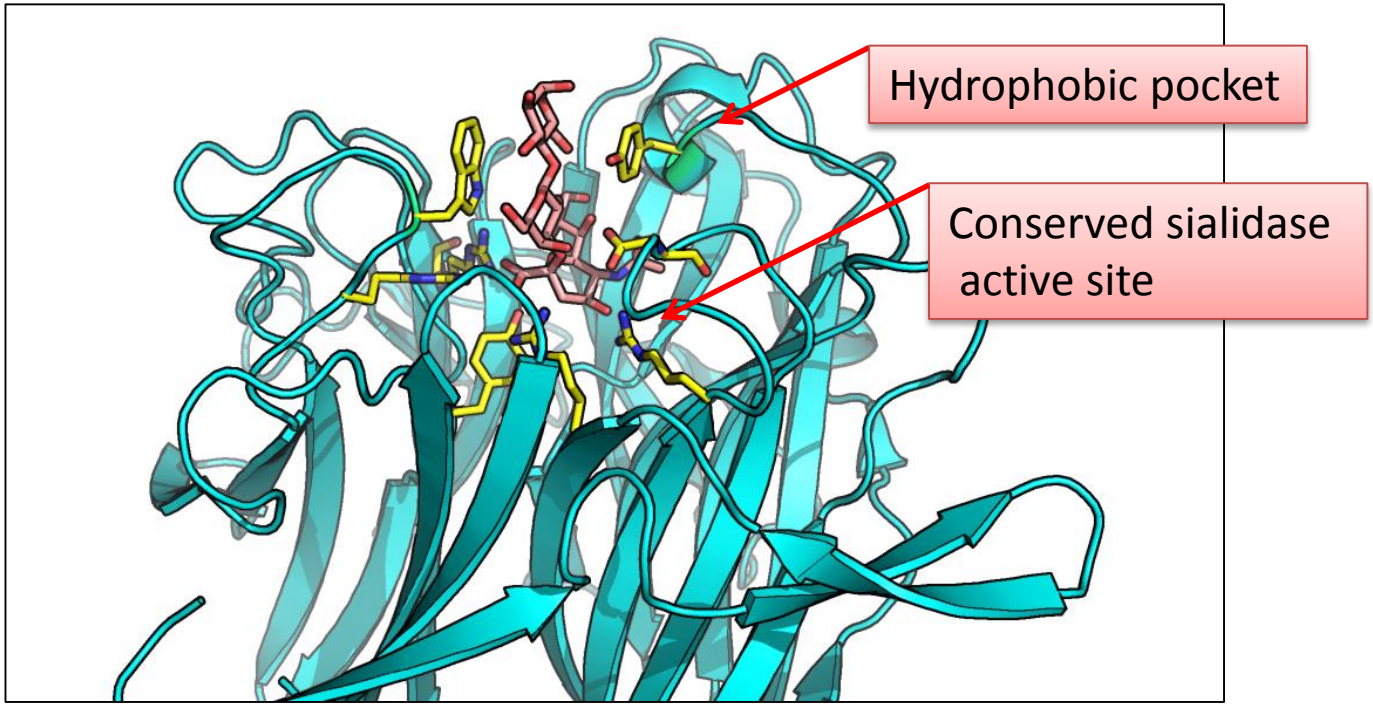
- New knowledge about trans-sialidases needed.
- No trans-sialyllation screening assays.
- Hard to find conserved AA when TcTS is the only trans-sialidase.
- What to do?

Reaquaint with TcTS



TcTS model based on Amaya M.F., et.al. (2004) Structure 12: 775–784

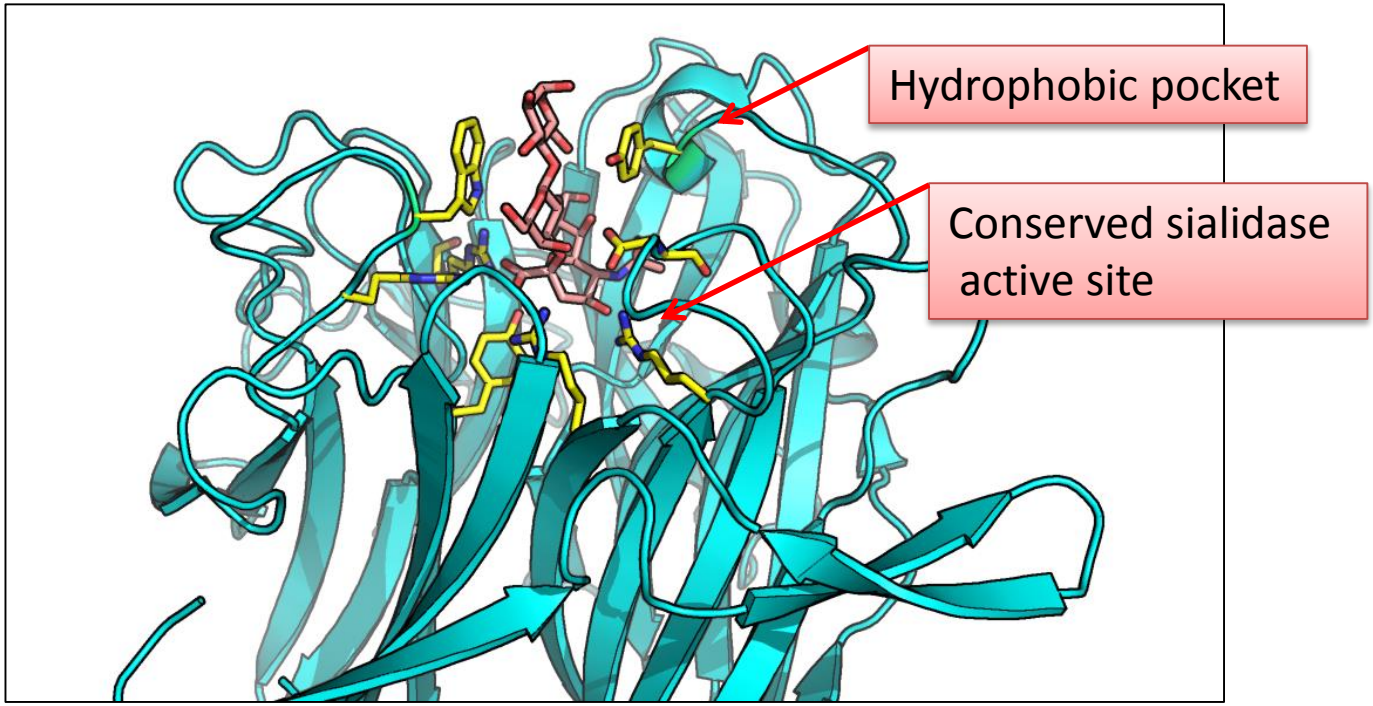
Enzyme identification strategy development



TcTS model based on Amaya M.F., et.al. (2004) Structure 12: 775–784

Hypothesis 1: The family of sialidases is extremely conserved and it can be speculated that a trans-sialidase could have developed in parallel with the TcTS inferring an aromatic sandwich in a similar position as it is found in TcTS.

Enzyme identification strategy development

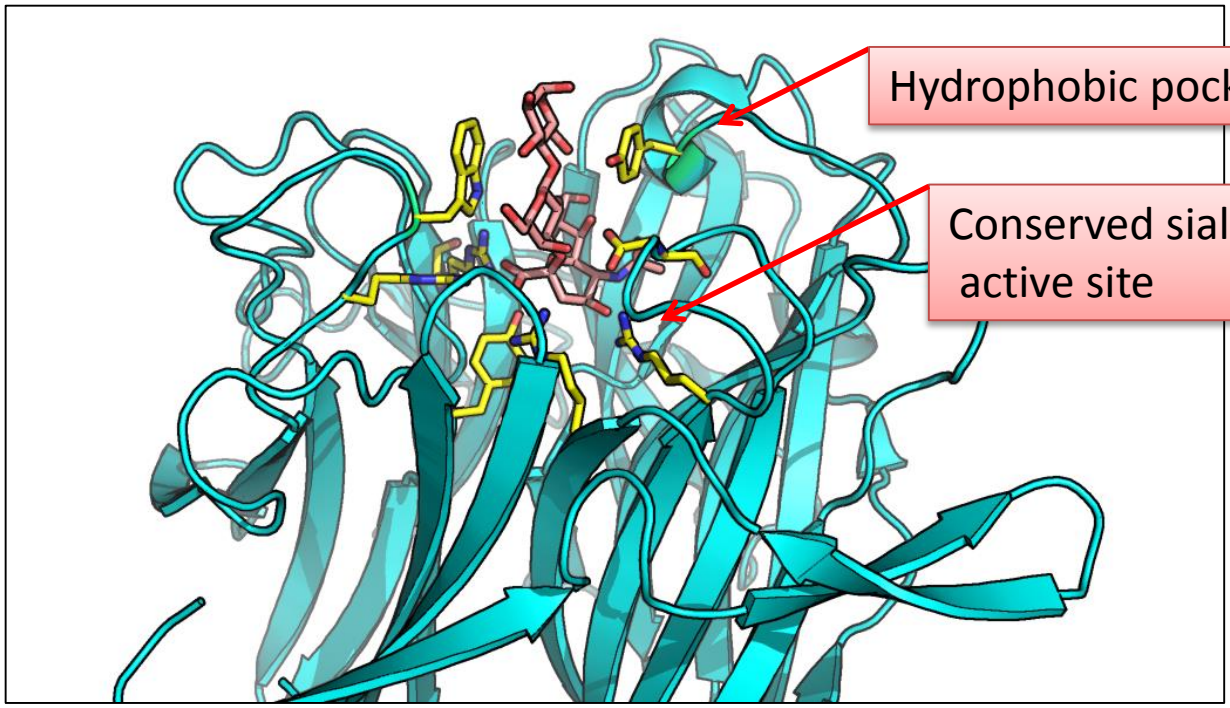


TcTS model based on Amaya M.F., et.al. (2004) Structure 12: 775–784

Hypothesis 2: If hypothesis 1 is correct, sialidases with an aromatic sandwich above the active site
will be a good candidates for identifying sialidases with trans-activity.

Enzyme identification strategy development

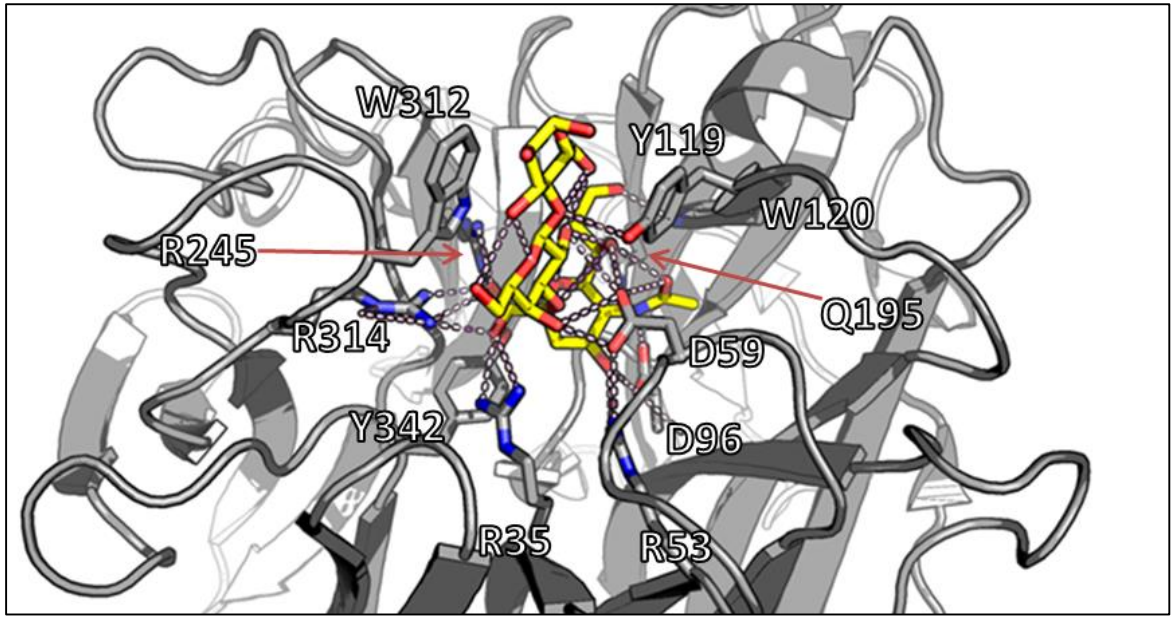
2909 Genes are labeled as Sialidases in GenBank



TcTS model based on Amaya M.F., et.al. (2004) Structure 12: 775–784

Search for motif: [W/Y/F-x-R-D-R]

A closer look at the TcTS active site

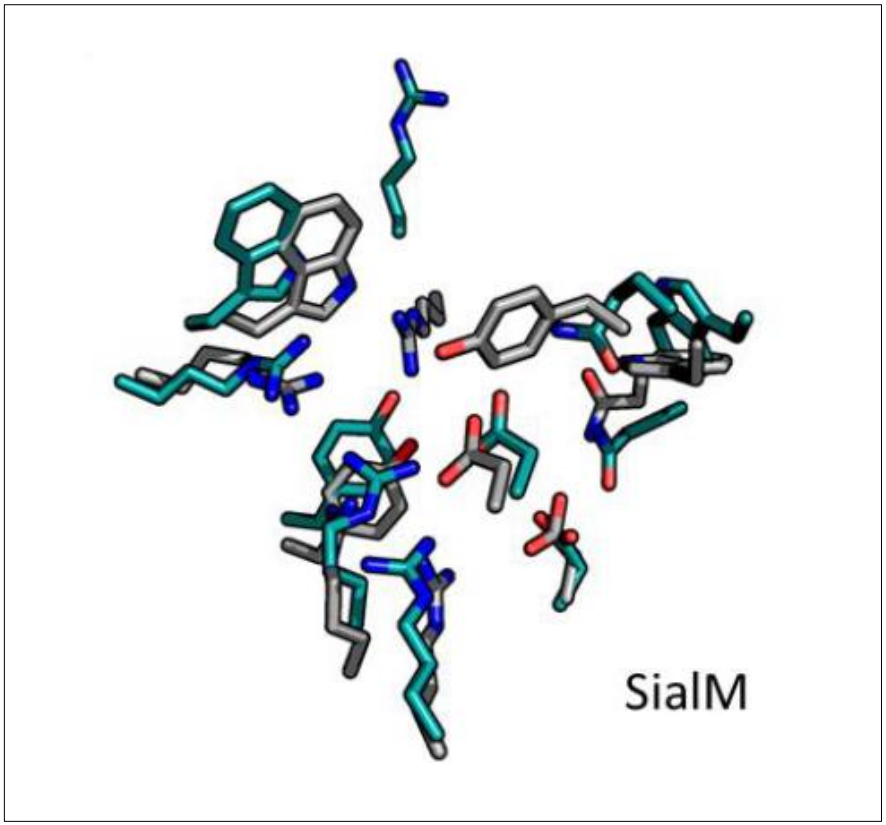


TcTS model based on Amaya M.F., et.al. (2004) Structure 12: 775–784

TcTS properties	Highlighted residues	Role of residues	Hydrogen bonds (to substrate)	Hydrophobic interactions
Sialic acid moiety fixation	Arg35			-
	Arg245	Carboxylate fixation	SA_O1A, SA_ O1B	-
	Arg314			-
	Asp96	Acetamide fixation	SA_N5	-
	Trp120	Glycerol fixation	SA_O9	-
	Gln195		SA_O8, SA_O9	-
	Arg53	Ring fixation	SA_O4	-
Catalysis	Asp59	Acid/base catalyst	SA_O2	-
	Tyr342	Enzymatic nucleophile	(covalent binding to C2)	-
Lactose moiety fixation	Tyr119	Aromatic sandwich	-	+
	Trp312		-	+

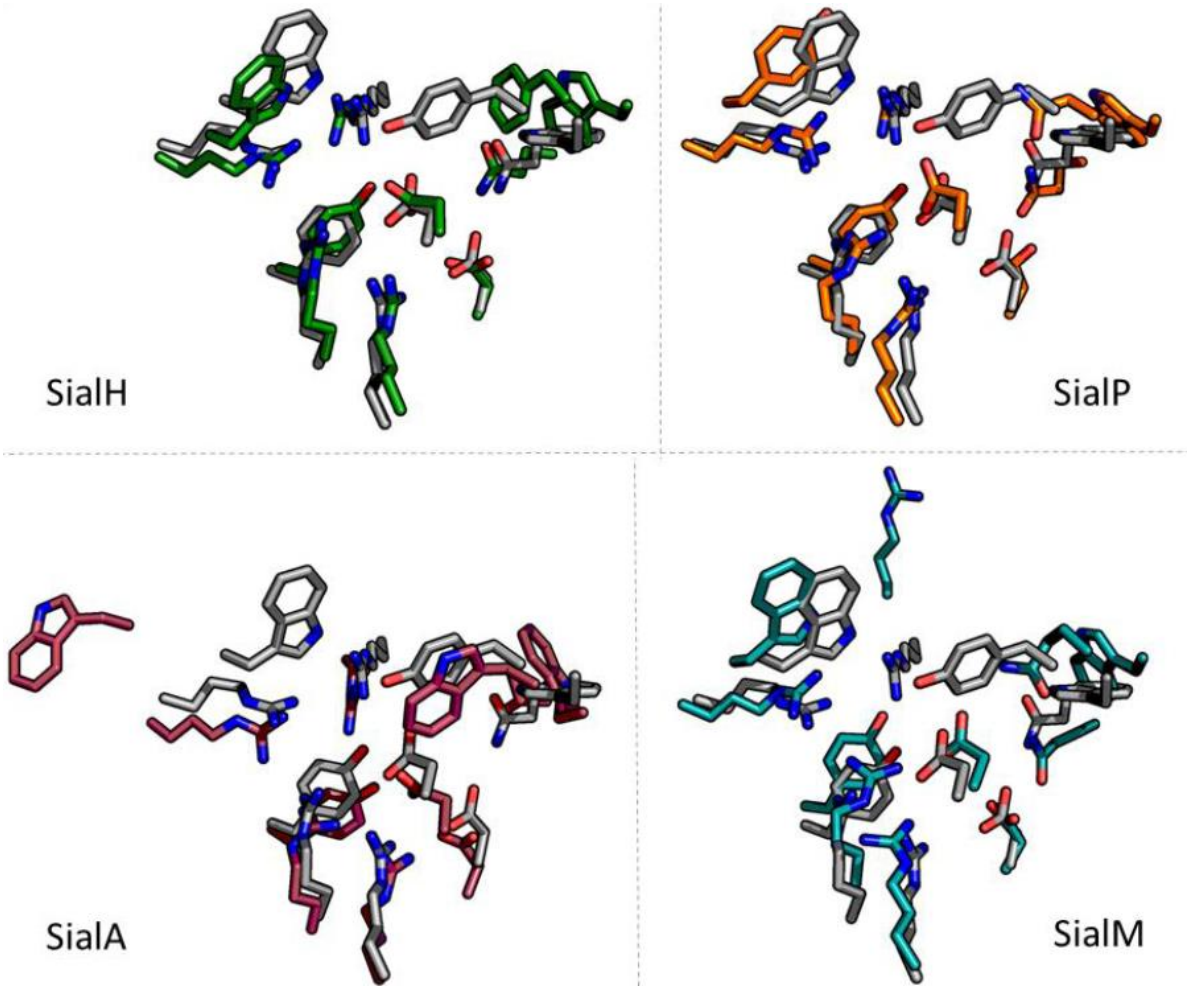
Candidate selection

- 3D homology moddeling using HHpred and CPHmodels
- Alignment of active site residues
- Visual inspection

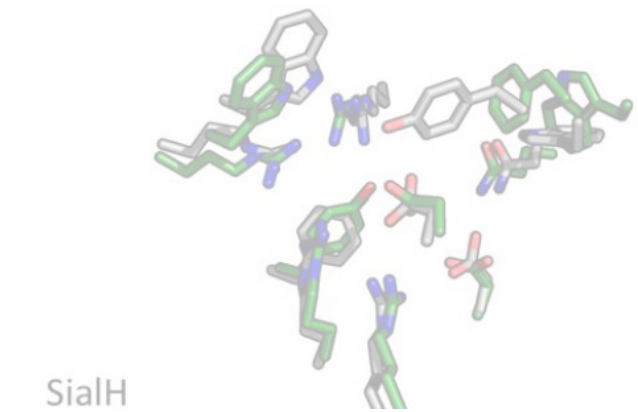


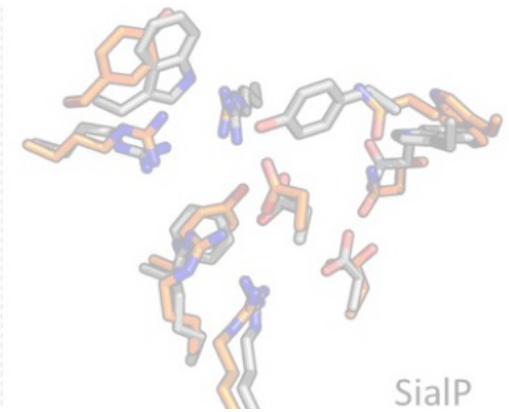
Aligned active site residues for 1 of 15 candidate enzymes

4 candidates enzymes model and align well

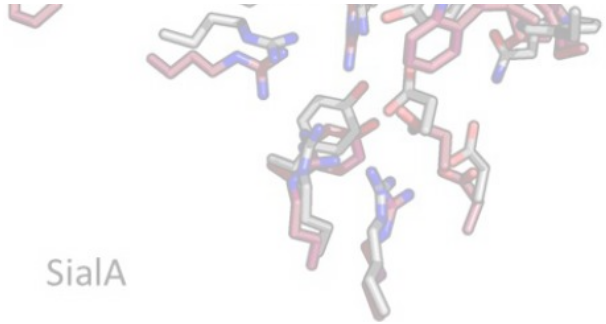


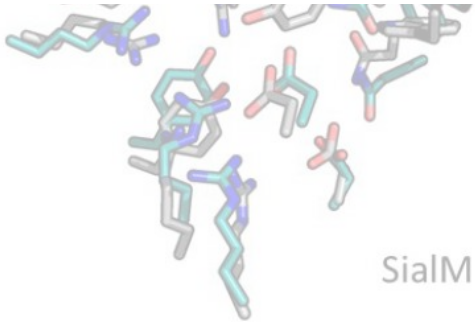
4 candidate enzymes selected for in vitro analysis





Candidate	Organism name	Uniprot ID	Submitted Name	Mw	Length
SialA	*Actinomyce Oris*	Q44562	NanH Sialidase	73,089	685 AA
SialH	*Haemophilus parasuis*	I7AJQ9	NanH Sialidase	89,948	803 AA
SialM	*Manheimia Heamolytica*	E2NYK4	NanH Sialidase	89,245	795 AA
SialP	*Pasteurella Multosida*	I1VL53	NanH Sialidase	89,217	798 AA





Candidate alignment overview

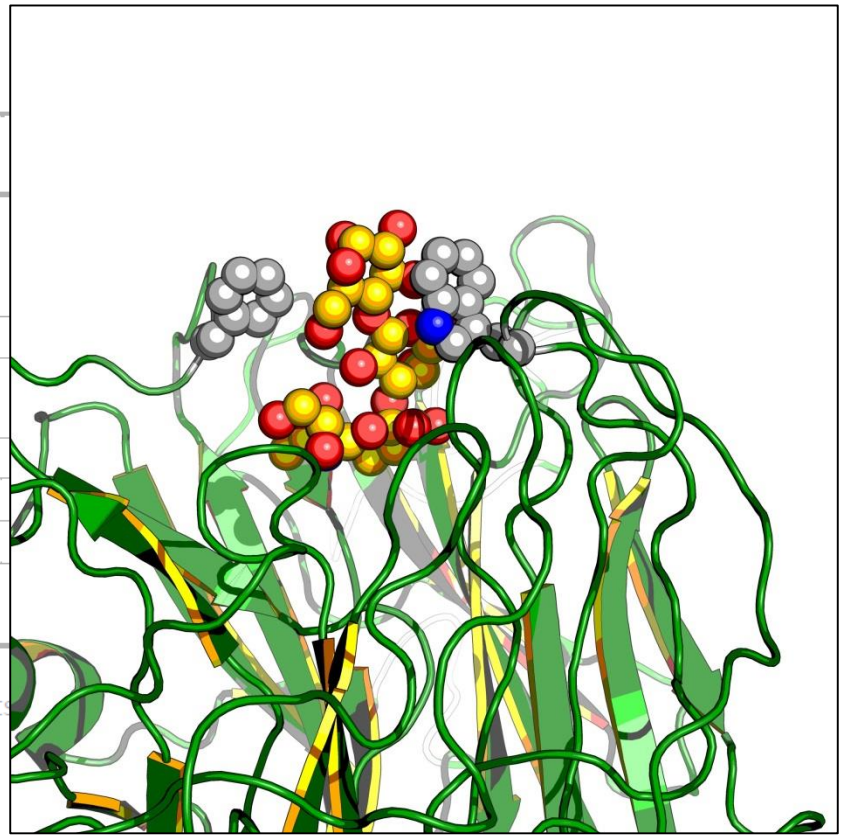
TcTS AC properties	TcTS	SialH	SialP	SialA	SialM
Sialic acid moiety fixation	R35	R80	R73	R311	R79
	R245	R298	R291	R524	G296 (R297)[1]
	R314	R368	R359	R592	R360
	D96	D143	D136	D379	D142
	W120	W169	W162	W403	W168
	Q195	Q245	Q238	T473 (Q472)[1]	Q243
	R53	R99	R92	R330	R98
Catalysis	D59	D105	D98	D340	D104
	Y342	Y402	Y393	Y620	Y394
Lactose moiety fixation	Y119	F168[3]	N161[2]	G404 (W403)[1]	Q167[2]
	W312	W366	Y357[3]	W590	W358

[1] Modelling error suspected - alternative alignment in brackets, [2] Non-aromatic residue, [3] Alternative aromatic residue

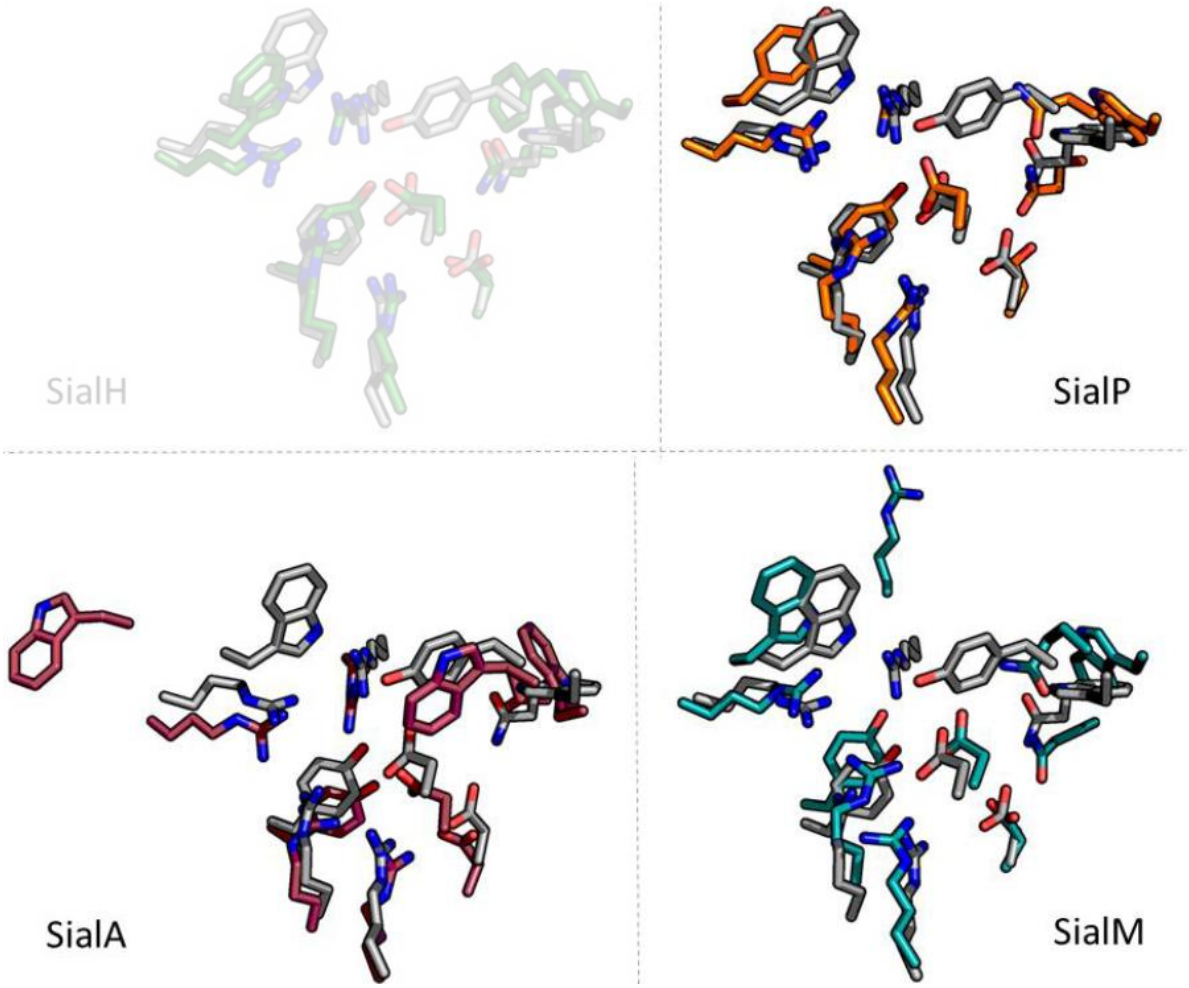
One candidate stands out

TcTS AC properties	TcTS	SialH
Sialic acid moiety fixation	R35	R80
	R245	R298
	R314	R368
	D96	D143
	W120	W169
	Q195	Q245
	R53	R99
Catalysis	D59	D105
	Y342	Y402
Lactose moiety fixation	Y119	F168[3]
	W312	W366

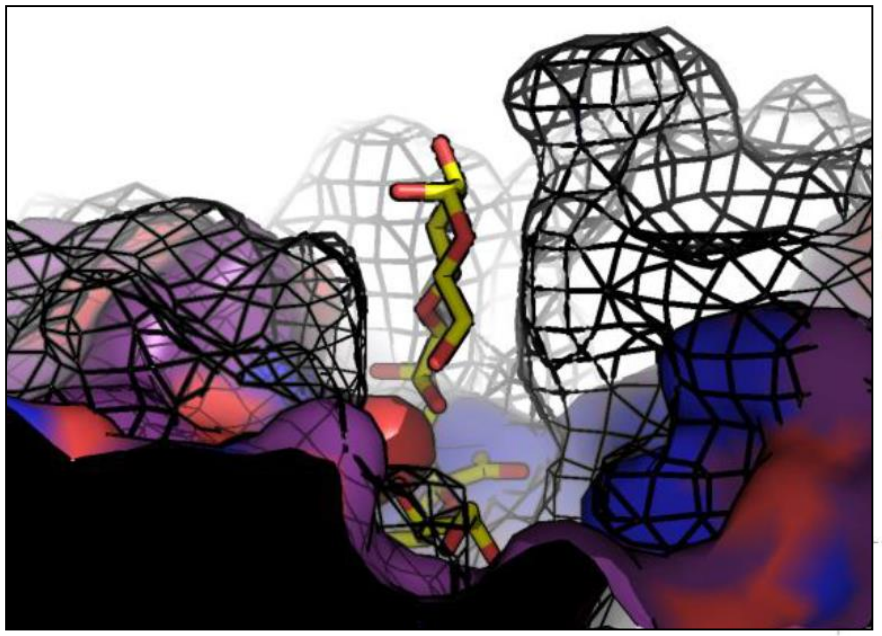
[1] Modelling error suspected - alternative alignment in brackets

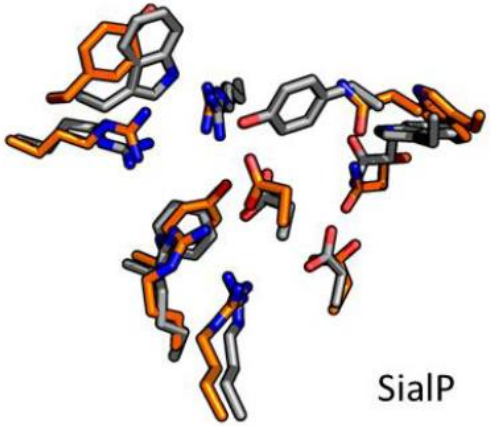


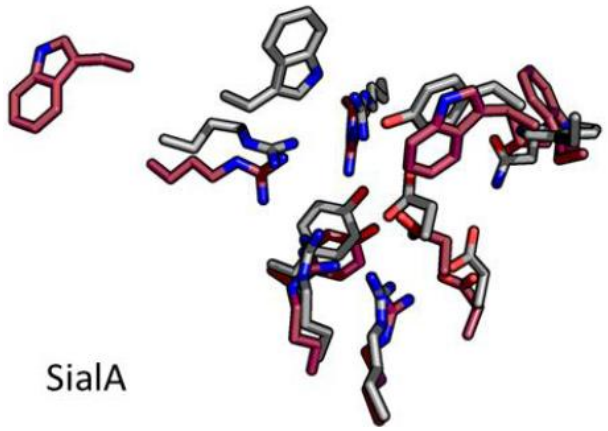
The remaining 3 candidates included
- Deep narrow binding pockets

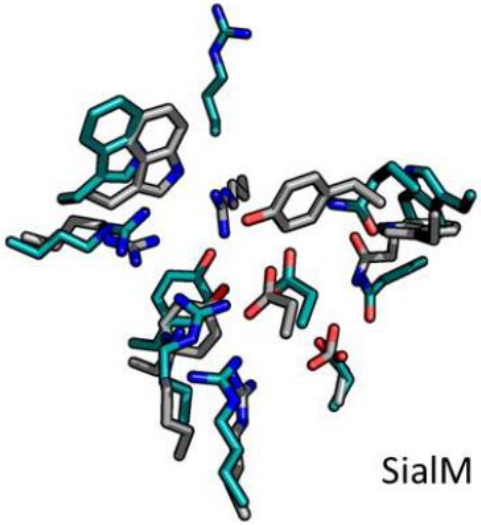


The remaining 3 candidates included
- Deep narrow binding pockets









Enzyme expression setup

- Gene sequences synthesized (DNA2.0)
  - With N-terminal His-taq
  - T7 promotor expression system vector
- Transformed into *E. coli* BL2 (DE3)
- Overnight 30º C autoinduction expression
- Enzyme recovery
  - Sonication
  - Äkta purification

Applying the candidate enzymes to the trans-sialidase setup

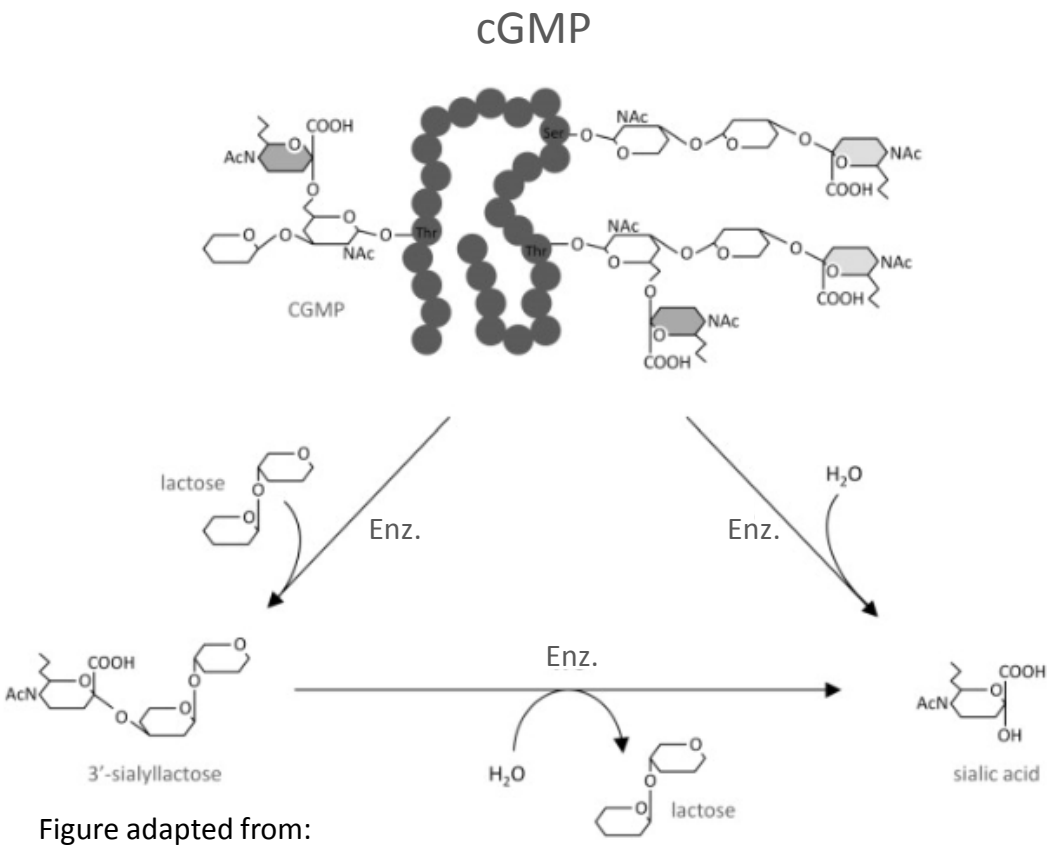


Figure adapted from:
Zeuner B, *et al.* 2014 Enzyme and Microbial Technology 55:85-93

Reaction conditions

Volume:	1ml (Eppendorf tubes)
Lactose:	100 g/L
cGMP:	40 g/L
Temperature:	30^{O}C (Thermo mixer)
pH:	6.4
Mixing:	700 rpm (Thermo mixer)
Reaction stop:	Heat inactivation 10 min.
Reaction time:	2 hours

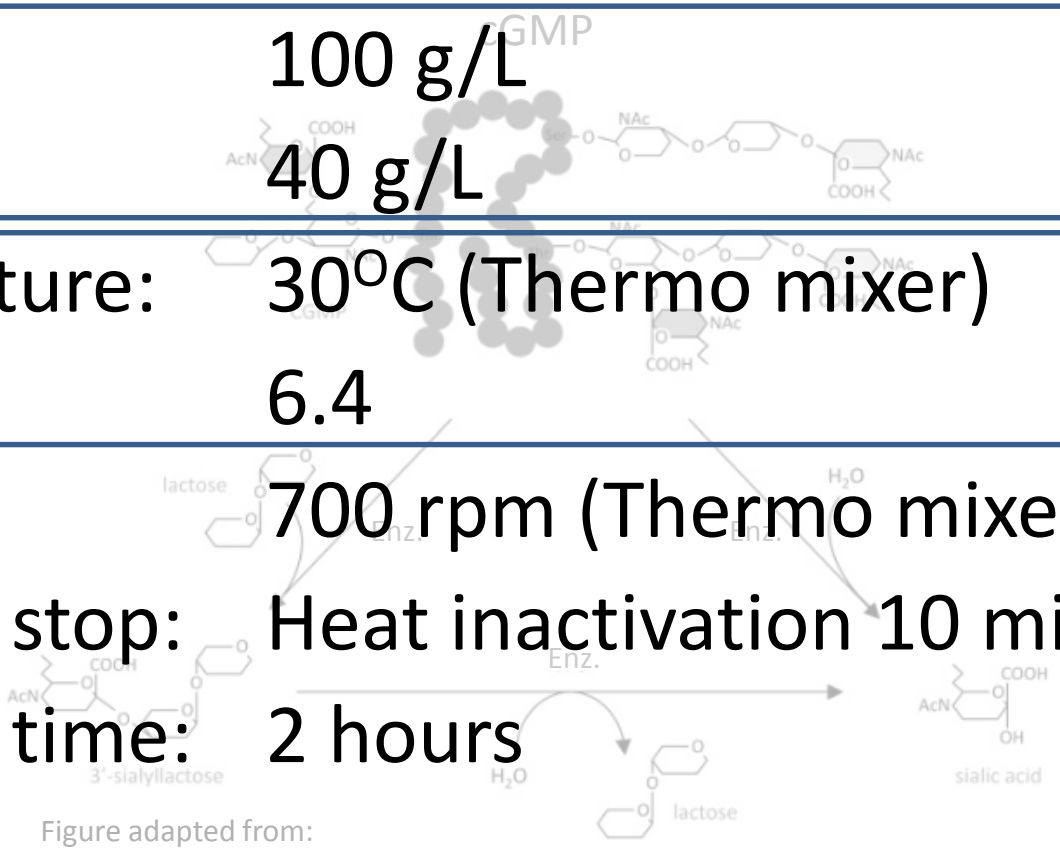
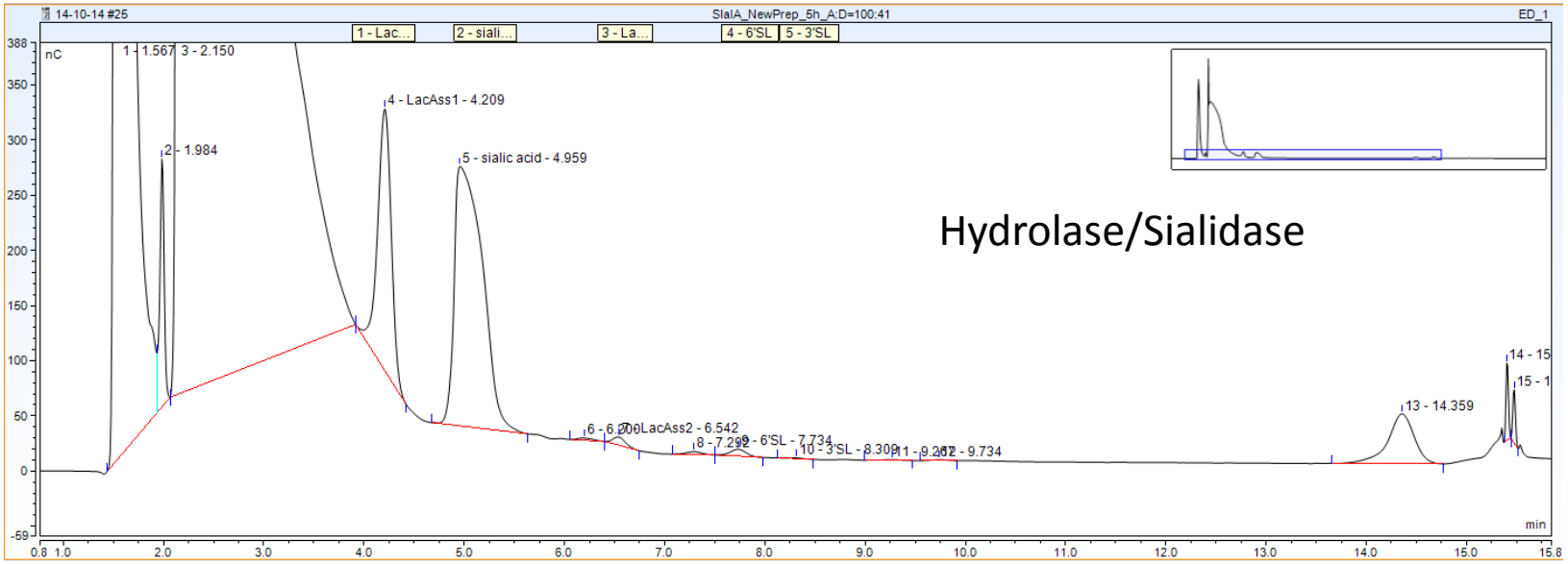


Figure adapted from:
Zeuner B, *et al.* 2014 Enzyme and Microbial Technology 55:85-93

HPLC - Analysis

Sial A

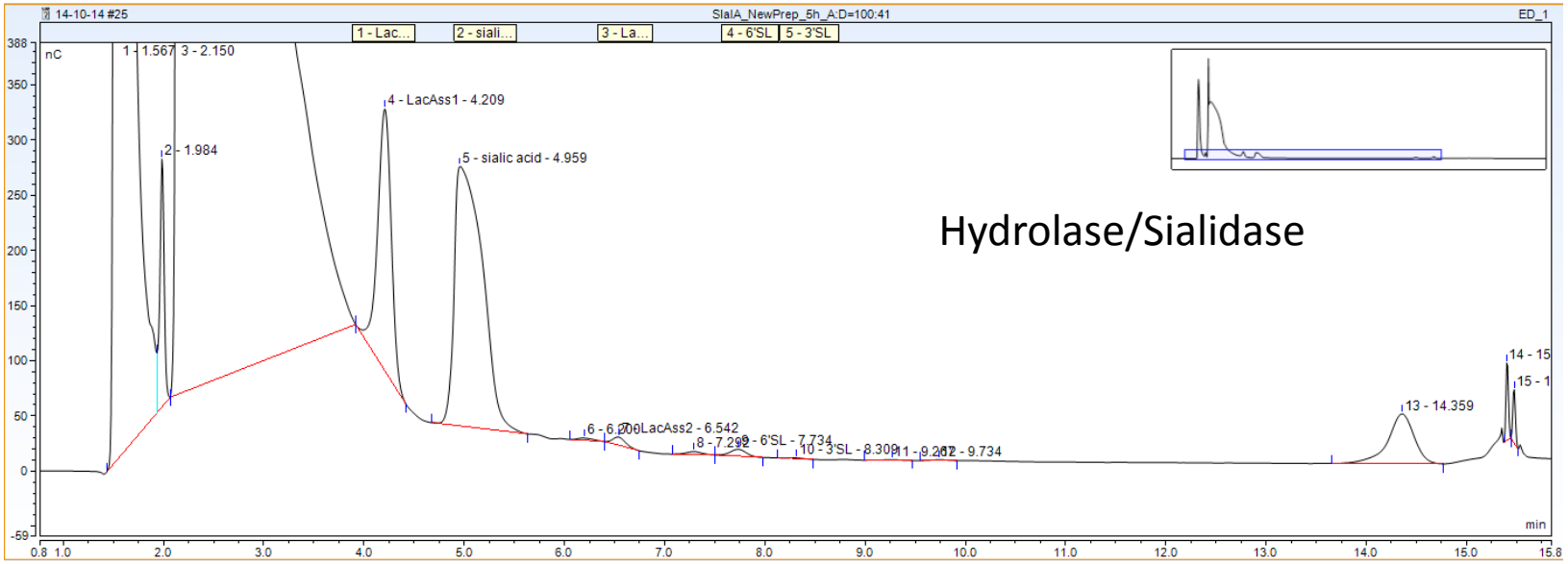


Type: HPAEC-PAD
Column: CarboPac™ PA100
Machine: ICS 3000

(Dionex Corp., Sunnyvale, CA)

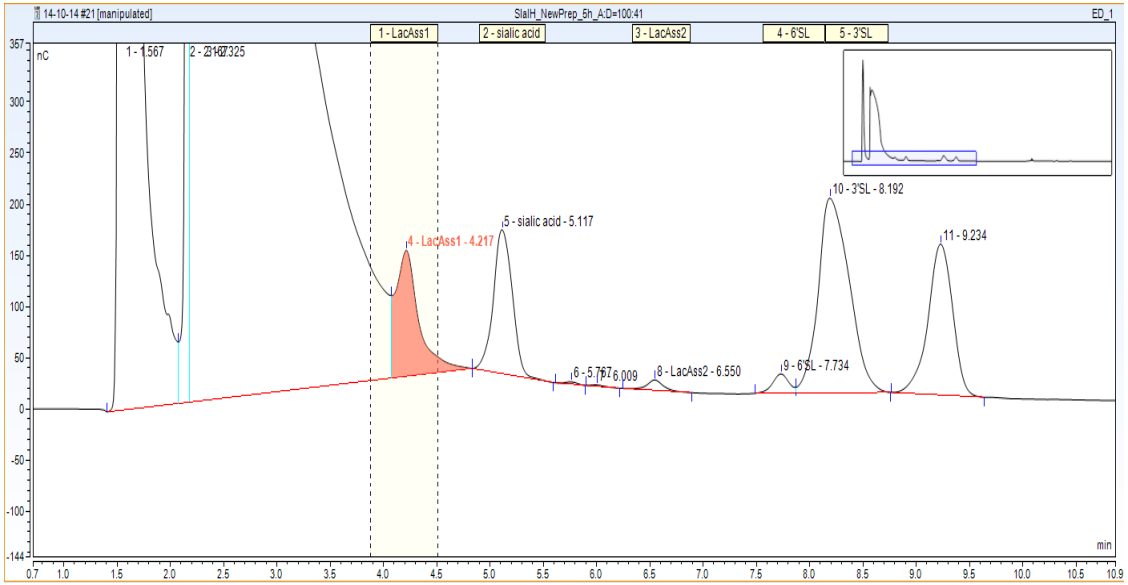
HPLC - Analysis

Sial A



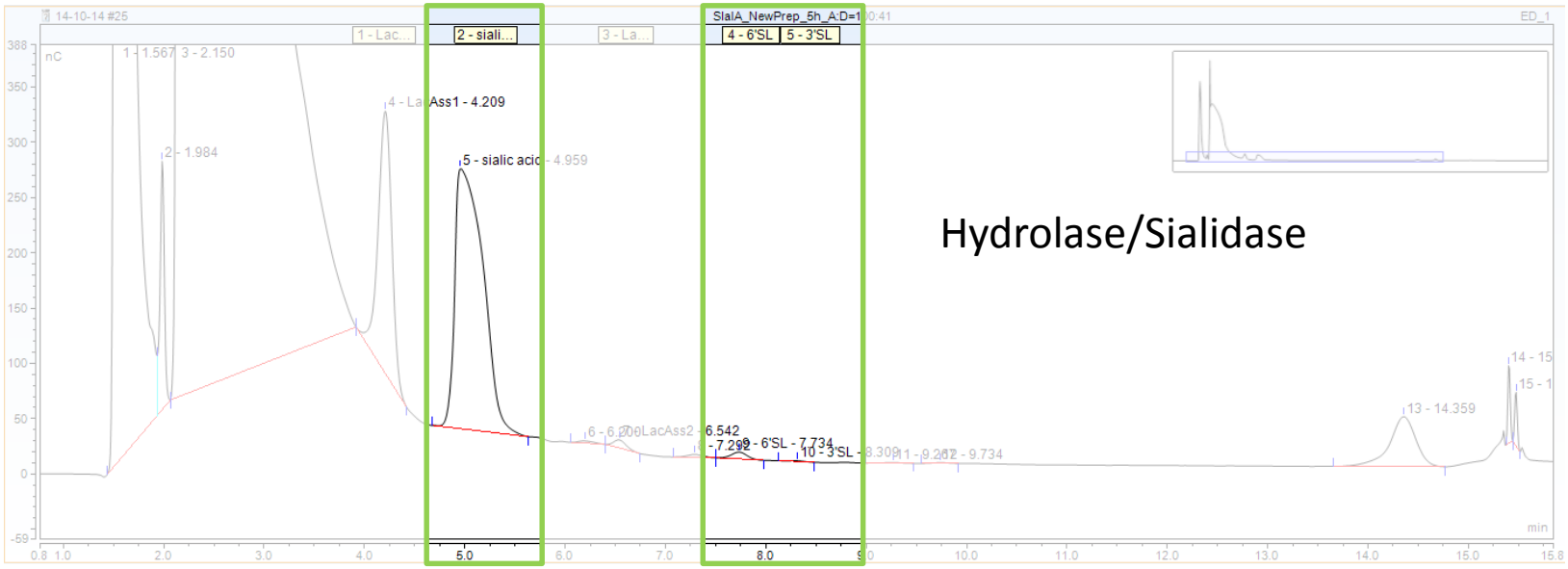
Hydrolase/Sialidase

Sial H



HPLC - Analysis

Sial A

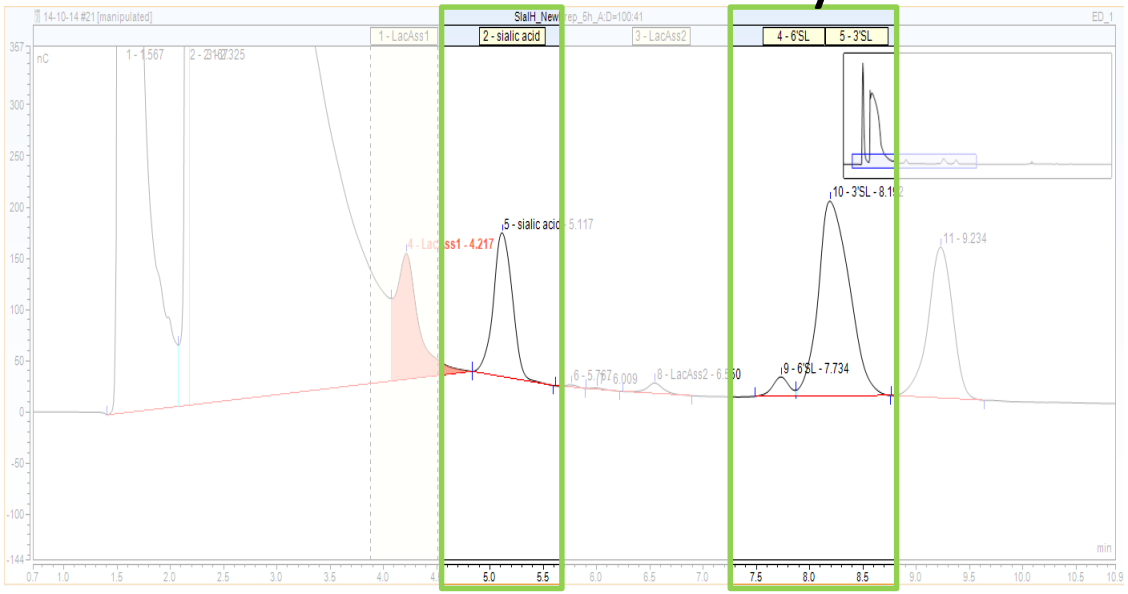


Hydrolase/Sialidase

Sialic acid

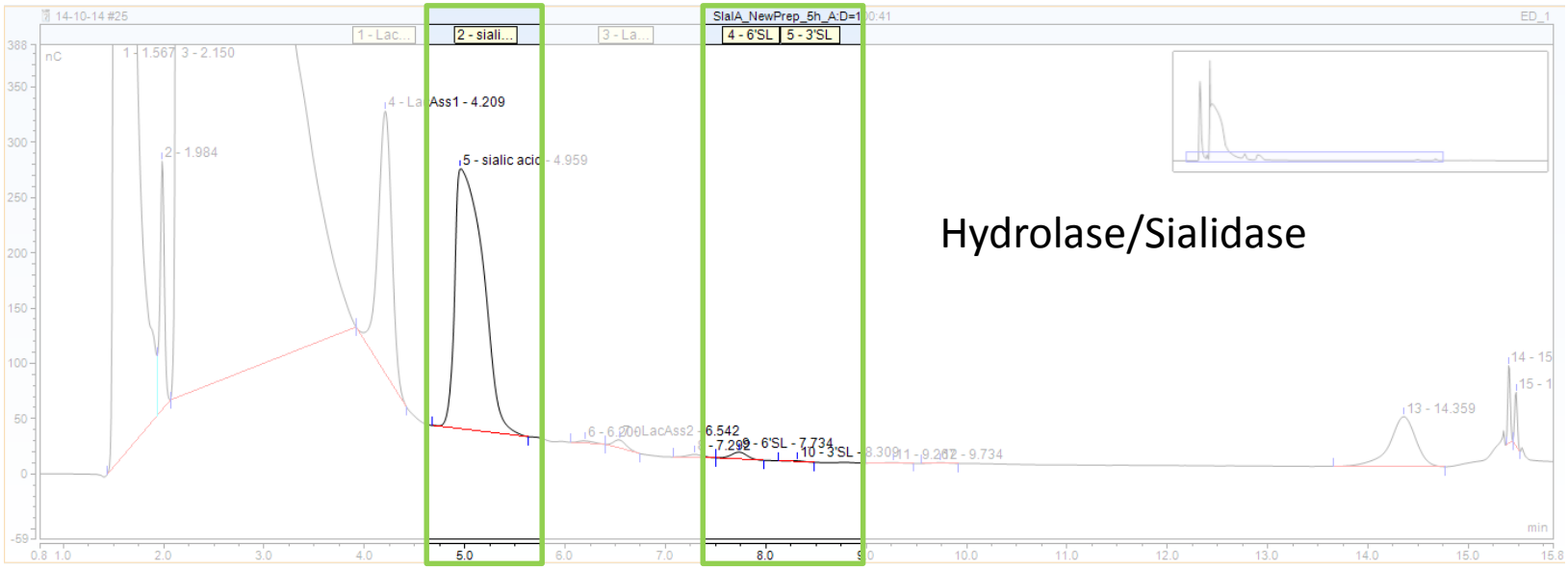
Sialyllactose

Sial H



HPLC - Analysis

Sial A

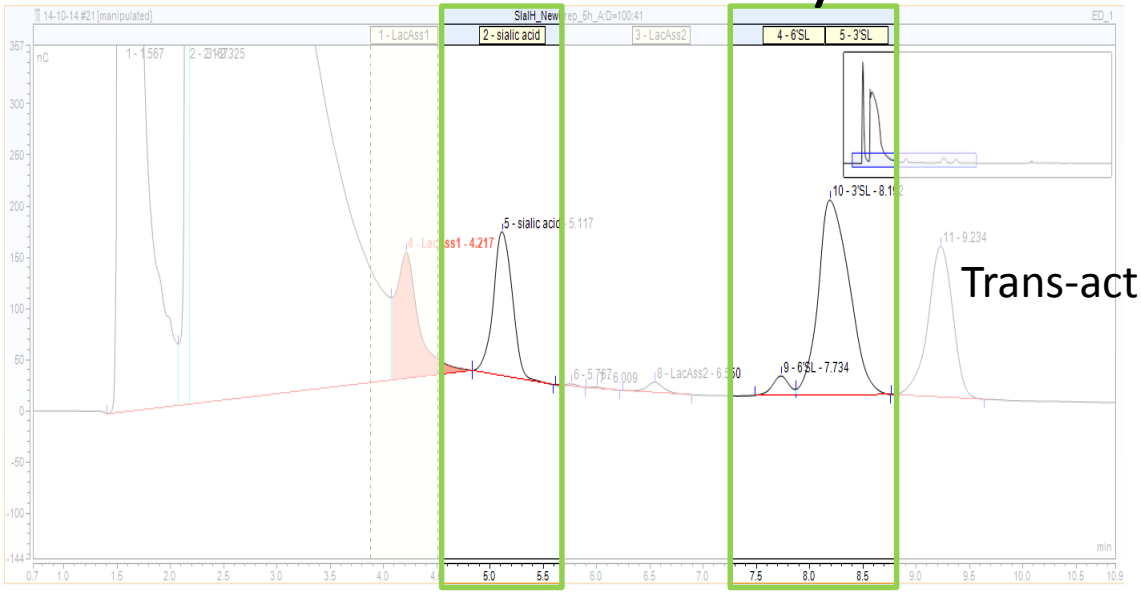


Hydrolase/Sialidase

Sialic acid

Sialyllactose

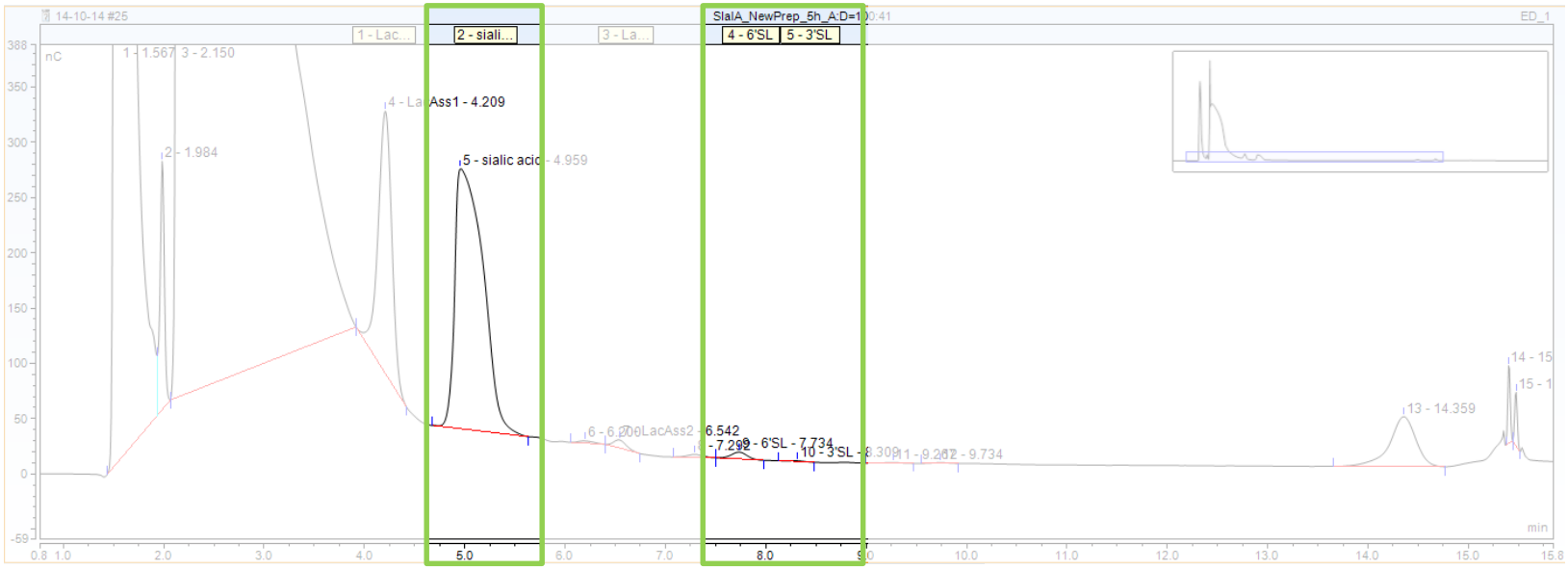
Sial H



Trans-activity!!!

HPLC - Analysis

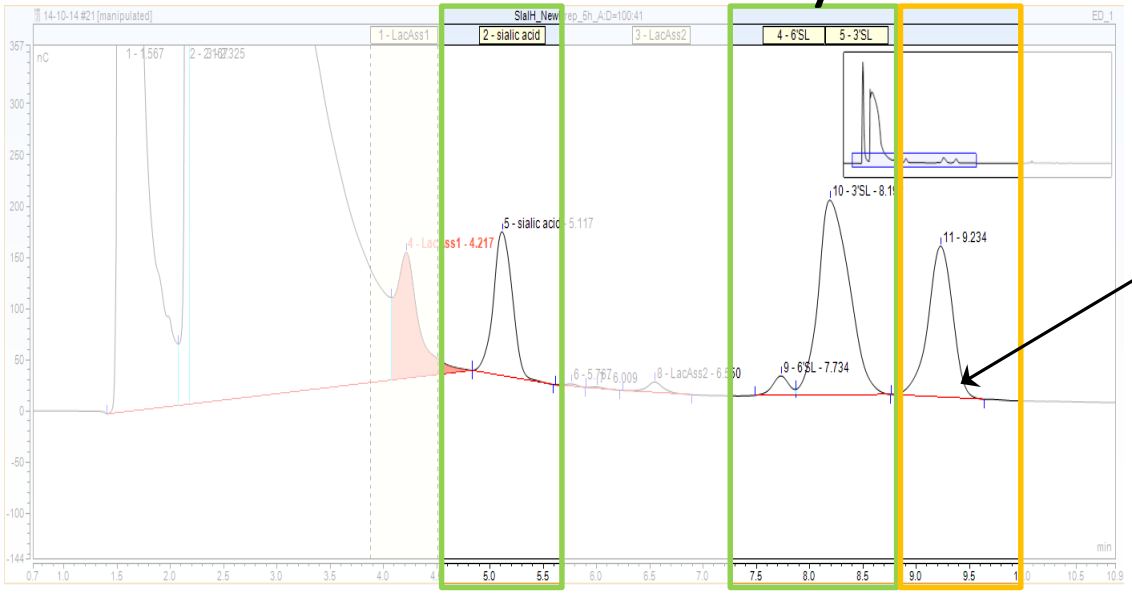
Sial A



Sialic acid

Sialyllactose

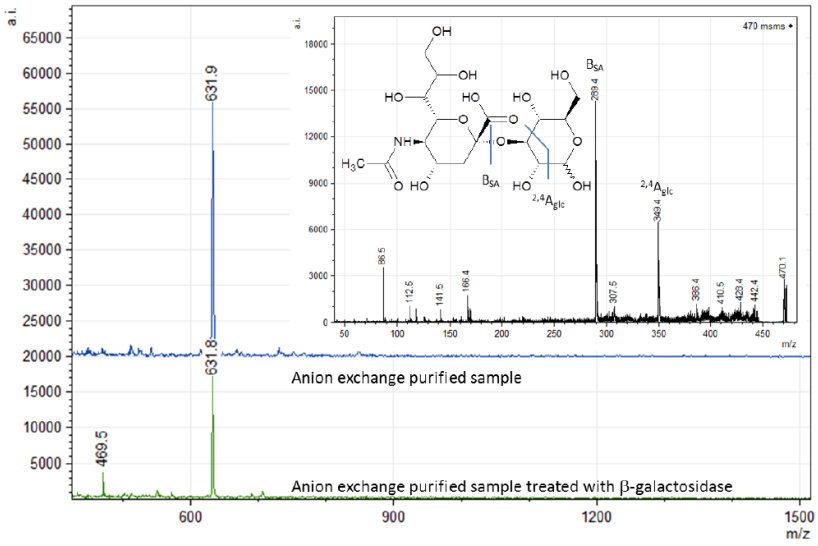
Sial H

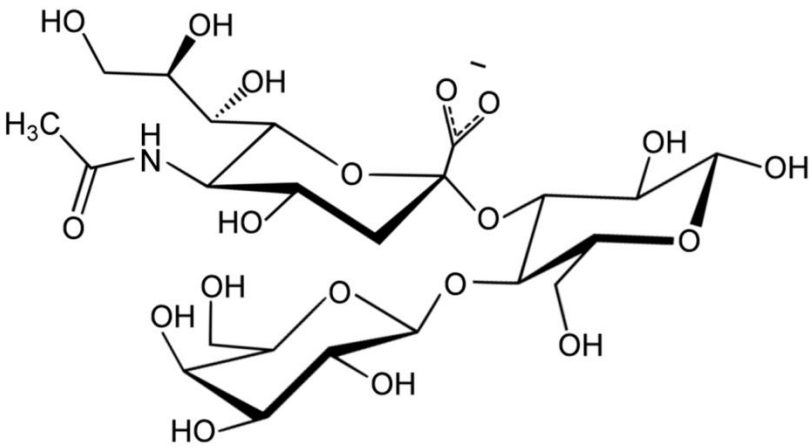


Mystery peak 2

Product identification

MS and β-galactosidase treatment

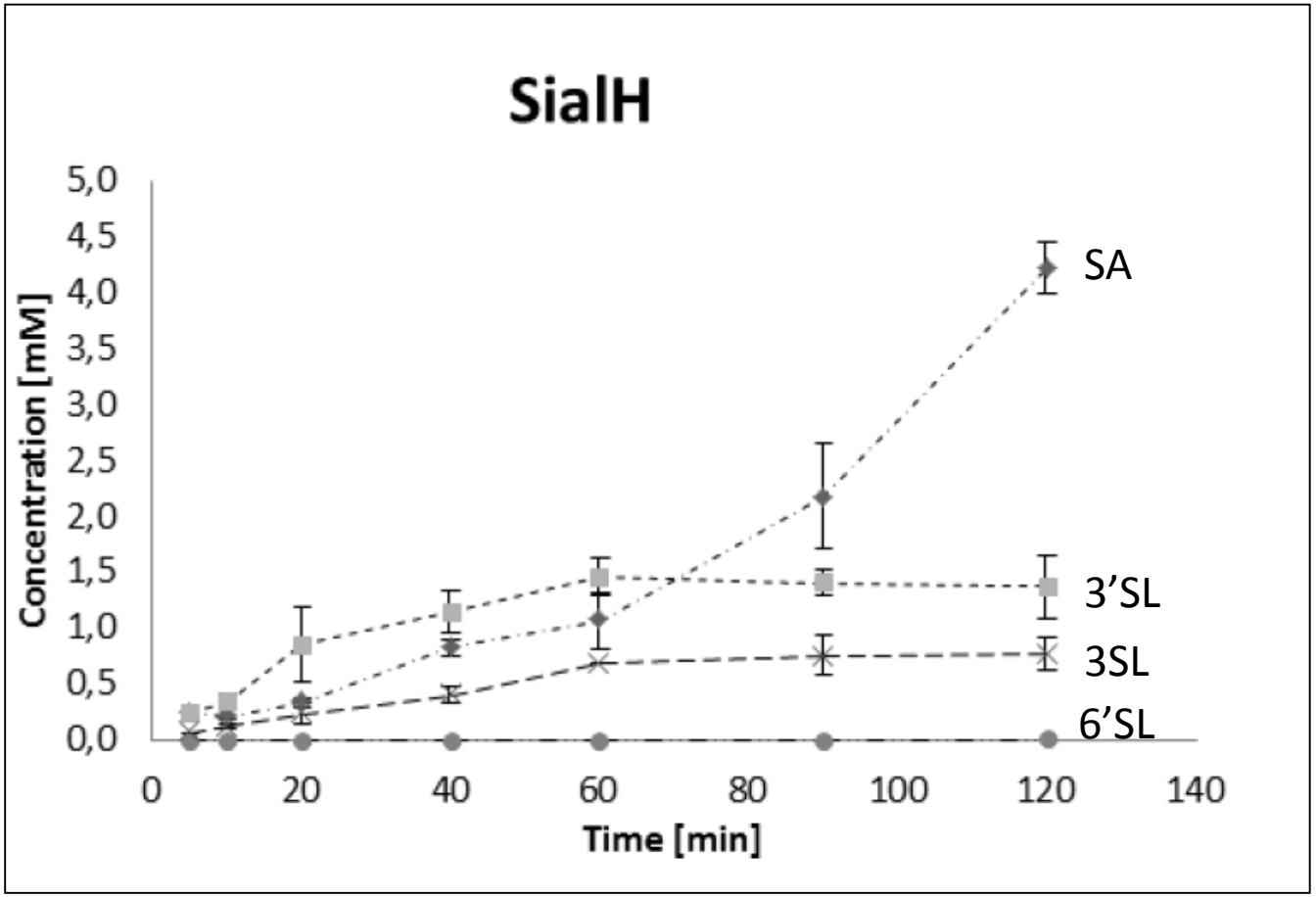




3-sialyllactose

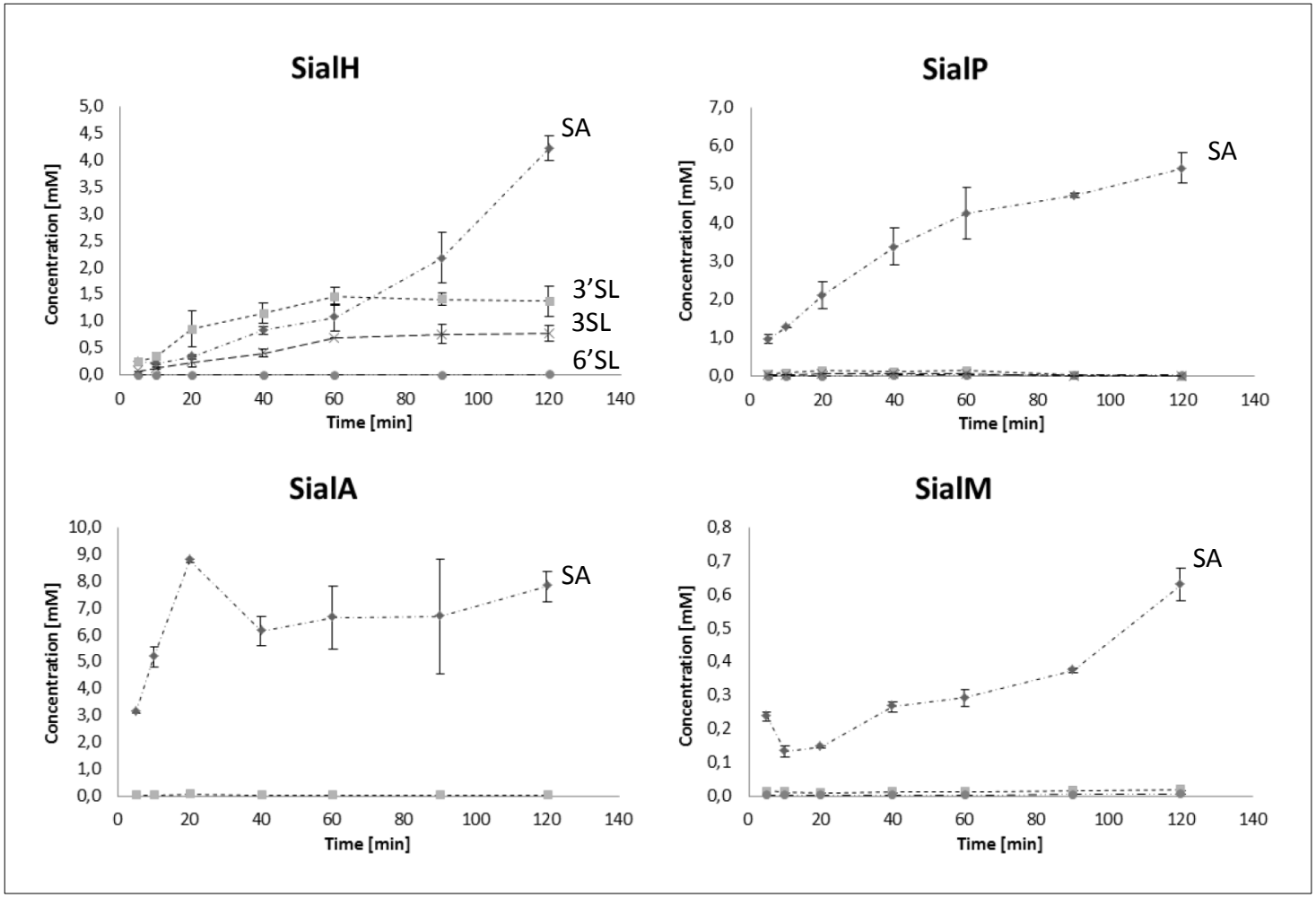
Confirmed by NMR

Time study SialH

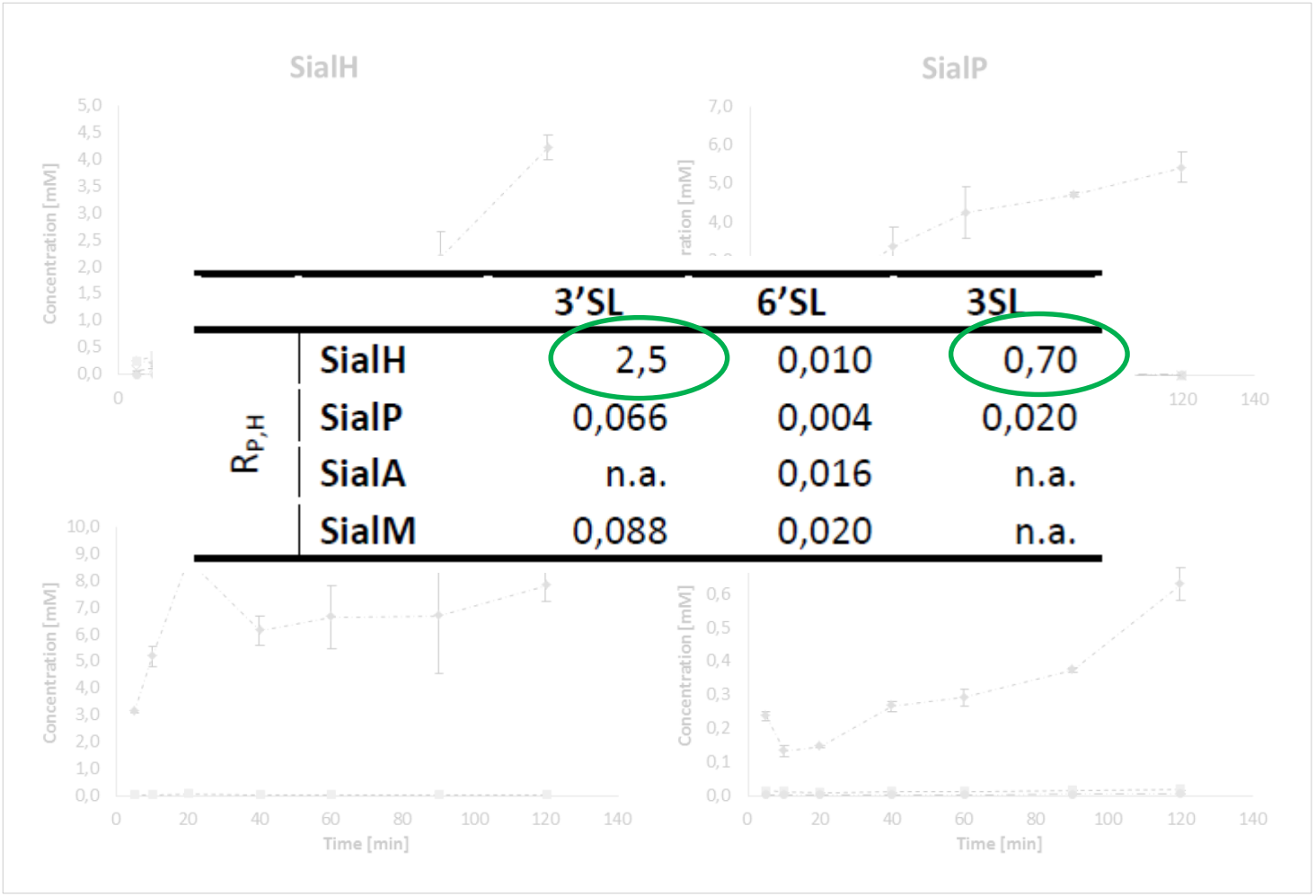


Products 3'SL and 3SL reaches levels of 1.5 mM and 0.9 mM respectively

Time study (all candidates)



Time Study (all candidates)



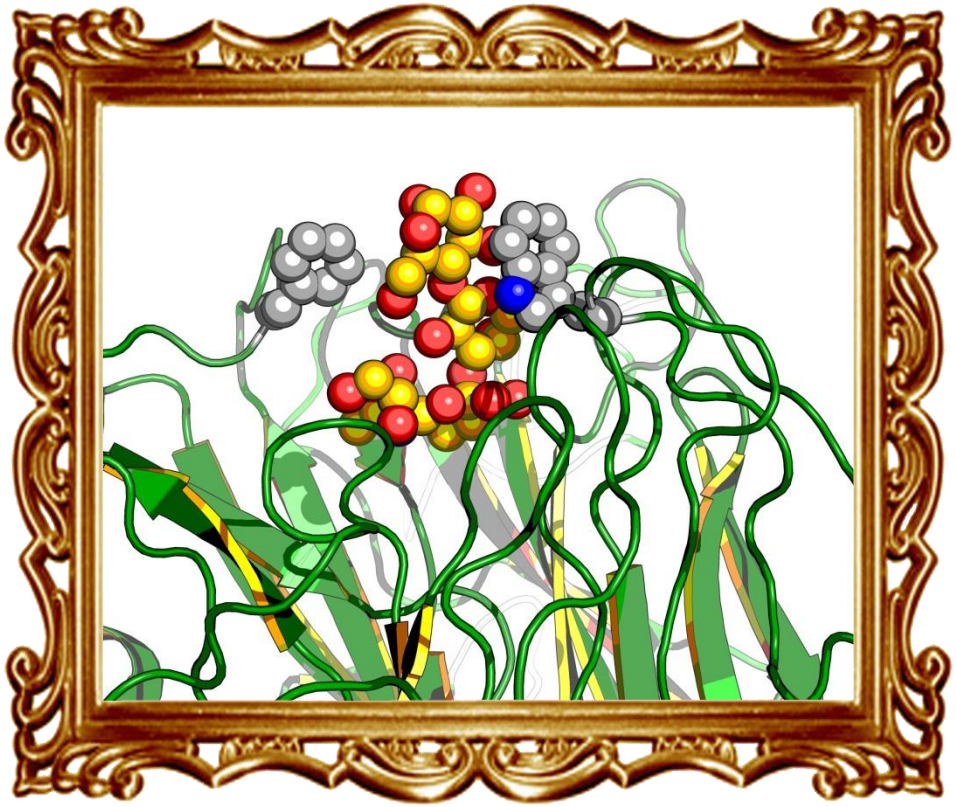
		3'SL	6'SL	3SL
$R_{P,H}$	SialH	2,5	0,010	0,70
	SialP	0,066	0,004	0,020
	SialA	n.a.	0,016	n.a.
	SialM	0,088	0,020	n.a.

Conclusion

”We give to you”

Conclusion

The trans-sialidase of Haemophilus parasuis (HpTS)



Conclusion

Novel trans-sialidase identified

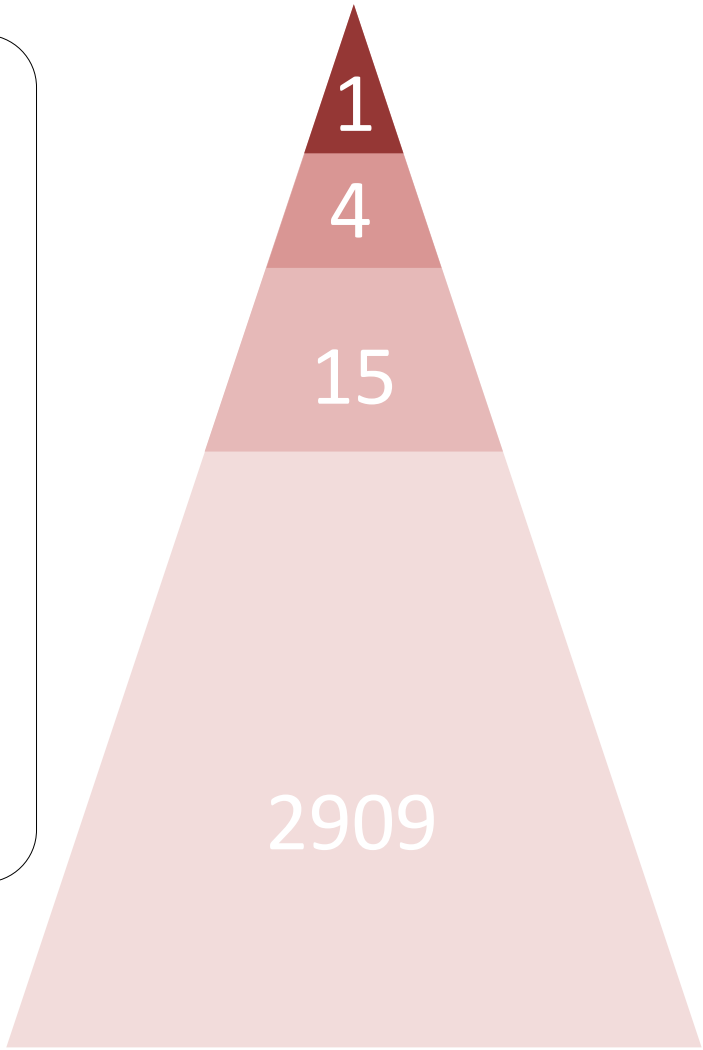
- From a database of 2909 sialidases
- Identified by rational sequence analysis strategy
- First confirmed bacterial trans-sialidase
- Trans-sialidase of H. parasuis

Novel sialylation product identified

- New type of 3-sialyllactose

4 candidates showed trans-activity

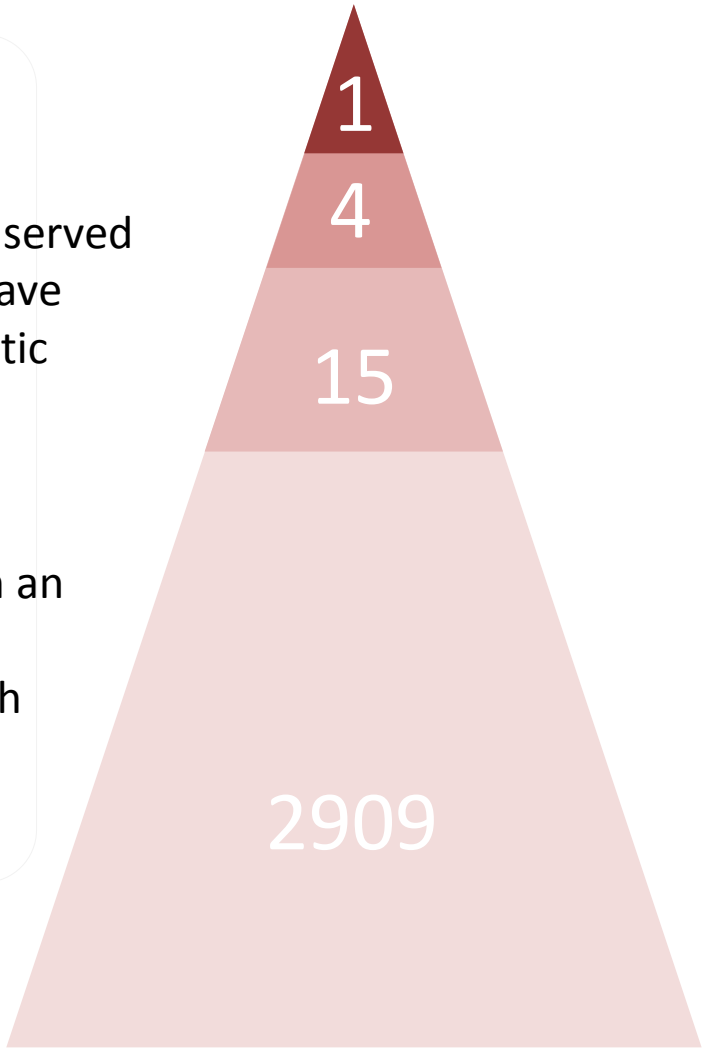
- Although higher hydrolysis rates were observed



Conclusion

Hypothesis 1: The family of sialidases is extremely conserved and it can be speculated that a trans-sialidase could have developed in parallel with the TcTS inferring an aromatic sandwich in a similar position as it is found in TcTS.

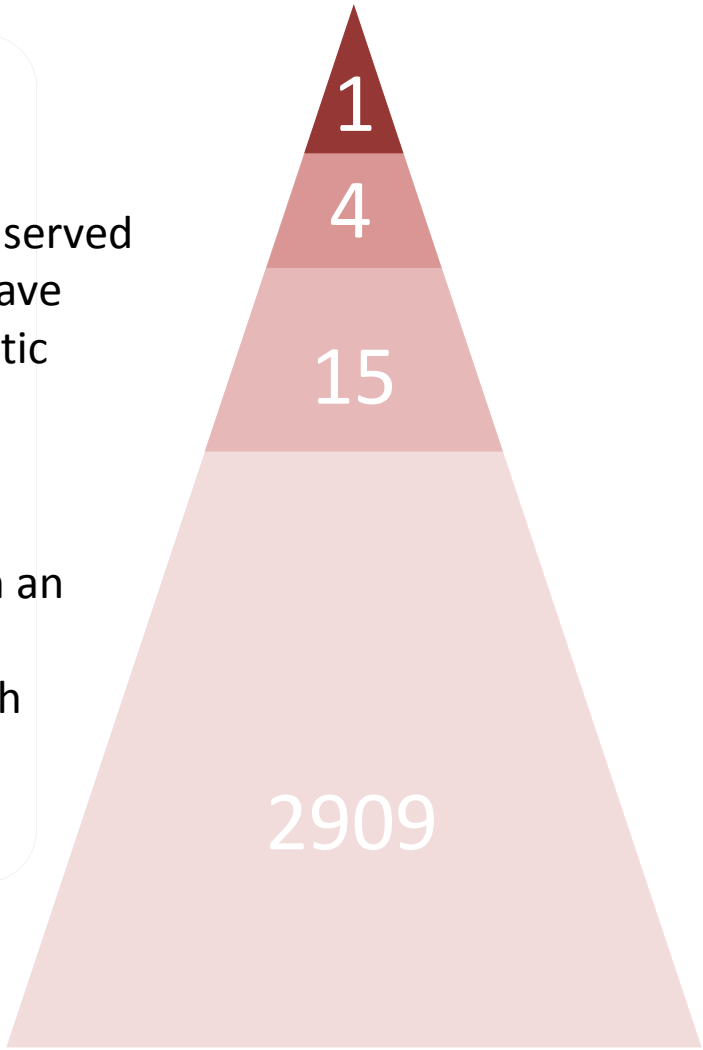
Hypothesis 2: If hypothesis 1 is correct, sialidases with an aromatic sandwich above the active site will be a good candidates for identifying sialidases with trans-activity.



Conclusion

Hypothesis 1: The family of sialidases is extremely conserved and it can be speculated that a trans-sialidase could have developed in parallel with the TcTS inferring an aromatic sandwich in a similar position as it is found in TcTS.

Hypothesis 2: If hypothesis 1 is correct, sialidases with an aromatic sandwich above the active site will be a good candidates for identifying sialidases with trans-activity.



Perspectives

- Crystallography of HpTS
  - Common traits between TcTS and HpTS
  - Further improvement of Tr13?
  - Identification of more native trans-sialidases
- Identification of other trans-glycosidases
  - ”Where can I find a sandwich ?”

Acknowledgements

- Colleagues:

(Supervisors)

Prof JD Mikkelsen

Asst Prof M. Pinelo

PhD C. Nyffenegger

PhD C. Jers

PhD B. Zeuner

PhD J. Holck

PhD Candidate S Jamek

PhD M. Lezyk

Prof AS Meyer

- Arla Foods A.M.B.A for providing substrate for donating the trans-sialylation reaction substrates

Questions please

Thank you for your attention