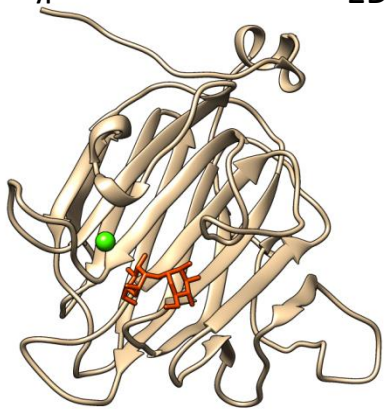
Critical Issues: Aspects of VIP36 Carbohydrate Interactions

Klaus Fiedler

Roles of lectins: How can bound carbohydrates best be studied?

VIP36
L-type lectin

2DUR-A



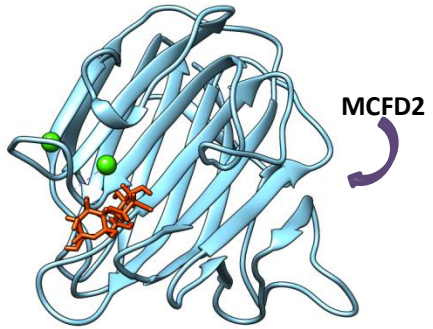
VIP36 stimulates secretion of clusterin
Hara-Kuge et al. (2002)

VIP36 stimulates α-amylase secretion
Hara-Kuge et al. (2004)

→ the pH-dependence is controversial or small relative to VIPL or ERGIC53

ERGIC53
L-type lectin

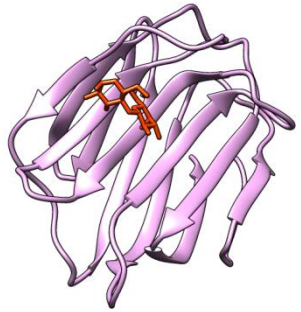
4GKX-A



MCFD2 binds coagulation factors V and VIII; some proteins bind directly to the lectin
Kamiya et al. (2008)

Galectin-9
Galectin

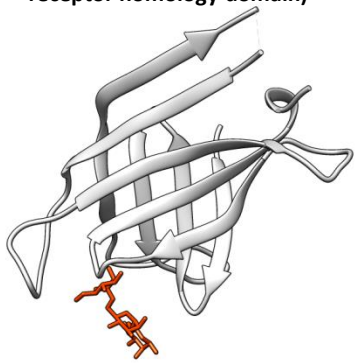
3LSE-A



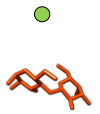
Recognizes internal N-acetyllactosamine units
Nagae et al. (2009)

OS-9
P-type lectin (mannose-6-phosphate-receptor homology domain)

3AIH-A



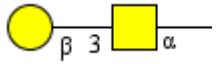
Binds M8-M5 high mannose glycans via α6 / D3 / C arm tri-mannose
Hosokawa et al. (2009)
Satoh et al. (2010)

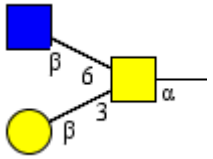


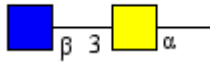
Carbohydrates

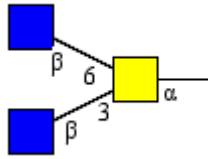
Gal GalNAc Man Glc GlcNAc Fuc NeuAc

O-glycan core

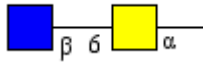
1  **Ser/Thr**

2  **Ser/Thr**

3  **Ser/Thr**

4  **Ser/Thr**

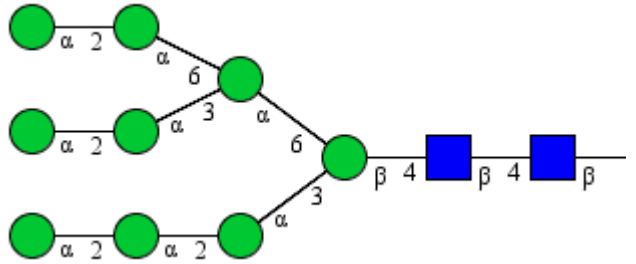
5  **Ser/Thr**

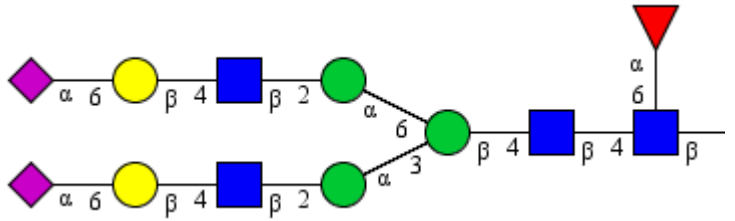
6  **Ser/Thr**

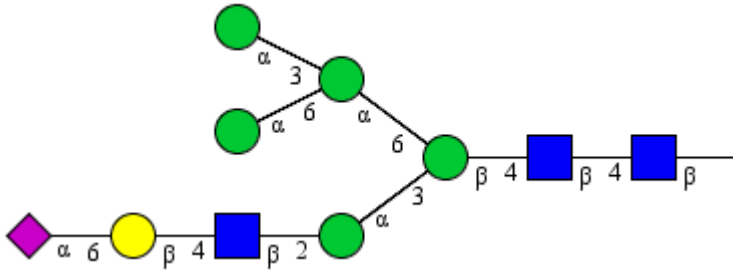
7  **Ser/Thr**

8  **Ser/Thr**

N-glycan

 **Asn** High mannose

 **Asn** Complex

 **Asn** Hybrid

Carbohydrate binding: 2DUR-A

VIP36

Similarity of VIP36 to plant lectins was first described based on primary structure relative to the lectin from *Bauhinia*
Fiedler et al. (1994)

The *Bauhinia* lectin was yet not retrieved in this search

Krissinel

Structural Search Programme PDBeFold

Protein	Structure	Q-Score	RMSD	Species
VIP36	2dur	1	0.00	*Canis lupus familiaris*
ERGIC53	4gkx	0.7	1.08	*Homo sapiens*
EMP47	2a71	0.52	1.80	*Saccharomyces cerevisiae*
EMP46	2a6w	0.48	1.95	*Saccharomyces cerevisiae*
Pea Lectin	2bqp	0.43	2.01	*Pisum sativum*
Soybean Agglutinin	2sba	0.41	1.88	*Glycine max*
Basic Agglutinin	2zml	0.41	1.97	*Psophocarpus tetragonolobus*
Seed Lectin	4u2a	0.40	2.07	*Vatairea macrocarpa*
Isolectin B4	1n47	0.40	1.94	*Vicia villosa*
Lectin DB58	1lul	0.40	1.92	*Vigna unguiculata*
Lectin	2eig	0.40	2.21	*Lotus tetragonolobus*
…				
yesU	1oq1	0.33	2.39	Prokaryotic, *Bacillus subtilis*
Galectin-9	3lse	0.31	2.41	*Homo sapiens*
Galectin-7	5gal	0.31	2.07	*Homo sapiens*
Galectin-8	3ap4	0.30	2.31	*Homo sapiens*
Galectin-3	3zsl	0.30	2.26	*Homo sapiens*
Galectin-4	3i8t	0.29	2.44	*Mus musculus*
Galectin-1*	4no4	0.29	2.51	*Rattus norvegicus*
Galectin LEC-6	3vv1	0.29	2.24	*Caenorhabditis elegans*
Galectin-1	1sla	0.29	2.53	*Bos taurus*
Galectin-2	2ymz	0.29	2.52	*Gallus gallus*
Galectin-10 Charcot-Leyden	1g86	0.28	2.49	*Homo sapiens*
…				
Calreticulin	3o0w	0.26	2.63	*Mus musculus*
Sialidase	2w68	0.24	2.33	Prokaryotic, *Vibrio cholerae*
…				
Calnexin	1jhn	0.15	2.99	*Canis lupus familiaris*
VP4	1kqr	0.13	3.98	Virus, *Rhesus rotavirus*
Concanavalin A	1vam	0.12	1.91	*Canavalia ensiformis*
COMP	3fby	0.11	2.62	*Homo sapiens*

*mutant

Protein glycosylation: Fucosylation

N-glycan chains are modified by adding fucose residues to their terminal chains (fucosyltransferase 2; Fut2) or to the N-glycan core (fucosyltransferase 8; Fut8)

Fut2 activity has a protective role and was suggested to alter host-microbial interactions by terminal modifications (Pickard et al. 2014; Goto et al. 2014)

Core fucosylation by Fut8 has been described as possible sorting signal in protein traffic in hepatocytes to the bile duct (apical traffic)(Nakagawa et al. 2006)

HNF1α is involved in the regulation of fucosylation (Lauc et al. 2010)

Regulatory role of IKZF1 in GlcNAc bisection versus core-fucosylation (Lauc et al. 2013)

Changes in protein glycosylation

… in Cancer

Fut8 is upregulated in epithelial-mesenchymal transition and was, furthermore, shown to regulate metastasis in nonsmall cell lung cancer (NSCLC) (Chen et al. 2013)

In breast, colorectal and ovarian cancer Fut8 is upregulated (Christiansen et al. 2014)

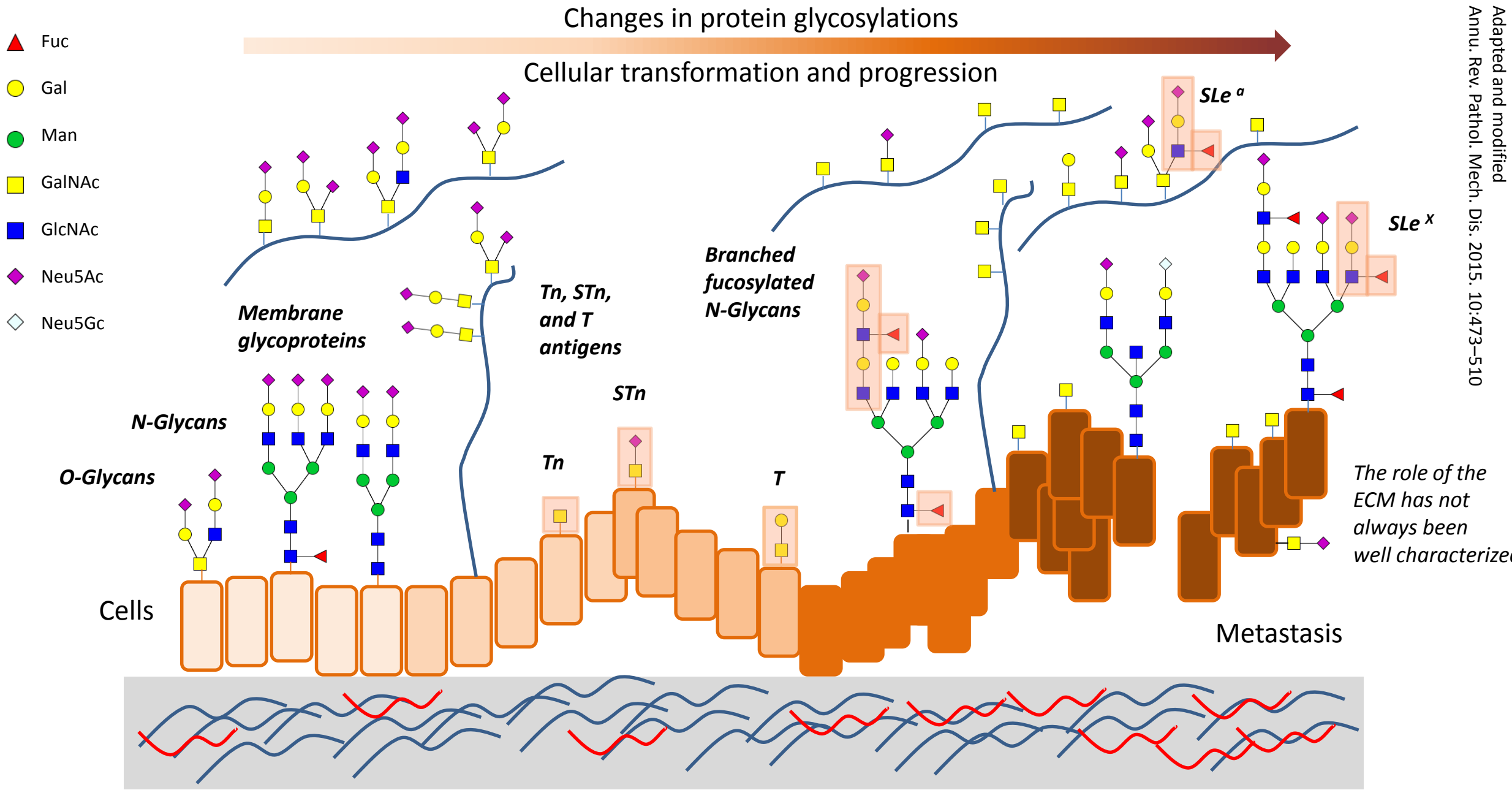
Fut8 overexpression in hepatoma cells (Hep3B) was demonstrated to suppress, however, intrahepatic metastasis in athymic mice (Miyoshi et al. 1999)

Absence of core fucose up-regulates GnT-III in mouse embryonic fibroblasts (Kurimoto et al. 2014)

In melanomas expression of fucokinase attenuates growth (Lau et al. 2015)
(these results refer to both types of fucosylation)

First detailed Fut8 suppression in a systems approach shows the requirement for melanoma metastasis (Agrawal et al. 2017)

Transformation in cells and glycosylation



Adapted and modified
Annu. Rev. Pathol. Mech. Dis. 2015. 10:473–510

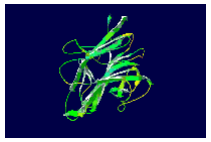
VIP36 cellular localizations and early tissue data

Although evidence has been obtained for certain localization with the GFP (green fluorescent protein)-tagging approach (Stadler et al. 2013) for proteins such as e.g. caveolin-1/VIP21 it has been shown, that these lead to aberrant localization (Han et al. 2015; Tiwari et al. 2016) and tagged constructs do not incorporate in natural endogenous complexes of proteins.

VIP36 has been analyzed in several cell types, endogenous VIP36 was found at the plasma membrane, the Golgi apparatus and intermediate compartment, and can be discerned in the Golgi apparatus in tissue sections (Human Protein Atlas; Uhlén et al. 2015). GFP-tagging approaches have failed on VIP36 and did not yield results consistent with the localization of the non-tagged protein obtained by raising antibodies against the extracellular domain or against natural peptides (Fiedler et al. 1994; Fiedler and Simons 1995; Füllekrug et al. 1999; Shimada et al. 2003). N- or C-terminal tagging approaches differed in resulting localization.

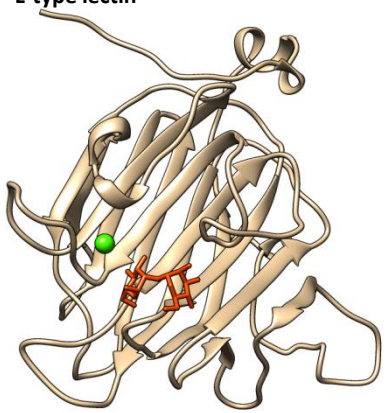
VIP36 structure: Carbohydrate binding

VIP36 simulation
(pre-structure data
could be compared)



VIP36 binds to 2 calcium-ions when tested and simulated

VIP36
L-type lectin



2DUR

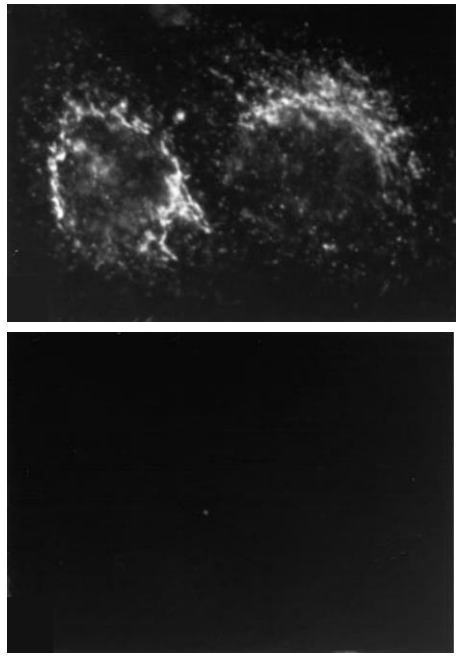
VIP36

Top scoring library glycans

MCS search

http://www.glycome-db.org

VIP36 localization

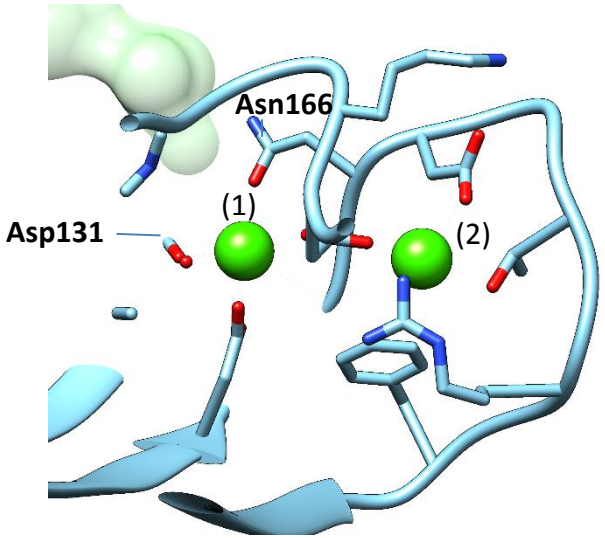


Fiedler and Simons (1995)

VIP36 structure: Calcium binding retest in simulation

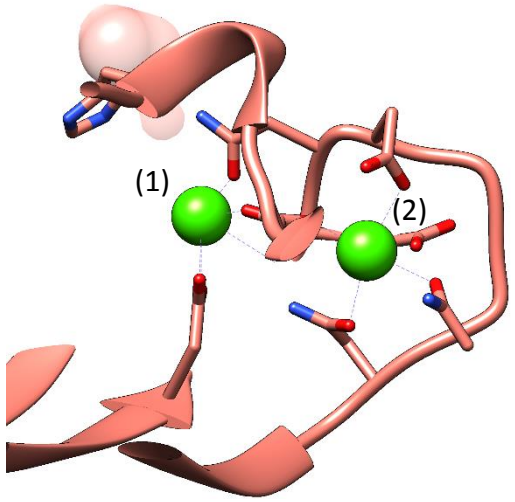
Previous data showed, that VIP36 binds to "1.8" calcium ions when tested in equilibrium dialysis and Scatchard analysis.
Calcium-independent affinity to carbohydrates has yet not been explained, the structural analysis, however, displayed calcium binding site (1) that is very close to the bound carbohydrate, and a distant cryptic calcium binding site (2).
We had not found any dependence on manganese in the simple dialysis that would have been expected based on the similarity to leguminous lectins (Fiedler and Simons, 1994, 1995).

carbohydrates



VIP36
2DUR

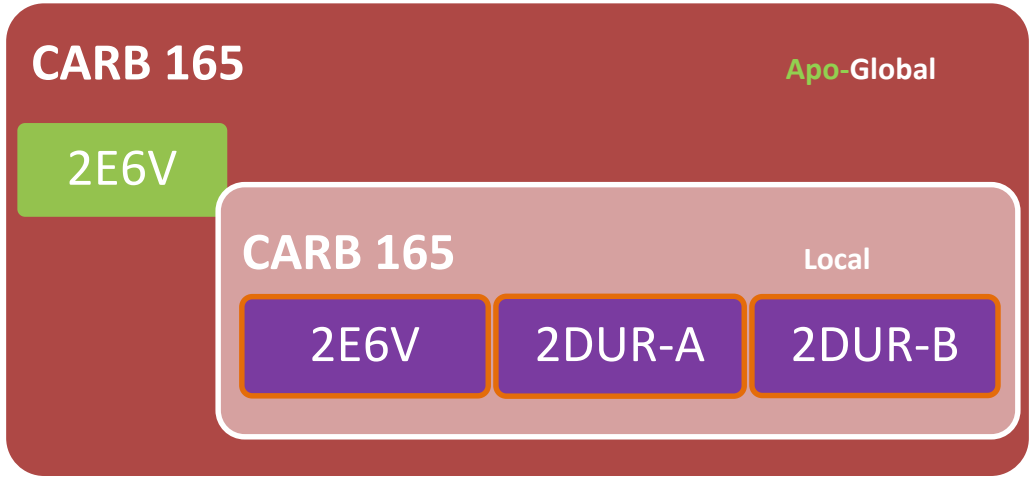
carbohydrates



ERGIC53
3WHU

VIP36 binding to carbohydrates is affected by Ca^{2+}, Ca^{2+} binding itself may be experimentally affected by pH and the variable conditions used of pH 6.5 (crystal) versus 7.4 (solution) and associated alteration of partial charges of the aspartate groups (~0.4e unit charge).

Carbohydrate library



Library of Carbohydrates

N-glycan high mannose	19
N-glycan hybrid	17
N-glycan complex	9
Sialoside	27
Fucoside	25
O-glycan core	17
Other	51

The library was assembled from internet sources: GLYCAM (http:/www.ccrc.uga.edu) and glycoSCIENCES.DE (www.glycosciences.de)

VIP36 structure: Carbohydrate binding

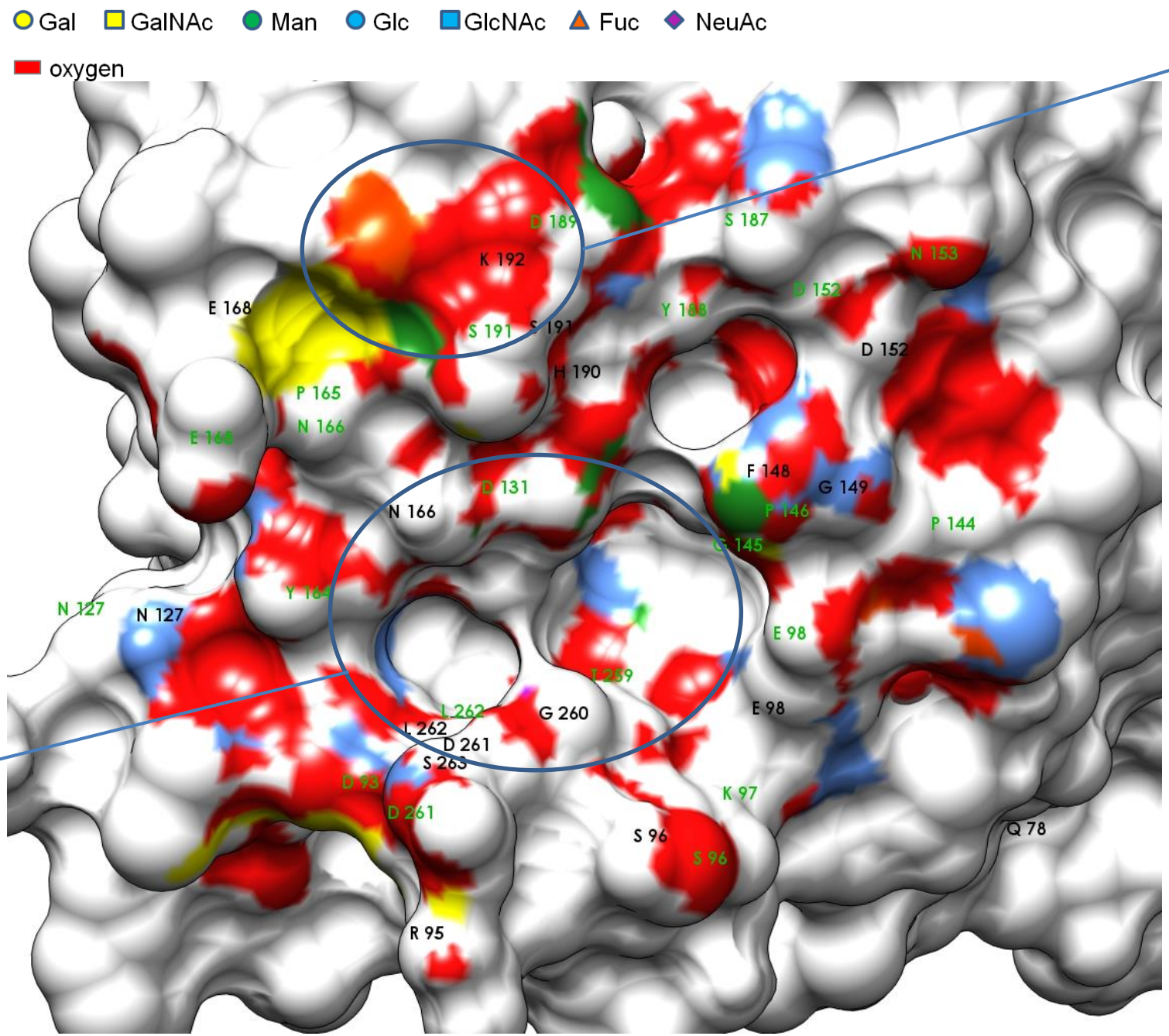
2DUR

carbohydrate projection

Carb165 (15·9 Models) at 3 Å

The central Man area is contacted via hydrogen-bonds

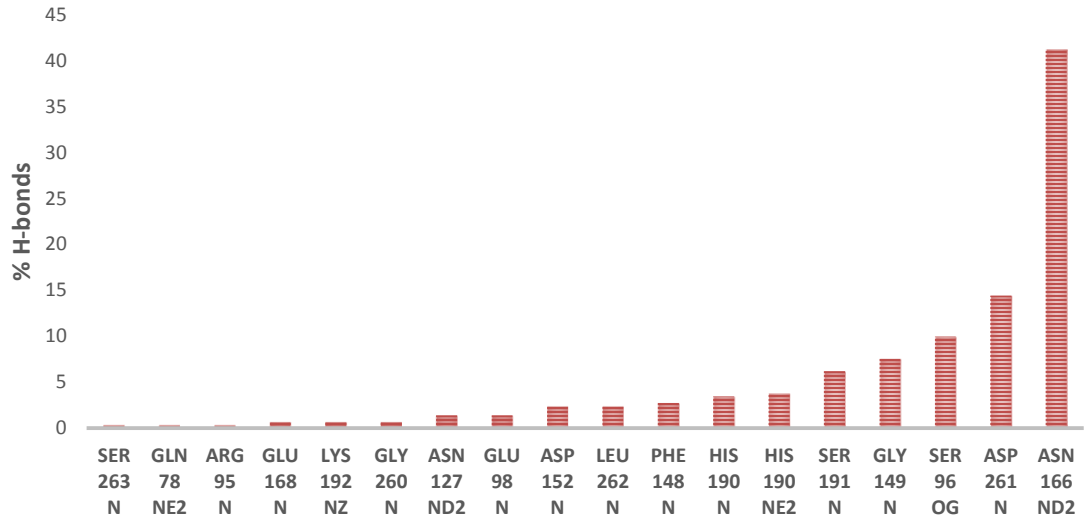
A top scoring glycan interacts via Fucose at S191 and W196



VIP36 structure: Carbohydrate binding

The similarity of VIP36 to the lectin from Bauhinia *may predict that glycan interactions depend on surface density. This may switch their select interaction. This is unexpected for a distinct binding site but for lipid converting enzymes it has been shown, that these also are not always e.g. in register with distinct substrates.*
Horan et al. (1999)

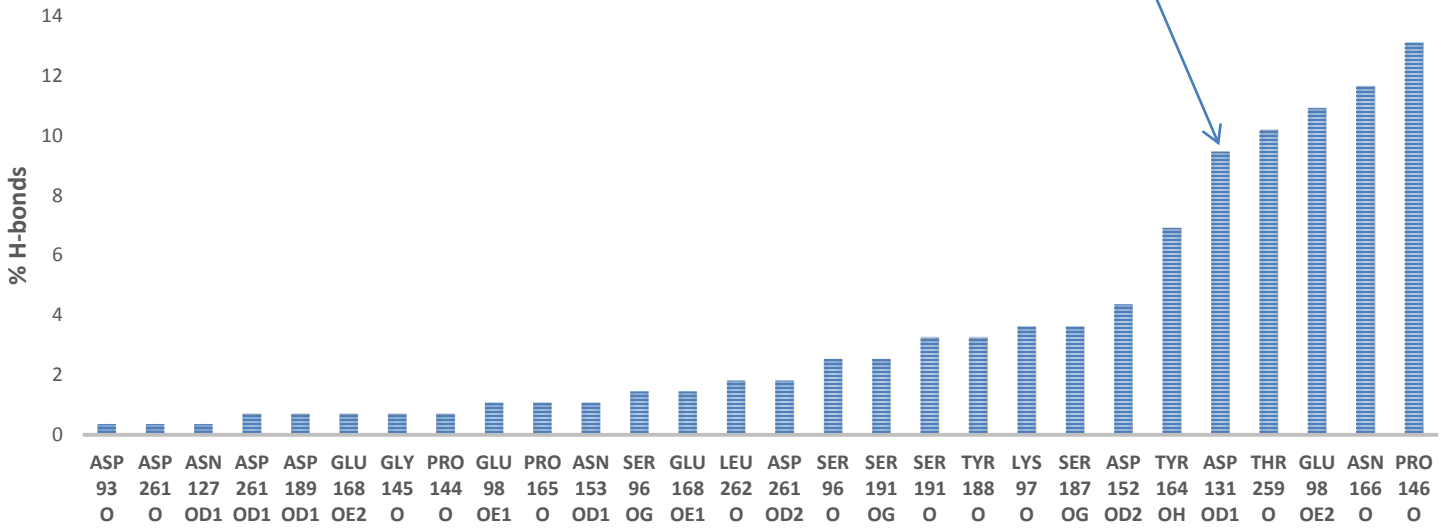
H-bond donor



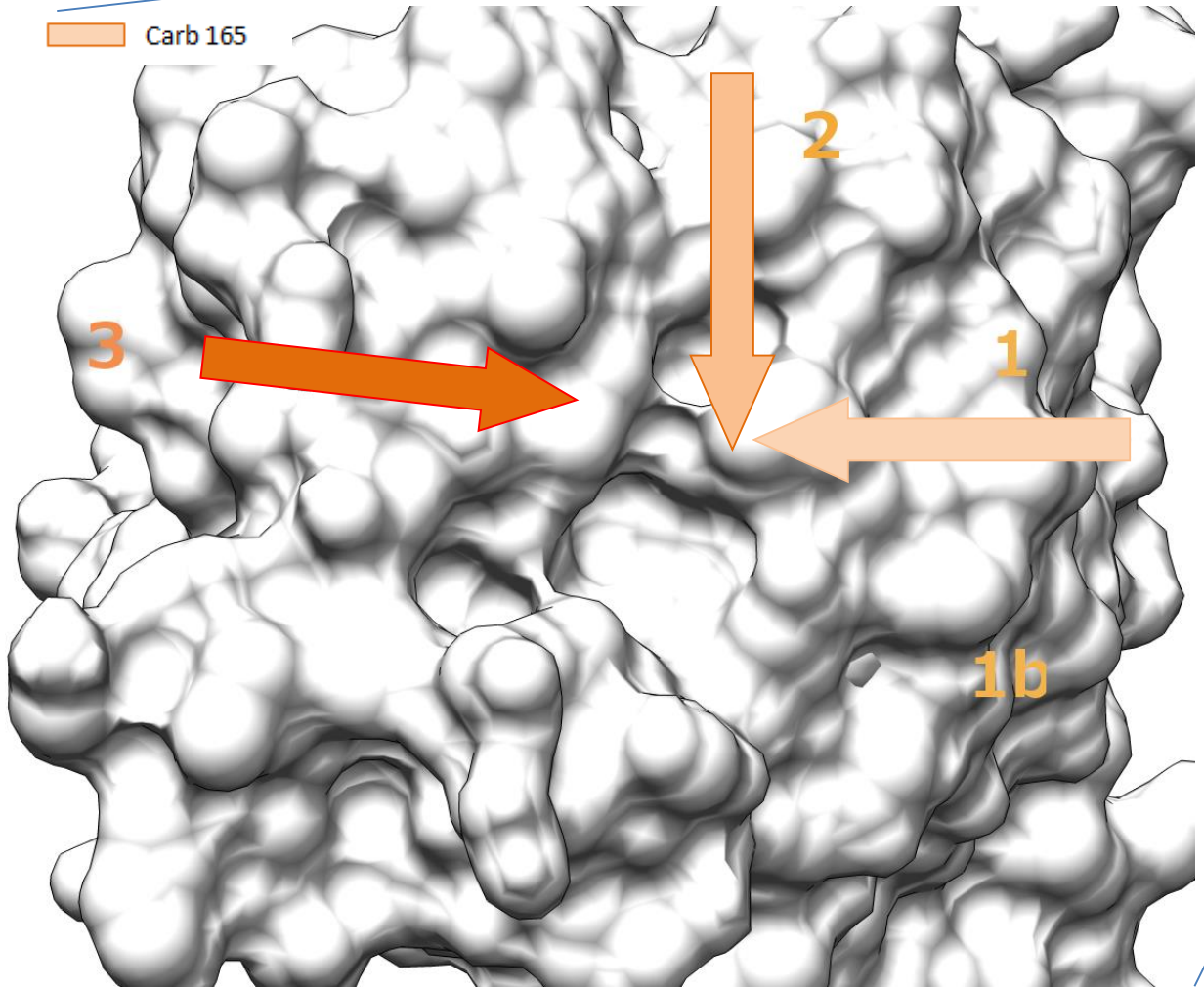
reduced binding in mutants

Kawasaki et al. 2007
Kamiya et al. 2008

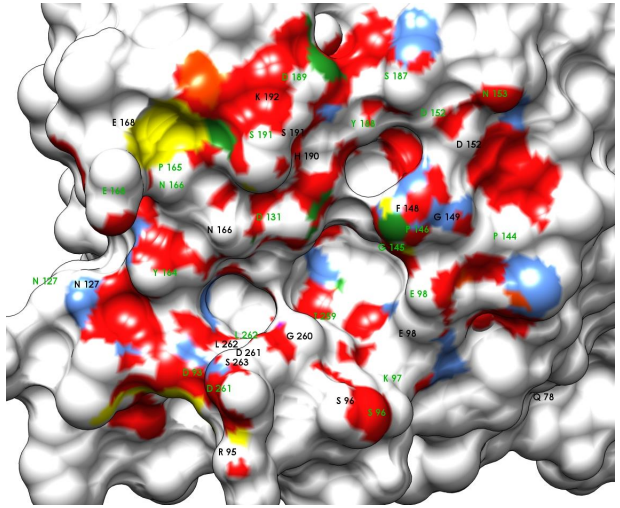
H-bond acceptor



Carbohydrate binding: Topology



Disulfide (C202-C239) distant from binding patch was not closed

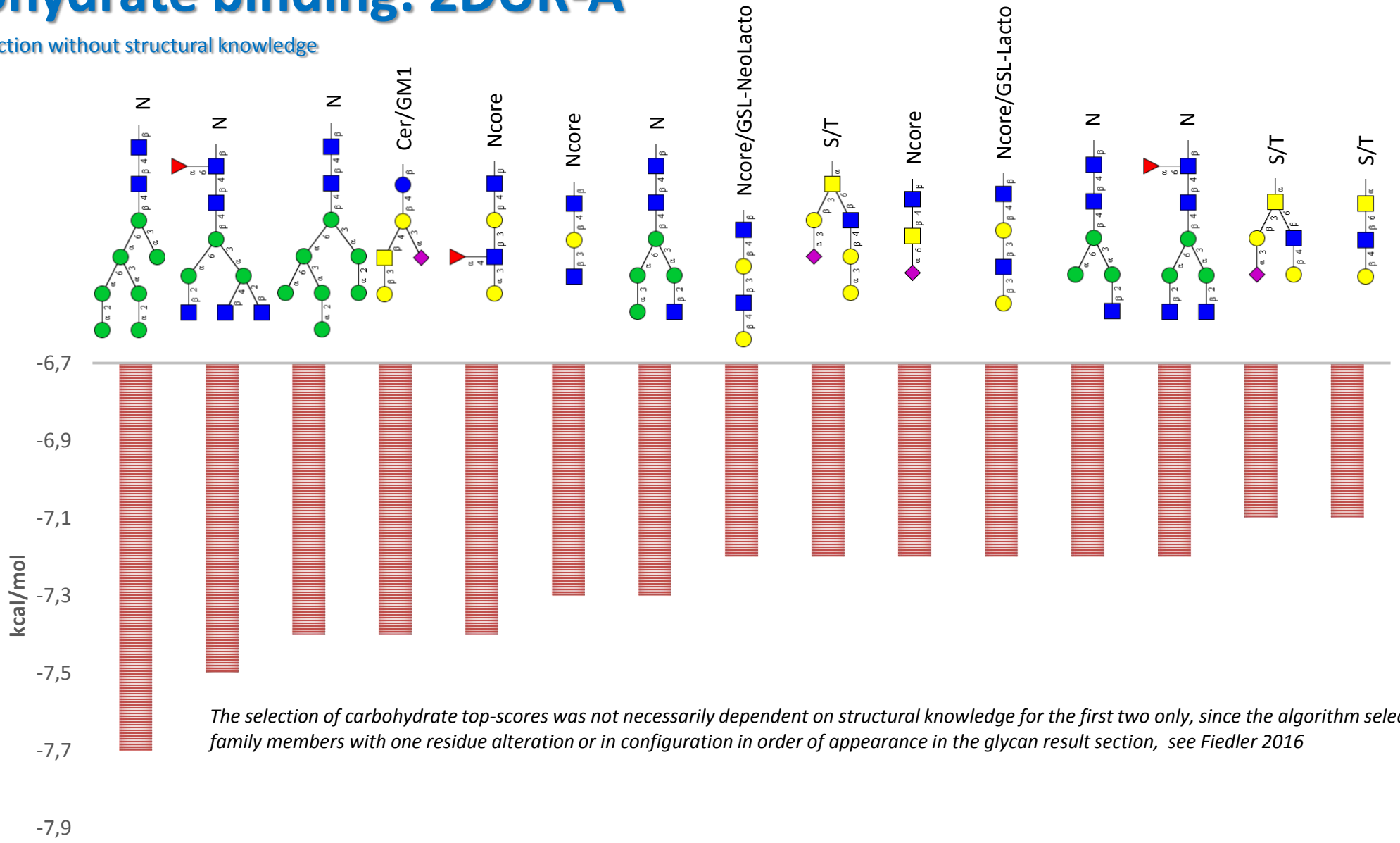


Topologies combining
15 top-scores of Carb165

Examples of glycan analogues in switched topology for some lectins are known (axial rotations)

Carbohydrate binding: 2DUR-A

VIP36 Selection without structural knowledge

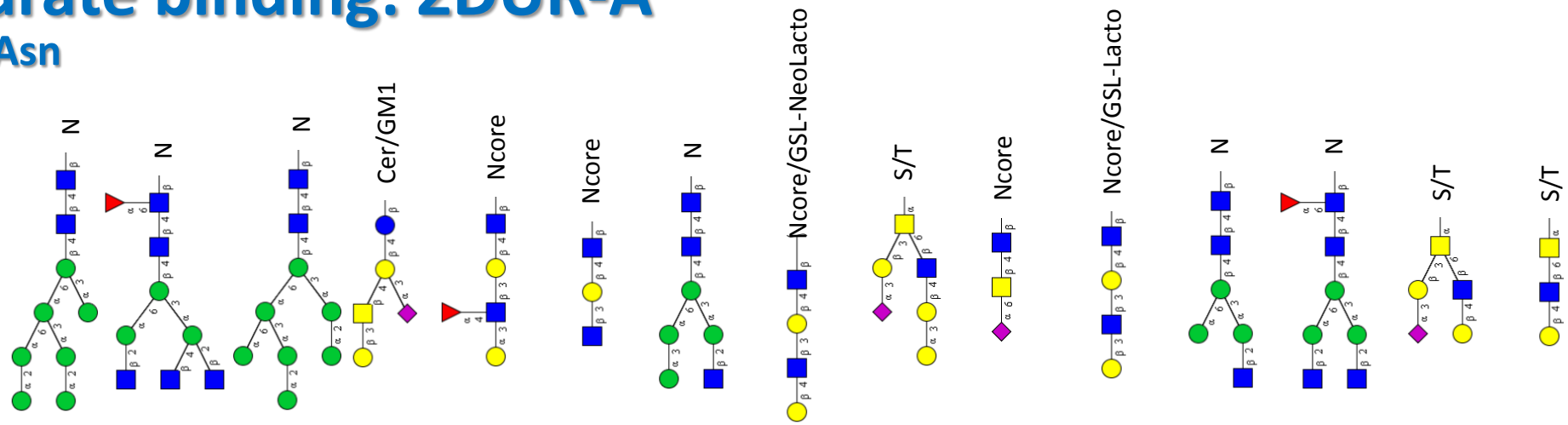


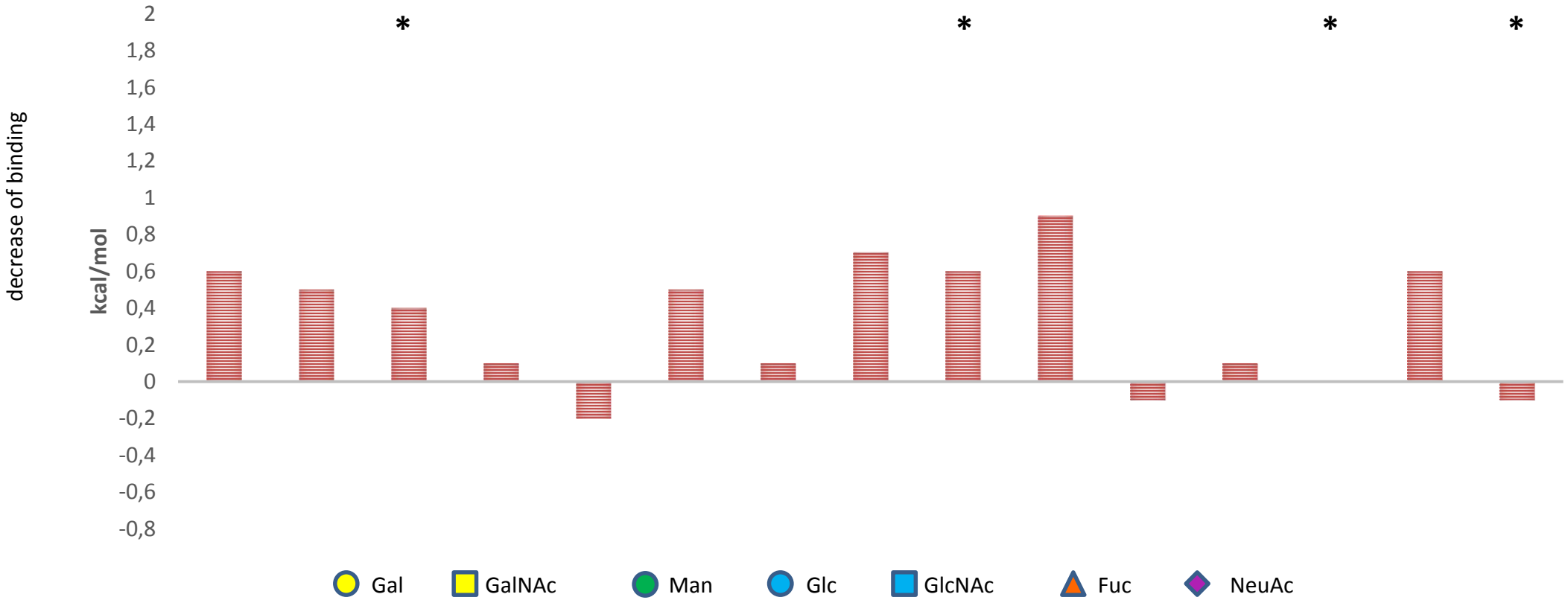
The selection of carbohydrate top-scores was not necessarily dependent on structural knowledge for the first two only, since the algorithm selected the correct family members with one residue alteration or in configuration in order of appearance in the glycan result section, see Fiedler 2016

Carbohydrate binding: 2DUR-A

VIP36-Asp131Asn

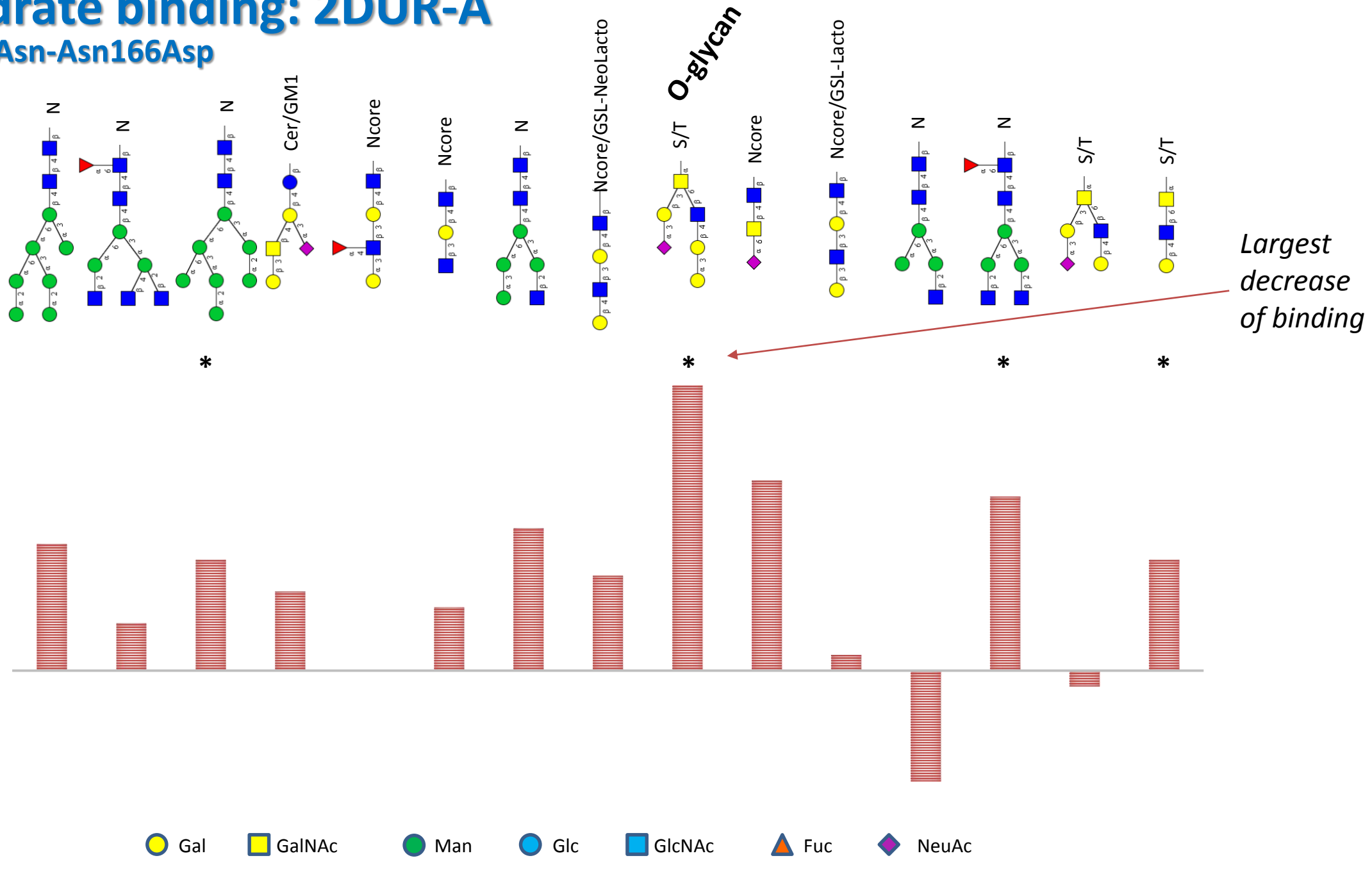
*
Ca^{2+}-dependent binding presumed based on double-H-bonds Asp131 and Asn166 (cut-off -7 kcal/mol)





Carbohydrate binding: 2DUR-A

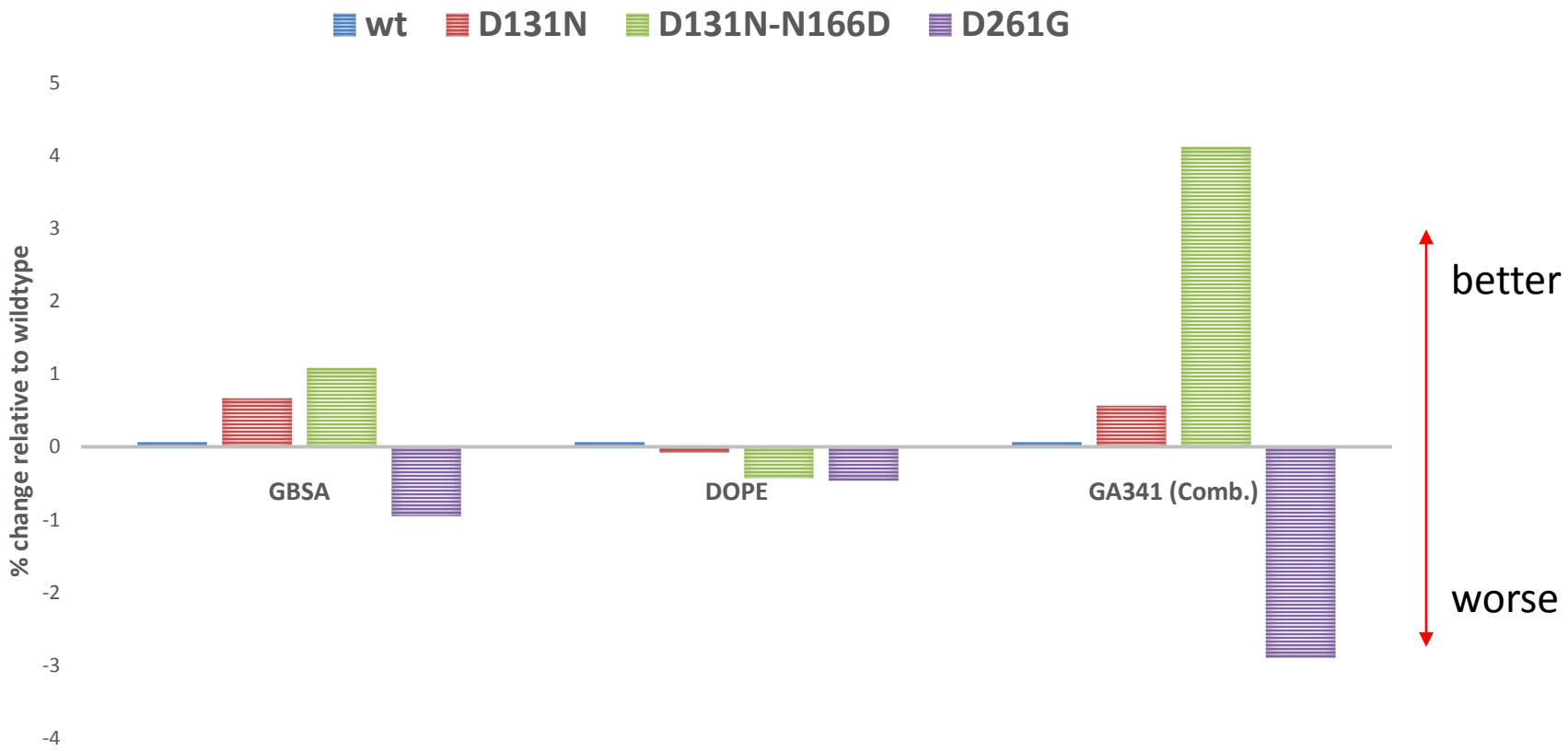
VIP36-Asp131Asn-Asn166Asp



Carbohydrate binding: Mutant molecular folding functions

Scores from Fiser and Sali 2003, Shen and Sali 2006

See the binding site (colored figure) for an overview of the carbohydrate interaction

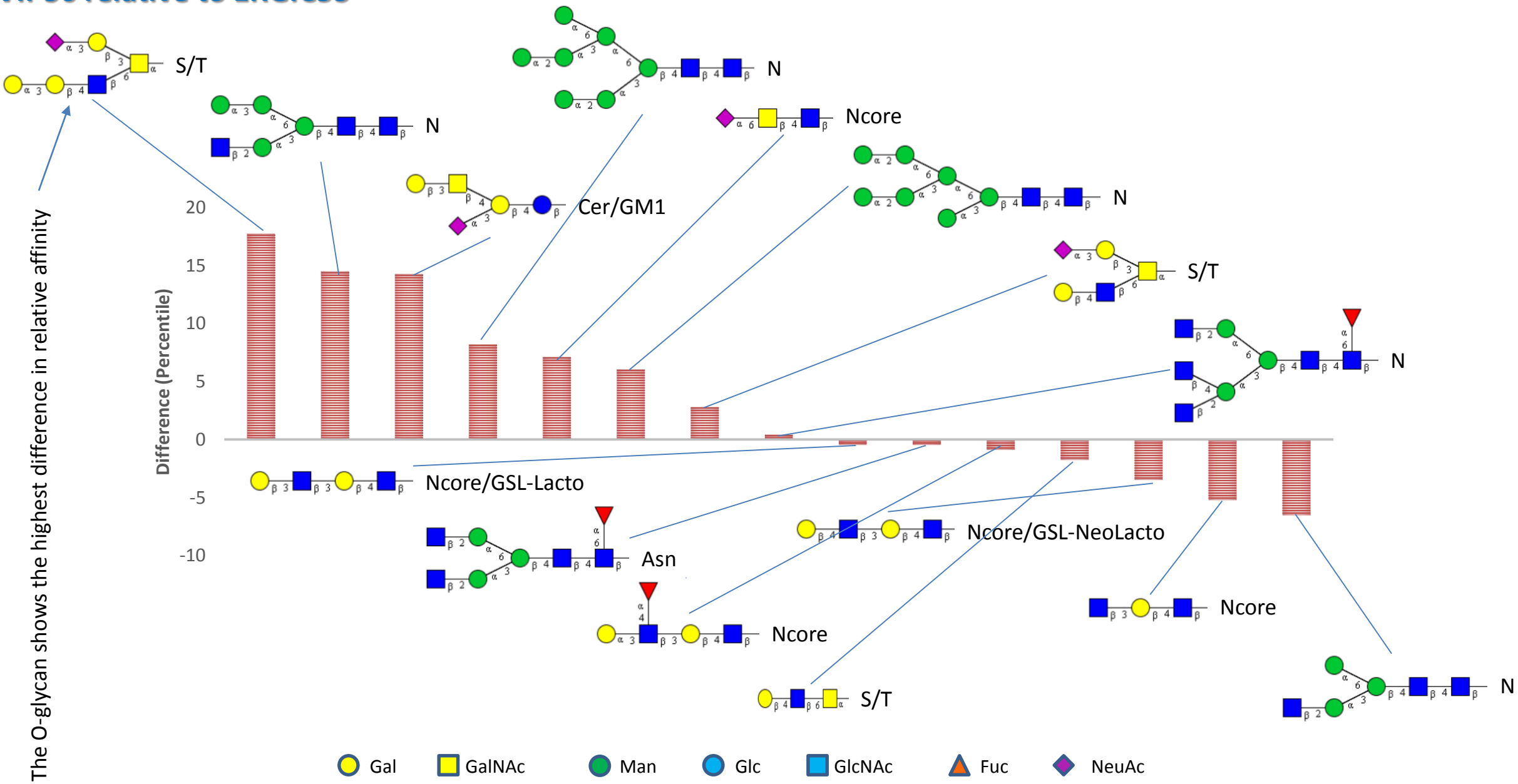


The DOPE score indicates good overall folding (D261G shows enhanced change)

Scores determined after energy minimization

Carbohydrate binding: wt2DUR-A and wt4GKX-A

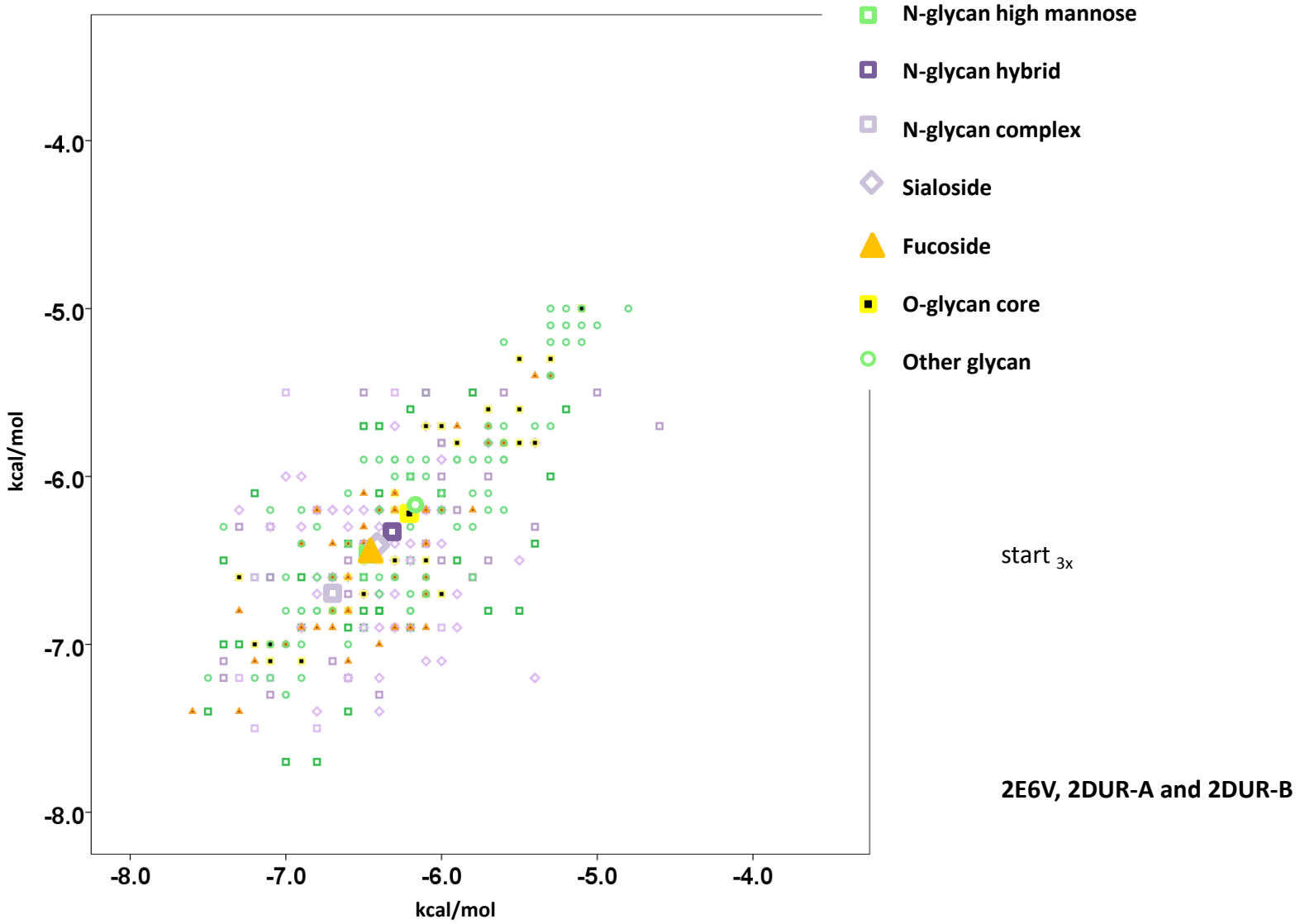
VIP36 relative to ERGIC53



Carbohydrate binding: Summary

VIP36

Own library data



start $_{3x}$

pairwise comparisons

2E6V, 2DUR-A and 2DUR-B

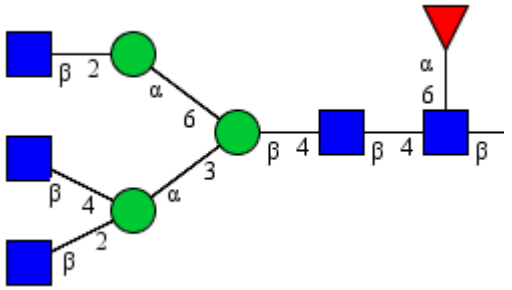
Carbohydrates interacting with VIP36: Top scores

high — relative affinity — low

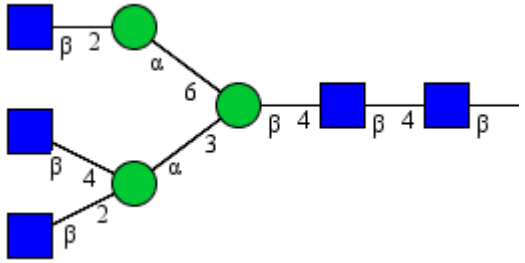
Fucosylated N-glycans show the highest relative interaction (identical hybrid glycans were so far not tested with or without fucose)

- Gal
- GalNAc
- Man
- Glc
- GlcNAc
- Fuc
- NeuAc

38



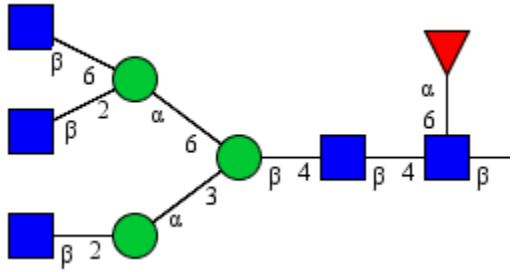
Asn -7.5



Asn -6.8

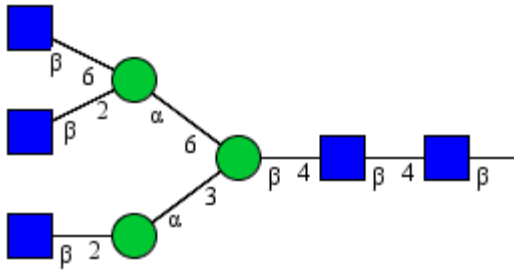
confirmed in independent screen

41



Asn -6.6

44



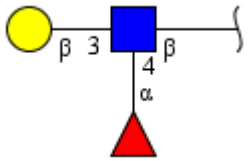
Asn -5.5

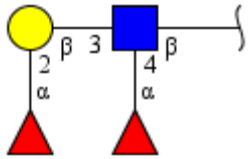
The α 1-6 linked fucose increases binding affinity by ≤ -0.7 kcal/mol

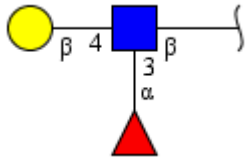
Carbohydrates interacting with VIP36: Top scores

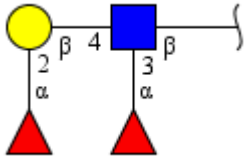
high — relative affinity — low

- Gal
- GalNAc
- Man
- Glc
- GlcNAc
- Fuc
- NeuAc

51  -6.9

56  -6.1

53  -6.7

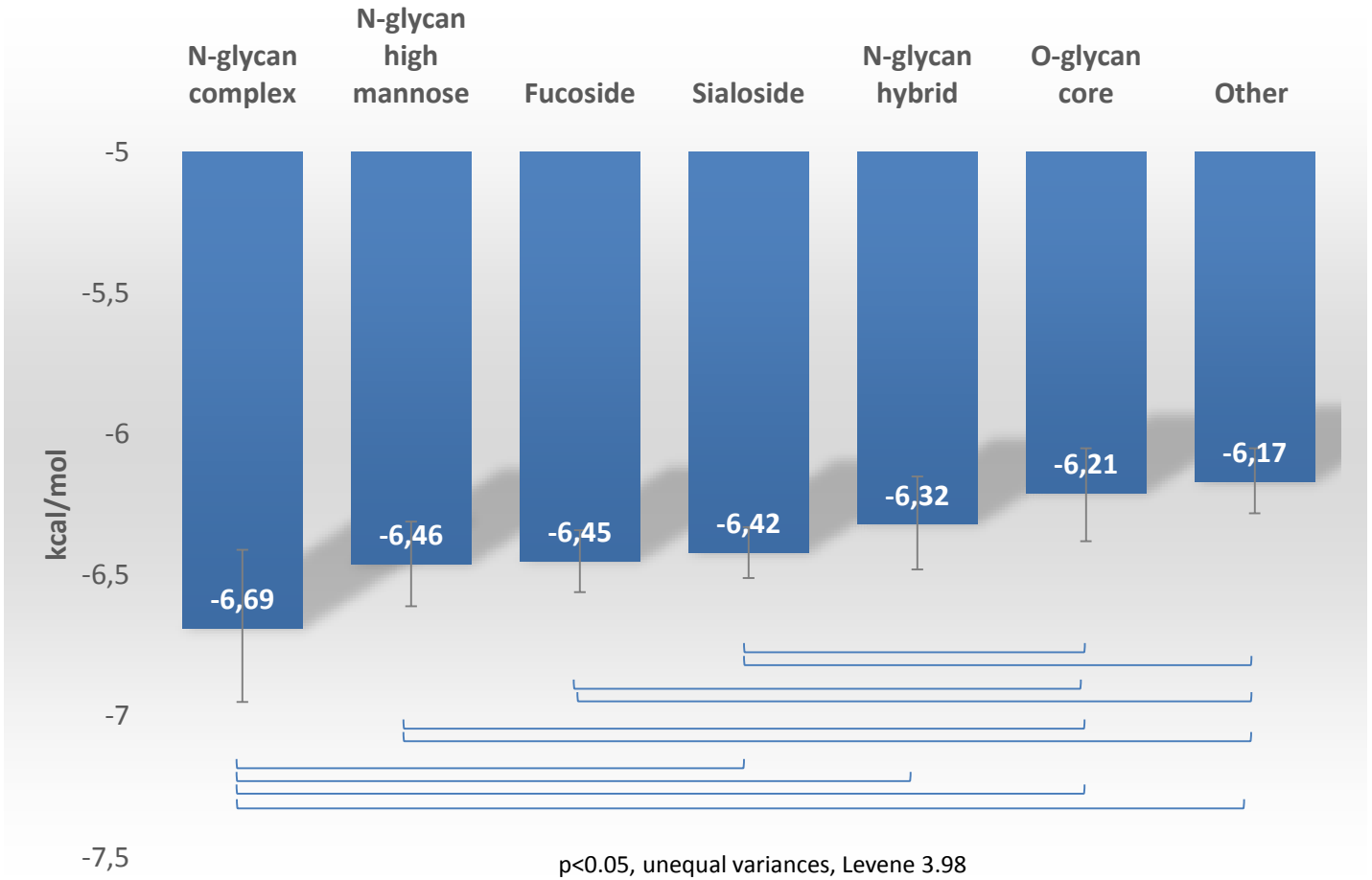
54  -6.6

N-core

Other single fucoses do not increase binding affinity

Carbohydrate binding: Summary of VIP36 binding

Group differences, Library data

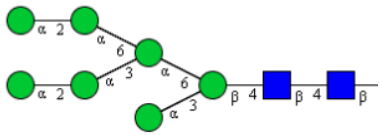


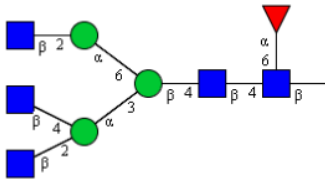
p<0.05, unequal variances, Levene 3.98

Confidence interval 95 %

Carbohydrate interactions: Top scores

- Gal
- GalNAc
- Man
- Glc
- GlcNAc
- Fuc
- NeuAc


Asn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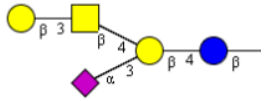
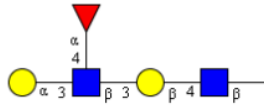
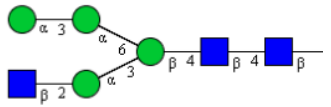
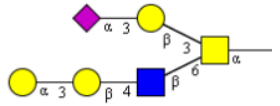

Cer


N-glycan core


Asn

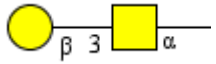
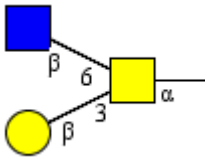
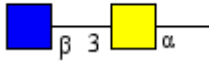
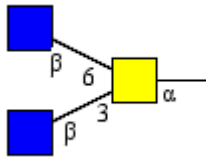
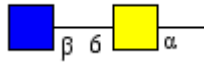

Ser/Thr

Including / name / biosynthetic origin	kcal/mol	No.
N-glycan high mannose: Location, medial Golgi	-7,7	I
N-glycan core basic: Location, medial/trans Golgi	-7,5	II
Glycosphingolipid-ganglio series: GM1; location: Medial-/trans-Golgi	-7,4	IV
Lactosamine motif	-7,4	V
N-glycan core basic: precursor of paucimannose-N-glycans or complex N-glycans; Golgi hexosaminidase removes GlcNAc / GlcNAcT-I required for viability; location, medial Golgi	-7,3	VII
O-glycan core 2, Gala1-3Gal epitope, Lactosamine motif: frequent in many tissues; location: Golgi	-7,2	IX

Carbohydrates interacting with VIP36: Top scores

O-glycan core including extension

- Gal
- GalNAc
- Man
- Glc
- GlcNAc
- Fuc
- NeuAc

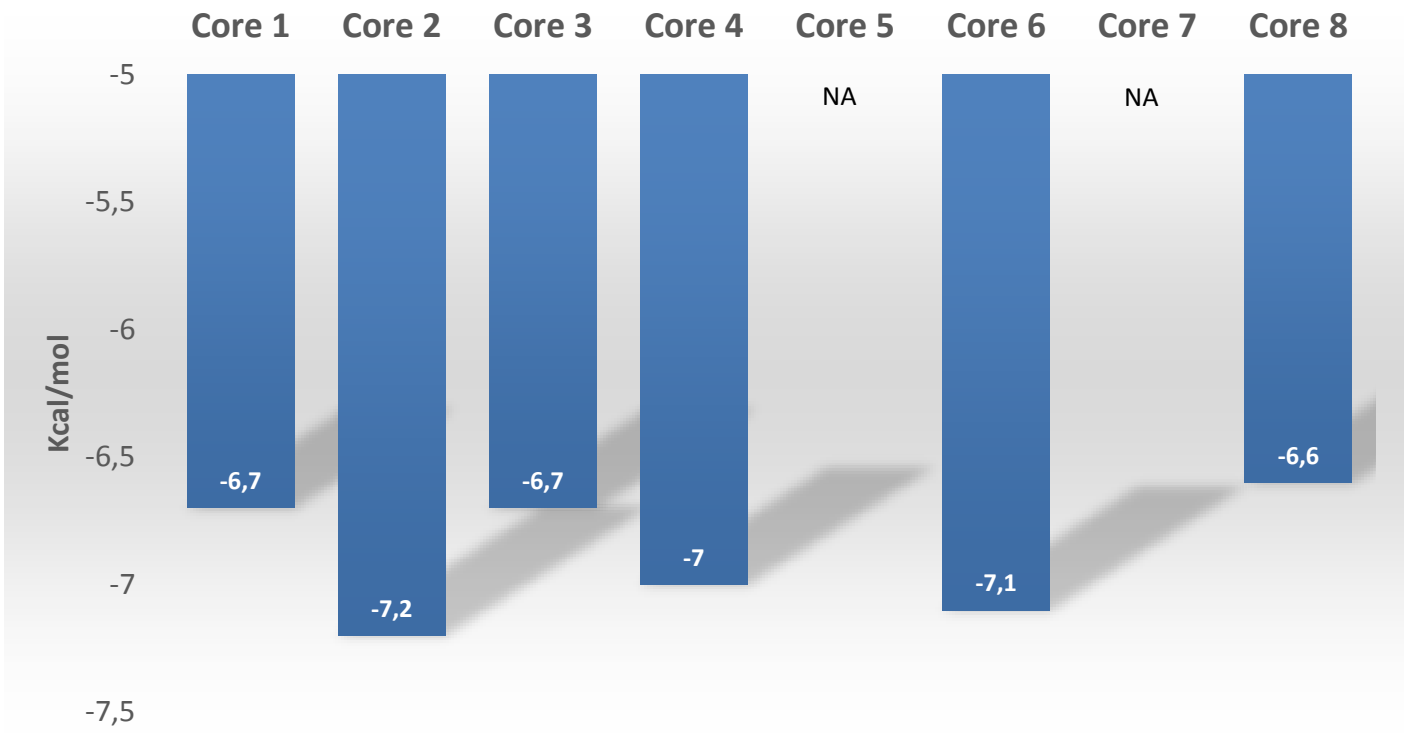
1		Ser/Thr	-6,7		
2		Ser/Thr	-7,2		
3		Ser/Thr	-6,7		
4		Ser/Thr	-7,0		
5		Ser/Thr	NA		
6		Ser/Thr	-7,1		
7		Ser/Thr	NA		
8		Ser/Thr	-6,6		

core 2 O-glycan only, and T antigen show lower binding affinity

Carbohydrates interacting with VIP36: Top scores

O-glycan core including extension,

Library data



Fut8 in mice knockout strains

Fut8 knockout ($Fut8^{-/-}$) mice show 70% mortality in the first 3 post-natal days

In surviving mice VEGFR-2 ist downregulated and the mice show emphysema-like changes of the lung

TGF-β1 receptor, EGF receptor and integrin activation show dysregulation

Wang et al. (2005, 2009)

The metastatic machinery in glycosylation?

In *Fut8*$^{-/-}$*/MEFs* the N-cadherin-β-catenin complex is not sufficiently degraded

common substrate

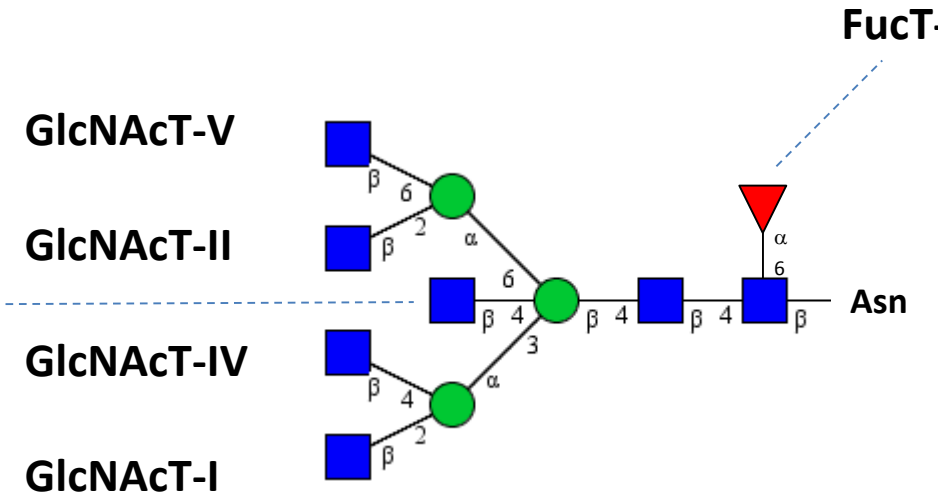
GlcNAcT-III

GlcNAcT-V

GlcNAcT-II

GlcNAcT-IV

GlcNAcT-I

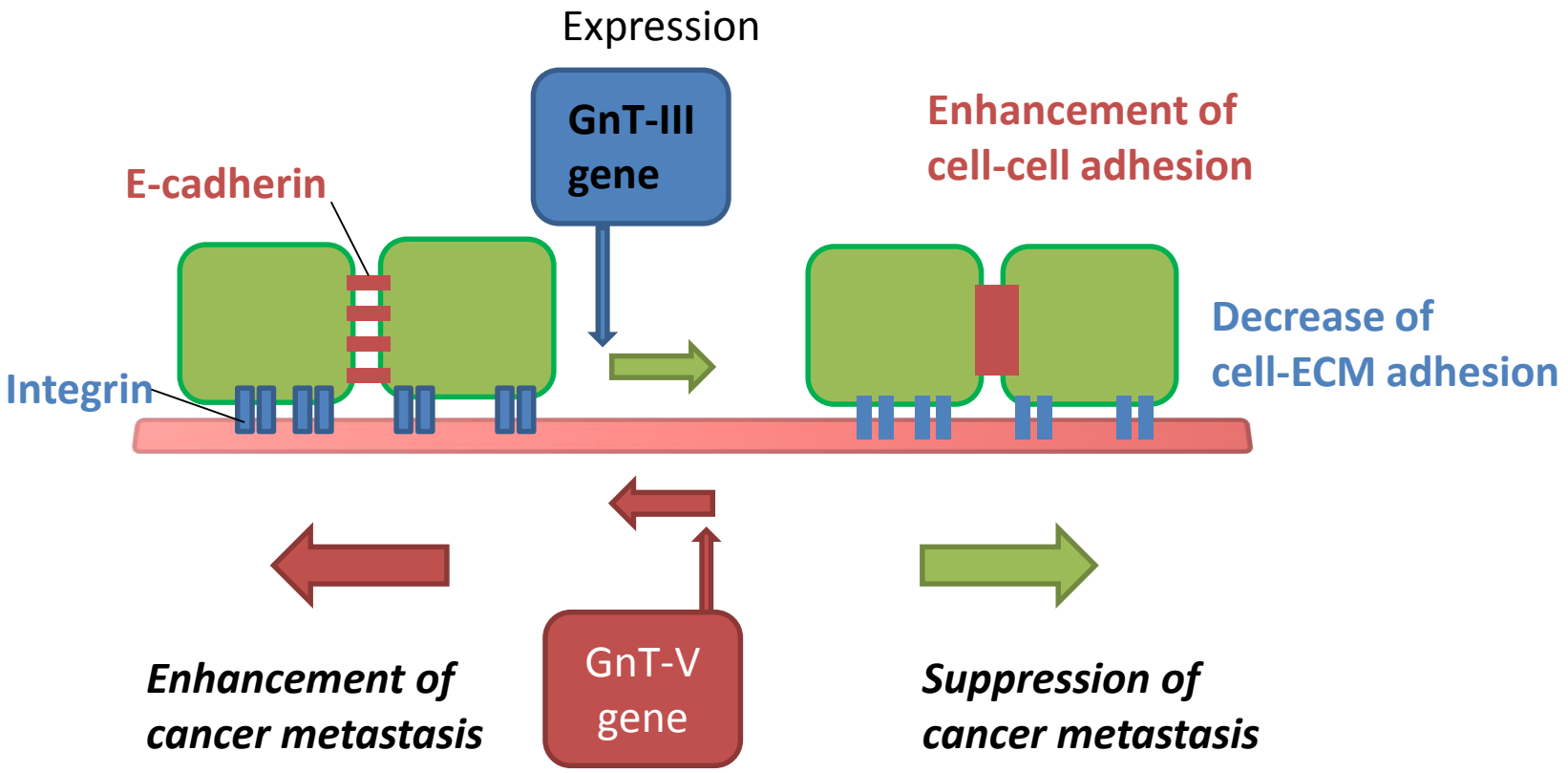


Wnt target	Fut8 KO/WT x change (microarray)
Growth arrest-specific 1 (GAS1)	12.1
Frizzled homolog 6 (FZD6)	2.3
Glutathione S-transferase, type1 (GSTM1)	11.3
Cytochrome P450, CYP1B1	9.8
Mitochondrial aldehyde dehydrogenase 2 (ALDH2)	2.8
GlcNAcT-III	~3.0 (PCR)
GlcNAcT-V	0.3 (PCR)

Wnt-signal inhibitor (IWP-2) studies suggest that changes in Wnt/β-catenin signalling are associated with the upregulation of GlcNAcT-III

Kurimoto et al. (2014)

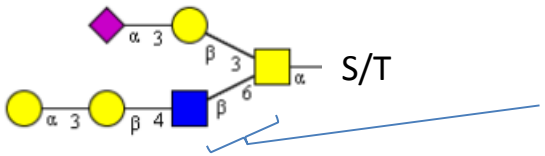
Several roles of glycosyl-transferases in cancer



Adapted from Zhao et al. (2008)

According to this view, Fut8 would also indirectly affect metastasis via regulation of GlcNAc-transferases

Several roles of glycosyl-transferases in cancer



Core 2 O-glycans allow tumorous cells to evade Natural-Killer (NK) cells

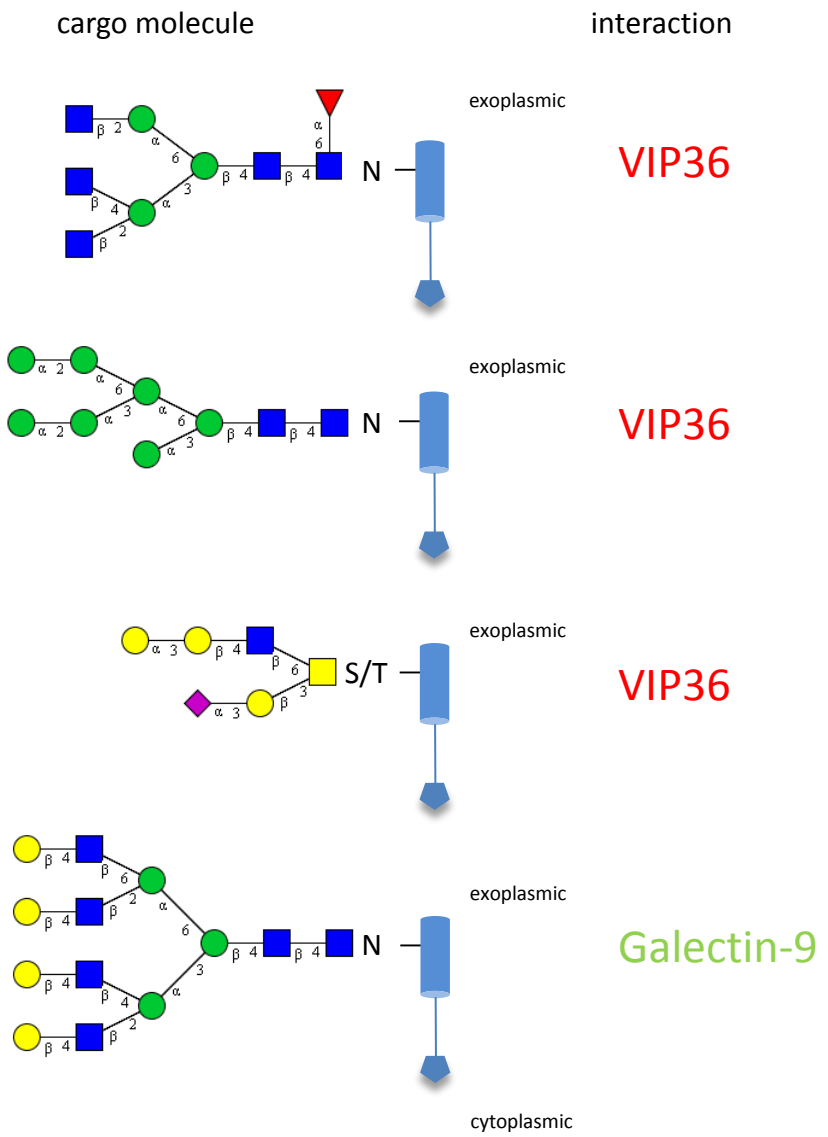
Benson et al. (2010)

Kidney tubule cell specific expression of core 2 GlcNAc-transferase

Suzuki et al. (2004)

May mucin transport in intestinal epithelia (core 3) and possibly kidney tubule specific O-glycans relate to VIP36 function?

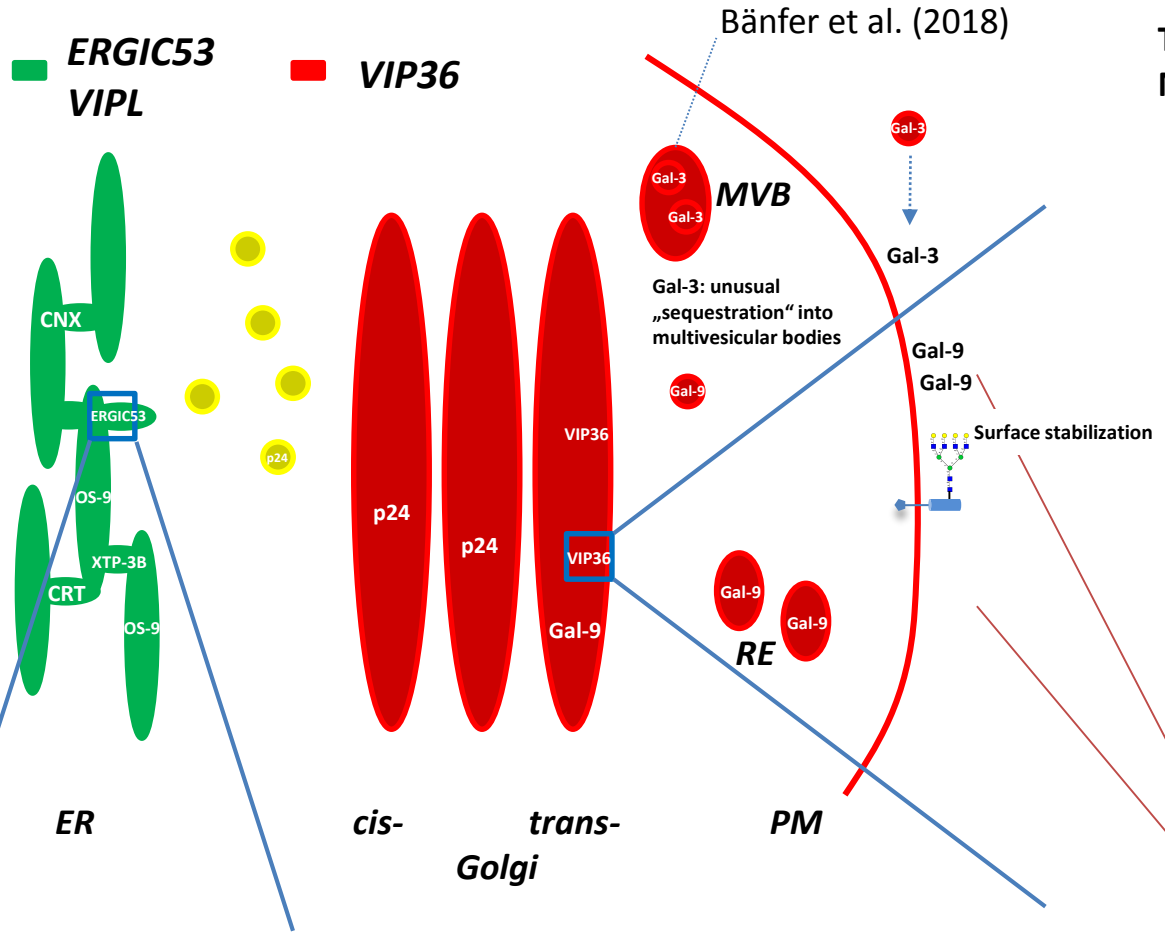
Multiple possibilities of sorting/inclusion into transport carriers in the constitutive secretory pathway



- **Regulated inclusion of cargo-receptors by a cytoplasmic coat**
- **Sorting in the Golgi lumen by domain formation and luminal matrix**
- **Cargo sequestering by substrate enzymatic conversions and release**
- **Surface/domain formation by stabilization**

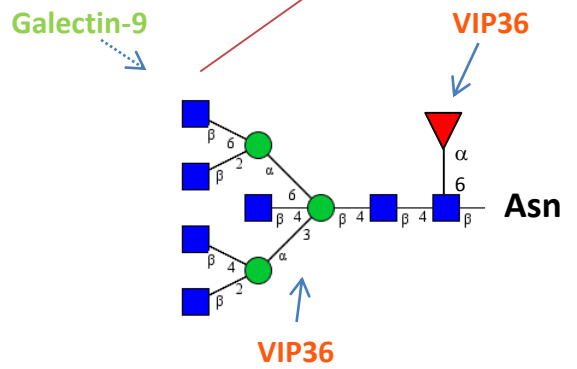
The capacities of a sorting machinery cannot be predicted off hand, therefore, the inverted U-shaped, or other dependence on cargo concentration has to be analyzed

Current summary: Secretory pathway and some lectins



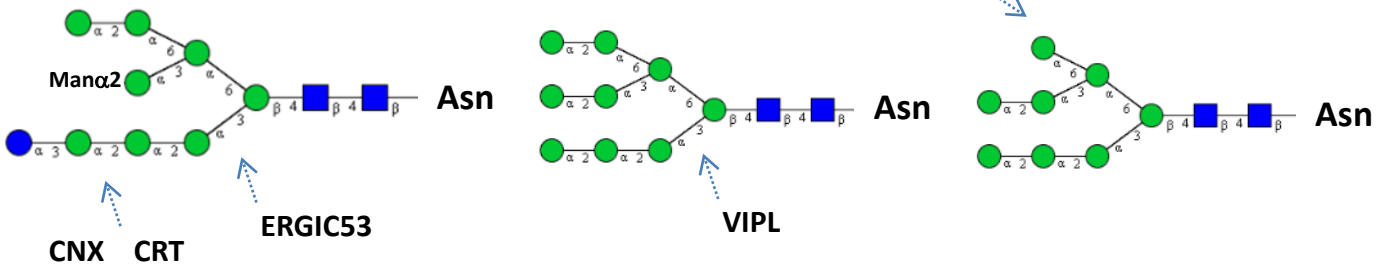
Trafficking:
N-Glycans in the trans-Golgi

these extensions may increase in metastasis



Galectins may bind to the termini of α6 / D3 arms of complex glycans, on the other hand, VIP36 may bind to the α3 / D1 arm and to core fucose and may be required for targeting

Recognition of N-Glycans in the ER



Conclusions

- The select affinity of VIP36 relative to ERGIC53 for O-glycans has been clearly shown in this *in silico* docking method
- Ca^{2+}-dependent binding mimicked and strongly reduced in the the proposed D131N-N166D mutant applies to the core2 O-glycan in particular and a few others, similar to the lectin from *Bauhinia purpurea* an expanded binding site with specific interactions on the perimeter of the central binding cavity can be expected
- Fucosylation by Fut8 may present one signal for enhanced cancer progression with fucosylated N-glycans, the role of core-fucosylation machinery has been demonstrated in melanomas
- Cancer progression has been shown to entail VIP36 as a prognostic marker in renal cancer (Uhlen et al. 2017)