Druggability of Lectins – Mission Possible?

Ischia Advanced School of Organic Chemistry Ischia, (Naples)
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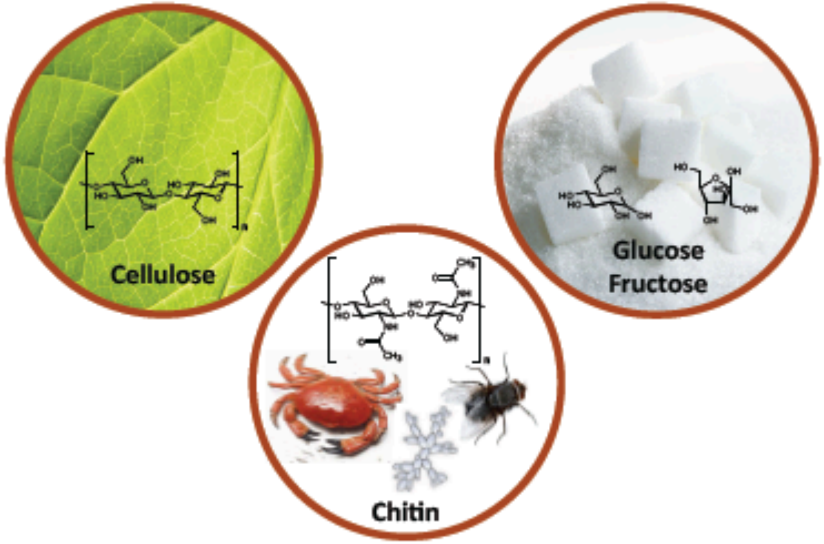
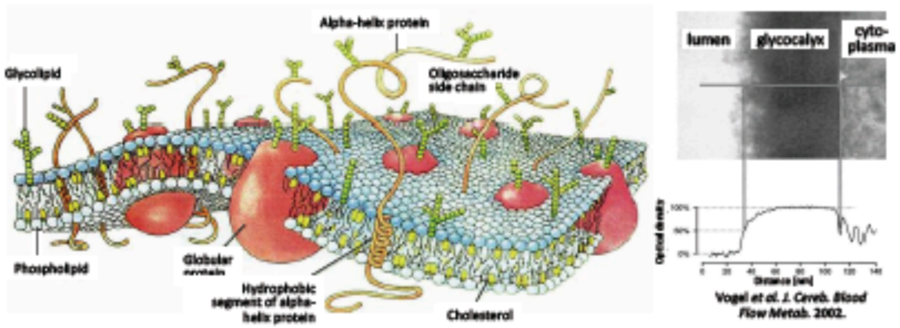
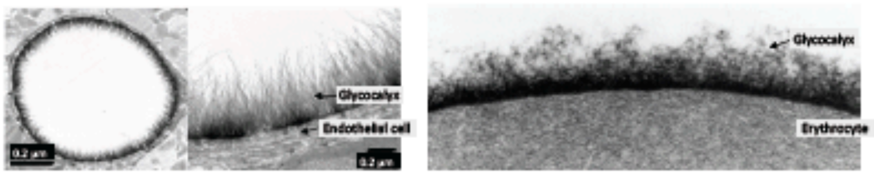
Biological Functions of Carbohydrates



The Glycocalyx



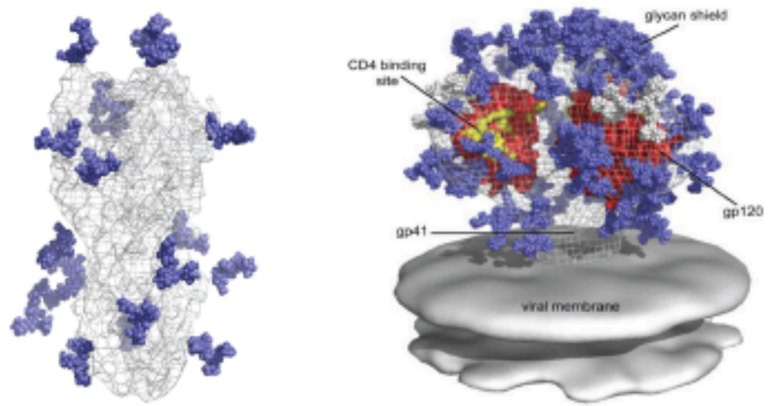
Vogel *et al. J. Cereb. Blood Flow Metab.* 2002.



http://www.hubrecht.eu/research/dekoning/research.html

Roseman, *J. Biol. Chem.* 2001, *276*, 41527-41542.

Large Oligosaccharide Structures Determine Protein Function

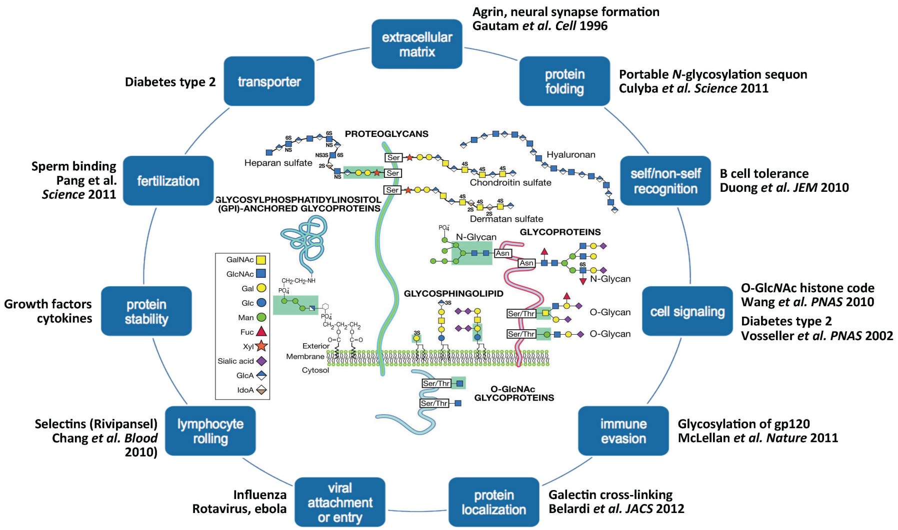


8% of hemagglutinin (H5N1 influenza virus) is attributed to carbohydrates

50% of HIV gp120 molecular weight is attributed to carbohydrates.

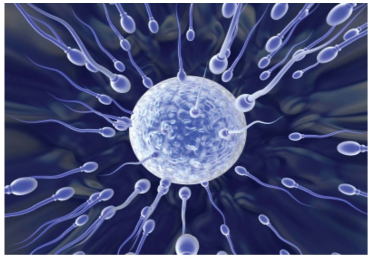
Wong *et al. PNAS* 2009; Schief, *Nat. Res. Council* 2012, Transforming Glycoscience

Glycans in Health and Disease



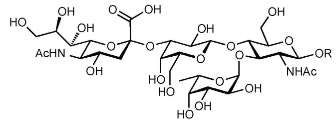
Varki *et al.* **(2009)** Essentials of Glycobiology 2nd Ed.

Your First Carbohydrate-Lectin Interaction

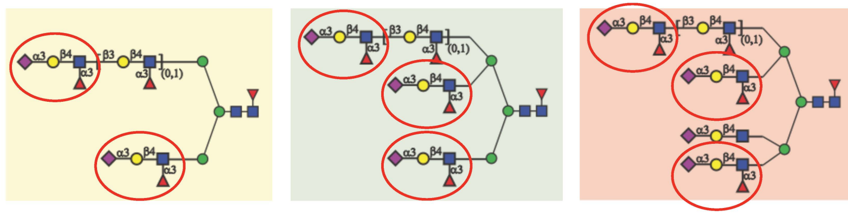


Human fertilization begins with spermatoza bind to the zona pellucida.

- Most abundant terminal sequence on N- and O-glycans of human ZP: sialyl Lewis^x



- Sperm-ZP binding largely inhibited by desialylation or anti-sLe^x antibodies.
- sLe^x epitope represents the major CH-ligand for human sperm-egg binding.



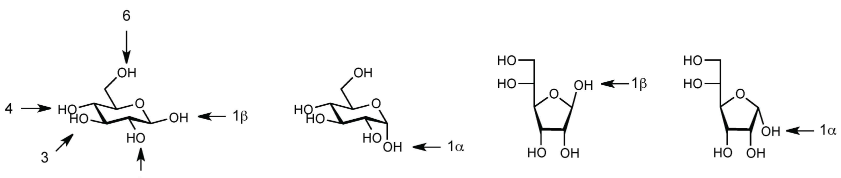
Pang *et al. Science* **2011;** http://blog.f1000.com/2011/12/16/how-egg-greets-sperm/

Glycocode

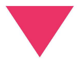
Ability to form large (rigid?) polymers with an enormous coding capacity

- Anomeric configuration (α or β)
- Linkage position (1 → 1, 2, 3, 4, 6 for a hexapyranose)
- Ring size (pyranose / furanose)
- Branching
- Site specific modifications (e.g. acetylation, sulfation, phosphorylation...)

➔ From 4 hexoses: 393'216 tetrasaccharides (R. Laine, *Glycobiology* **1994**, *4*, 759)
From 5 hexoses: 31'457'280 pentasacchrides



Carbohydrate-Lectin vs Glycomimetic-Lectin Interactions

Carbohydrate 

 **Glycomimetic**

low affinity (µM to mM) and insufficient PK properties; however high specificity

mimics carbohydrate and shows high affinity and a drug-like PK profile

Lectin: A carbohydrate-binding protein playing a role in biological recognition phenomena.

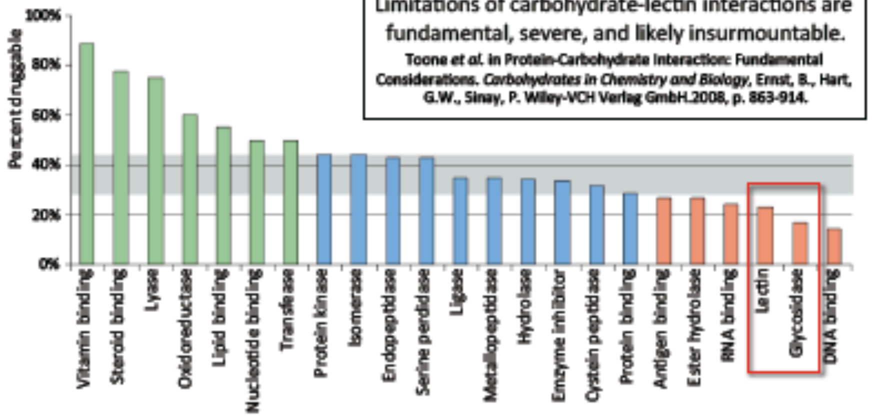
Glycomimetics for lectin targets:

Selectins, Siglecs, Galectins, DC-SIGN, ASGP-receptor, bacterial lectins

Beat Ernst, John L. Magnani, From carbohydrate leads to glycomimetic drugs, *Nature Reviews Drug Discovery* **2009**.

Druggability of Lectins - Mission Possible?

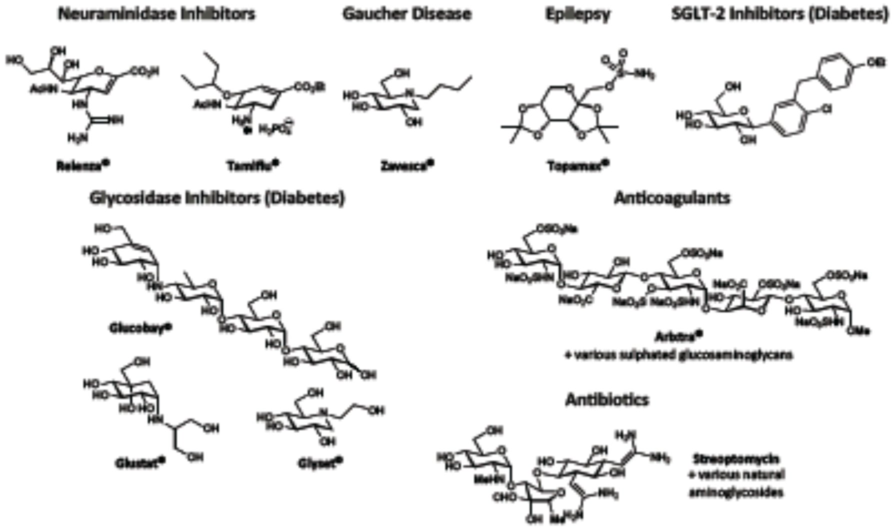
Limitations of carbohydrate-lectin interactions are fundamental, severe, and likely insurmountable.
Toone *et al.* In Protein-Carbohydrate Interaction: Fundamental Considerations. *Carbohydrates in Chemistry and Biology*, Ernst, B., Hart, G.W., Sinay, P. Wiley-VCH Verlag GmbH.2008, p. 863-914.



Hajduk *et al. Drug Discovery Today* 2005.

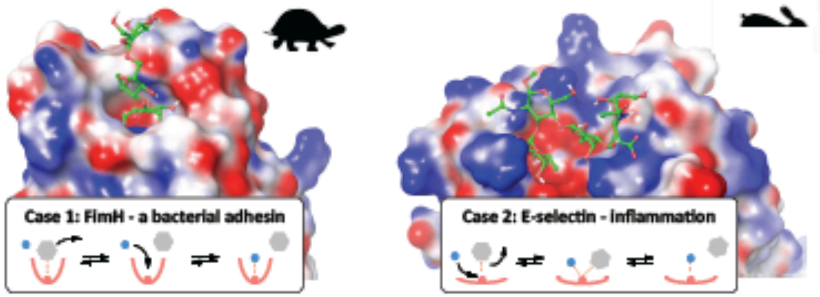
Druggability is the likelihood of modulating a therapeutic target with an orally available small molecule drug.
Hopkins & Groom, *Nat. Rev. Drug Discov.* 2002.

Carbohydrate-based Drugs



B. Ernst, J. Magnani, *Nat. Rev. Drug Disc.* 2009

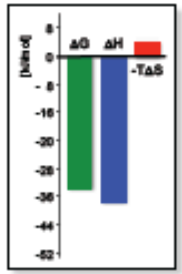
Druggability of Lectins - Mission Possible?

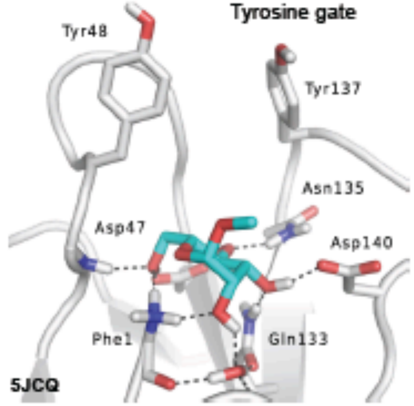


Properties	FimH		E-selectin	
Depth of binding site	deep	☺	flat	☹
Total size of contact area	210 Å²	☺	340 Å²	☹
Hydrophobic fraction thereof	43 Å²	☺	12 Å²	☹
Affinity of physiological ligand	trimannoside; µM	☺	sialyl Lewis^x; mM	☹
Residence time ($t_{1/2}$ of complex with physiol. ligand)	> min	☺	< 1 s	☹
Pharmacokinetic properties of CH ligands	log$D$ < 1, logP_e < - 5.7	☹	log$D$ < 1, logP_e < - 5.7	☹

Carbohydrate Recognition Domain of the Bacterial Lectin FimH

Methyl mannoside – K_D: 1 µM

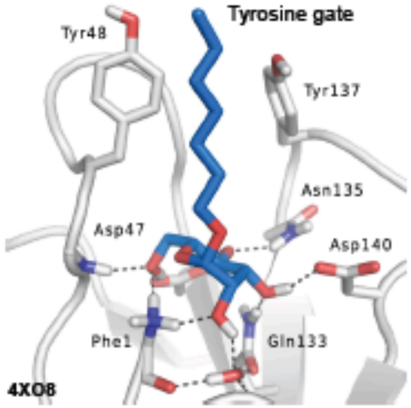


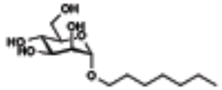


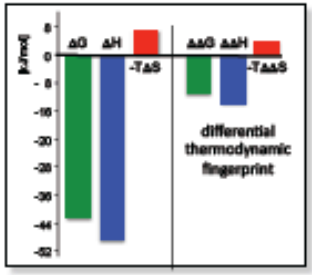
Pascal Zihlmann, Xiaohua Jiang, submitted.

Carbohydrate Recognition Domain of the Bacterial Lectin FimH

n-Heptyl mannoside – K_D: 29 nM







Pascal Zihlmann, Xiaohua Jiang, submitted.

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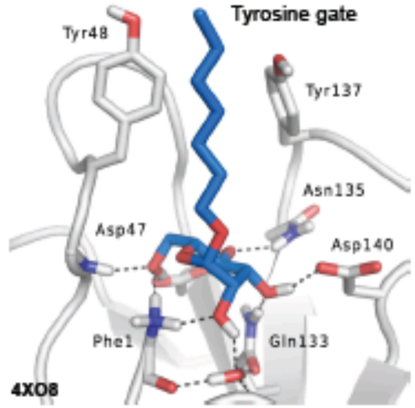
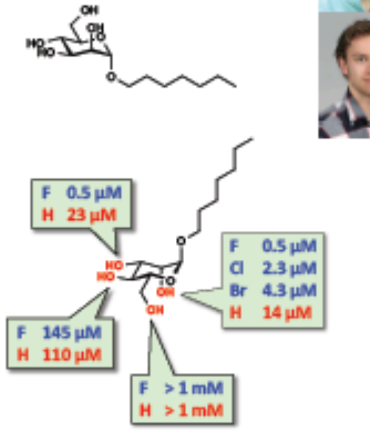
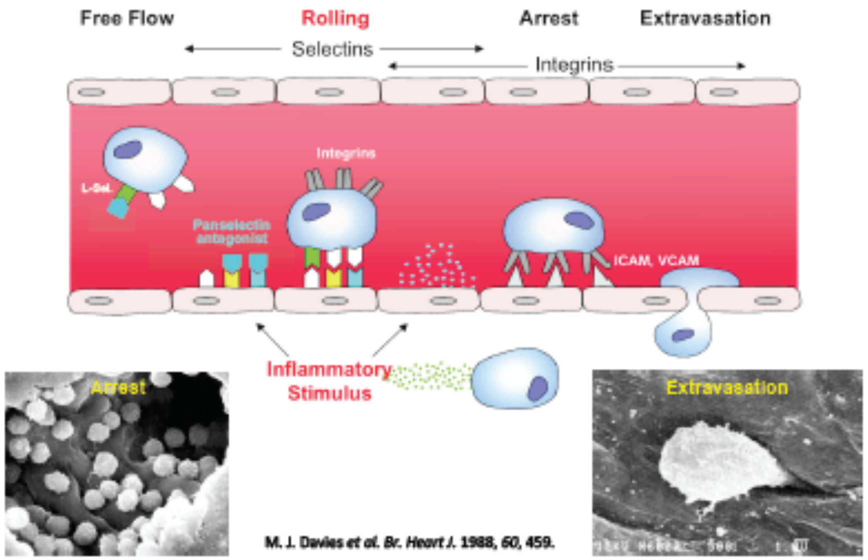




Other pyranoses (Glc, Gal, Tal....): K_D > 1 mM

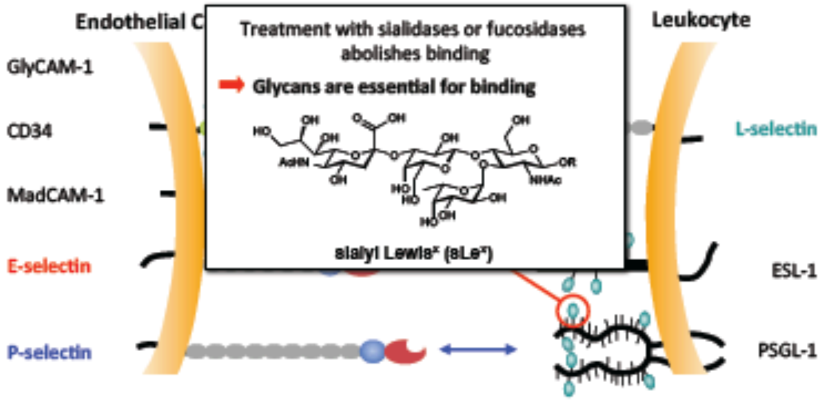
Pascal Zihlmann, Xiaohua Jiang, submitted.

Case Study: Selectin Antagonists



M. J. Davies *et al. Br. Heart J.* 1988, *60*, 459.

Origin of the Affinity Glycoprotein Ligands

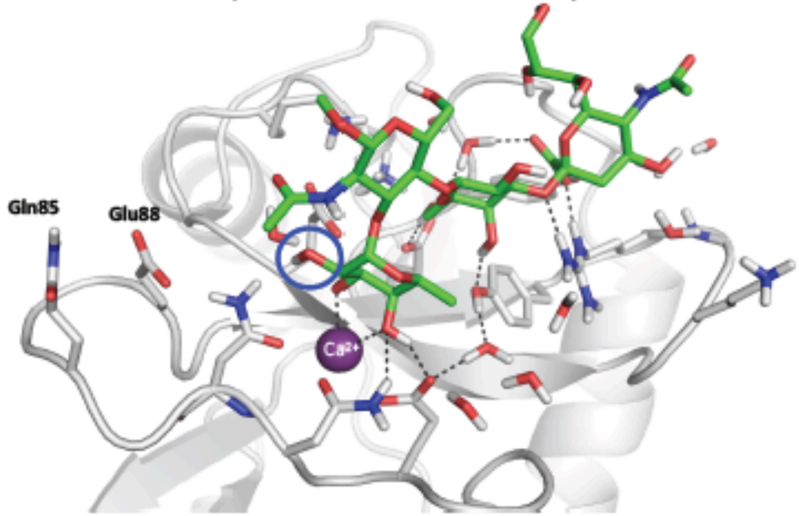


	K_D [µm]	$t_{1/2}$ [s]		K_D [µm]
L-selectin/CD34	108	0.06	L-selectin/sLe^x	≈ 3-7000
E-selectin/ESL-1	62	0.2	E-selectin/sLe^x	≈ 1000
P-selectin/PSGL-1	0.3	0.5	P-selectin/sLe^x	≈ 3-7000

D. Vestweber *et al. JBC* 2001, *34*, 31602-31612.

The Pharmacophore Dilemma: 2-Hydroxy of Fucose

(E-selectin soaked with sLe^x)

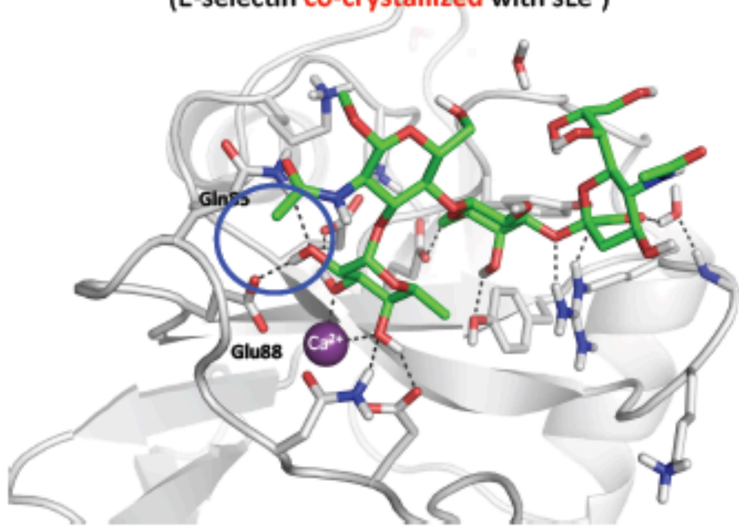


PDB ID: 1G1T

W.S. Somers *et al.* *Cell* 2000.

The Pharmacophore Dilemma: 2-Hydroxy of Fucose

(E-selectin co-crystallized with sLe^x)

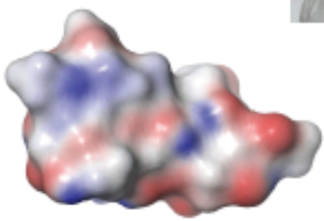


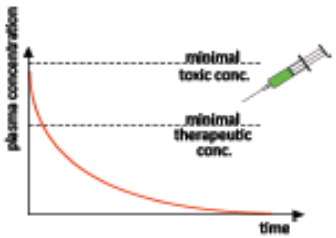
PDB ID: 4CSY

Roland C. Preston *et al.* *J. Mol. Chem. Biol.* 2015.

Druggability of E-selectin

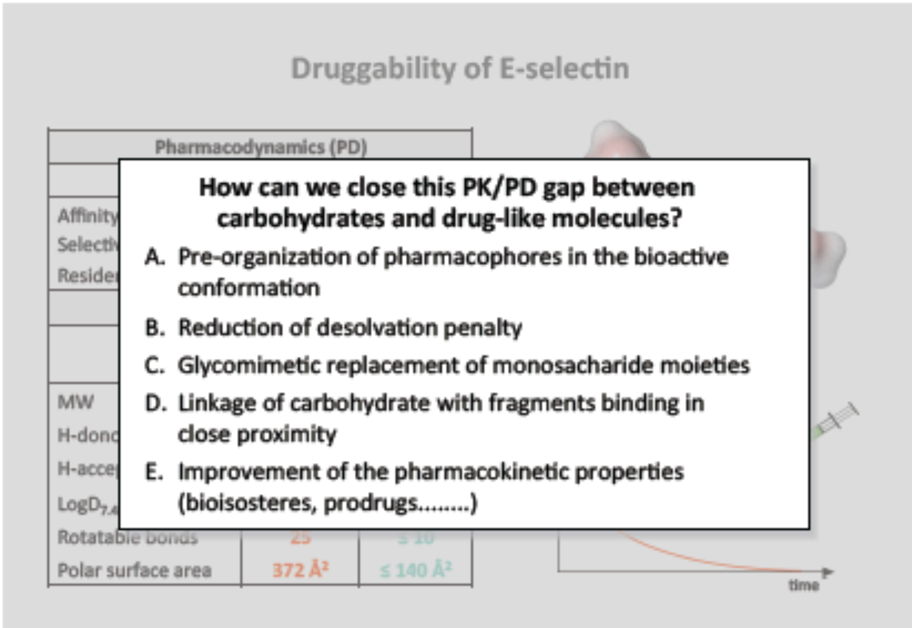
Pharmacodynamics (PD)		
	sialyl Lewis^x	Drug-like
Affinity (E-selectin)	1 mM	1 nM
Selectivity	Low	High
Residence time ($t_{1/2}$)	< 1 sec	> 10 min
Pharmacokinetics (PK)		
	sialyl Lewis^x	For oral availability
MW	834	500
H-donors	14	≤ 5
H-acceptors	25	≤ 10
$LogD_{7.4}$	- 6.1	< 5 (1 - 3)
Rotatable bonds	25	≤ 10
Polar surface area	372 Å²	≤ 140 Å²





Philipp Dätwyler

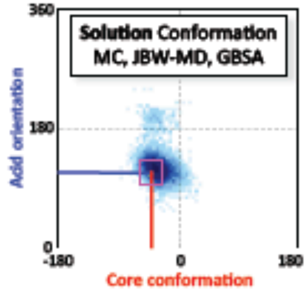
Druggability of E-selectin

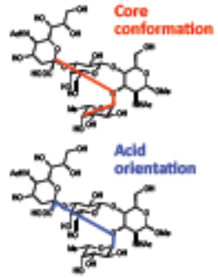


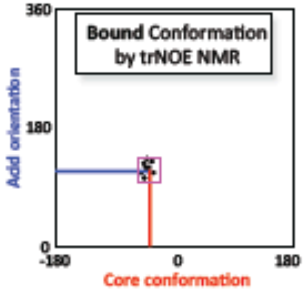
How can we close this PK/PD gap between carbohydrates and drug-like molecules?

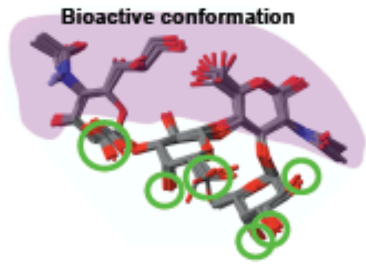
A. Pre-organization of pharmacophores in the bioactive conformation
B. Reduction of desolvation penalty
C. Glycomimetic replacement of monosacharide moieties
D. Linkage of carbohydrate with fragments binding in close proximity
E. Improvement of the pharmacokinetic properties (bioisosteres, prodrugs........)

A. Pre-Organization of Bioactive Conformation









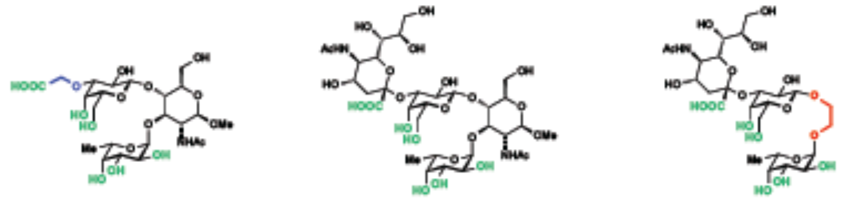
Pharmacophores by synthesis and STD-NMR

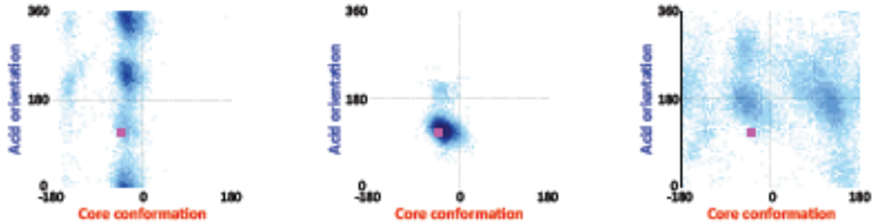
Not involved in binding; spacial task

Chem. Eur. J. 1997;
Pure Appl. Chem. 1997;
Angew. Chem. 1999;
J. Am. Chem. Soc. 2013.

Angew. Chem. 1995;
J. Biomol. NMR 1997;
J. Am. Chem. Soc. 1999.

The Importance of Pre-organization



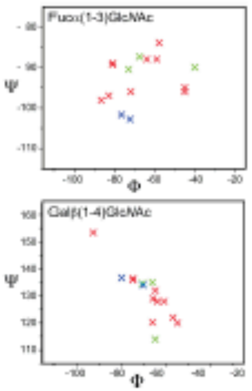


$K_D \geq 10$ mM | $K_D \approx 1$ mM | $K_D \geq 10$ mM

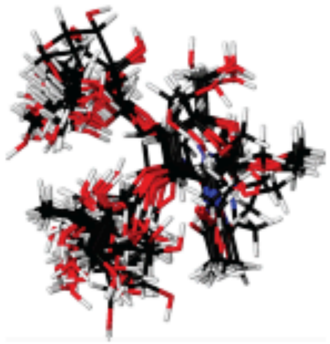
Total loss of affinity although all pharmacophores are still present!

What are the Stabilizing Forces for the Lewis^x Core?

Literature value for the two glycosidic bonds of Lewis^x

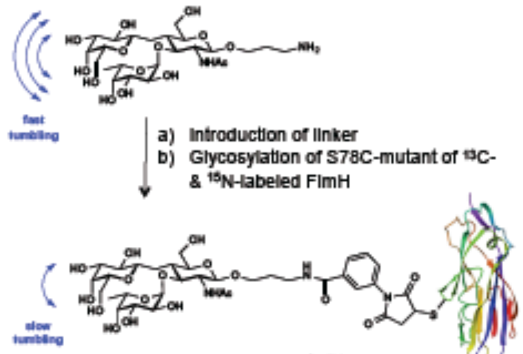


Solution structure of Lewis^x determined by 1H-NMR

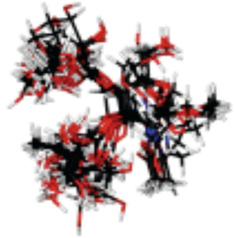


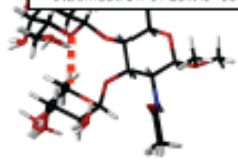
M. Zierke *et al. JACS* 2013, *135*, 13464.

Increase of Tumbling Rate by Linking to ^{13}C,^{15}N-labeled Protein



9 inter-residual NOEs define Lewis^x structure in solution

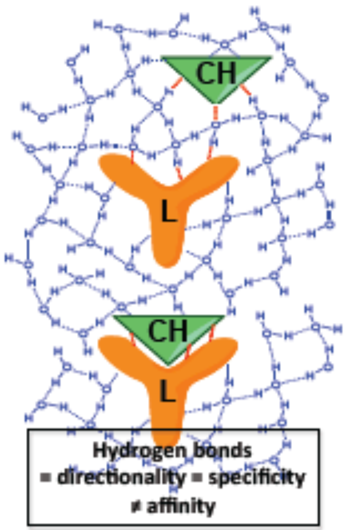




Non-conventional H-bond
- H5–O5 distance is 2.50Å (sum of van der Waals radii: 2.61Å)
- Major contribution to the stabilization of Lewis^x core: 40%

M. Zierke *et al. JACS* 2013, *135*, 13464.

B. Desolvation Costs and Water Reorganization



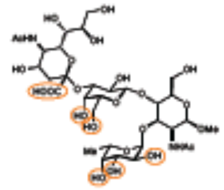
Group	ΔG (kJ/mol)	ΔH (kJ/mol)	-TΔS [kJ/mol]
OH	25.95	36.38	-10.43
NH_2	24.13	33.21	-9.08
SH	13.55*		
CH	1.06	3.07	-2.01
CH_3	-3.17	2.39	-5.56
F	5.14	1.87	3.27
Cl	7.26	6.90	0.36
Br	7.75	9.27	-1.52

S. Cabani *et al. J. Solution Chem.* 1981
* Minnesota Solvation Database

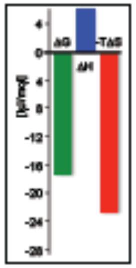
Hydrogen bond	ΔG° [kJ/mol]
O-H---O	-19.8
O-H---N	-18.6
N-H---O	-16.4

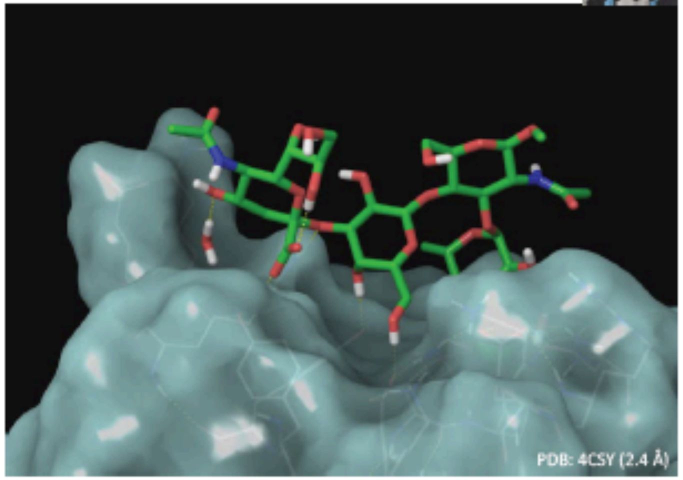
Vedani & Dunitz *J. Am. Chem. Soc.* 1985, *107*, 7653.

B. Desolvation Costs and Water Reorganization



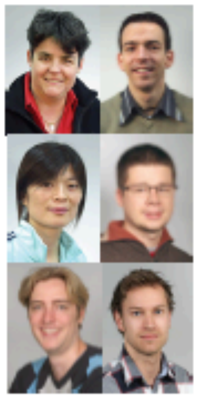
sLe^x: K_D 877 µM
Angew. Chem. 2012.

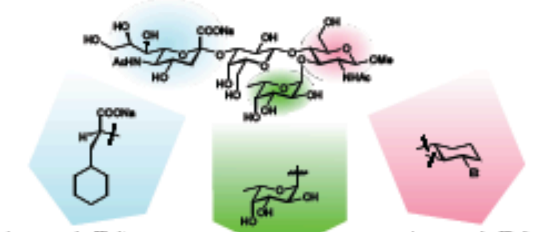




Roland C. Preston *et al. J. Mol. Cell Biol.* 2015.

C. Glyomimetic Replacement of Monosaccharide Moieties





Improved affinity by a factor of 5

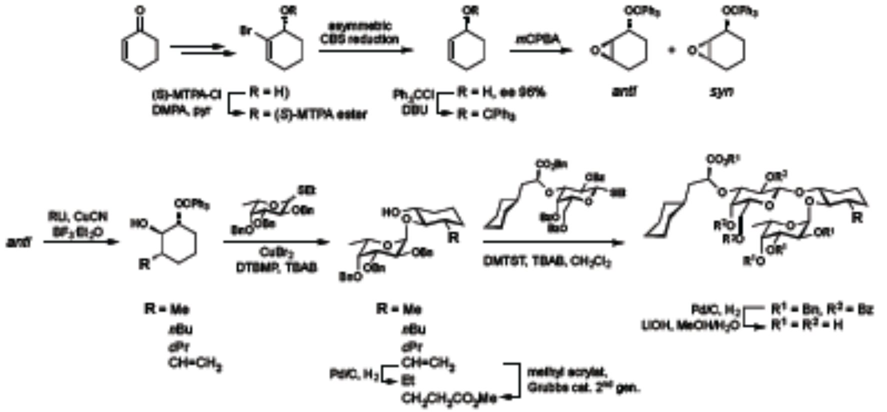
No improvement of affinity

Improved affinity by a factor of 10

H. C. Kolb, WO 97/01569, prior. date 26.6.96.
A.Titz *et al. Eur. J. Org. Chem.* 2012, 5534.
D. Schwizer *et al. Chem. Eur. J.* 2012, *18*, 1342.

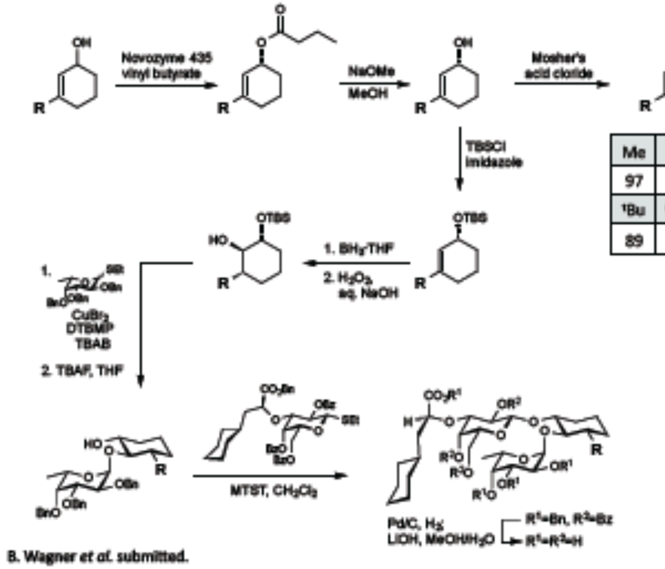
Beatrice Wagner
Daniel Schwizer
Xiahua Jiang
Norbert Varga
Pascal Zihlmann
Roland C. Preston

Glc*N*Ac-Mimetics – Chemical Approach

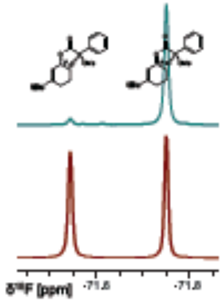


D. Schwizer *et al. Chem. Eur. J.* 2012, *18*, 1342.

GlcNAc-Mimetics – Enzymatic Approach (1)

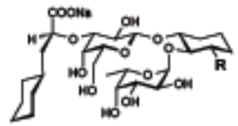


Me	Et	Pr	nBu	iBu
97	96	85	90	95 ee
tBu	nHex	Ph	CH_2Ph	$CH_2C_6H_{11}$
89	94	98	97	97 ee



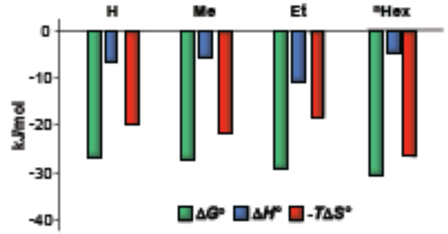
B. Wagner *et al.* submitted.

GlcNAc-Mimetics – Enzymatic Approach (2)



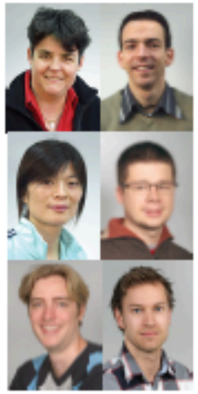
R on GlcNAc mimic	H	Me	Et	Pr	nBu	iBu	tBu	nHex	Ph	CH_2Ph	$(CH_2)_2Ph$	$CH_2C_6H_{11}$
K_D [nM]	61	14	10	7	5.4	6	51	4	8.8	18	28	14

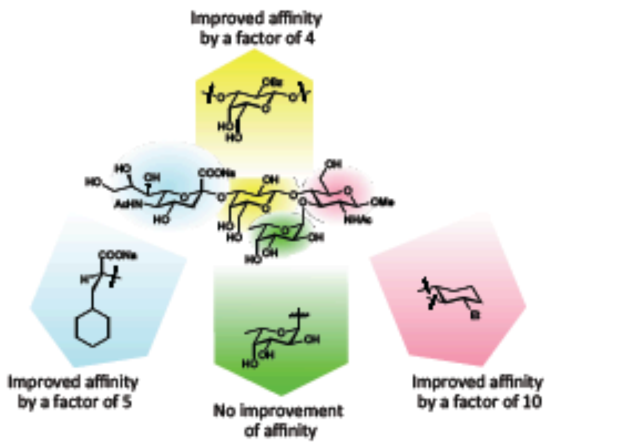
Ligand	K_D [nM]	$\Delta G°$ [kJ/mol]	$\Delta H°$ [kJ/mol]	$-T\Delta S°$ [kJ/mol]
H	61	-24.1	-5.3	-18.8
Me	14	-27.1	-5.9	-21.2
Et	10	-29.0	-10.7	-18.3
nHex	4	-30.8	-4.7	-26.1



B. Wagner, X. Jiang, P. Zihlmann *et al.* submitted.

C. Glyomimetic Replacement of Monosaccharide Moieties

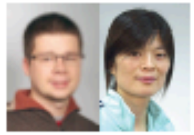


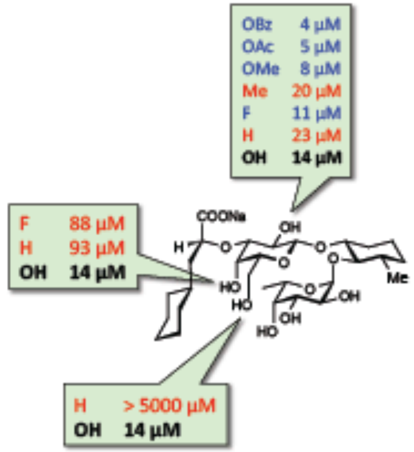


H. C. Kolb, WO 97/01569, prior. date 26.6.96.
A.Titz *et al. Eur. J. Org. Chem.* 2012, 5534.
D. Schwizer *et al. Chem. Eur. J.* 2012, *18*, 1342.
G. Thoma *et al. Angew. Chem.* 2001, *113*, 3756.

Beatrice Wagner
Daniel Schwizer
Xiahua Jiang
Norbert Varga
Pascal Zihlmann
Roland C. Preston

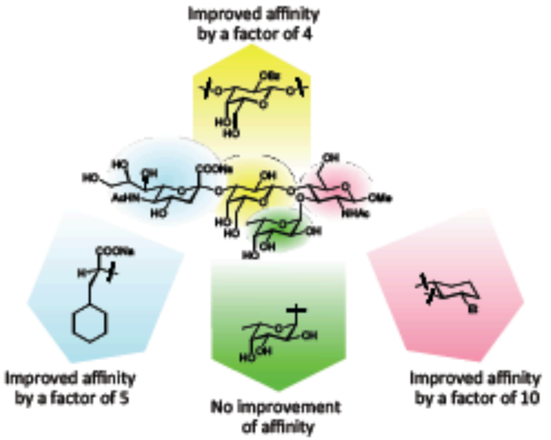
Gal-Mimetics

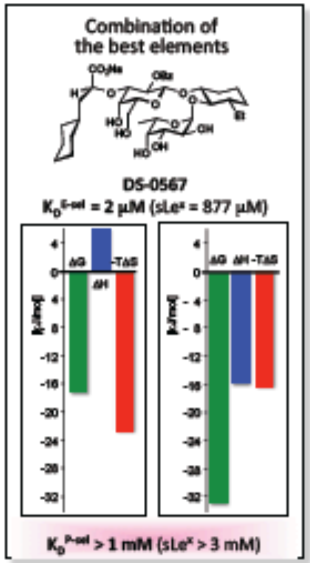




N. Varga, X. Jiang *et al.* submitted.

C. Glyomimetic Replacement of Monosaccharide Moieties





H. C. Kolb, WO 97/01569, prior. date 26.6.96.
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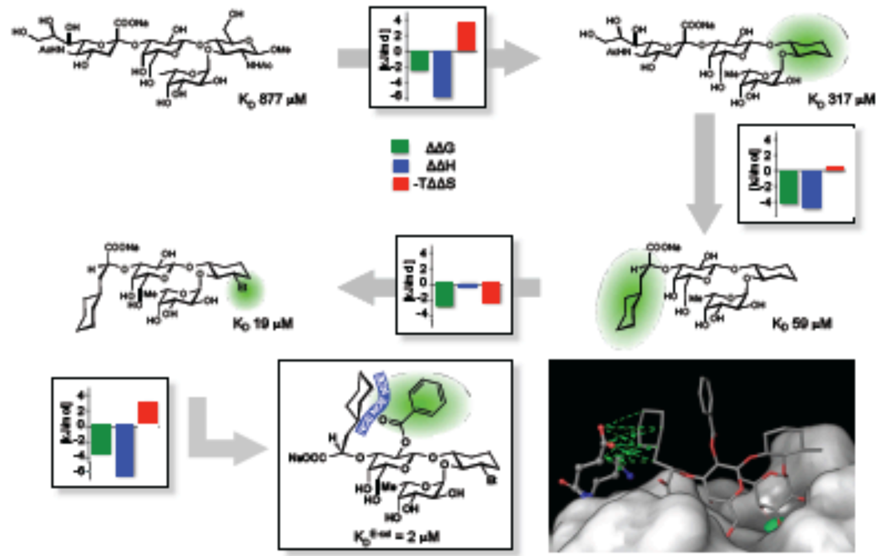
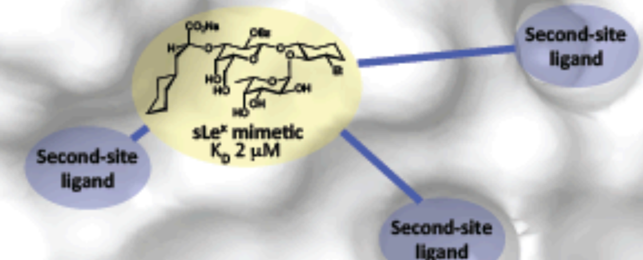
A.Titz *et al. Eur. J. Org. Chem.* 2012, 5534.
D. Schwizer *et al. Chem. Eur. J.* 2012, *18*, 1342.

ITC for Mimetics of sLe^x/E-selectin: ΔΔH & -TΔΔS



Florian Binder, Katrin Lemme, Roland Preston, *Angew. Chem.* 2012.

D. Linkage of CH with Fragments Binding in Close Proximity



Thermodynamic additivity principle[1]

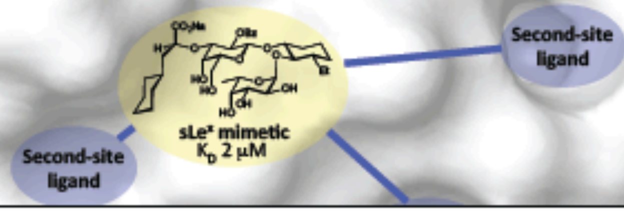
When two independent fragments A and B are linked, then the total change in free energy is the sum of the individual free energies; $\Delta G = \Delta G^A + \Delta G^B$; $K_D = K_D^A \cdot K_D^B$

In addition, linking of two fragments leads to:

- **Reduction of translational and rigid body rotational entropy costs** (binary vs. ternary complex, approx. 10 kJ/M)
- **Favorable/unfavorable interactions** caused by linker
- **Entropy costs for rotatable bonds**[2] added by the linker (two rotatable bonds add approx. 2 to 3 kJ/M to the entropy costs; factor 5)

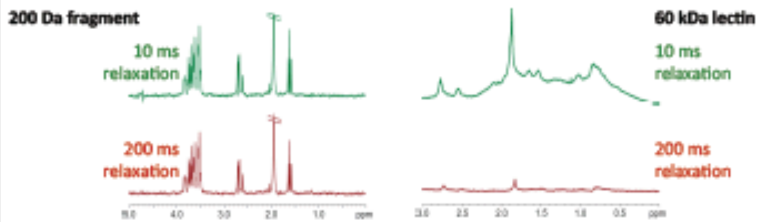
[1] W.P. Jencks, *PNAS USA* 1981, *78*, 4046; [2] G.M. Whitesides, *J. Org. Chem.* 1998, *63*, 3168.

D. Linkage of CH with Fragments Binding in Close Proximity



1st Step: Identification of Second-site Ligands

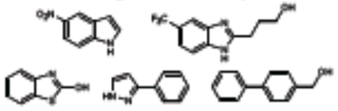
Transverse Magnetization Decay Depends on the Molecular Weight

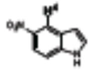


B. Ernst, B. Cutting, S. Shelke, PCT WO 2007/105094 A1; Jonas Egger *et al. J. Am. Chem. Soc.* 2013, *135*, 9820.

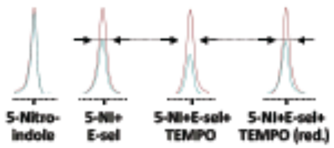
2nd: Identification of "Real" Second-site Ligands

Second-site ligands identified by NMR-screening based on transverse magnetization decay

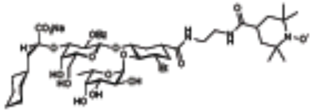




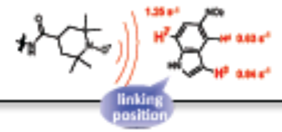
green: 10ms relaxation
red: 200ms relaxation



Glycomimetic with TEMPO

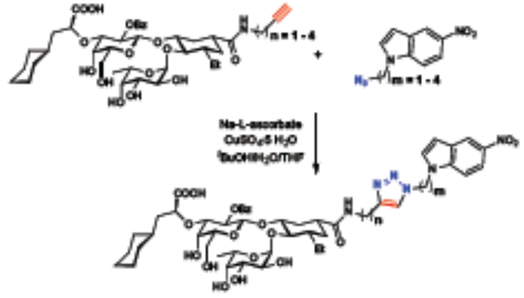


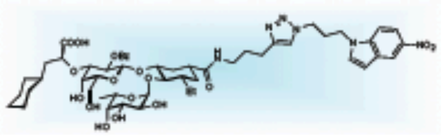
Orientation of second-site ligand



Jonas Egger, Céline Weckerle *et al. J. Am. Chem. Soc.* 2013, *135*, 9820.

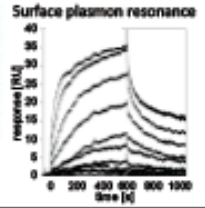
3rd Step: Linking Glycomimetic & Real Second-Site Ligand

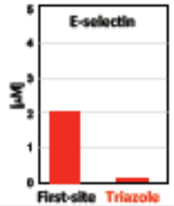




K_D for E-selectin: 30 nM
P-selectin: > 1 mM

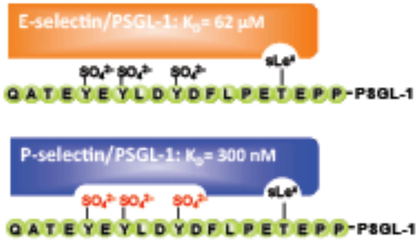
k_{on}: 1.42·10⁵ $M^{-1}s^{-1}$
k_{off}: 0.0026 s^{-1}
$t_{1/2}$: 250 s





Jonas Egger, Céline Weckerle *et al. J. Am. Chem. Soc.* 2013, *135*, 9820.

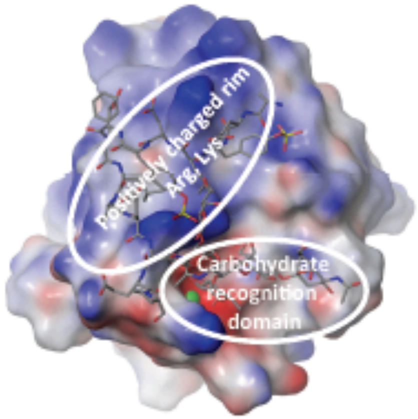
Pan-Selectin Antagonists: Screening a Thematic Library of Fragments



Binding to P-selectin

Glyco-sulfopeptide	rel. binding	Glyco-sulfopeptide	rel. binding
sLex	100 %	sLex	3 %
sLex	15 %	Lex	6 %
sLex	6 %	Sia-LacNAc	0 %

Leppänen *et al. J. Biol. Chem.* 2003, *278*, 26391-26400.

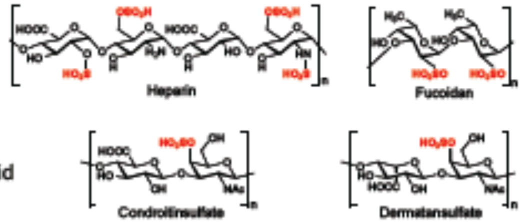


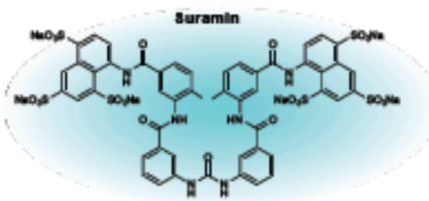
P-selectin co-crystallized with PSGL-1 fragment
W.S. Somers *et al. Cell* 2000.

Unspecific P-selectin Antagonists

A wide range of structurally diverse, negatively charged molecules have been reported to bind to P- and L-selectin:

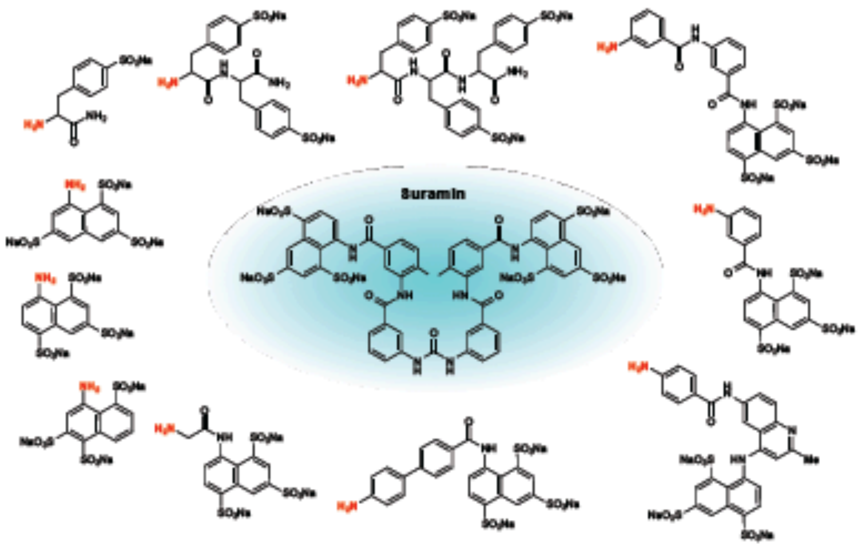
- Sulfatides
- Heparins
- Fucoidan
- Sulfated dextran
- Condroitin sulfate
- Dermatan sulfate
- Sulfated hyaluronic acid
- Sulfogalabiose
- Suramin



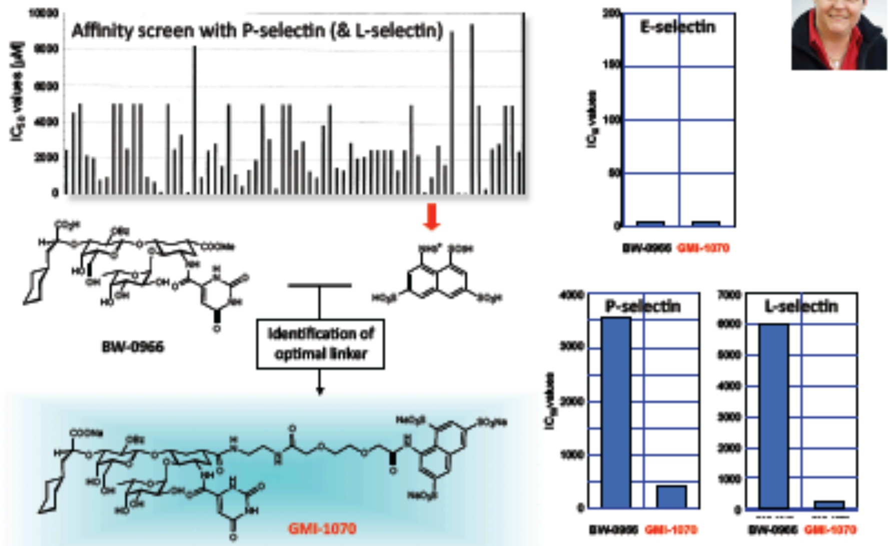


- Polysulfonated naphthylurea
- Paraciticide (sleeping sickness)
- Anticancer agent that inhibits tumor cell proliferation
- **Binds to P- & L-selectin, not to E-selectin**
- **Plasma half-life: > 50 h!**

Thematic Library of Suramin Fragments – Some Examples

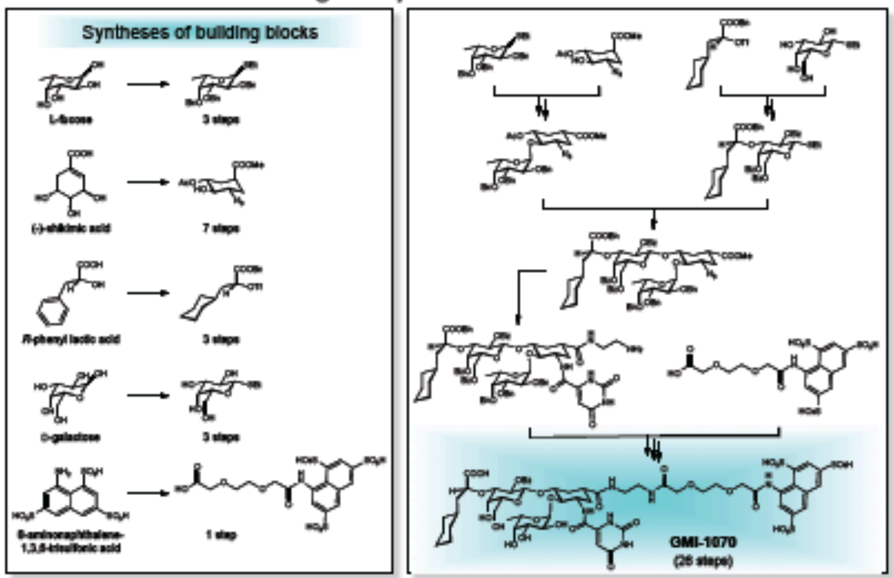


Pan-Selectin Antagonist



Beatrice Wagner; John L. Magnani, Arun Sarkar, John T. Patton/GMI

Convergent Synthesis of GMI-1070



Effect of GMI-1070 in Preclinical Animal Models with an inflammatory component

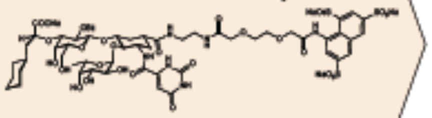
GMI-1070 inhibits thrombus formation	✓
GMI-1070 increases survival in multiple myeloma	✓
GMI-1070 inhibits relapse in multiple sclerosis (EAE)	✓
GMI-1070 reduces infarct size in a myocardial infarct model	✓
GMI-1070 protects mice treated with chemotherapy	✓
GMI-1070 resolves vaso-occlusive crisis in sickle cell disease mice	✓

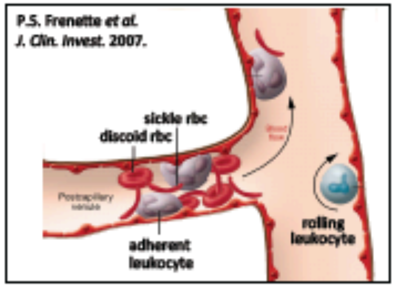
John L. Magnani, GlycoMimetics, Inc.

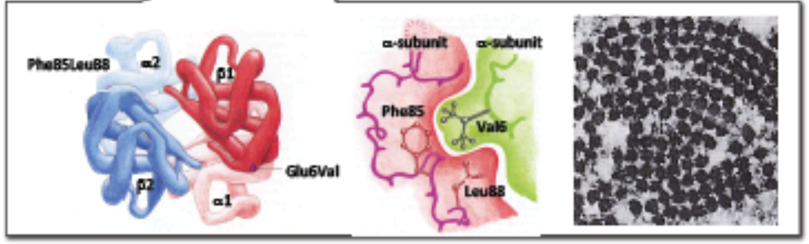
Effect of GMI-1070 in Sickle Cell Mice

Pre-clinical Animal Models (with an inflammatory component)
Treatment of vaso-occlusive crisis in sickle cell mice

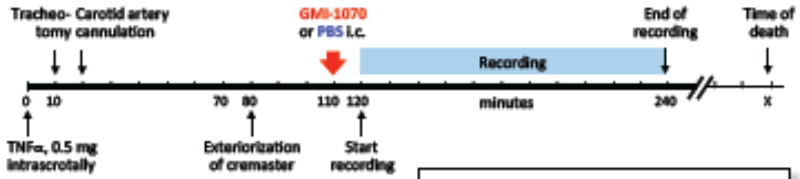
Clot formation is dissolved by GMI-1070

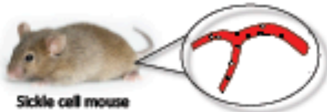






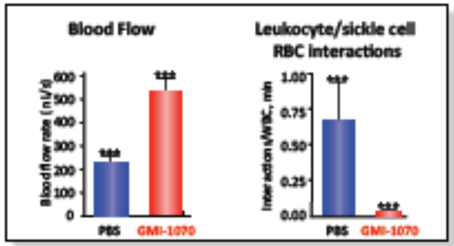
Effect of GMI-1070 in Sickle Cell Mice

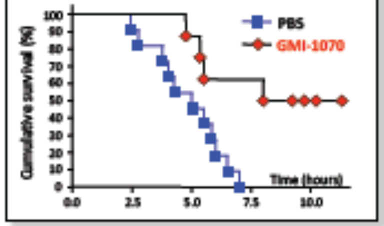




Treatment leads to

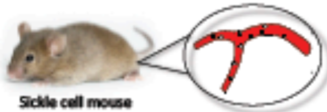
- improved blood flow
- reduced leukocyte/sickle RBC interaction
- reduced leukocyte/endothelial interaction
- increased survival



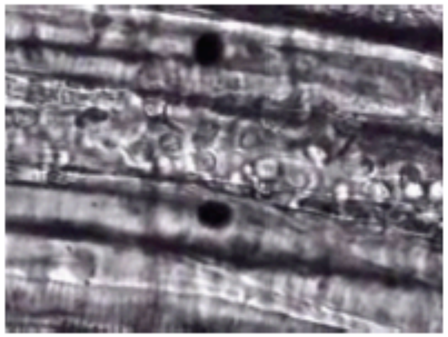


Paul Frenette *et al. Blood* 2010.

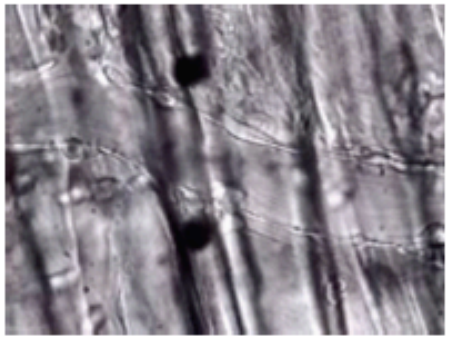
Sickle Cell Mouse In a Vasoocclusive Crisis



PBS treated



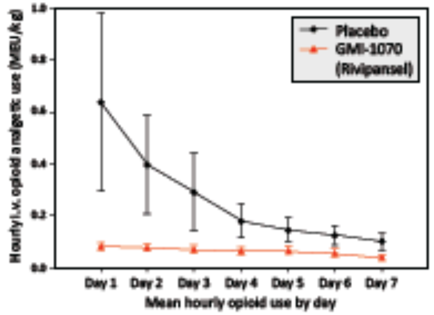
GMI-1070 treated



GlycoMimetics Announces Top Line Results from Phase 2 Trials with GMI-1070 in Sickle Cell Disease

Patients Treated with GMI-1070 Experience Improved Outcomes in Vaso-Occlusive Crisis
Press Release: GlycoMimetics, Inc. - Tue, April 16, 2013 6:00 AM EDT

3 year Phase 2 clinical trial in 22 hospital sites in the US and Canada on the treatment of Sickle Cell Disease patients in Vaso-occlusive Crisis (VOC).



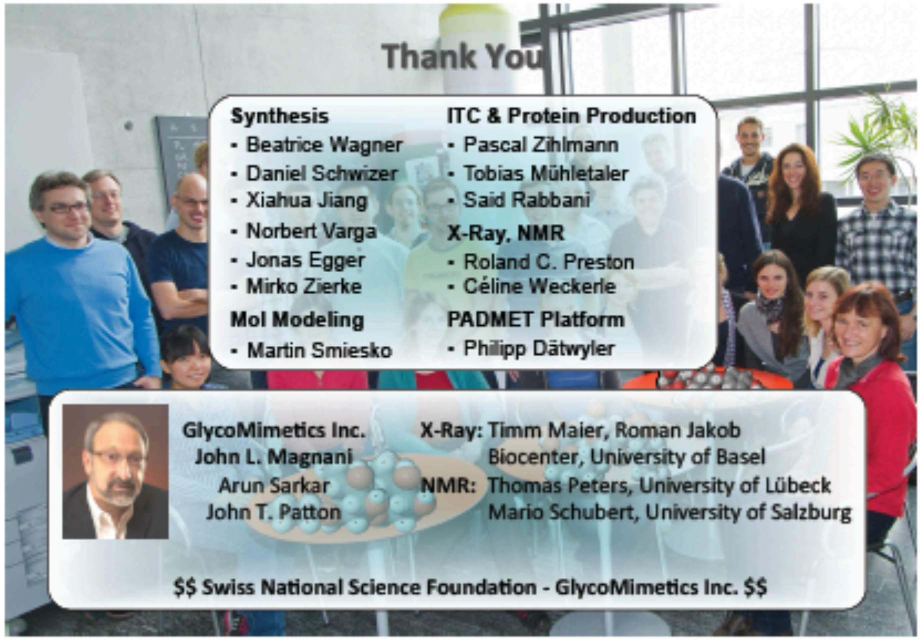
Double-blinded and placebo-controlled.

Reduction of multiple **Clinical Endpoints:**

- Reduction in duration of vaso-occlusive crisis
- Reduction in length of hospital stay
- Reduction in the use of narcotic pain relief
- **Reduction of irreversible, cumulative organ damage**

Blood 2015, *125*, 2656-2664.


Pfizer Announces Enrollment Of First Patient In Phase 3 Trial
In Sickle Cell Disease
RESET Trial to Assess Effectiveness and Safety of Rivipansel (GMI-1070) in the
Treatment of Vaso-Occlusive Crisis in Hospitalized Individuals with Sickle Cell Disease
June 23, 2015
NASDAQ: GLYC
2014
NASDAQ
Druggability of Lectins - Mission Possible?


Thank You
Synthesis
- Beatrice Wagner
- Daniel Schwizer
- Xiahua Jiang
- Norbert Varga
- Jonas Egger
- Mirko Zierke
Mol Modeling
- Martin Smiesko
ITC & Protein Production
- Pascal Zihlmann
- Tobias Mühletaler
- Said Rabbani
X-Ray, NMR
- Roland C. Preston
- Céline Weckerle
PADMET Platform
- Philipp Dätwyler
GlycoMimetics Inc.
John L. Magnani
Arun Sarkar
John T. Patton
X-Ray: Timm Maier, Roman Jakob
Biocenter, University of Basel
NMR: Thomas Peters, University of Lübeck
Mario Schubert, University of Salzburg
$$ Swiss National Science Foundation - GlycoMimetics Inc. $$