CEITEC
Central European Institute of Technology
BRNO | CZECH REPUBLIC

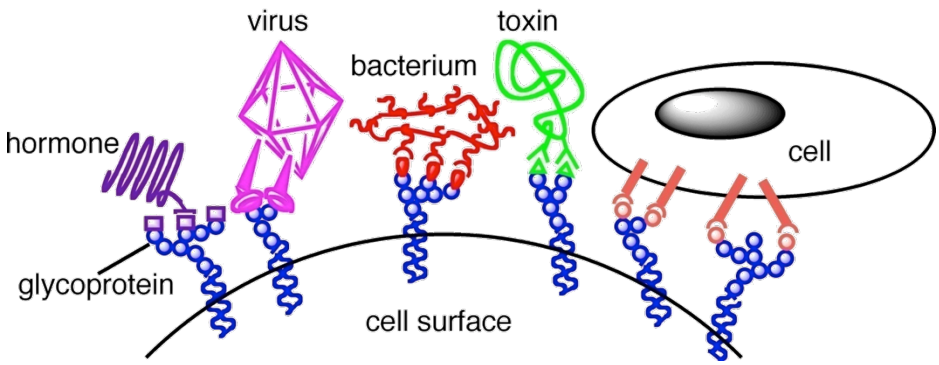
Combination of various structural and biophysical approaches to study lectins from pathogens

Michaela Wimmerová
Glycobiochemistry group

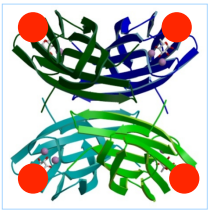
MASARYK UNIVERSITY
CENTRAL EUROPEAN INSTITUTE OF TECHNOLOGY

BRNO, CZECH REPUBLIC

INTERACTION BETWEEN PROTEINS AND GLYCO-CONJUGATES

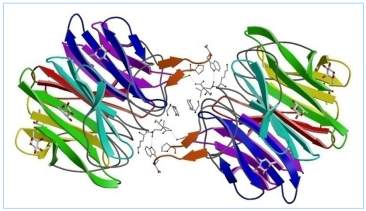


LECTINS



- Sugar binding proteins
- Ubiquitous
- Generally **week affinity** toward monosaccharides

- Avidity effects through multivalency
  - Tandem repeat
  - Oligomerisation
  - Surface presentation

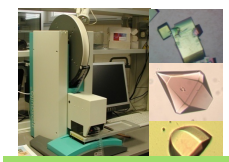


KEY INFORMATION IN PROTEIN/CARBOHYDRATE INTERACTION

Molecular biology and Biochemistry

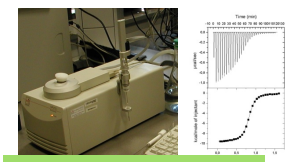
Gene → Protein ← Mutagenesis

Structure	Specificity	Affinity
Crystallography AUC, CD, NMR,..	hemagglutination, ELLA, glycochips, SPR, MST, ..	ITC, SPR, MST, FA,..



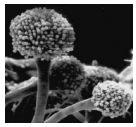
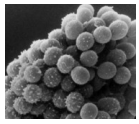
Mosquito, Rigaku UV, Tecan EVO

BiaCore 3000, T200,NT.114

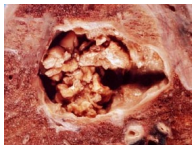


VP-ITC, VP-DSC, ITC_{200}, AutoITC

ASPERGILLUS FUMIGATUS

- Saprophytic mold
- Human opportunistic pathogen
- spores ubiquitous in the environment
- The most common nosocomial fungal infection



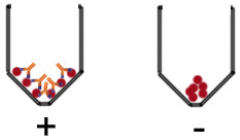
aspergilloma (fungus ball) in human lungs

invasive aspergillosis in the cerebrum

Alergic bronchopulmonar aspergillosis
aspergilloma
Invasive aspergillosis

BINDING SPECIFICITY – (HEM)AGGLUTINATION

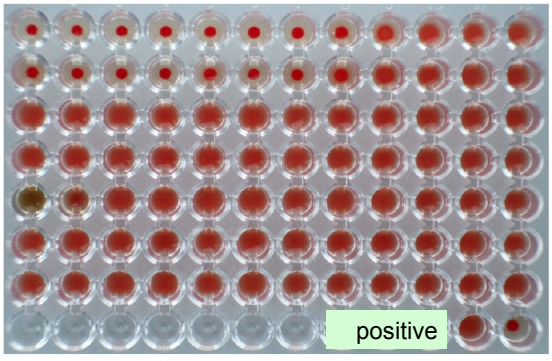
- agglutination of red blood cells
- multivalency needed



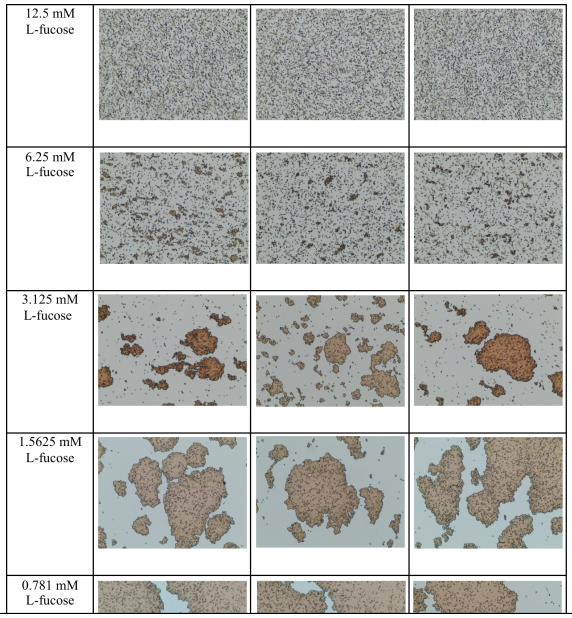
saccharide concentration

37,5 18,8 9,38 4,69 2,34 1,17 0,586 0,293 0,146 0,073 0,037 0,014

Methyl-α,L-fucoside
L-fucose
Methyl-α,L-mannoside
D-mannose
sialic acid
D-galactose
D-glucose

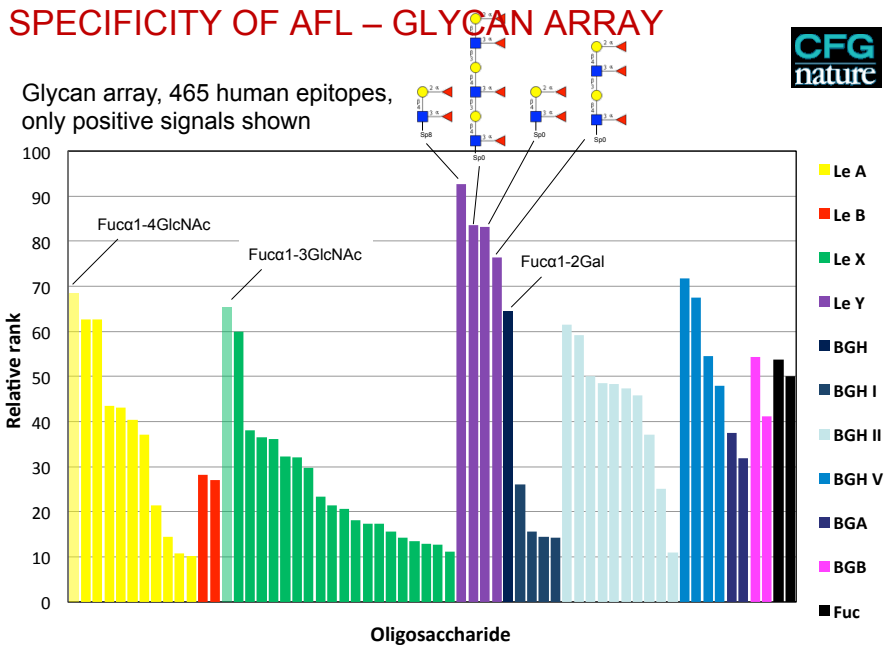


BINDING SPECIFICITY – (HEM)AGGLUTINATION



SPECIFICITY OF AFL – GLYCAN ARRAY

Glycan array, 465 human epitopes, only positive signals shown



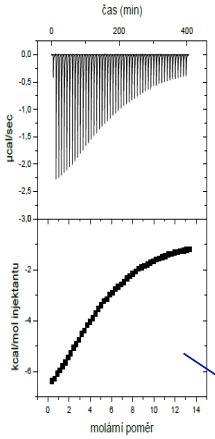
AFFINITY OF AFL TOWARDS FUCOSE APPARENT K_D

Isothermal titration calorimetry

Microscale thermophoresis

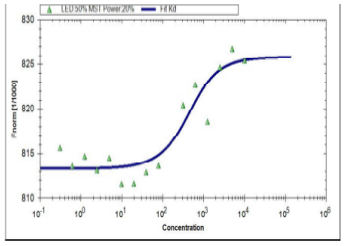
Surface plasmon resonance

VP-ITC



Kd approx. 165 µM

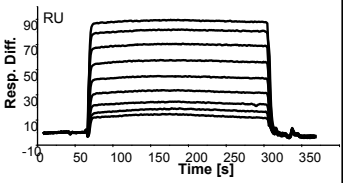
NT.115

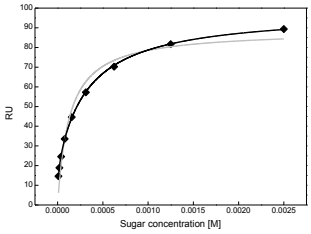


Kd 296 ± 51.3 µM

Impossible to measure on ITC200 due to precipitation (also cell volume matters)

Biacore3000





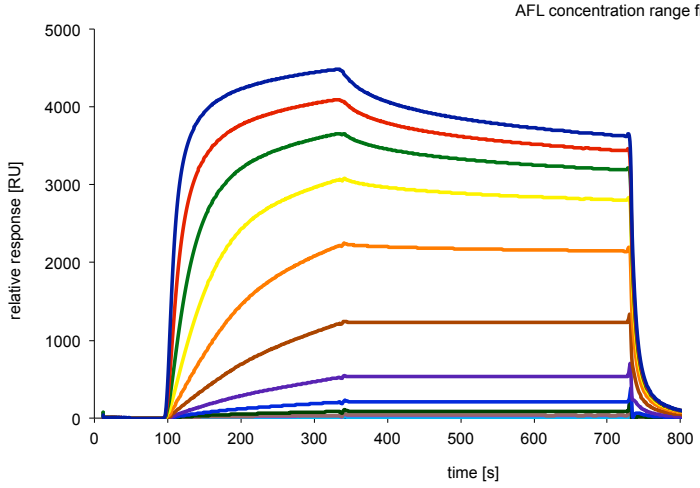
Kd 209 ± 15 µM

AVIDITY OF AFL TOWARDS SURFACE PRESENTED FUCOSIDES

SPR experiment in reverse arrangement (immobilized sugar)

AFL concentration range from 0.061 nM to 1 µM

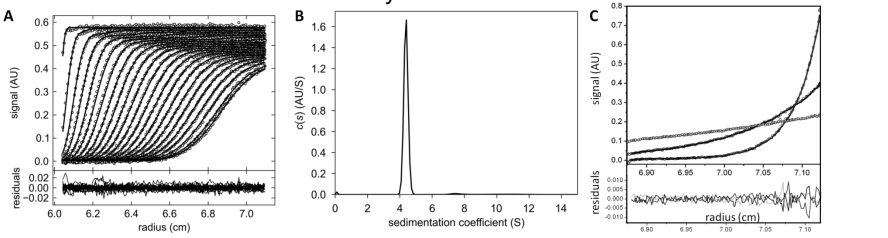
$K_{D\ av}$ = 96 nM

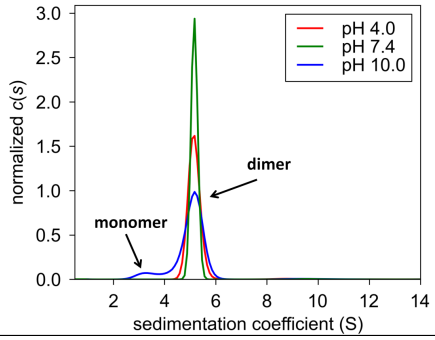


OLIGOMERIC STATE OF AFL - AUC

sedimentation velocity

sedimentation equillibrium

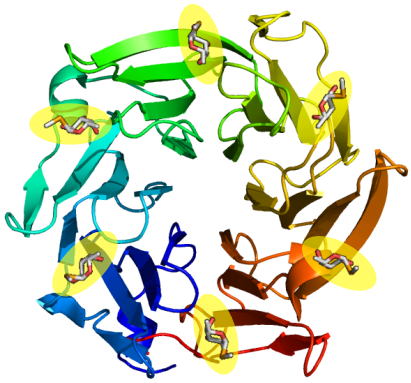


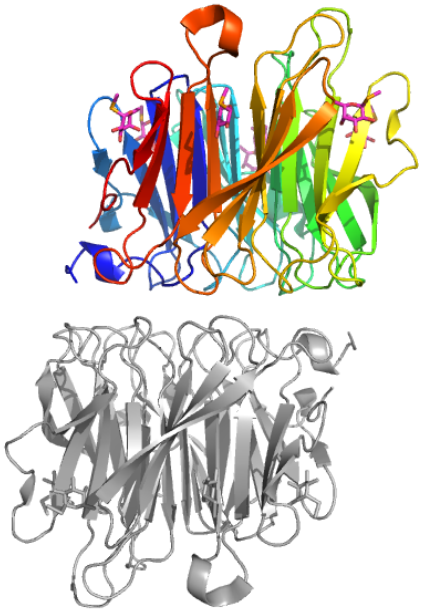


- AFL forms dimer
  - MW_{dimer}(AUC) = 67.3 kDa
  - MW_{dimer}(teor.) = 68.9 kDa
- Dissociation at basic pH

AFL STRUCTURE

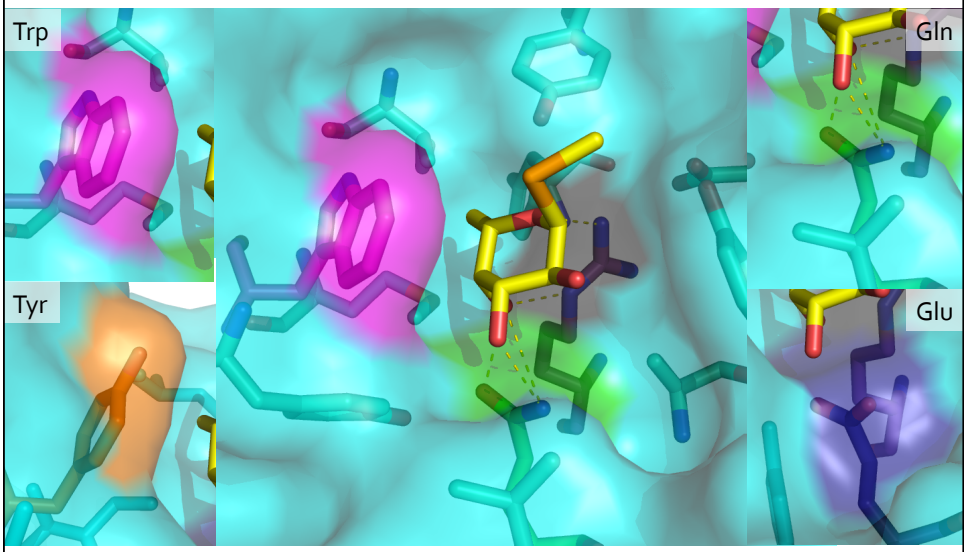
with Me-Se-α-L-fucopyranoside





Houser et al, Plos One 2003, Acta Cryst D 2015

AFL/SACCHARIDE INTERACTION

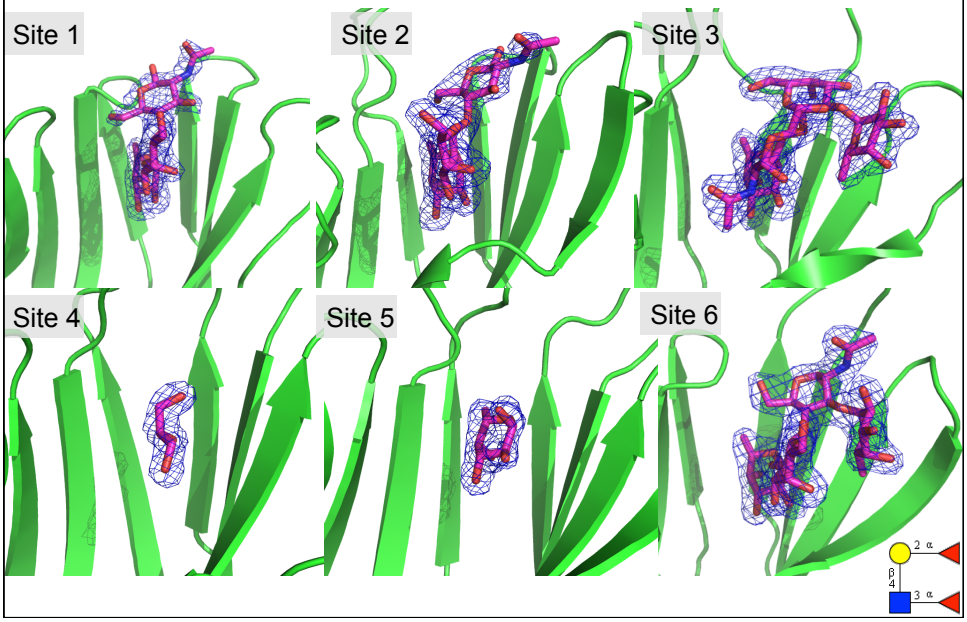


SURFACE PLASMON RESONANCE

Ligand	K_d (one site) [μM]	K_{d1} (two sites) [μM]	K_{d2} (two sites) [μM]
Fuc	110 ± 20	350 ± 20	7.1 ± 1.2
αMeFuc	120 ± 5.7	630 ± 450	80 ± 20
βMeFuc	260 ± 40	[a]	[a]
L-Gal	810 ± 70	[a]	[a]
αFuc(1-2)Gal	70 ± 3.4	100 ± 30	20 ± 20
αFuc(1-3)GlcNAc	60 ± 6	210 ± 20	7.5 ± 0.8
αFuc(1-4)GlcNAc	60 ± 7.6	240 ± 10	6.6 ± 0.5
αFuc(1-6)GlcNAc	90 ± 6.7	200 ± 30	10 ± 4
Blood group A trisaccharide	70 ± 5.7	440 ± 180	30 ± 4.8
Blood group B trisaccharide	60 ± 5.4	190 ± 30	10 ± 2.1
Blood group H II type trisaccharide	70 ± 7.7	200 ± 30	9.1 ± 2.1
Lewis a trisaccharide	90 ± 20	540 ± 160	9.7 ± 2.6
Lewis X trisaccharide	150 ± 20	280 ± 30	4.4 ± 1.9
Lewis b tetrasaccharide	140 ± 8.3	220 ± 20	10 ± 5.1
Lewis Y tetrasaccharide	70 ± 5.8	120 ± 10	3.1 ± 1.6

BINDING SITE NON-EQUIVALENCY

Le^Y tetrasaccharide



BINDING SITE NON-EQUIVALENCY

Equimolar mixture of Fucα1-2Gal, Fucα1-3GlcNAc, Fucα1-4GlcNAc, Fucα1-6GlcNAc

Site	SeMeFuc	L-Gal	β-MeFuc	Fuc (1-6) GlcNAc	Fuc (1-X) GlcNAc	BGA trisaccharide	Lewis Y tetrasaccharide
1					Fuc(1-4)GlcNAc		Fuc(1-2)Gal
2			glycerol		Fuc(1-4)GlcNAc		Fuc(1-2)Gal
3							Fuc(1-3)GlcNAc
4		PEG	glycerol				glycerol
5		PEG	glycerol				
6							Fuc(1-2)Gal

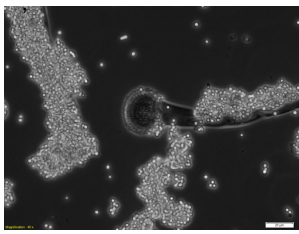
Glu Tyr Gln Trp

Whole monosaccharide recognized
Specific binding (second/third saccharide recognized)
Non-specific binding (only first saccharide recognized)
Specifically recognized motif
Undecided (binding stabilized by crystal formation)
Unoccupied site (no saccharide)

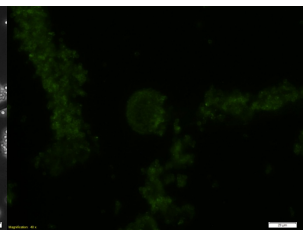
Josef Houser

AFL1 EXPRESSION ON *A. FUMIGATUS* CONIDIA

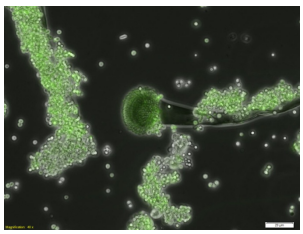
Cy3-goat anti-rabbit IgG, Rabbit AntiAFL



Phase contrast



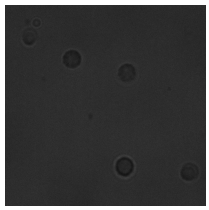
Fluorescence

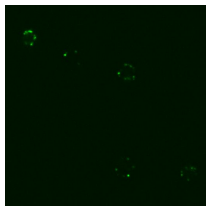


Superposition

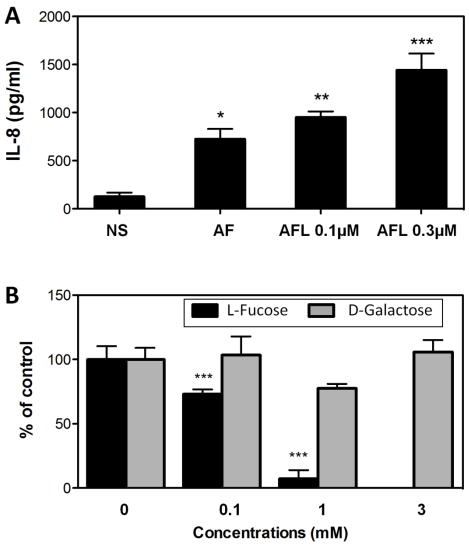
Permeable conidia

,



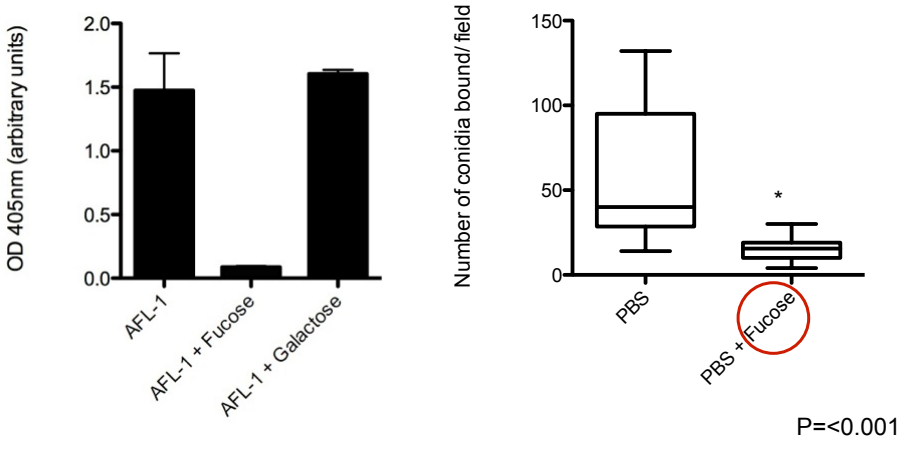


PROINFLAMATORY EFFECT OF AFL



Collaboration with M. Chignar, V. Balloy, Pasteur Institute, France

BINDING OF AFL AND *A. FUMIGATUS* CONIDIA TO HUMAN MUCINS



P=<0.001

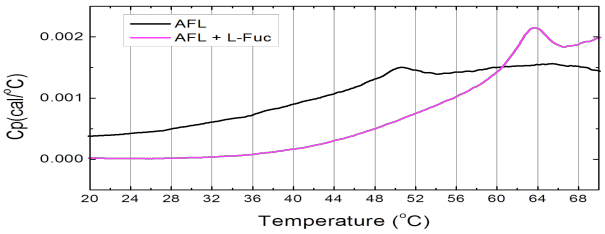
Collaboration with J. Fahy, S. Kerr, UCSF, USA

Methods for primarily different purposes can also be employed to search for protein ligands

most common approach – any method to measure protein **temperature stability**

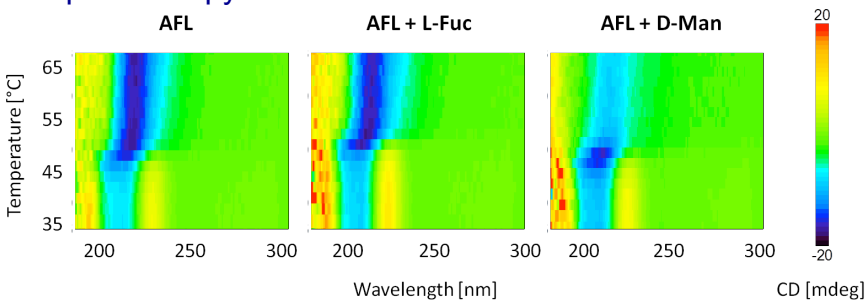
HEAT STABILITY OF AFL

Differential scanning calorimetry



Shift in Tm in the presence of **fucose** but not mannose

CD spectroscopy

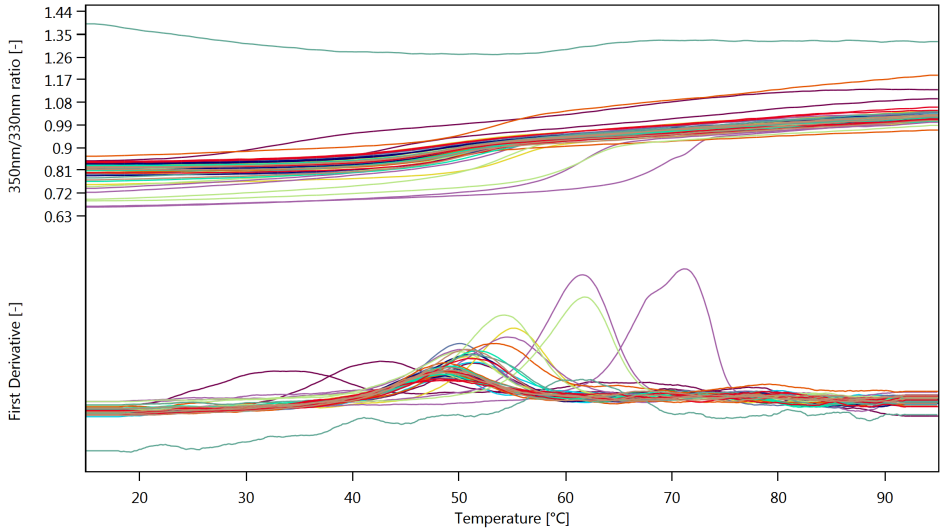


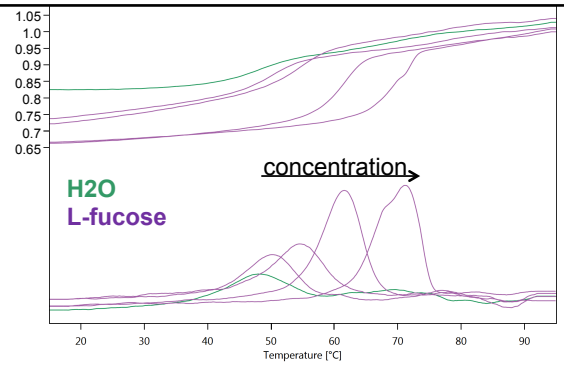
HEAT STABILITY OF AFL – SCREENING APPROACH: SUGAR/METAL SCREEN

		1	2	3	4	5	6	7	8	9	10	11	12
Sugars	A	H2O	**Sia**			**D-Gal**				**D-Man**			
			50 mM	5 mM	0.5 mM	0.5 M	50 mM	5 mM	0.5 mM	0.5 M	50 mM	5 mM	0.5 mM
	B	**L-Fuc**				**D-GlcNAc**				**D-Glc**			
		0.5 M	50 mM	5 mM	0.5 mM	0.5 M	50 mM	5 mM	0.5 mM	0.5 M	50 mM	5 mM	0.5 mM
	C	**L-Rha**				**D-Ara**				**L-Ara**			
		0.5 M	50 mM	5 mM	0.5 mM	0.5 M	50 mM	5 mM	0.5 mM	0.5 M	50 mM	5 mM	0.5 mM
	D	**D-Rib**				**D-Xyl**				**Glycerol**			
		0.5 M	50 mM	5 mM	0.5 mM	0.5 M	50 mM	5 mM	0.5 mM	0.5 M	50 mM	5 mM	0.5 mM
Ions (chlorides)	E	**Li+**				**Na+**				**K+**			
		2.5 M	250 mM	25 mM	2.5 mM	2.5 M	250 mM	25 mM	2.5 mM	2.5 M	250 mM	25 mM	2.5 mM
	F	**Mg2+**				**Ca2+**				**Mn(II)**			
		2.5 M	250 mM	25 mM	2.5 mM	2.5 M	250 mM	25 mM	2.5 mM	2.5 M	250 mM	25 mM	2.5 mM
	G	**Fe(II)**				**Co(II)**				**Ni(II)**			
		2.5 M	250 mM	25 mM	2.5 mM	2.5 M	250 mM	25 mM	2.5 mM	2.5 M	250 mM	25 mM	2.5 mM
	H	**Cu(II)**				**Zn(II)**				**Cd(II)**			
		2.5 M	250 mM	25 mM	2.5 mM	2.5 M	250 mM	25 mM	2.5 mM	2.5 M	250 mM	25 mM	2.5 mM

DIFFERENTIAL SCANNING FLUORIMETRY: AFL/SUGAR SCREEN

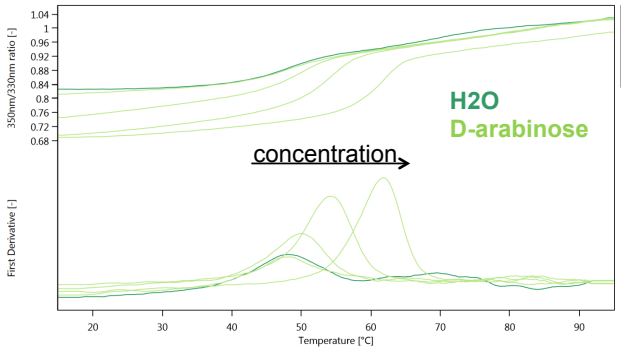
Prometheus (Nanotemper) with intrinsic protein fluorescence detection

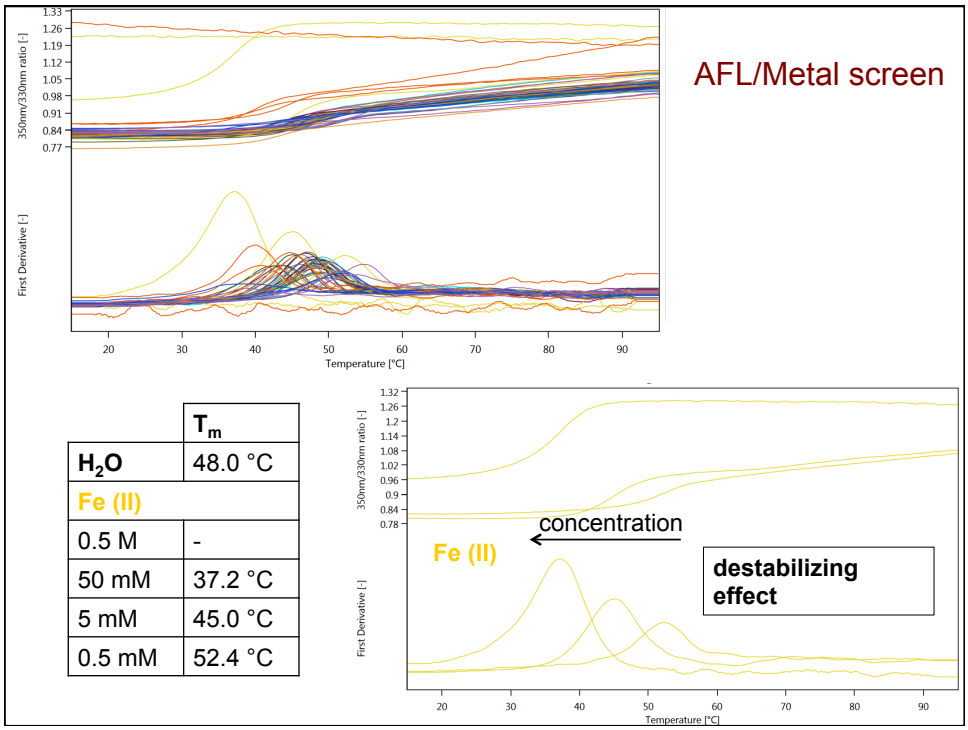




	T_m
H_2O	48.0 °C
L-fucose	
0.5 M	**71.8 °C**
50 mM	61.6 °C
5 mM	54.5 °C
0.5 mM	50.1 °C

	T_m
H_2O	48.0 °C
D-arabinose	
0.5 M	**61.8 °C**
50 mM	54.2 °C
5 mM	50.0 °C
0.5 mM	47.6 °C

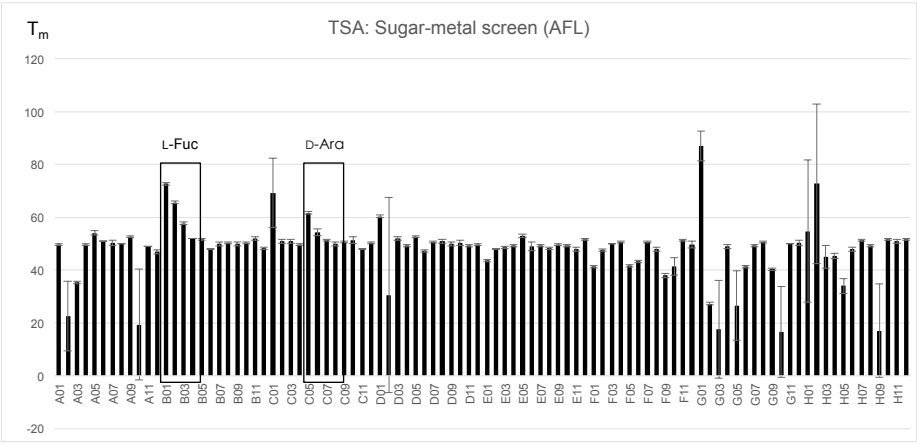




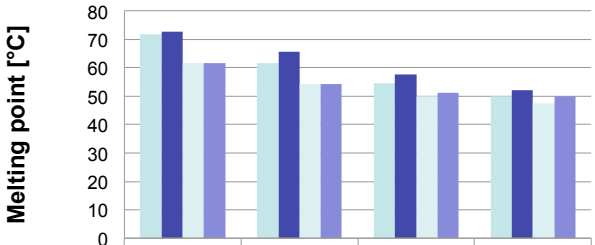
	T_m
H_2O	48.0 °C
Fe (II)	
0.5 M	-
50 mM	37.2 °C
5 mM	45.0 °C
0.5 mM	52.4 °C

THERMAL SHIFT ASSAY: SUGAR/METAL SCREEN

RT-PCR thermocycler with SYBRO ORANGE as a marker



COMPARISON OF DSF AND TSA RESULTS

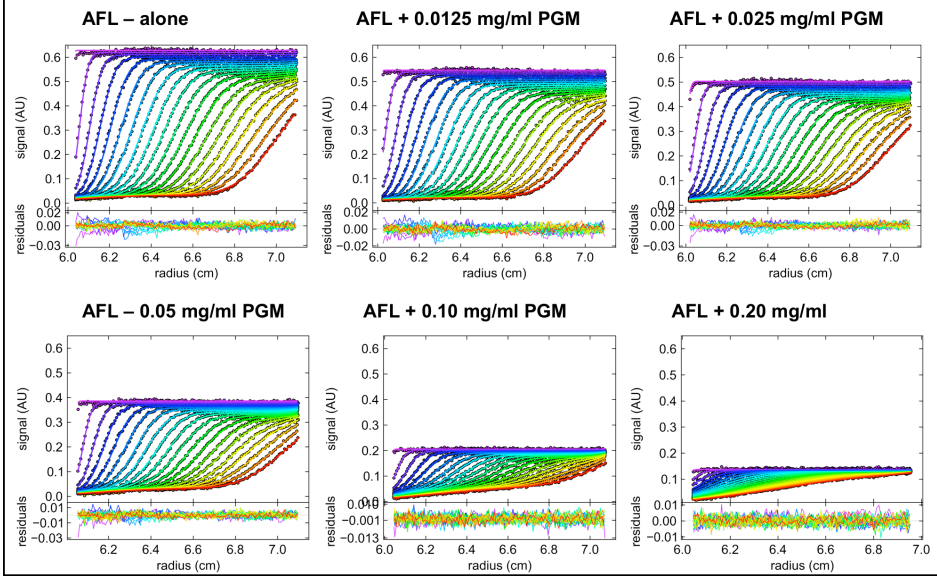


	0.5 M	50 mM	5 mM	0.5 mM
DSF L-Fuc	71.8	61.6	54.5	50.1
TSA L-Fuc	72.7	65.7	57.7	52.0
DSF D-Ara	61.8	54.2	50.0	47.6
TSA D-Ara	61.7	54.3	51.3	50.0

✓ comparable results

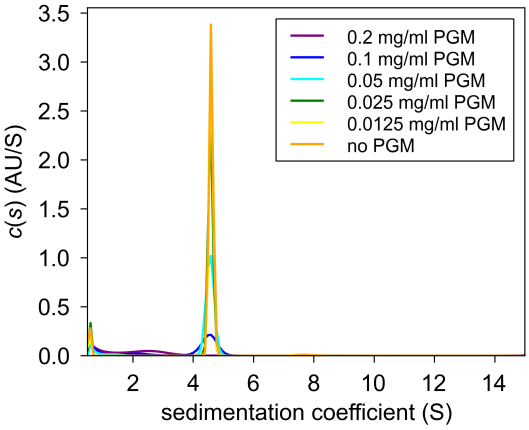
INTERACTION OF AFL WITH MUCIN USING AUC - SEDIMENTATION "FINGERPRINT"

0.24 mg/ml AFL in all cells

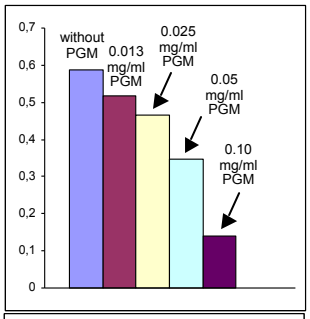


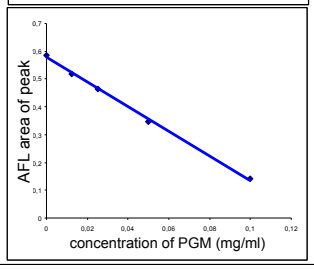
INTERACTION OF AFL WITH MUCIN USING AUC - SEDIMENTATION "FINGERPRINT"

0.24 mg/ml AFL in all cells



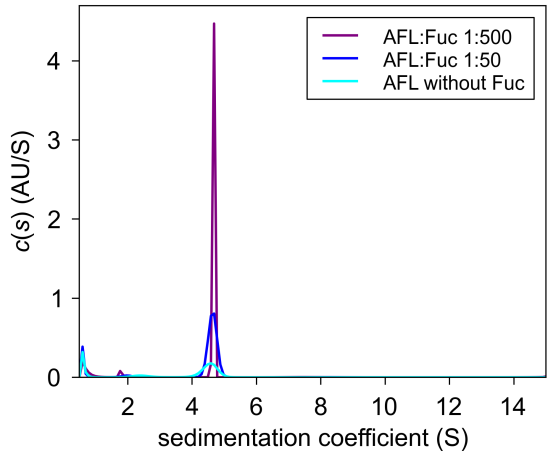
Remaining AFL in solution
(in signal units)

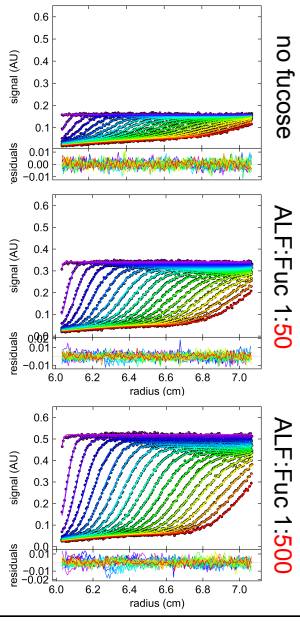




INTERACTION OF AFL WITH MUCIN USING AUC - SEDIMENTATION "FINGERPRINT"

0.24 mg/ml AFL in all cells





no fucose

ALF:Fuc 1:50

ALF:Fuc 1:500

DECIPHERING PROTEIN/ CARBOHYDRATE INTERACTION

PROTEIN/CARBOHYDRATE INTERACTION BY ITC

Erythrina corallodendron LECTIN / LACTOSE INTERACTION

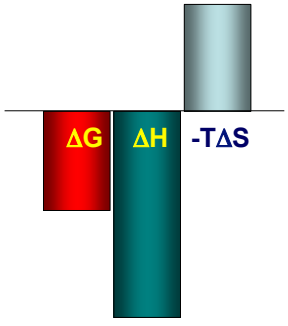
ITC microcalorimetry

K_a= 9.7 10^3 M^{-1}

K_d= 1 10^{-4} M

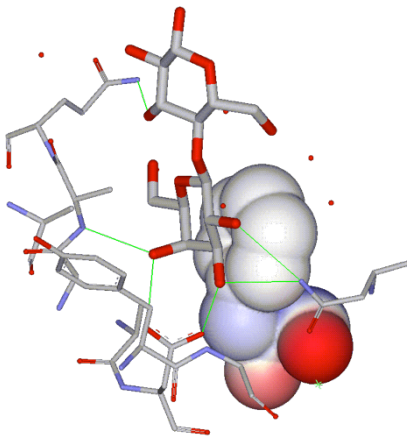
ΔH = - 47.1 kJ/mol

TΔS = -24.4 kJ/mol



Surolia et al. (1996) *J Biol Chem* 271, 17697-17703

Crystal structure



Shaanan et *al*. (1991) *Science*, 254, 862-866, 1991

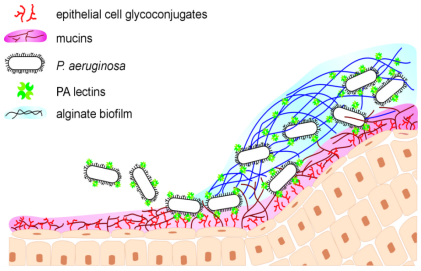
PSEUDOMONAS AERUGINOSA

- Gram-negative bacterium
- Opportunistic human pathogen
- Colonization – cystic fibrosis patients (90% mortality)
- Production of two soluble lectins - PA-IL and PA-IIL

Protein	gene	size	specificity	affinity
PA-IL	*lecA*	121 aa	D-Gal	medium (mM)
PA-IIL	*lecB*	114 aa	L-Fuc >>> D-Ara>D-Man	strong (μM)

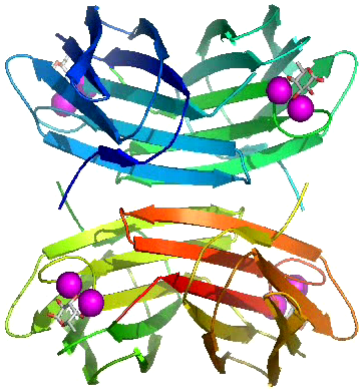
Virulence of bacterium –

- adhesion to host cell surface
- biofilm formation
- secretion of hydrolytic enzymes and toxic compounds

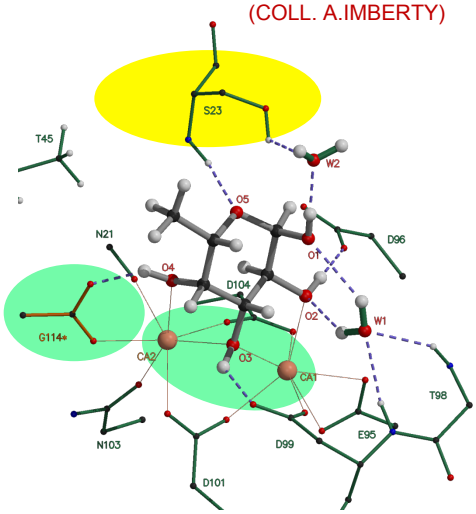


LECTIN PA-IIL (LECB)

(COLL. A.IMBERTY)



PA-IIL tetrameric arrangement



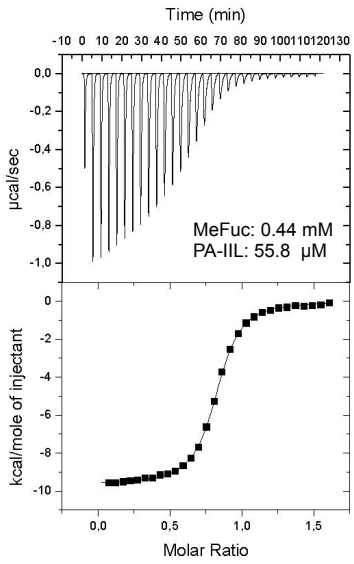
PA-IIL binding site with L-fucose (1 Å)

Mitchell *et al.*, *Nature Struct. Biol.* 9, 918 (2002),
Mitchell *et al.*, *Proteins*, 58, 735(2005)
Wimmerova *et al.*, *J Mol Model* ,15, 673 (2009)
Adam, Pokorná *et al.*, *BMC Struct Biol.* 7:36. (2007)

High affinity .....K_d ~ 10^{-7} M

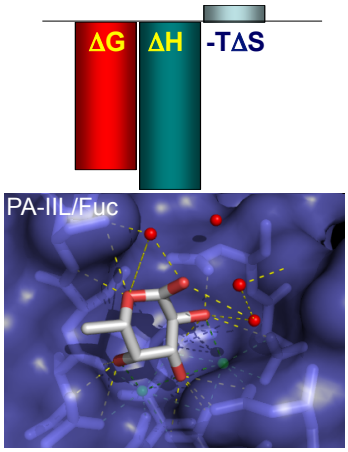
Specificity loop

THERMODYNAMICS OF PA-IIL/αMeFuc INTERACTION



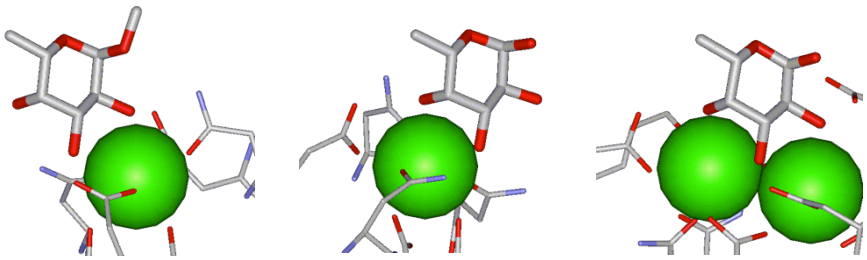
n = 0.98
K_A=2.3 10^6 M-1
K_D=4.3 10^{-7} M

ΔG= -36.3 kJ.mol^{-1}
ΔH= -41.1 kJ.mol^{-1}
-TΔS= 4.1 kJ.mol^{-1}



Mitchell E.P. et al., Proteins, 58, 735 (2005)

INTERACTION BETWEEN FUCOSE AND CALCIUM IN LECTINS



MBP-C/Fuc (1RDI)
MBP-A/Fuc (1KWW)

P-selectin/SLex (1G1T)

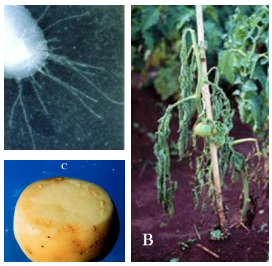
PA-IIL/Fuc (1GZT)

low affinity

high affinity

RALSTONIA SOLANACEARUM

- aggressive soil phytopathogen
- may infect animals and humans



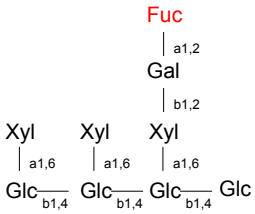
RSL – fucose-binding lectin

(Sudakevitz et al. (2002) J. Biochem. 132, 353-358,
Kostlánová et al. (2005) J. Biol. Chem. 280, 27839-27849)

Not calcium-dependent !!!

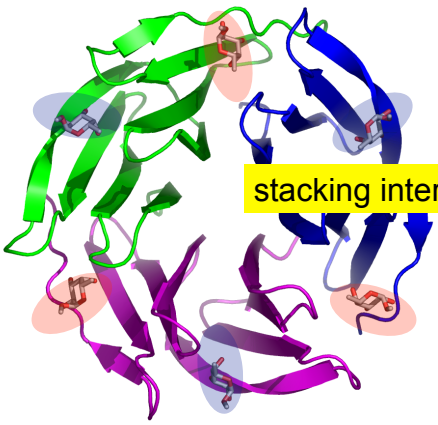
K_D for Me-α-Fuc = 7.3 10^{-7} M

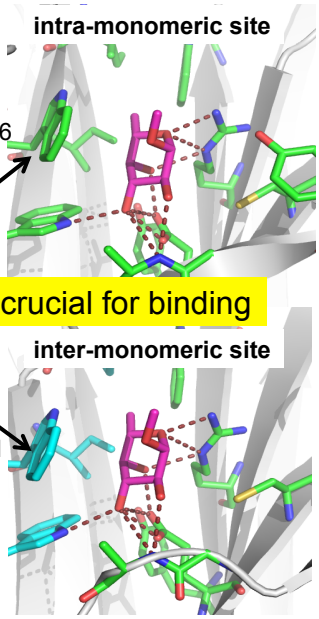
Xyloglucan :



RALSTONIA SOLANACEARUM LECTIN (RSL)

RSL/Me-α-L-Fuc trimer

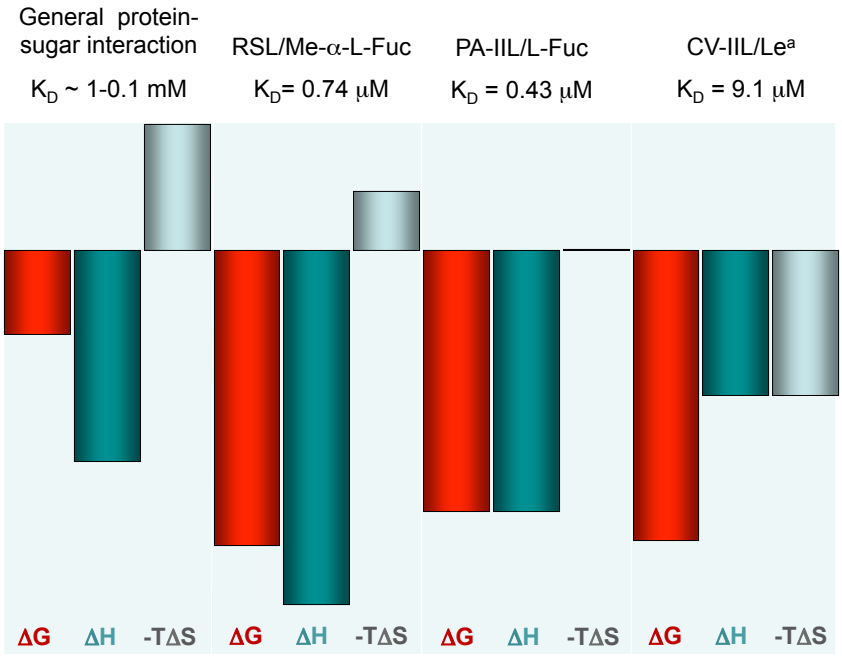




stacking interaction is crucial for binding

Both binding sites are equivalent with K_D= 7.4 10^{-7} M

Kostlánová et al., J. Biol. Chem., 2005



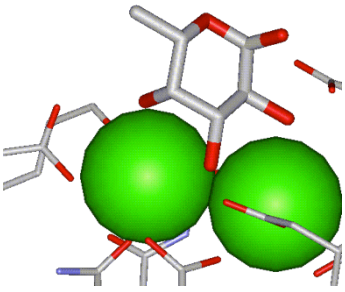
HIGH AFFINITY CAN BE REACHED BY DIFFERENT ARRANGEMENTS OF BINDING SITES

Correlation between number of the released water molecules:

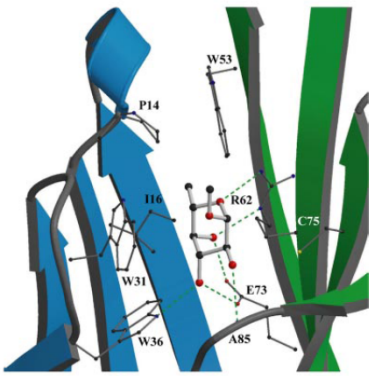
Pokorná et al, Biochemistry 2006

Contribution of CH-π interaction:

\Wimmerová et al, Plos One, 2012



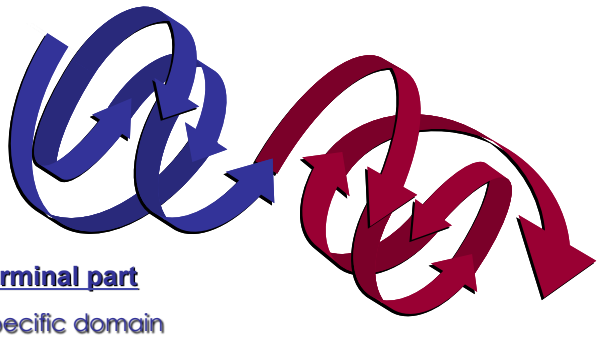
CV-IIL/Fuc



RSL/MeFuc

SOMETIMES, ATOMIC RESOLUTION DO NOT BRING ANSWERS…

BURKHOLDERIA CENOCEPACIA: BC2L-C LECTIN



N-terminal part

L-fucose specific domain

C-terminal part

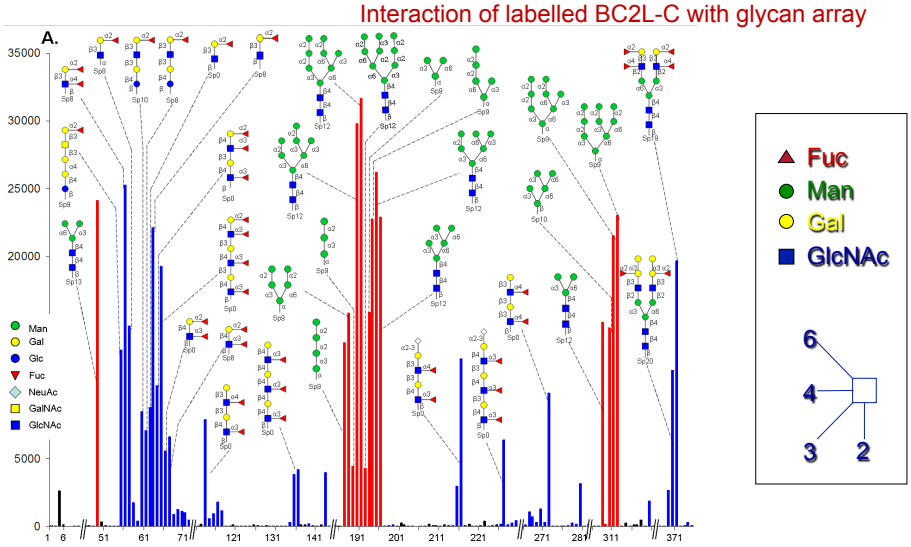
***Ca*$^{2+}$- dependent lectin domain**
sequence homology with lectins from **PAIIL superfamily**.

D-mannose specific domain

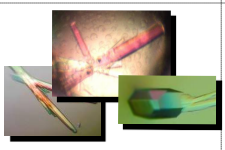
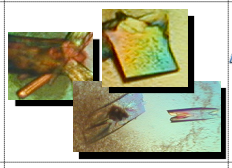
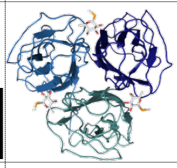
Šulák *et al.*, Structure 18, 59-72 (2010)

CONSORTIUM FOR FUNCTIONAL GLYCOMICS http://www.functionalglycomics.org

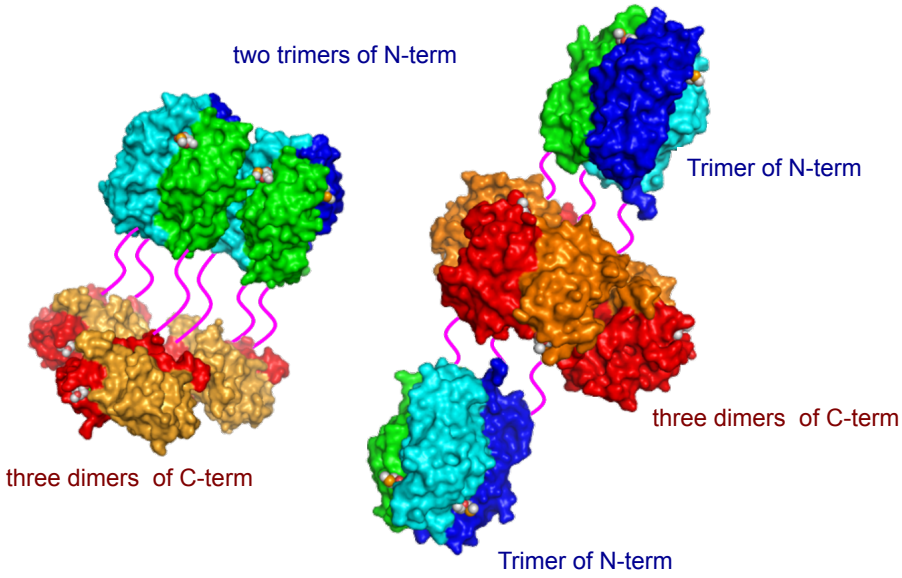
Interaction of labelled BC2L-C with glycan array



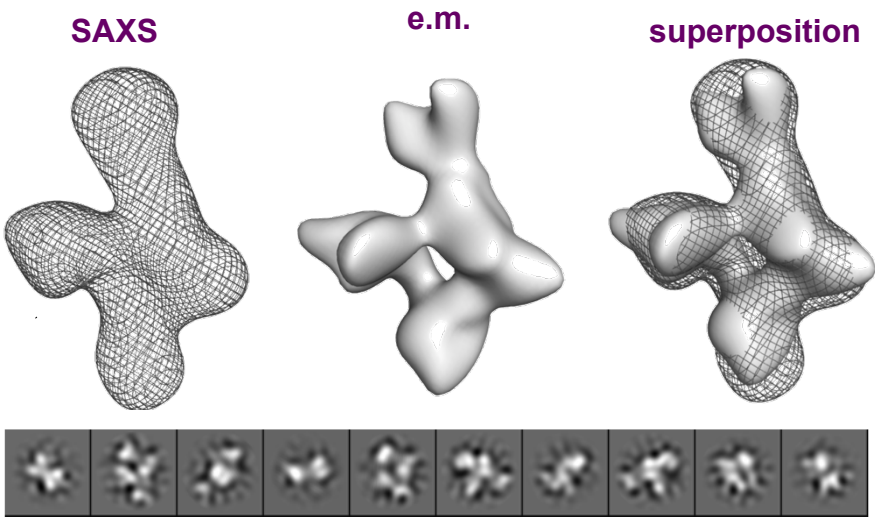
CRYSTALLISATION AND X-RAY OF BC2L-C

BC2L-C	crystals	structure	oligomeric state
C-terminal domain			**dimer**
N-terminal domain Se-Methionine modified protein			**trimer**
whole protein	✗	✗	must be hexamer !

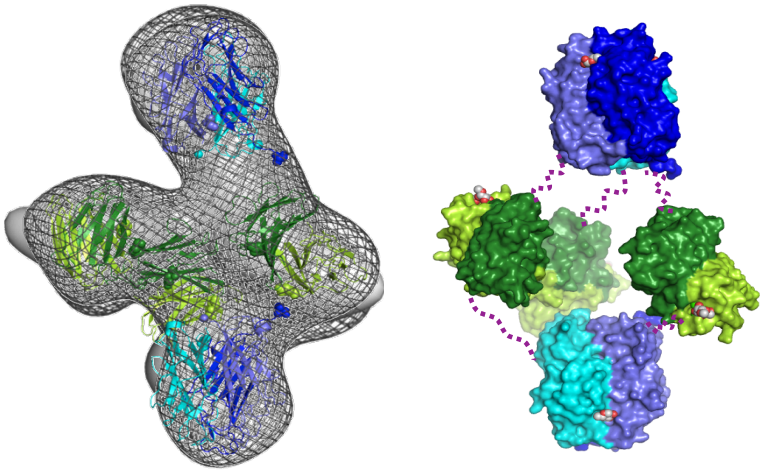
POSSIBLE ARCHITECTURE OF THE BC2L-C SUPER LECTIN ?



POSSIBLE ARCHITECTURE OF THE BC2L-C SUPER LECTIN ?



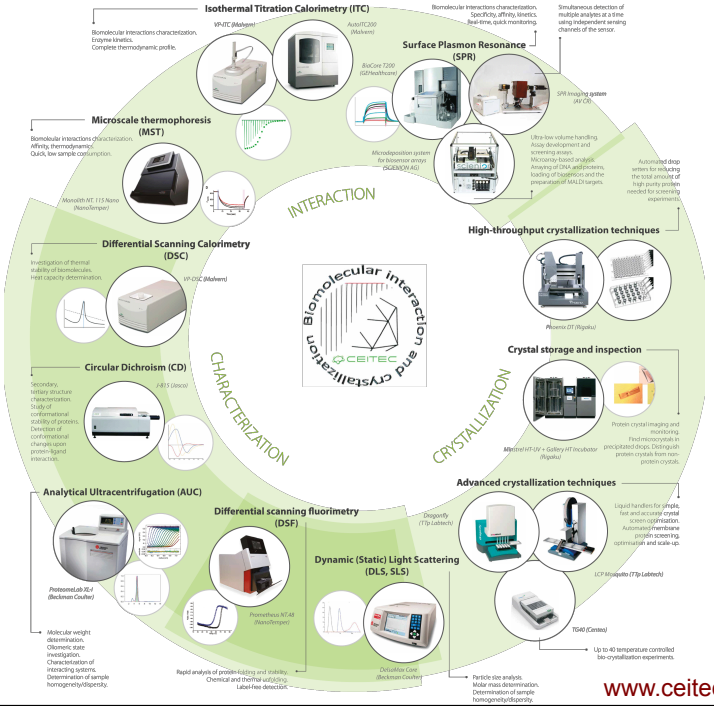
SOLUTION STRUCTURE OF BC2L-C HEXAMER CryoEM AND SAXS



Šulák et al., Structure 18, 59-72 (2010) ; Šulák et al., PLoS Pathog 7(9): e1002238 (2011)

GLYCOBIOCHEMISTRY GROUP

BIOMOLECULAR INTERACTIONS AND CRYSTALLIZATION CORE FACILITY



OPEN ACCESS AVAILABLE

www.ceitec.eu/bic