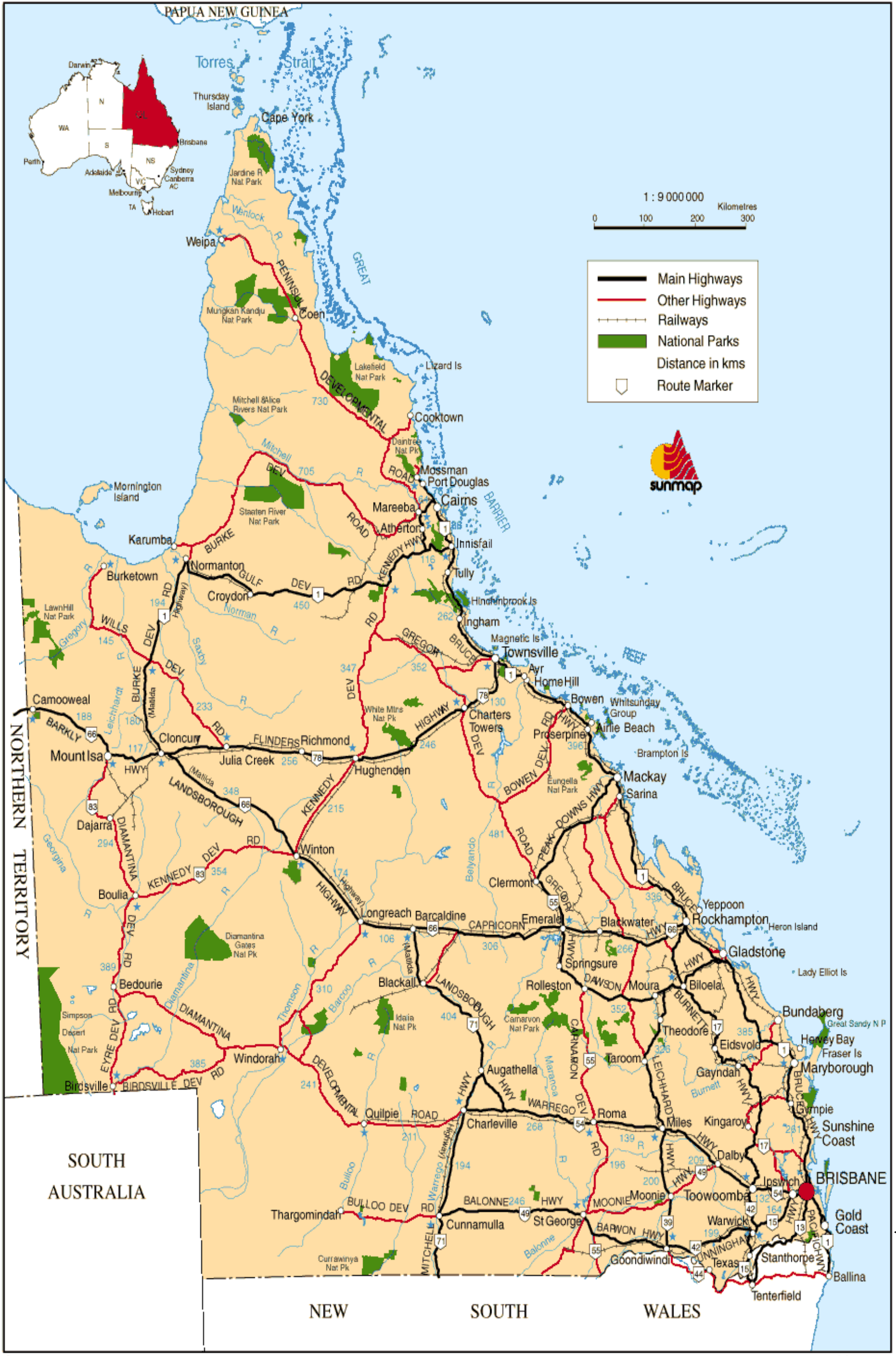
Griffith UNIVERSITY
Institute for Glycomics

Application of glycan array analysis in the discovery of novel bacterial-host interactions.

Michael Jennings

Griffith UNIVERSITY
Institute for Glycomics

Gold Coast, Queensland, Australia



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QS 097392



•Griffith University, Gold Coast Campus

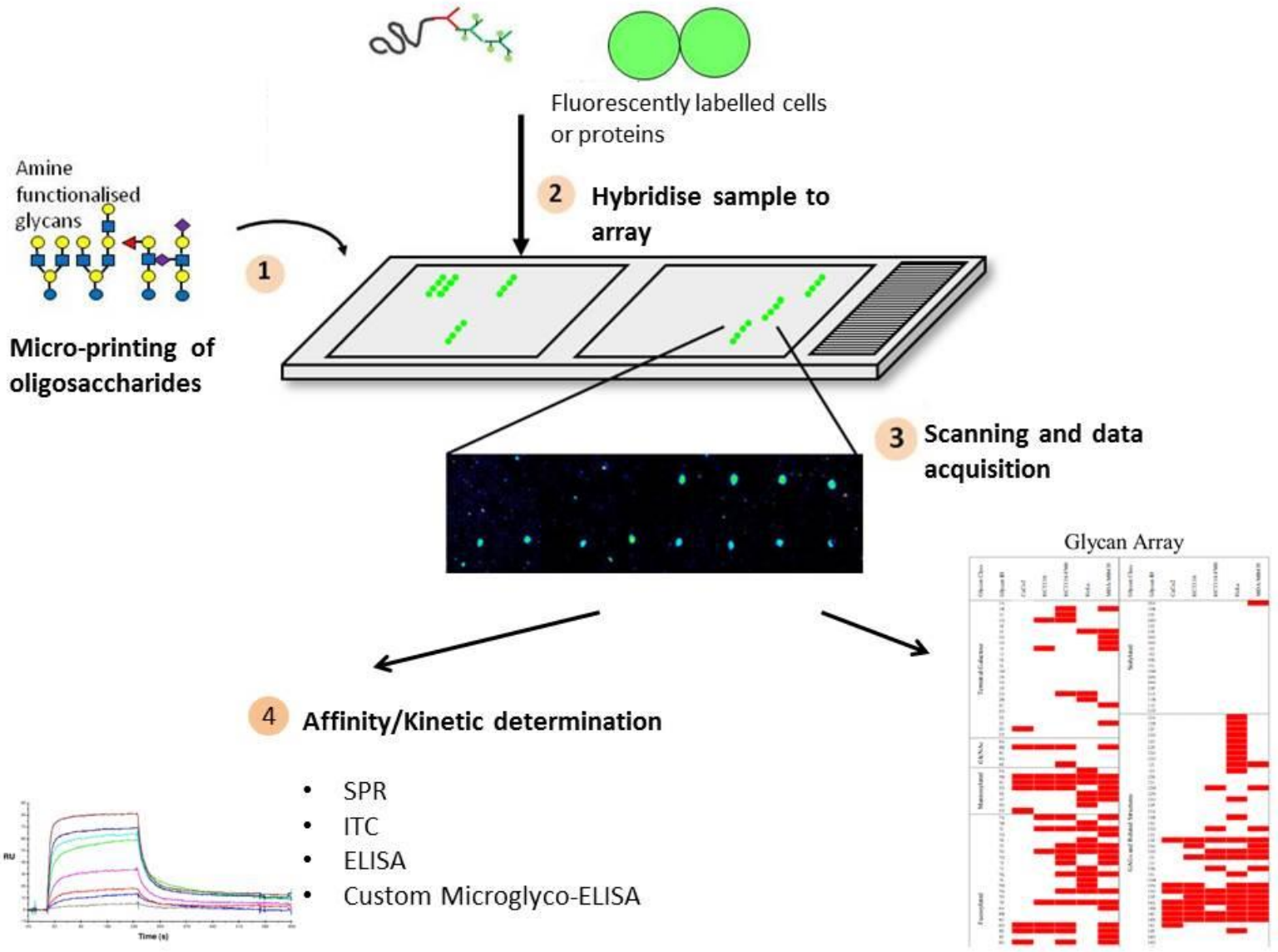
•Institute



Griffith UNIVERSITY
Institute for Glycomics



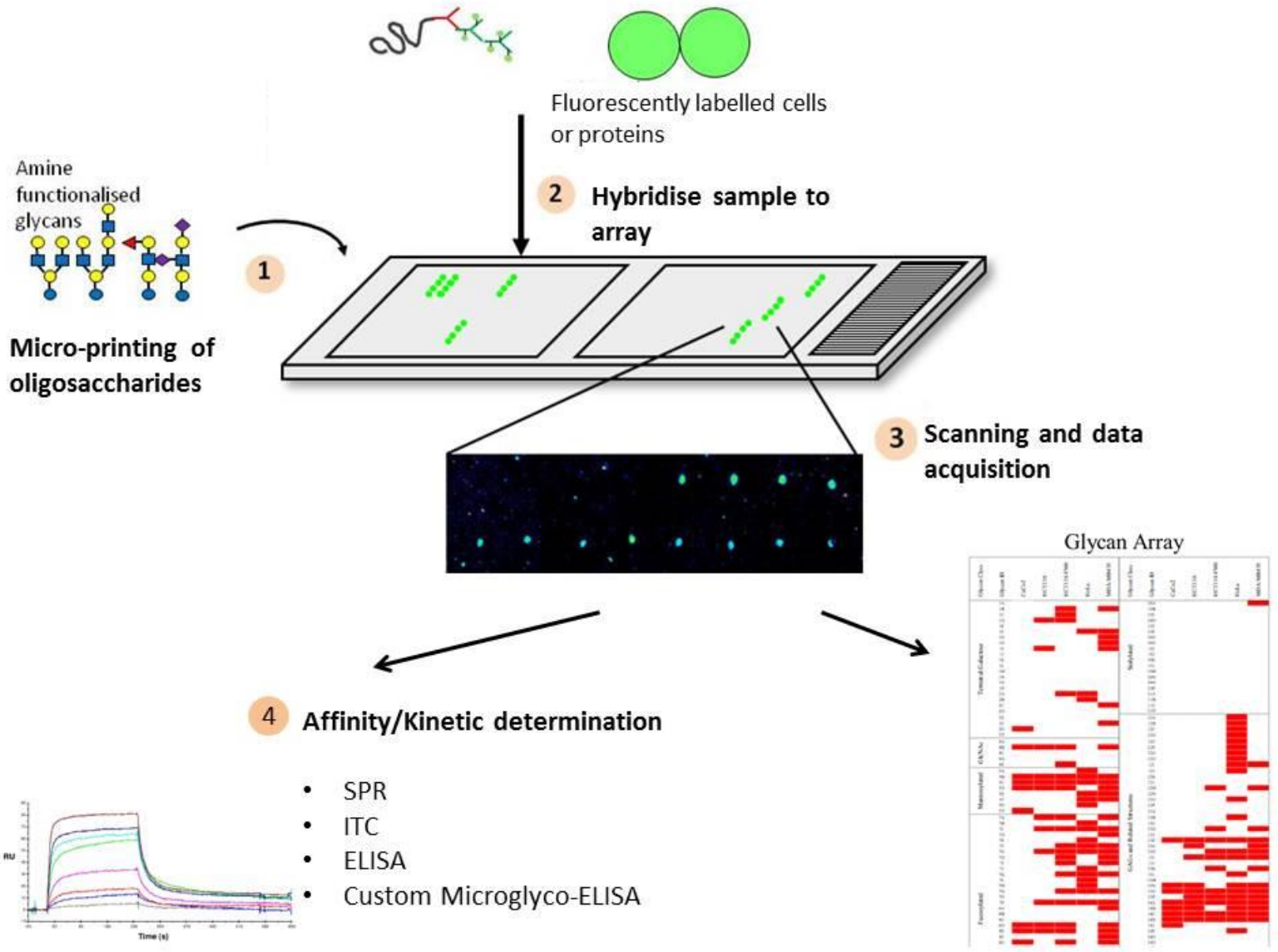
Glycan arrays:



Glycan arrays

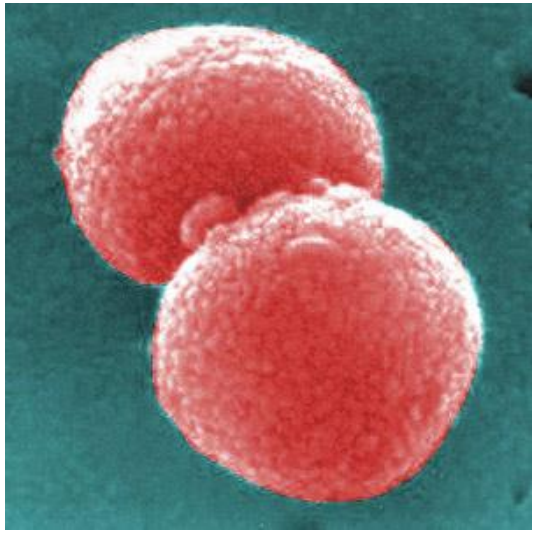
- Development began in 2001 by multiple groups
  - CFG has produced most diverse array
  - First published by Blixt *et al* (PNAS 2004 101:17033-8)
  - Featured 465 glycans
- IFG glycan arrays development began in 2006
  - First published in 2009 (Day *et al*, PLOS one 2009)
  - Featured 120 glycans
  - Current array has over 400 glycan.

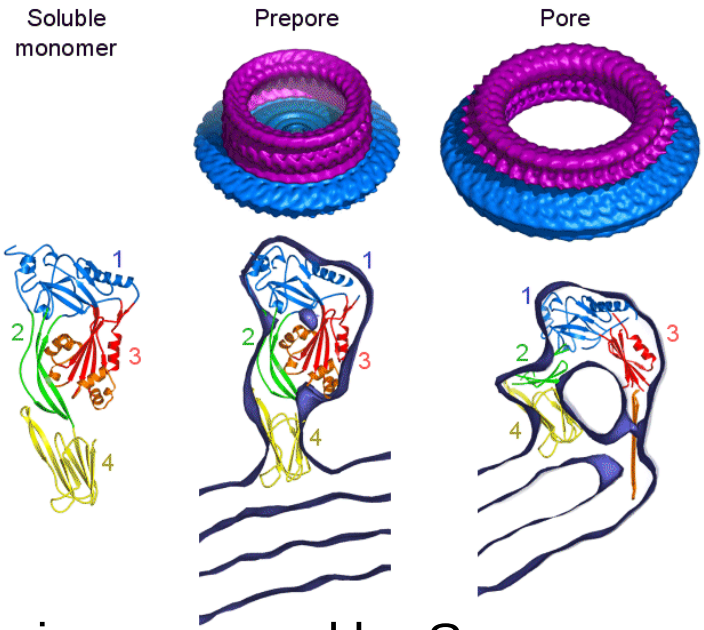
Glycan arrays:



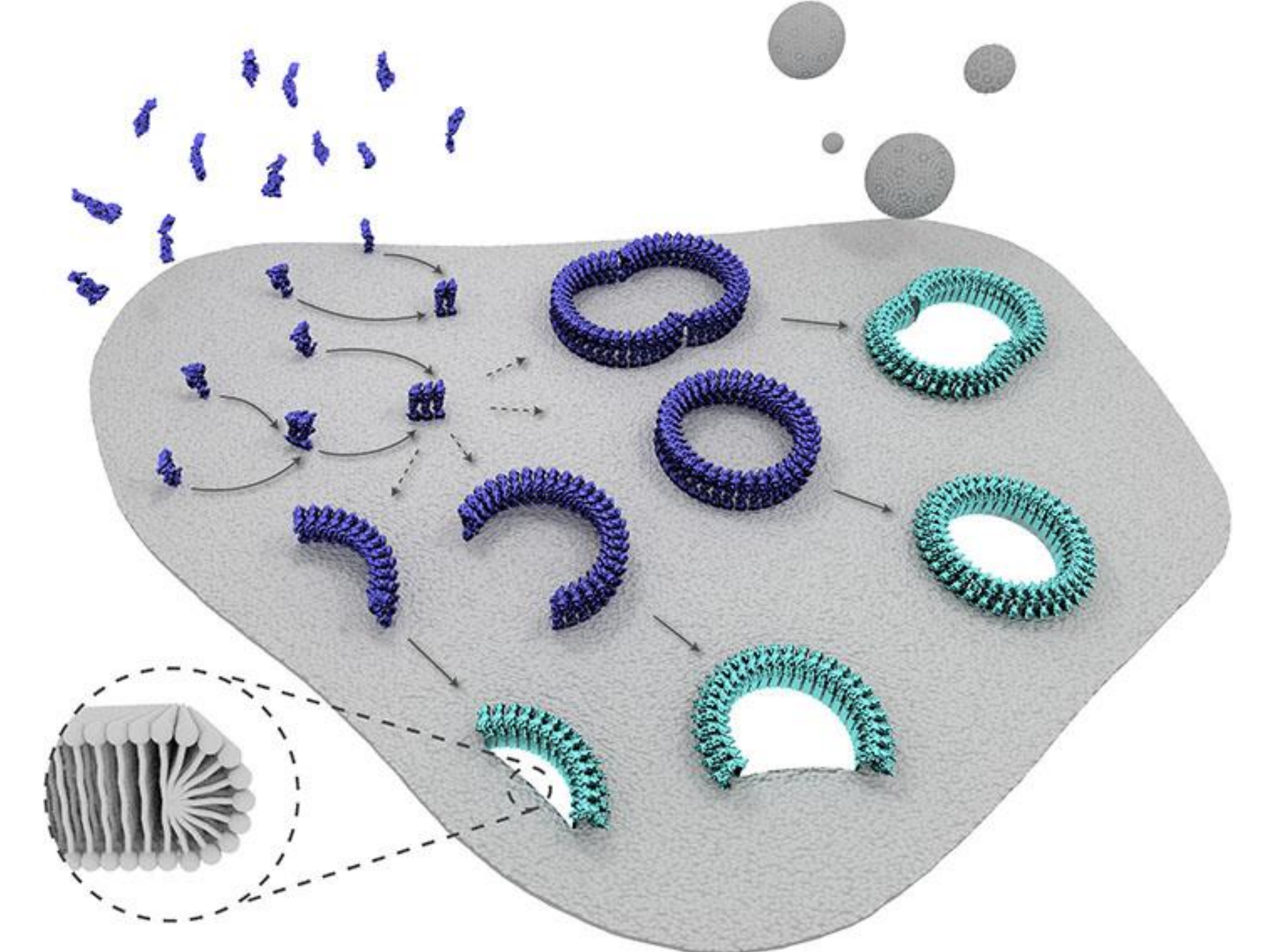
Cholesterol-dependent cytolysins (CDCs)

Streptococcus pneumoniae and Group A Streptococci are a leading causes of morbidity and mortality worldwide





- Pneumolysin Ply, is a pore-forming toxin expressed by *S. pneumoniae* and is a major virulence factor
- Streptolysin, SLO is a pore-forming toxin expressed by Group A Streptococci
- Both toxins are cholesterol-dependent cytolysins (CDC)



Cholesterol-dependent cytolysins (CDCs)

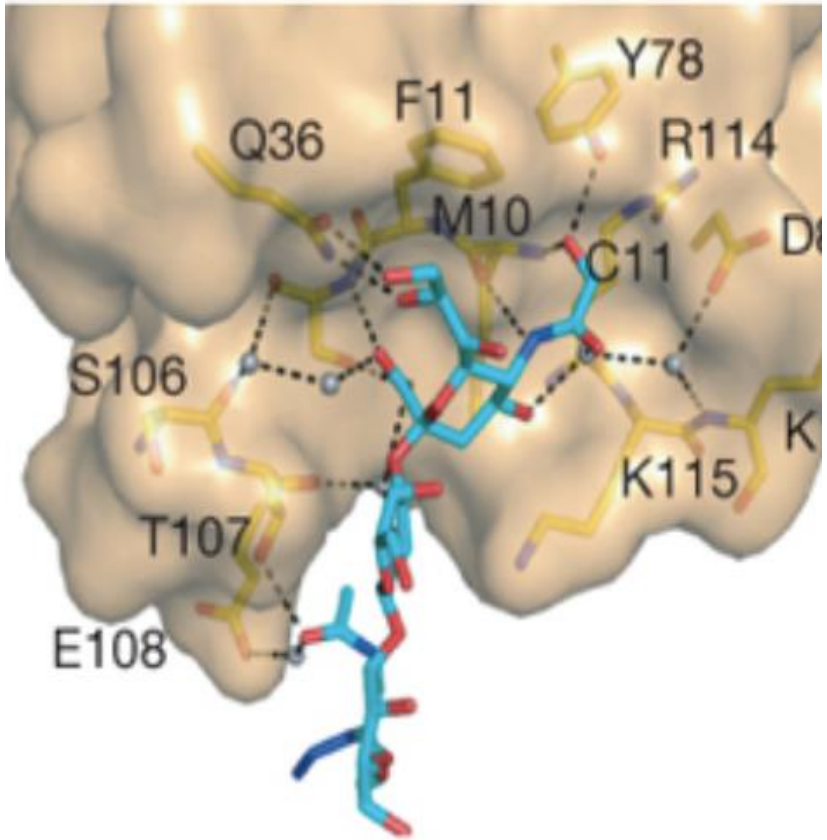
CDCs form pores in cholesterol containing membranes

Intermedilysin (ILY) binds human CD59 (hCD59) as a receptor - still requires cholesterol for insertion of pre-pore complex (Giddings *et al*, 2004, *Nat Struct Mol Biol* 11:1173-1178)

Proteinaceous receptors have recently been identified for membrane lipid-dependent, pore-forming cytotoxins of *Staphylococcus aureus* (DuMont *et al*, 2014, *Trends in microbiology* 22:21-27)

Could Ply and SLO also have a cellular receptor that contributes to target cell specificity?

Glycan structures as toxin receptors



A subunit of SubAB

Host glycans are a common class of receptor for bacterial toxins.

e.g. SubAB toxin of *E. coli* is selective for Neu5Gc terminated structures.

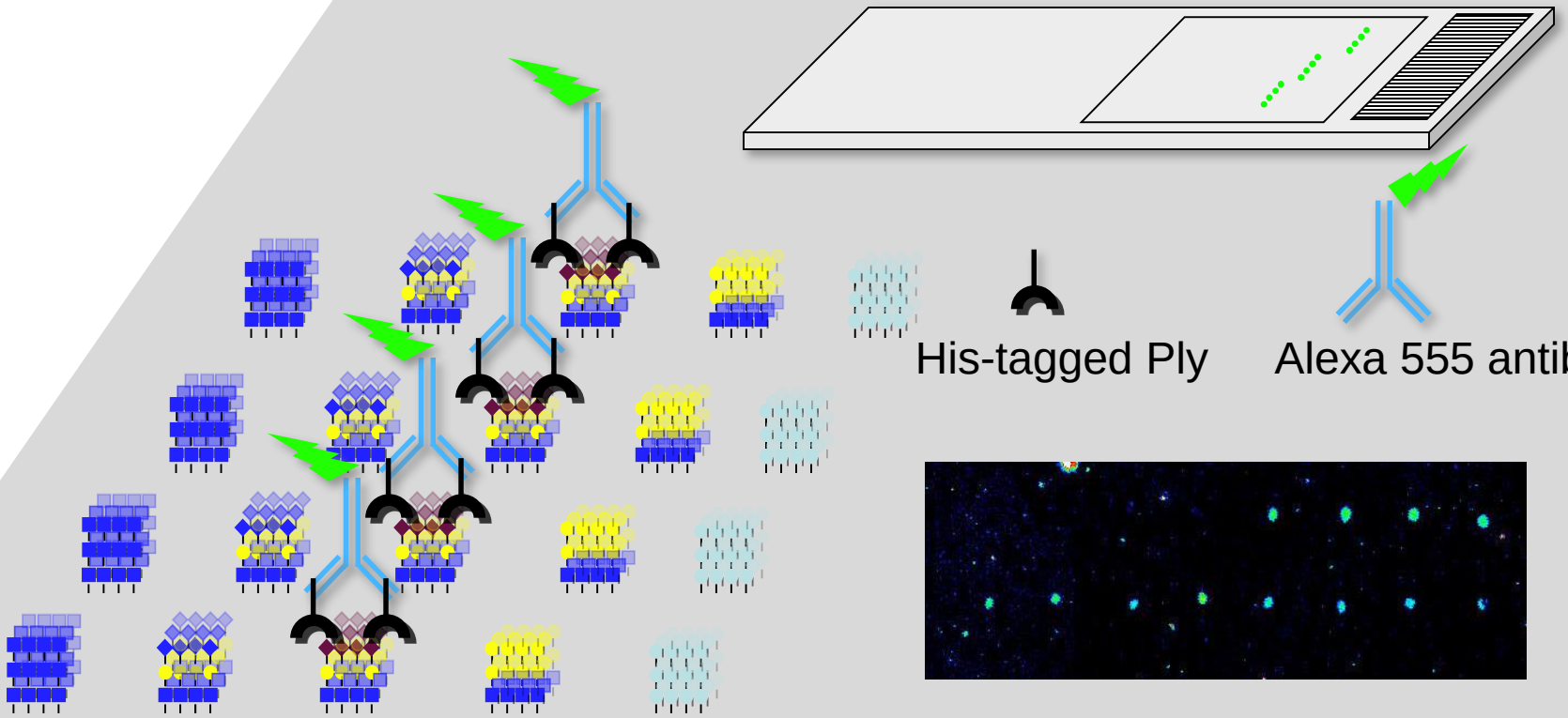
We sought to test the hypothesis that Ply and SLO may interact with host glycans as a cellular receptor.

Nature. 2008; 456(7222): 648–652

Glycan Arrays

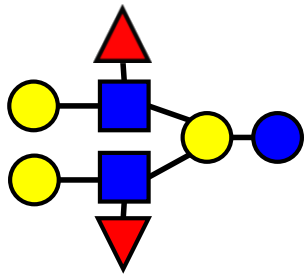
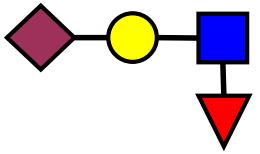
Array consisting of 400 glycans (mono- and oligo-saccharides) of known structures covalently immobilised onto glass slides.

Used to evaluate Ply for glycan recognition.

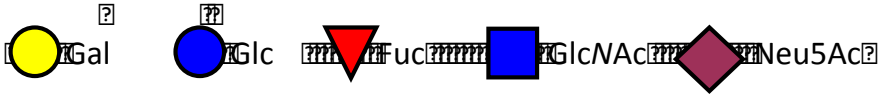


Ply binds to the Lewis histo-blood group antigens LeX and sLeX

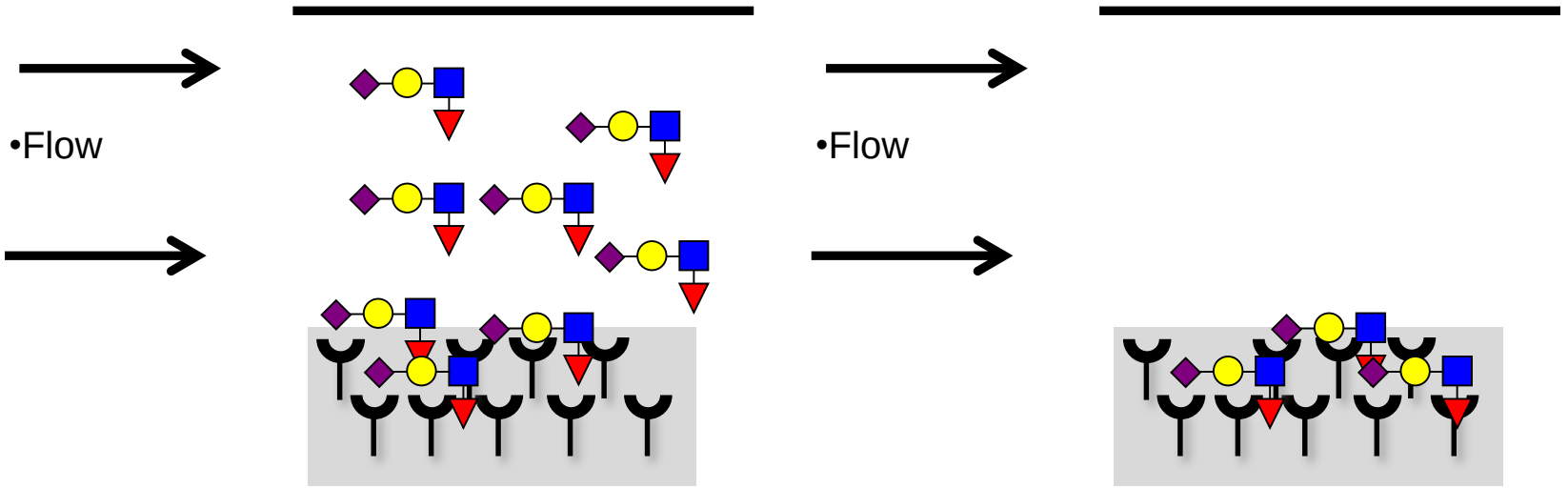
Glycan array analysis revealed significant binding of Ply to the fucosylated glycan divalent-LewisX (LeX) and the sialylated glycan sialyl LewisX (sLeX)

Code	Name	Formula	Structure
8L	DiLewisX	Galb1-4(Fucα1-3)Glc*N*Acb1-6(Galb1-4(Fucα1-3)Glc*N*Acb1-3)Galb1-4Glc	
10B	Sialyl LewisX	Neu5Acα2-3Galb1-4(Fucα1-3)Glc*N*Ac	

Symbol nomenclature



Surface Plasmon Resonance (SPR) analysis of Ply with LeX and sLeX

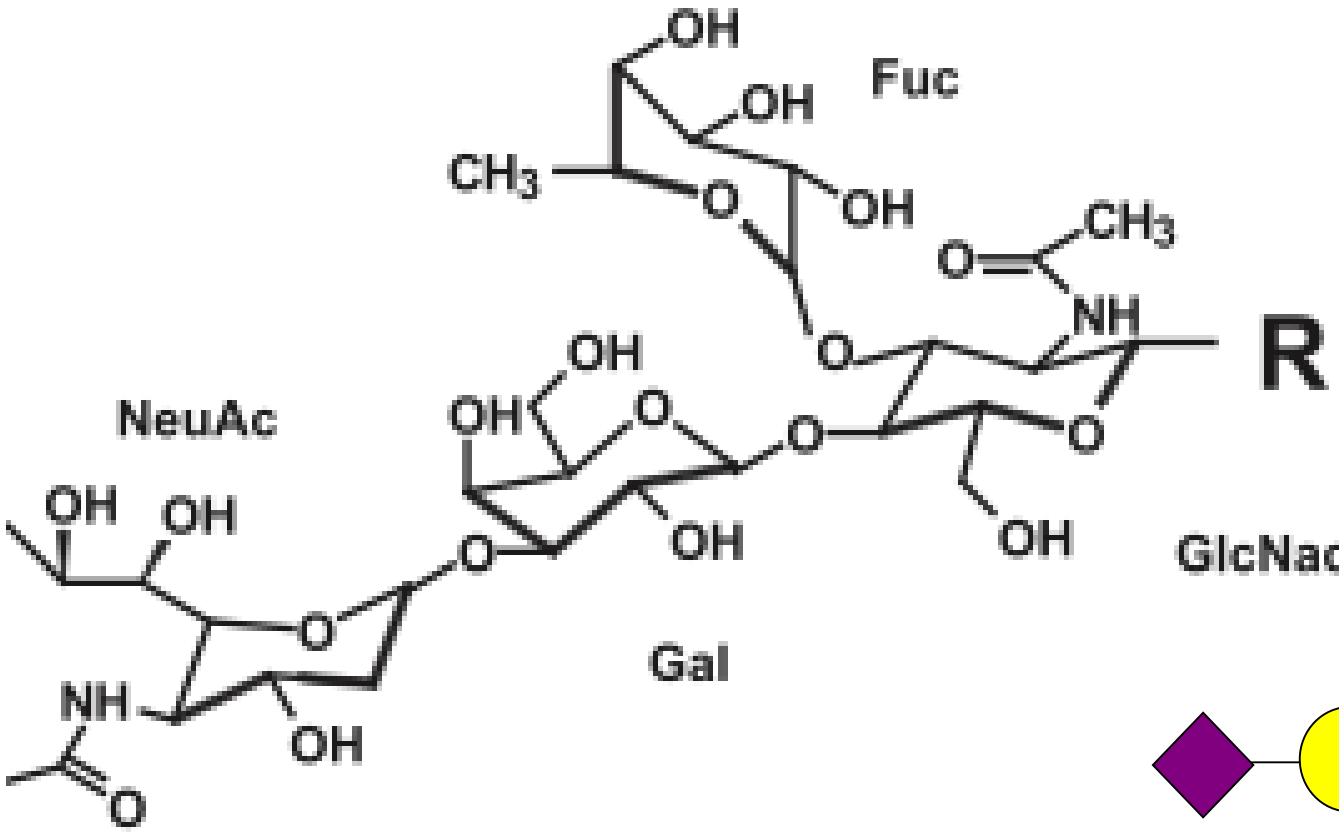


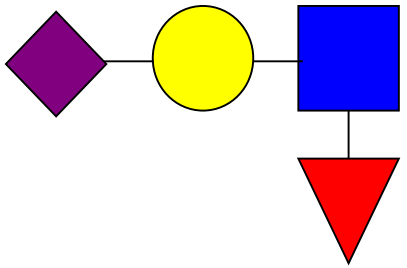
Ply binds to the Lewis histo-blood group antigens LeX and sLeX

SPR was used to validate glycan binding

Glycan	**Ply K_D**
LeX	31.7 µM
sLeX	18.8 µM

Sialyl Lewis X





Sialyl Lewis X

Detected on the surface of multiple cell types including neutrophils, monocytes, platelets, natural killer cells, activated lymphocytes and helper memory T cells, present as glycoprotein or glycolipid

Serves as essential component of the ligands for the P-, L- and E-selectins to mediate ‘tethering and rolling’ of neutrophils

Upregulated during inflammation on the surface of leukocytes

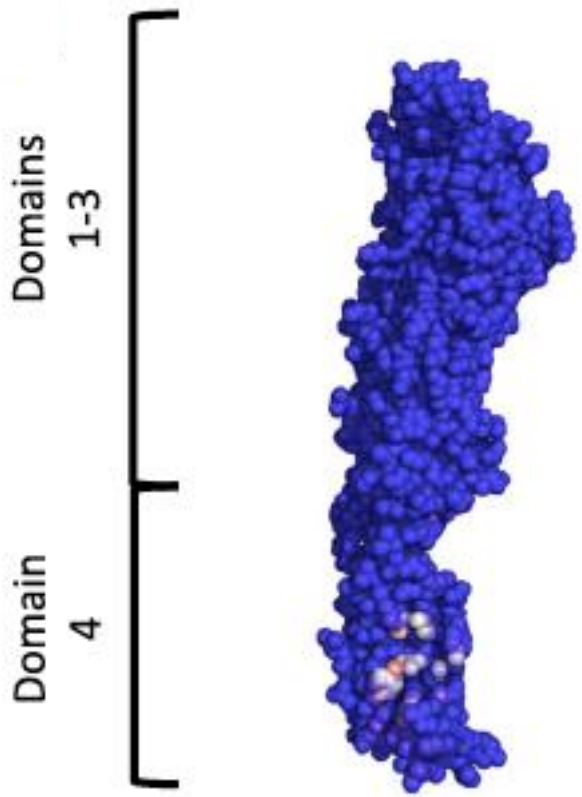
Originally identified on human RBCs, in plasma and in mucous secretions.

Later shown that RBCs passively acquire sLeX as glycosphingolipids that are incorporated into the RBC membrane.

Ply binds to the Lewis histo-blood group antigens LeX and sLeX

SPR was used to validate glycan binding

Glycan	Ply K_D
LeX	31.7 µM
sLeX	18.8 µM

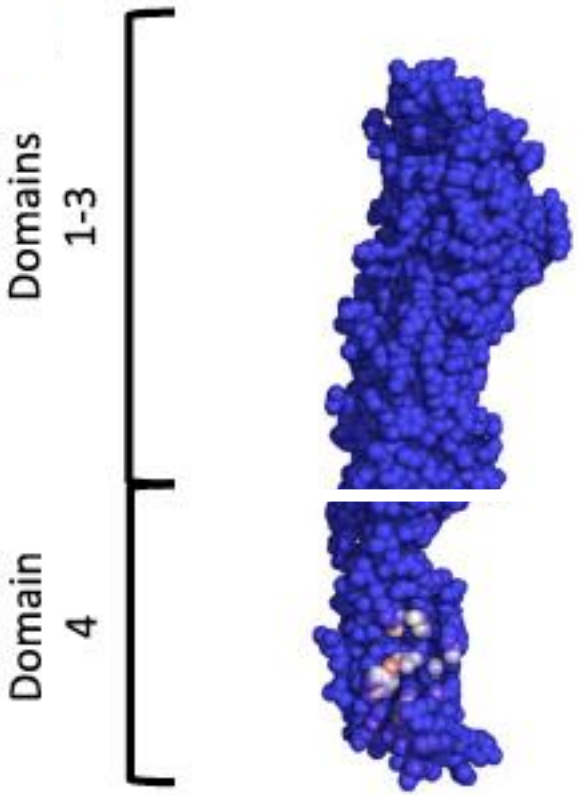


Ply binds to the Lewis histo-blood group antigens LeX and sLeX

SPR was used to validate glycan binding

SPR analysis was also conducted with Ply truncation mutants

Glycan	Ply K_D	PlyL K_D Domains 1-3	PlyS K_D Domain 4
LeX	31.7 µM	No interaction	26.2 µM
sLeX	18.8 µM	No interaction	43.0 µM

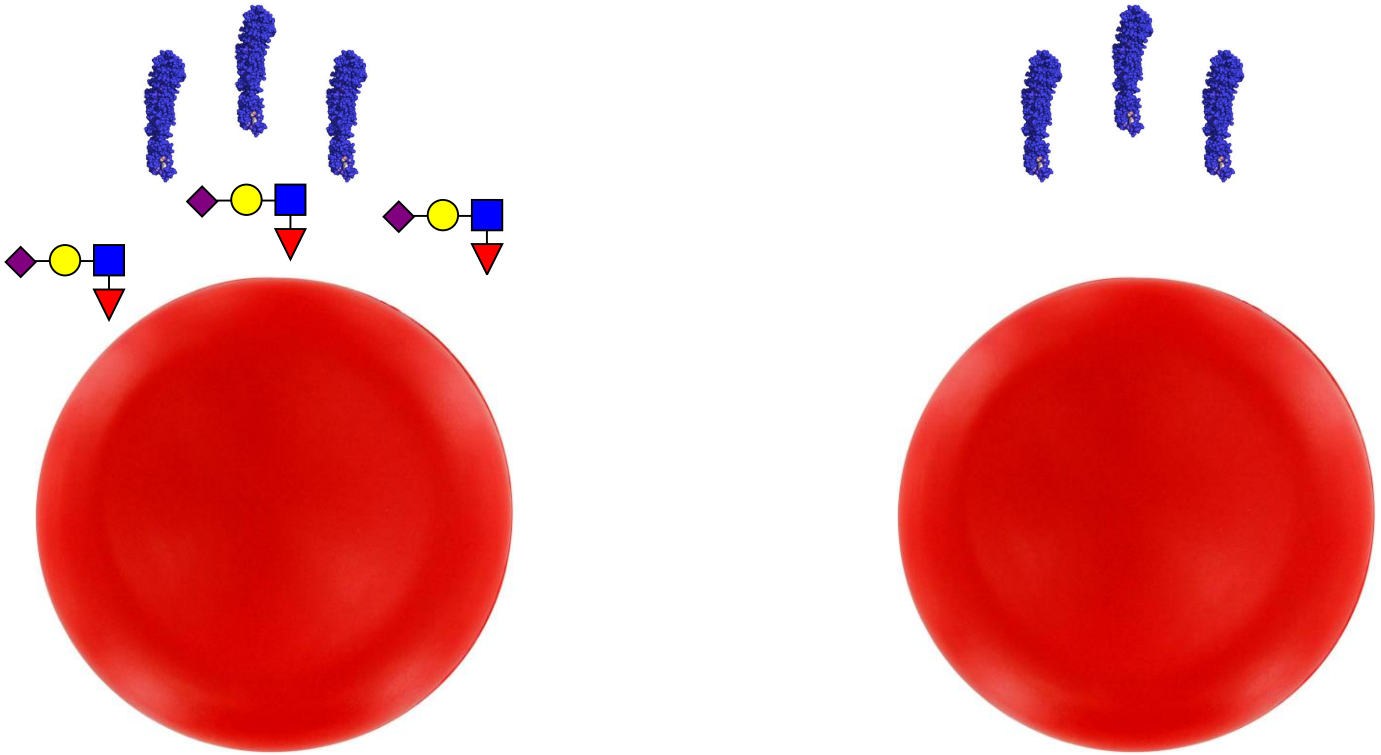


sLeX inhibits Ply hemolytic activity

Hemolysis is a classic Ply toxin activity

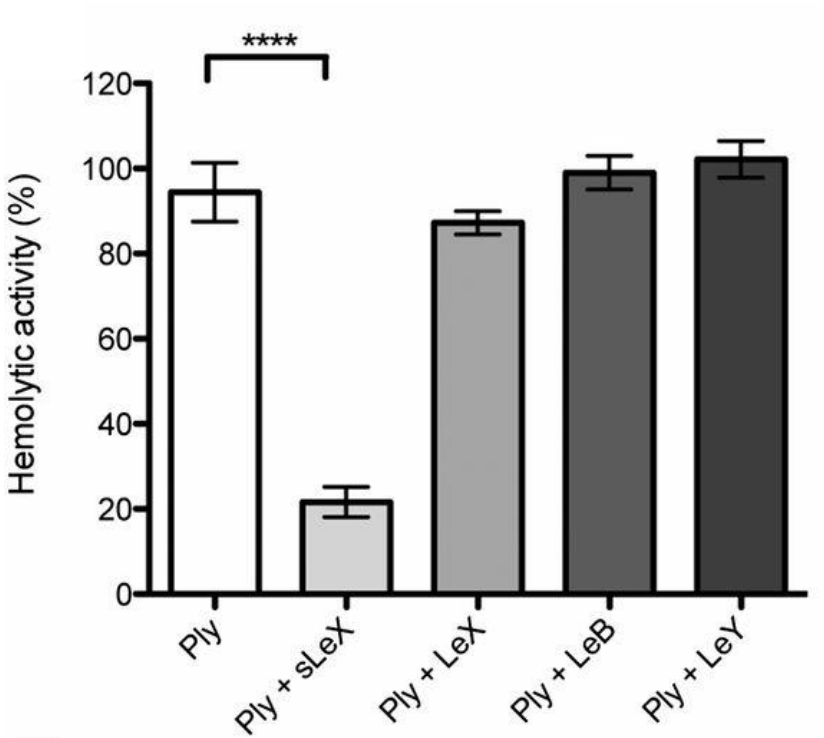
LeX and sLeX are histo-blood group antigens on RBCs and may be acting as toxin receptors

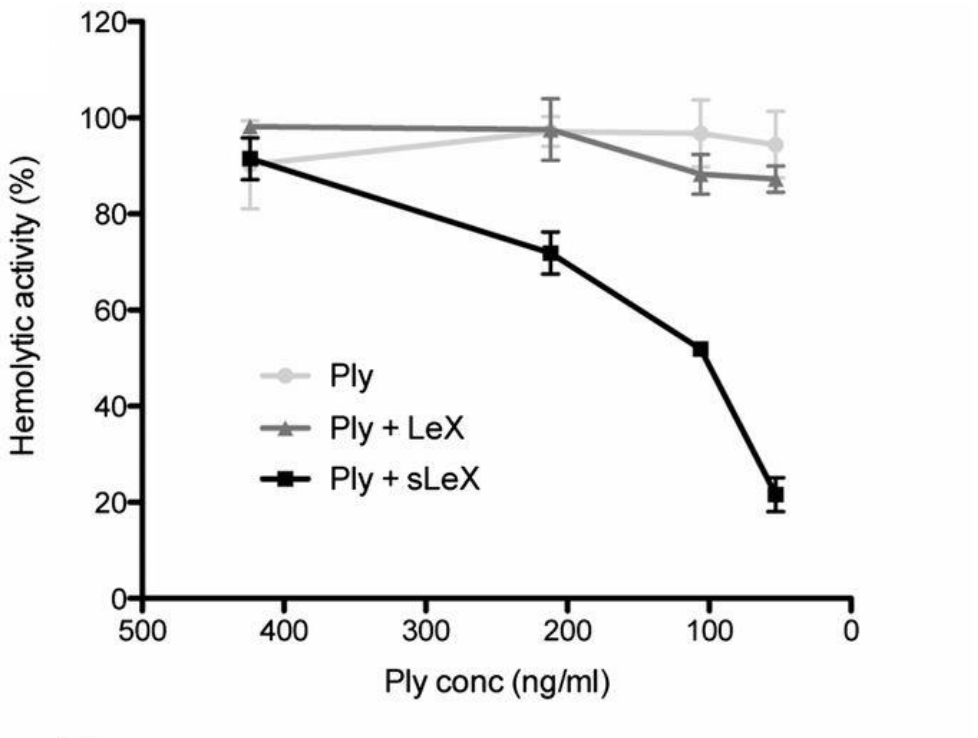
Can the lysis of RBCs be blocked by sLeX?



sLeX inhibits Ply hemolytic activity

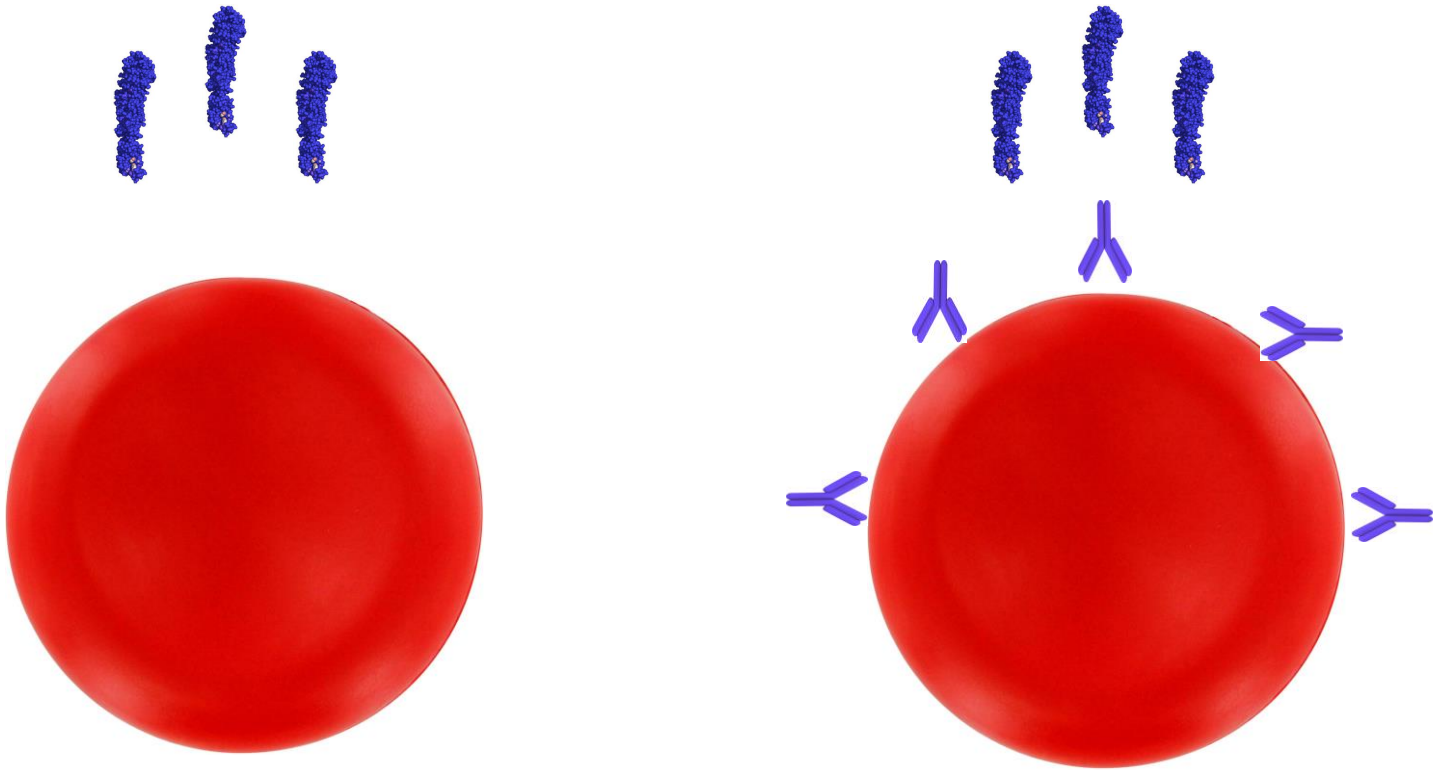
The presence of free sLeX can inhibit Ply mediated hemolysis against human Group O RBCs over a range of concentrations





Can monoclonal antibodies specific for sLeX and LeX block Ply hemolytic activity?

Pre-incubation of RBCs with anti-sLeX (a-sLX) and anti-LeX (a-LX) mAbs was followed by challenge with Ply

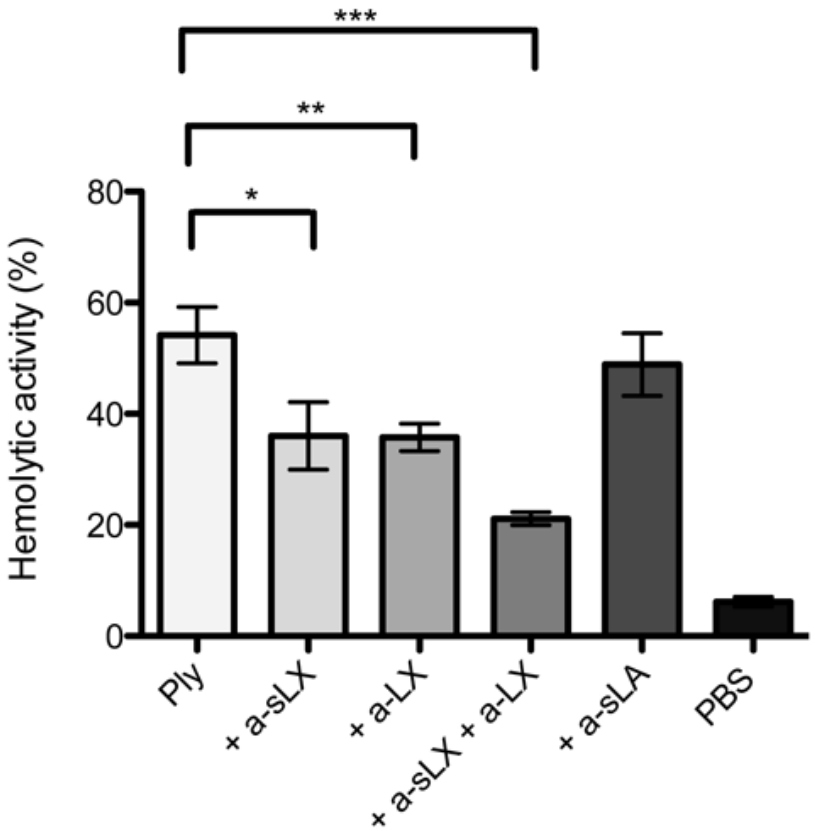


Monoclonal antibodies specific for sLeX and LeX can block Ply hemolytic activity

Pre-incubation of RBCs with anti-sLeX (a-sLX) and anti-LeX (a-LX) mAbs significantly reduced Ply hemolytic activity

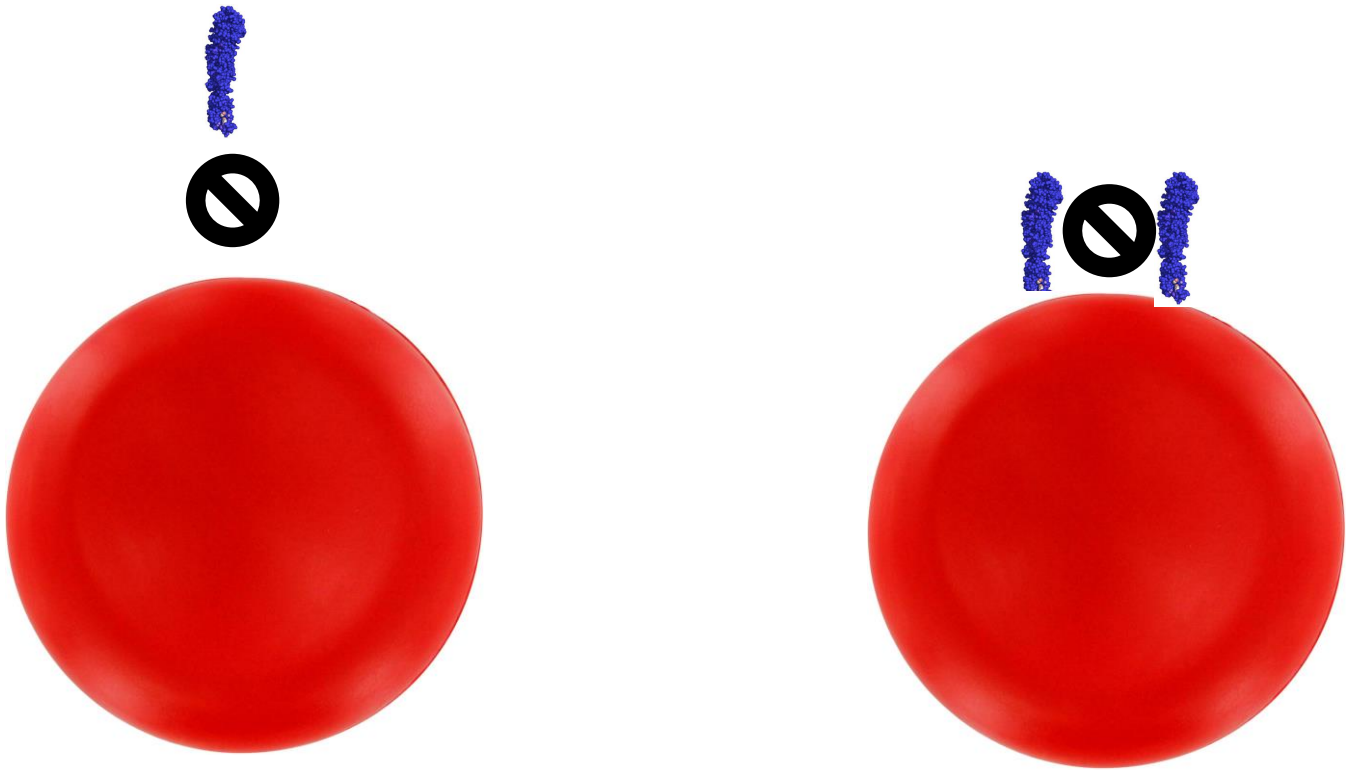
A combination of both mAbs caused a greater reduction

Anti-sLeA mAb (a-sLA) used as negative control



How does sLeX inhibit Ply hemolytic activity?

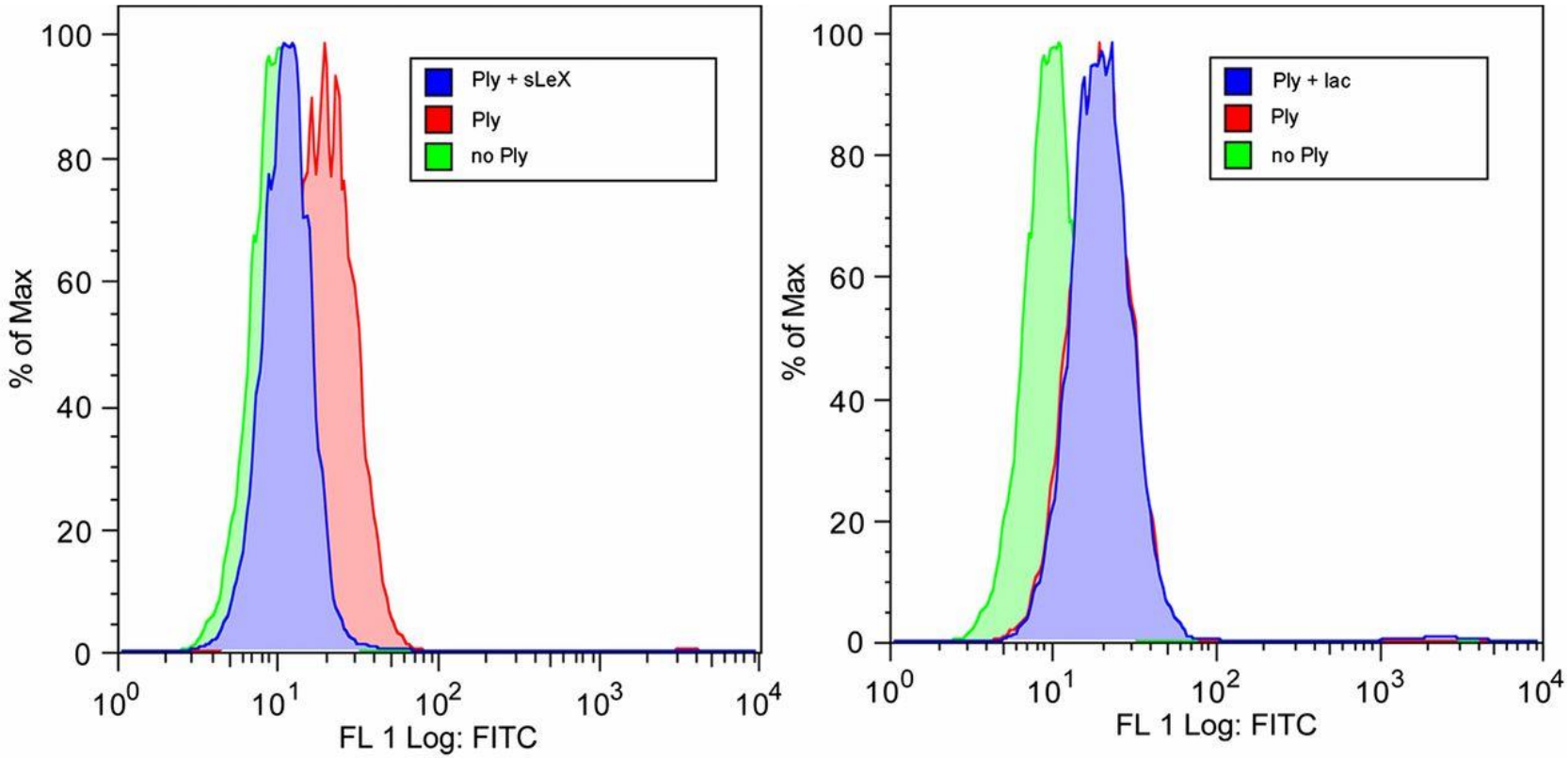
Free sLeX inhibitor may block deposition of Ply onto the RBC surface, or may interfere with some step in the pathway to pore formation.



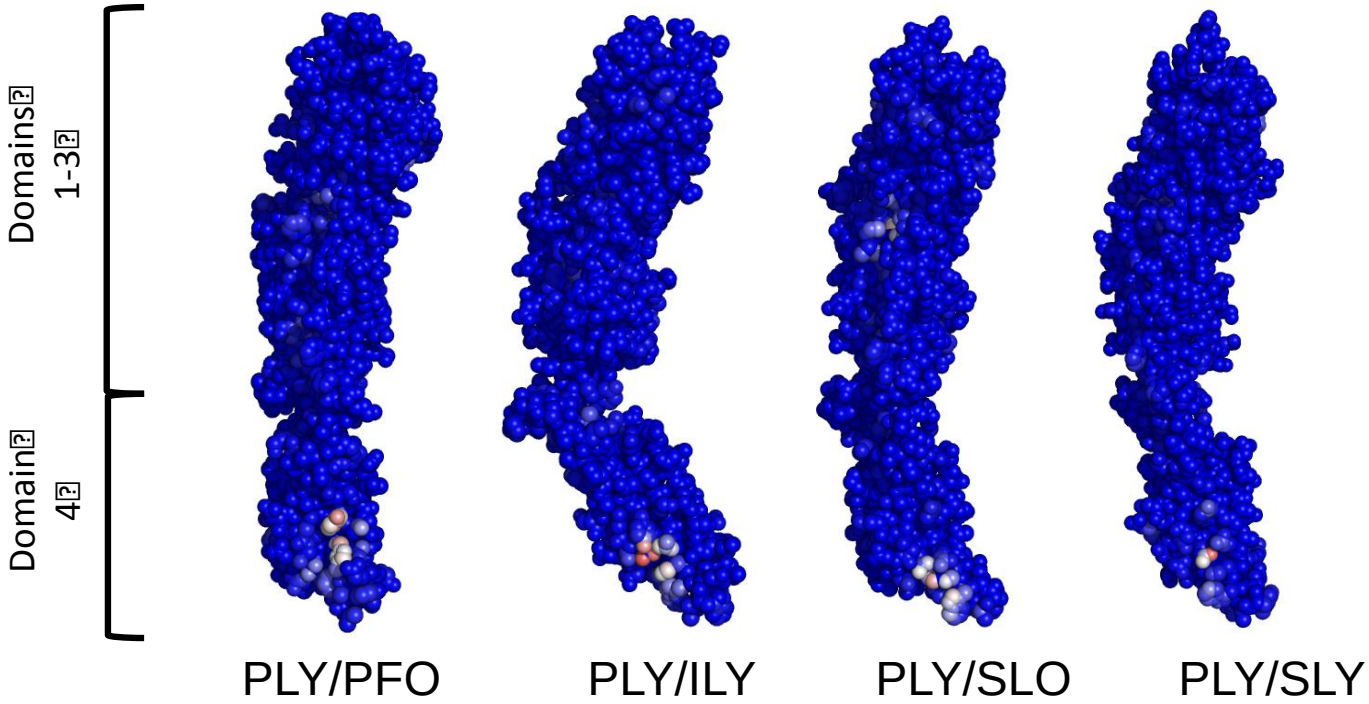
sLeX inhibits Ply hemolytic activity by blocking binding of the toxin to the RBC surface

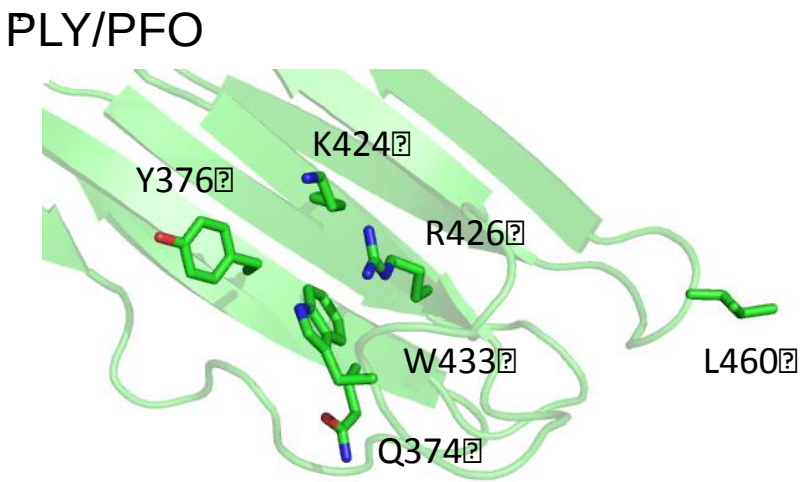
Free sLeX inhibits binding of Ply to the RBC surface

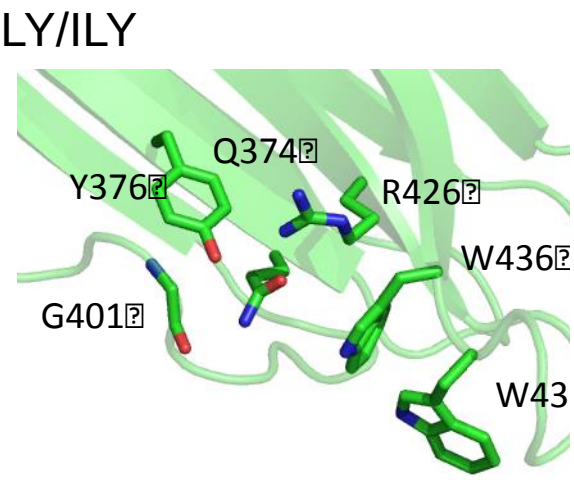
Shown by flow cytometry of unlysed RBCs with anti-Ply serum.



Modeling to identify Ply carbohydrate binding site





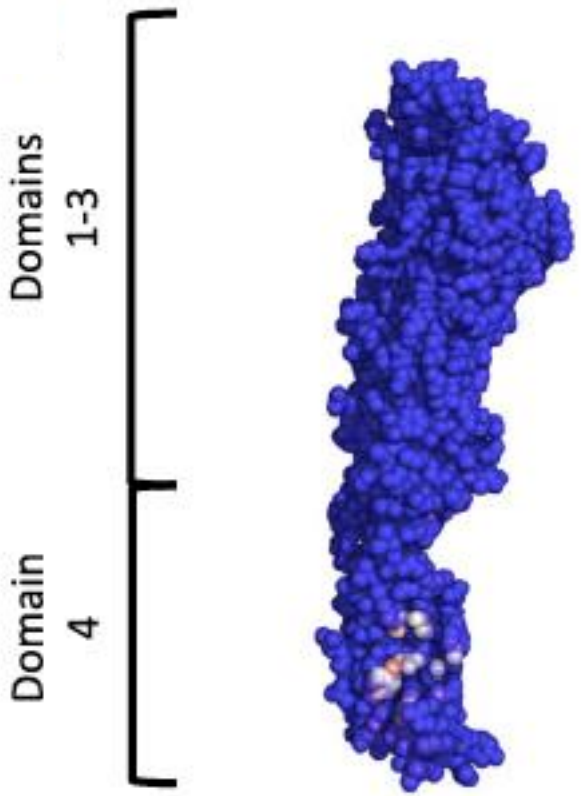


Protein-carbohydrate binding site prediction in domain 4 of Ply and mutagenesis

Site-directed mutagenesis was performed on predicted-carbohydrate binding residues in domain 4 to generate mutant Ply proteins PlyQ374A and PlyY376A.

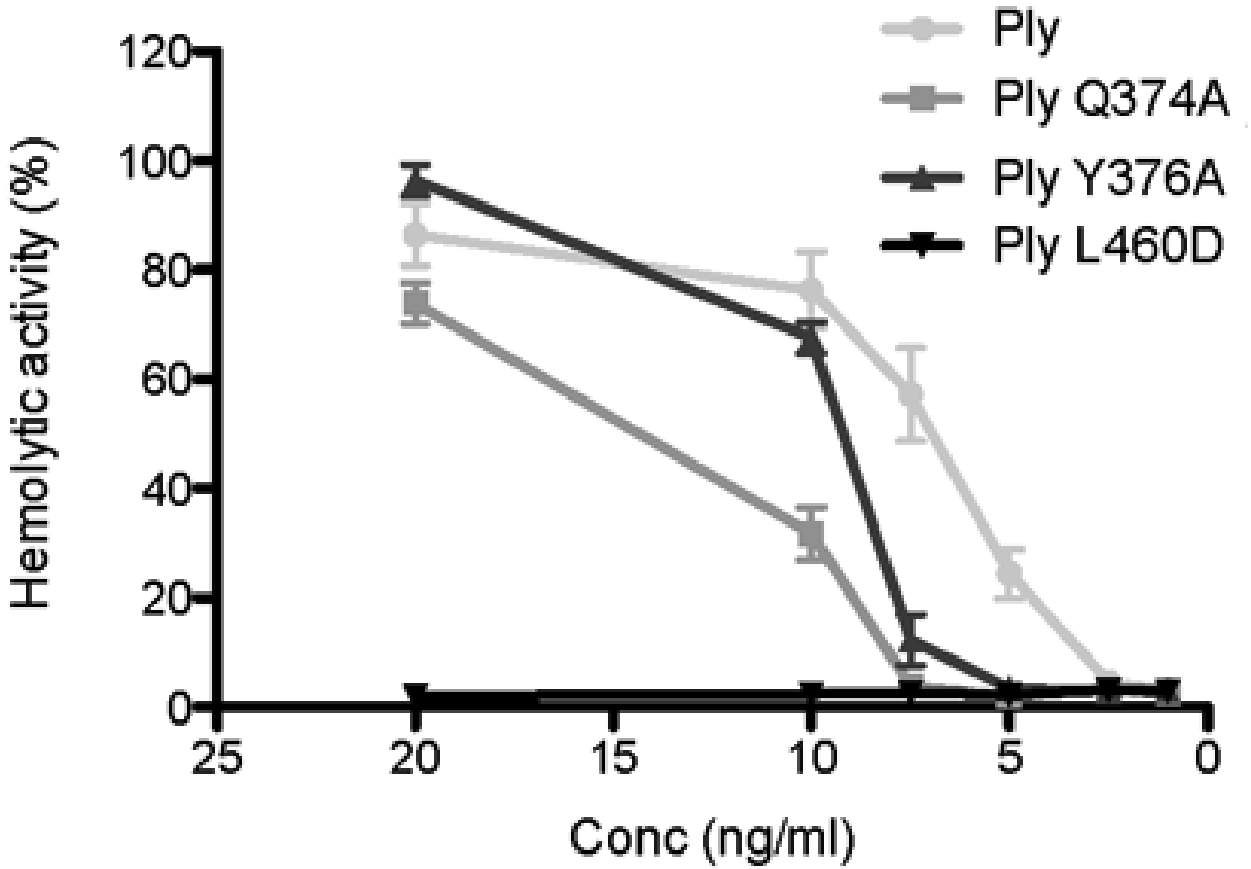
Both mutants had significantly reduced affinity for sLeX compared to wild-type as determined by SPR

Glycan	**Ply K_D**	**Ply Q374A K_D**	**Ply Y376A K_D**
sLeX	18.8 µM	137 µM	194 µM

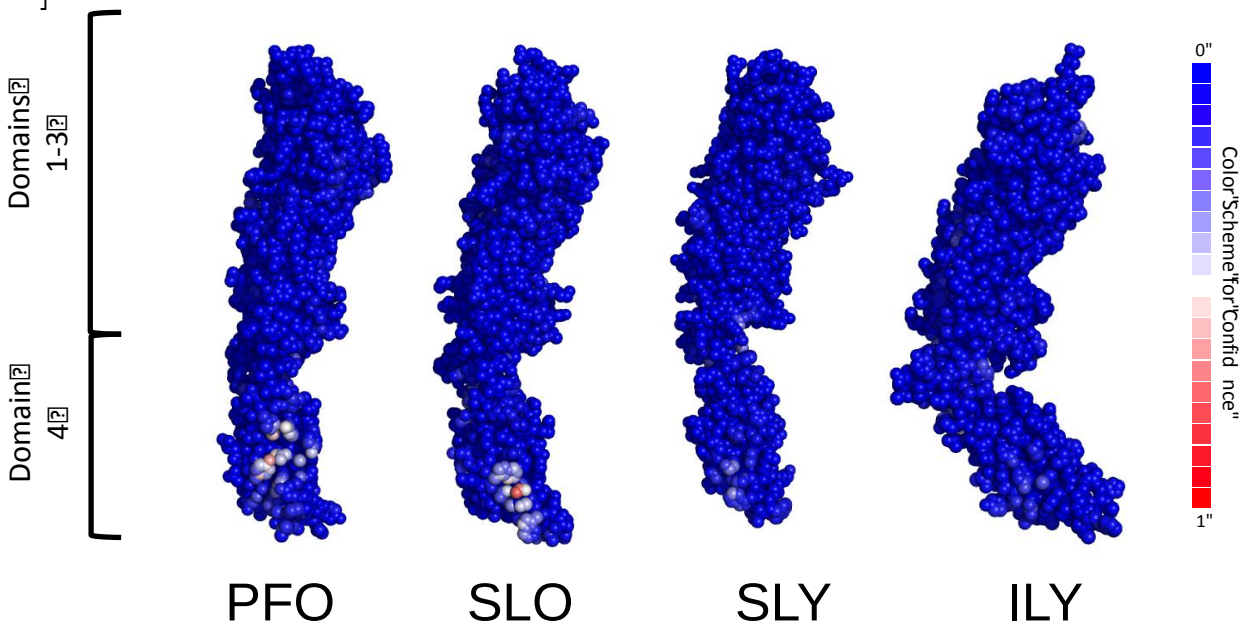


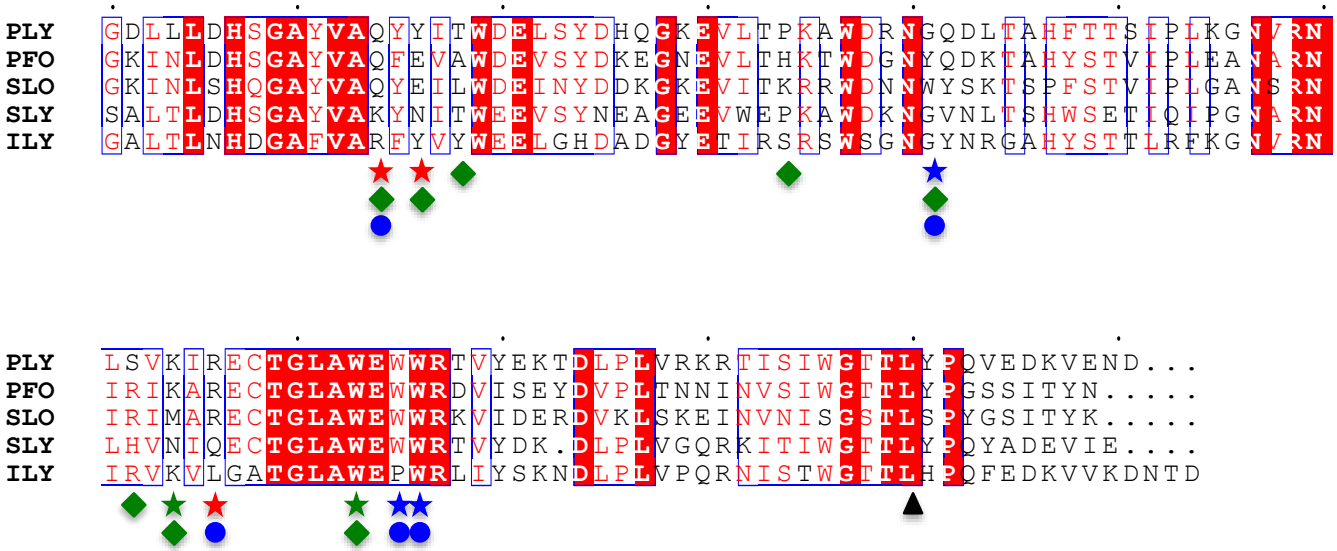
Protein-carbohydrate binding site prediction in domain 4 of Ply and mutagenesis

Both the PlyQ374A and PlyY376A mutants had reduced hemolytic activity against human RBCs.



Modeling to identify carbohydrate binding sites in other CDCs

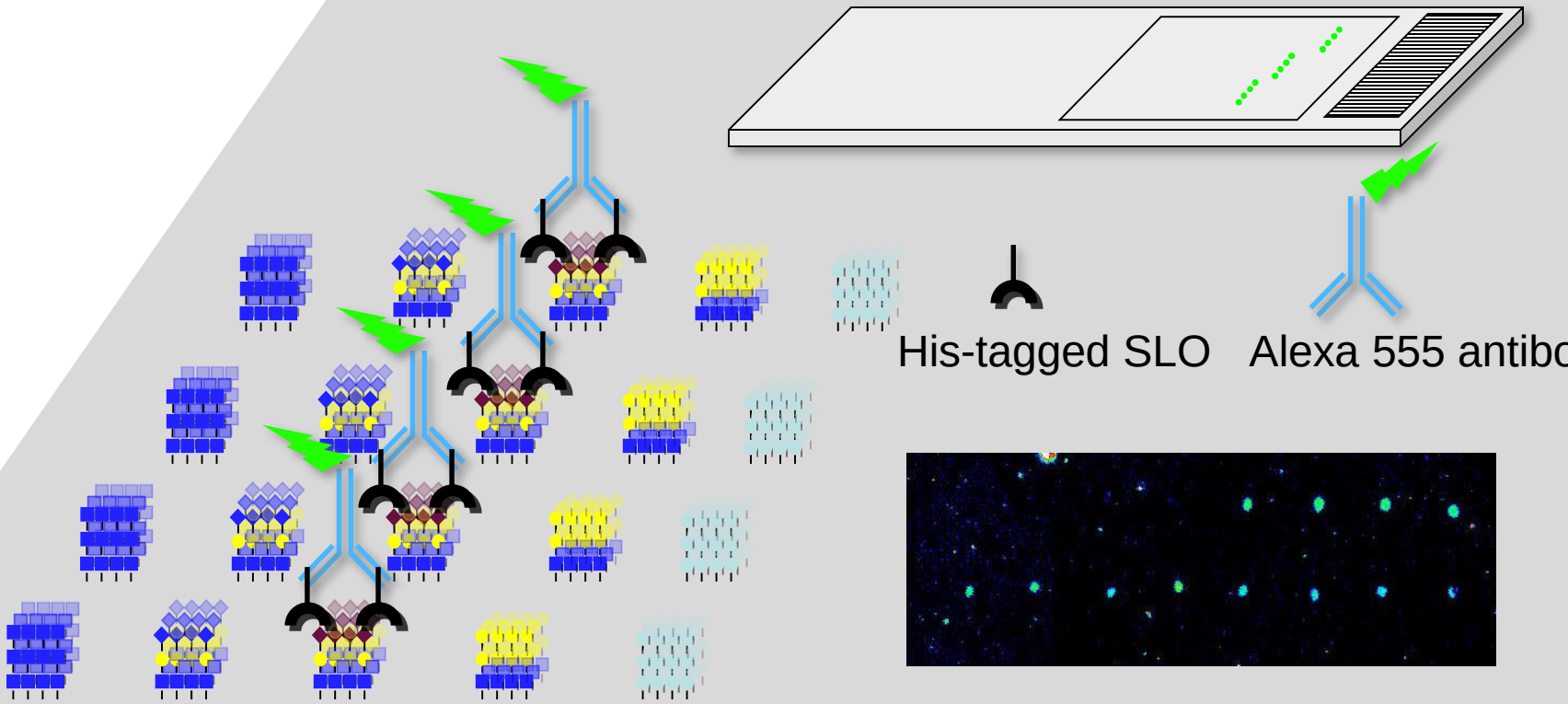




Glycan Arrays

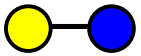
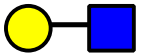
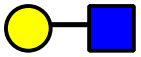
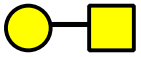
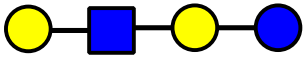
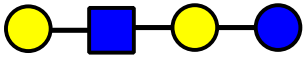
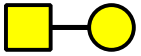
Array consisting of 400 glycans (mono- and oligo-saccharides) of known structures covalently immobilised onto glass slides.

Used to evaluate SLO for glycan recognition.

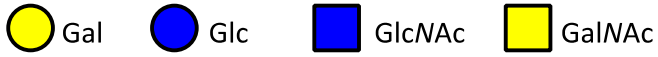


SLO also has lectin function

Glycan array analysis of SLO revealed binding to 47 glycan structures. SPR analysis further characterised and verified a selection of these glycan interactions.

Code	Name	Formula	Structure	K_D (M)
N/A	Lactose	Galβ1-4Glc		2.92×10^{-8} ($\pm 8.72 \times 10^{-9}$)
1A	Lacto-*N*-Biose I	Galβ1-3Glc*N*Ac		n.i.*
1B	*N*-Acetyllactosamine	Galβ1-4Glc*N*Ac		1.73×10^{-7} ($\pm 9.1 \times 10^{-9}$)
1E	β1-3galactosyl-*N*-acetylgalactosamine	Galβ1-3Gal*N*Ac		1.73×10^{-7} ($\pm 8.70 \times 10^{-10}$)
1G	Lacto-*N*-tetraose	Galβ1-3Glc*N*Acβ1-3Galβ1-4Glc		1.31×10^{-8} ($\pm 5.50 \times 10^{-10}$)
1H	Lacto-*N*-neotetraose	Galβ1-4Glc*N*Acβ1-3Galβ1-4Glc		6.44×10^{-10} ($\pm 1.82 \times 10^{-10}$)
1O	Linear B-2 Trisaccharide	Galα1-3Galβ1-4Glc*N*Ac		n. i.*
2C	Terminal disaccharide of globotetraose	Gal*N*Acβ1-3Gal		n. i.*

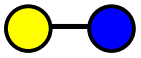
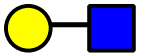
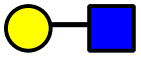
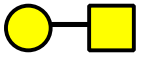
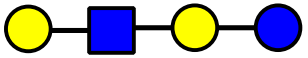
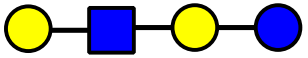
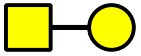
Symbol nomenclature



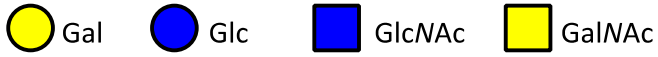
*n.i. = No interaction (K_D observed was greater than 1×10^{-3} M and/or the chi-square value was greater than 50% of the R_{max})

SLO also has lectin function

Glycan array analysis of SLO revealed binding to 47 glycan structures. SPR analysis further characterised and verified a selection of these glycan interactions.

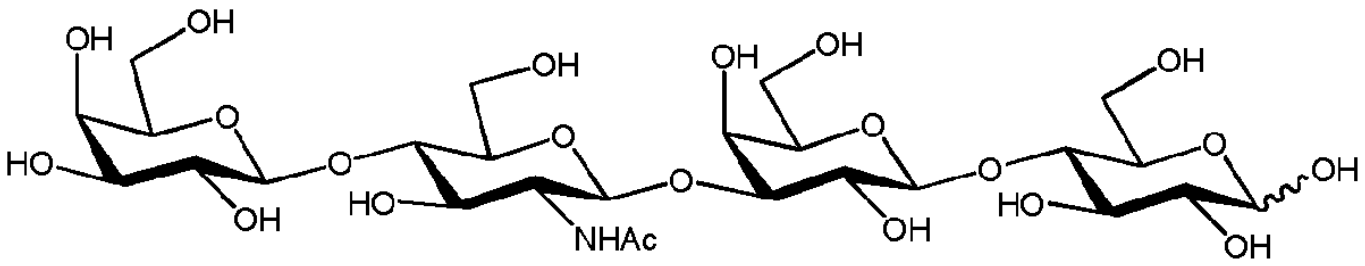
Code	Name	Formula	Structure	K_D (M)
N/A	Lactose	Galβ1-4Glc		2.92×10^{-8} ($\pm 8.72 \times 10^{-9}$)
1A	Lacto-*N*-Biose I	Galβ1-3Glc*N*Ac		n.i.*
1B	*N*-Acetyllactosamine	Galβ1-4Glc*N*Ac		1.73×10^{-7} ($\pm 9.1 \times 10^{-9}$)
1E	β1-3galactosyl-*N*-acetylgalactosamine	Galβ1-3Gal*N*Ac		1.73×10^{-7} ($\pm 8.70 \times 10^{-10}$)
1G	Lacto-*N*-tetraose	Galβ1-3Glc*N*Acβ1-3Galβ1-4Glc		1.31×10^{-8} ($\pm 5.50 \times 10^{-10}$)
1H	Lacto-*N*-neotetraose	Galβ1-4Glc*N*Acβ1-3Galβ1-4Glc		6.44×10^{-10} ($\pm 1.82 \times 10^{-10}$)
1O	Linear B-2 Trisaccharide	Galα1-3Galβ1-4Glc*N*Ac		n. i.*
2C	Terminal disaccharide of globotetraose	Gal*N*Acβ1-3Gal		n. i.*

Symbol nomenclature



*n.i. = No interaction (K_D observed was greater than 1×10^{-3} M and/or the chi-square value was greater than 50% of the R_{max})

lacto-*N*-neotetraose



LNnT found on human RBCs as the glycosphingolipid paragloboside, also known as *N*-neotetraosyl ceramide

Paragloboside is an intermediate in the biosynthesis of ABH blood group and P1 glycosphingolipid antigens.

Present on human polymorphonuclear leukocytes

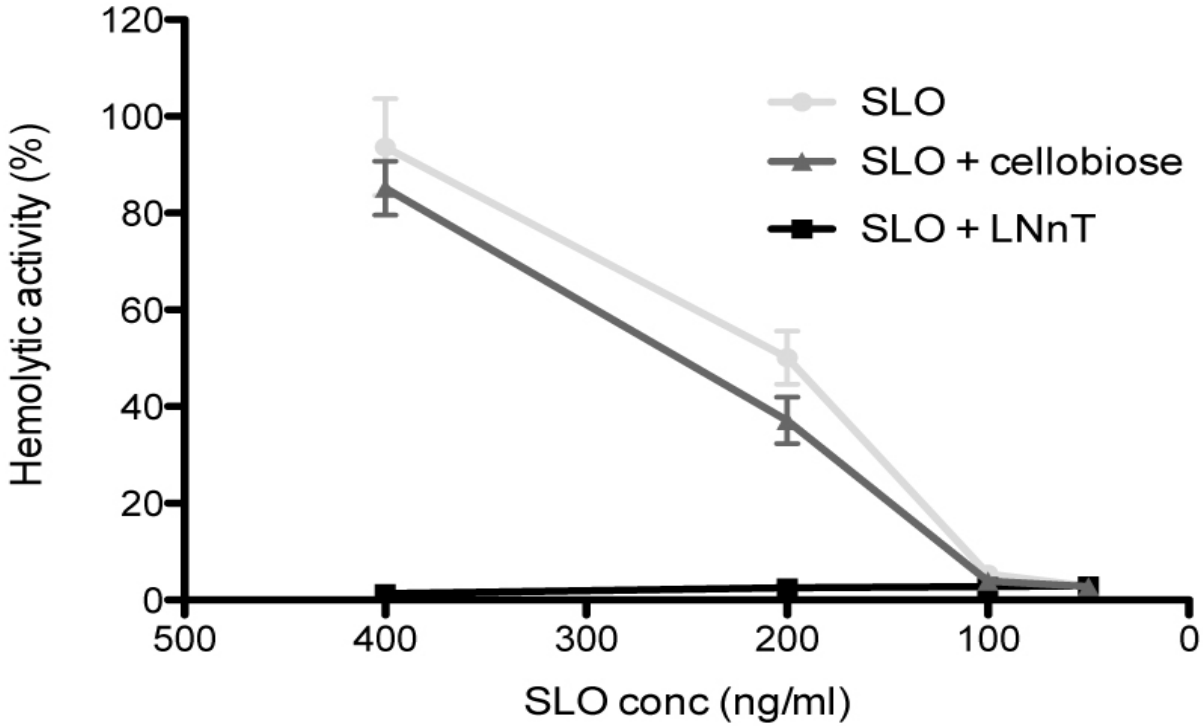


SLO glycan binding is required for hemolytic activity and deposition on RBC surface

Hemolysis assays and flow cytometric analysis of RBC binding were performed with SLO in the presence of lacto-*N*-neotetraose (LNnT) (highest affinity binding in SPR K_D=0.6nM)

D-cellobiose (Glcβ(1➔4)Glc) included as a negative control

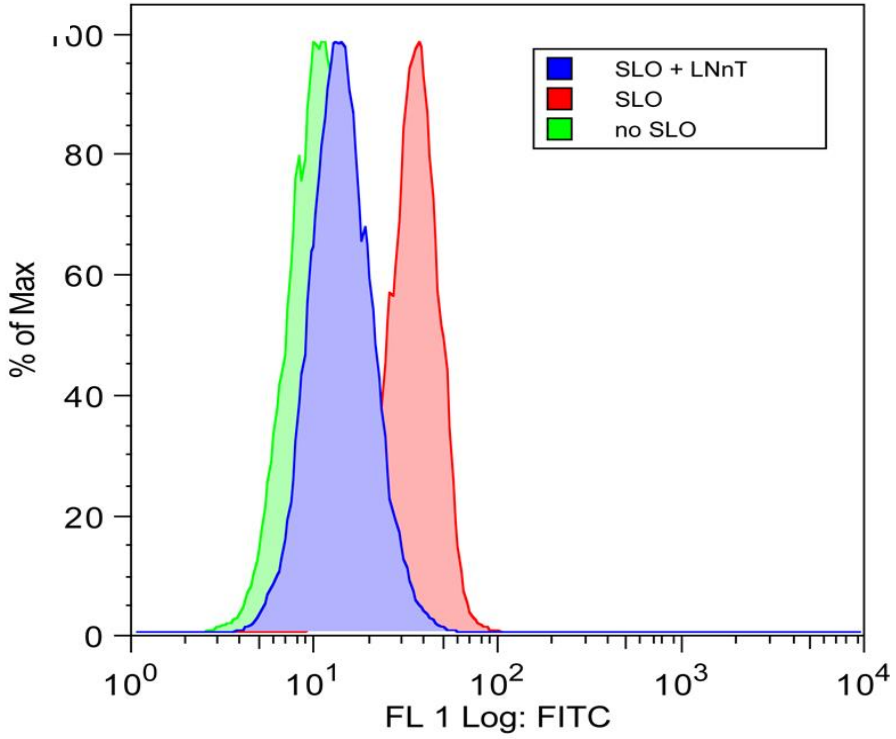
Free LNnT blocked SLO hemolytic activity.

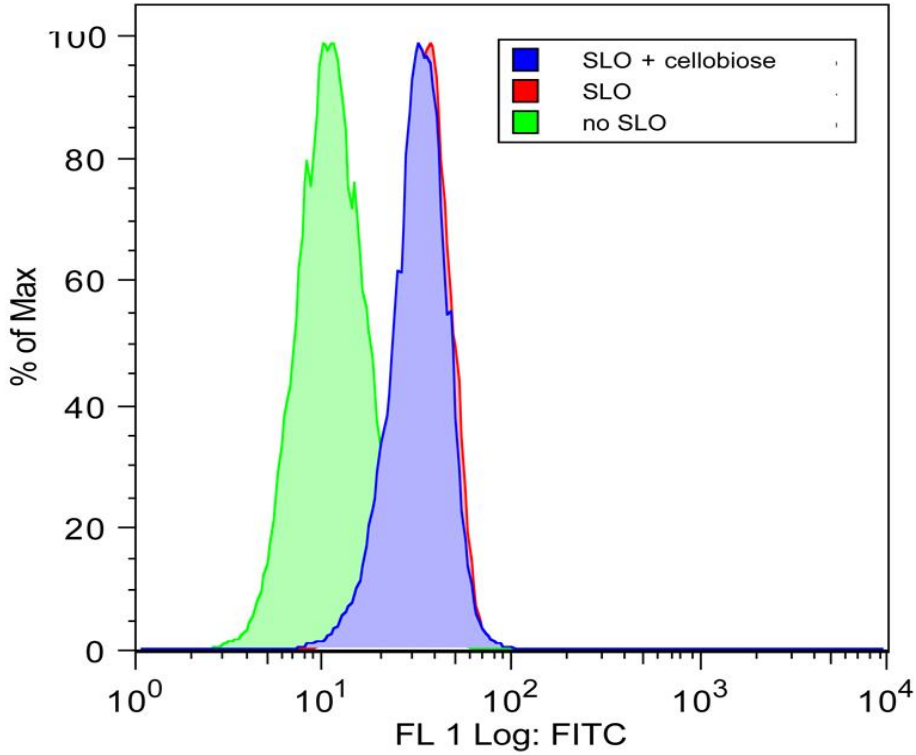


SLO glycan binding is required for hemolytic activity and deposition on RBC surface

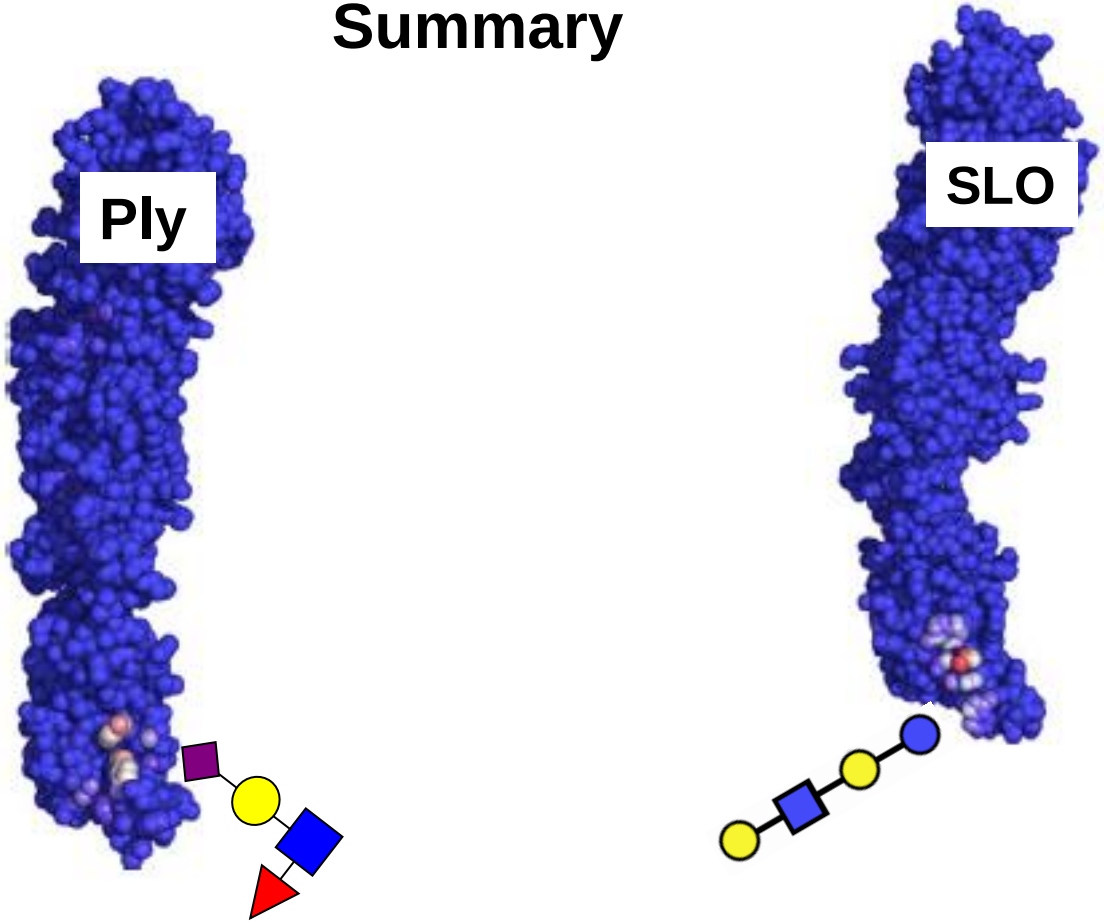
Hemolysis assays and flow cytometric analysis of RBC binding were performed with SLO in the presence of lacto-*N*-neotetraose (LNnT) (highest affinity binding in SPR K_D=0.6nM)

Free LNnT blocked SLO binding to the RBC surface.



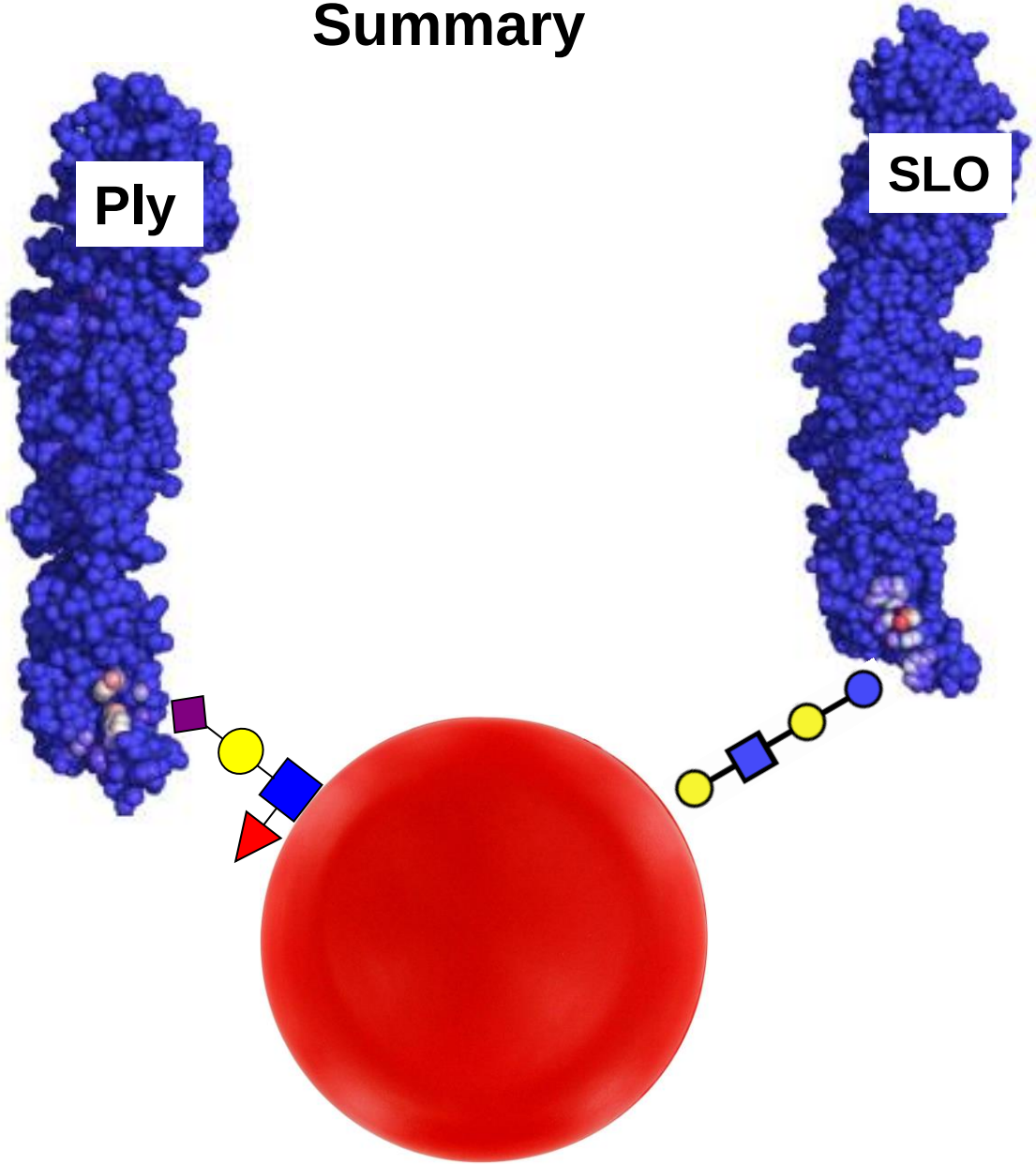


Summary



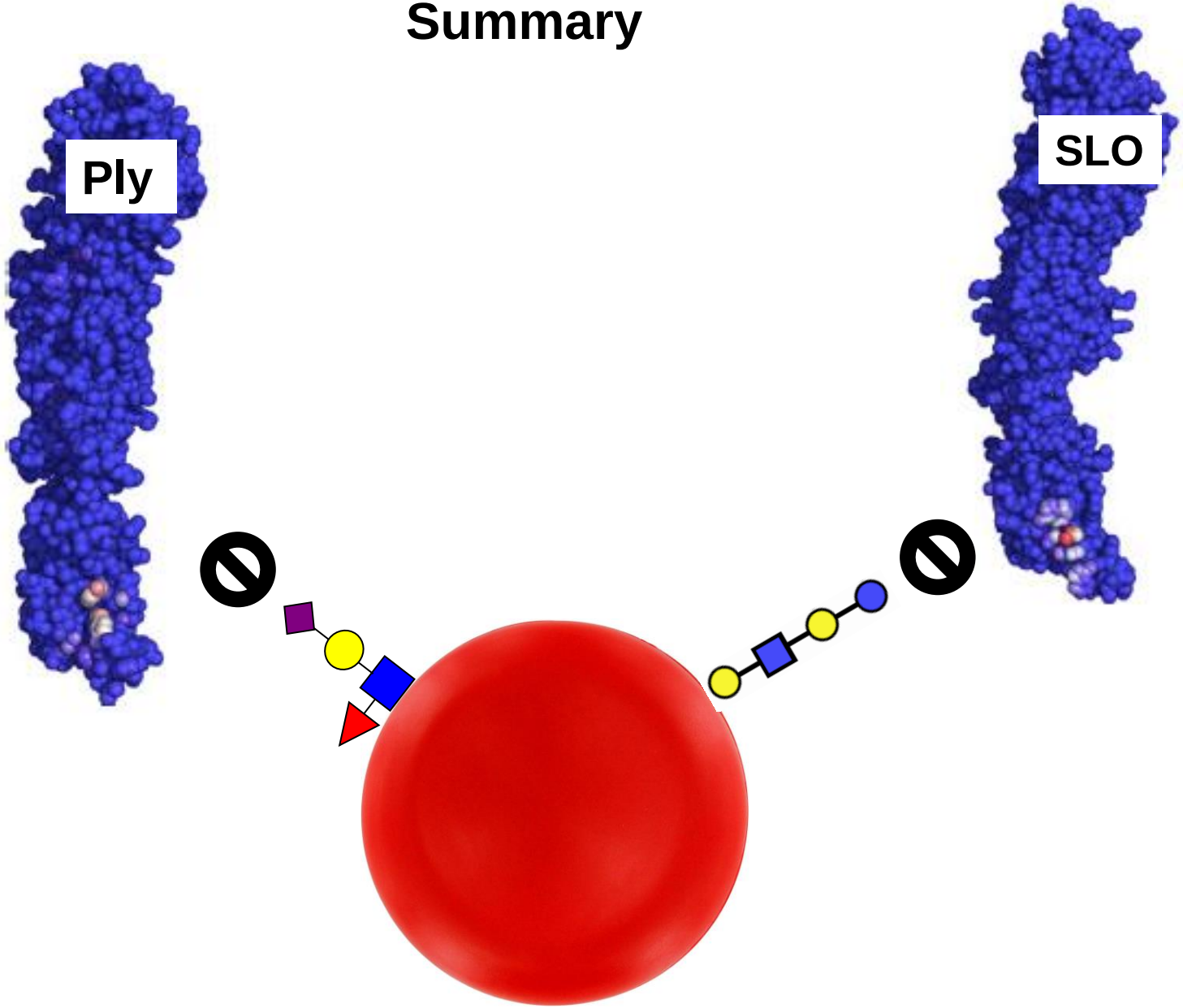
Shewell *et al PNAS E5312–E5320, doi: 10.1073/pnas.1412703111*

Summary



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Summary



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sLeX can inhibit Ply cytotoxicity against human alveolar basal epithelial cells

