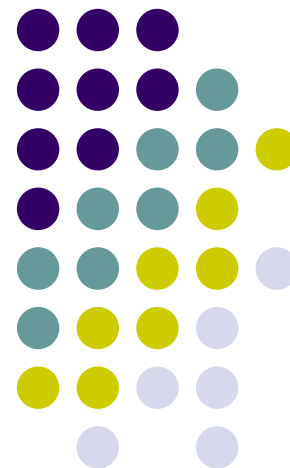


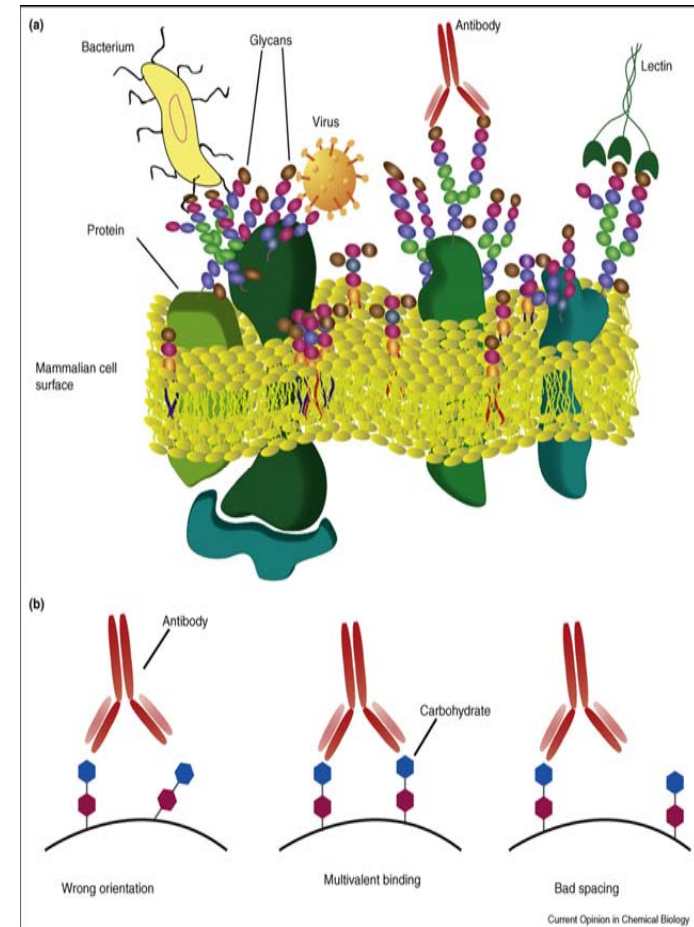
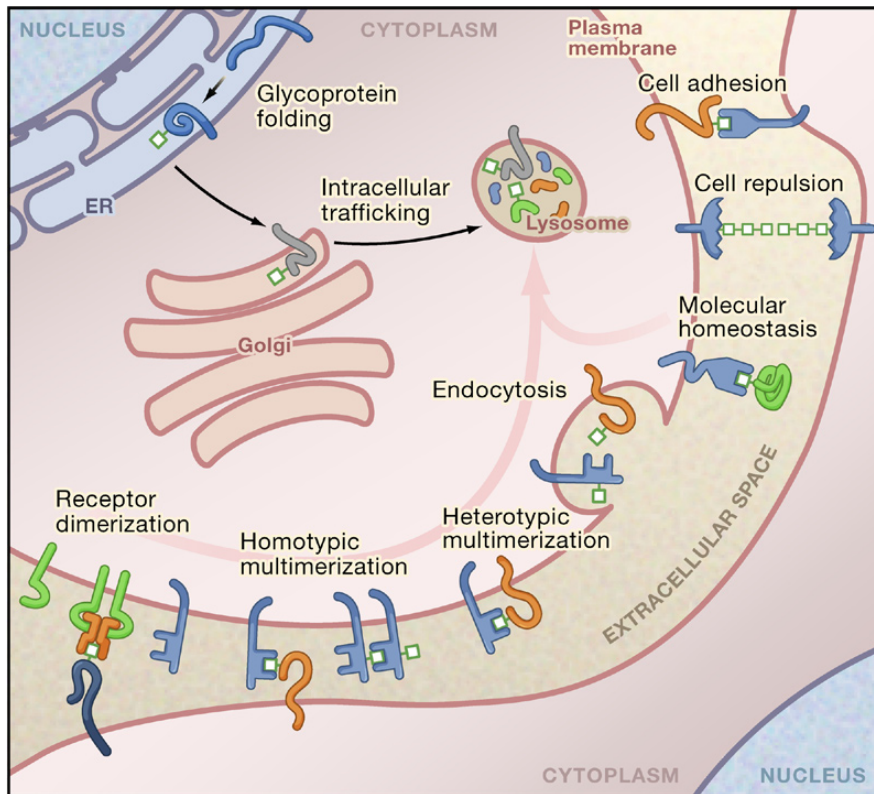
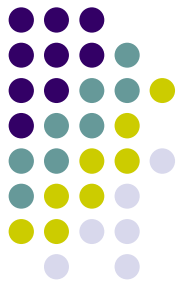
Quantitative Analysis of Carbohydrate – Protein Interactions Using Glycan Microarrays: Determination of Surface and Solution Dissociation Constants

Pi-Hui Liang, Sheng-Kai Wang, and Chi-Huey Wong.
J. Am. Chem. Soc., 2007, 129 (36), pp 11177–11184.
Contribution from the Department of Chemistry
the Scripps Research Institute

Presented by Yong Li
I690, Nov.18, 2009



Background: Carbohydrate-Protein Interactions



Background: Carbohydrate-Protein Interactions



- Mostly multivalent interactions
- Monovalent interactions are weak
 - Spacing of the glycans
- Low-specificity

Background:

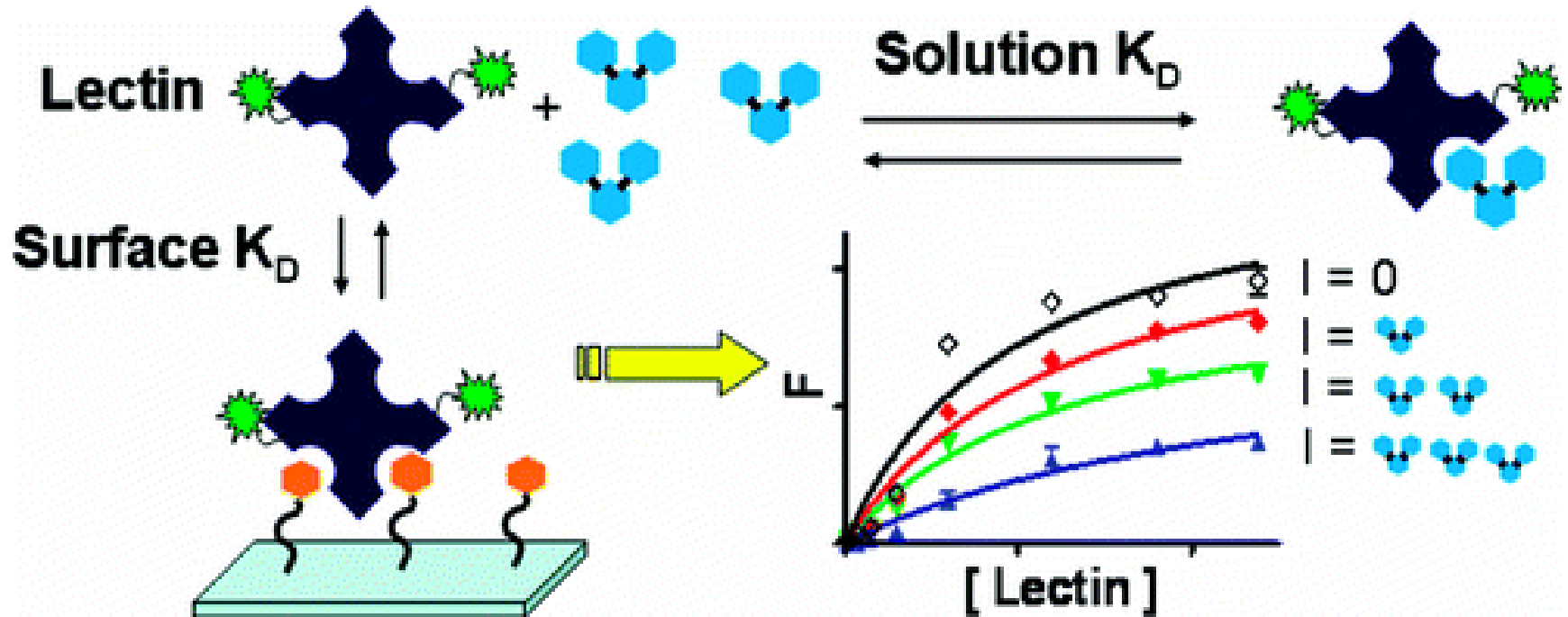
Quantify the Interactions



- Monosaccharide and oligosaccharide inhibition studies
- Isothermal calorimetry (ITC)
- Surface plasmon resonance (SPR)
- Enzyme-linked lectin assays (ELLAs)

- These techniques can be labor intensive and/or require large quantities of each carbohydrate.

Outline of this article



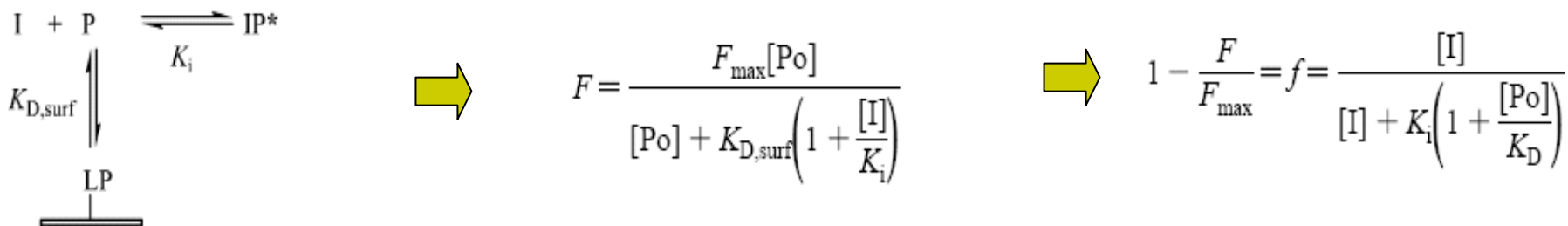
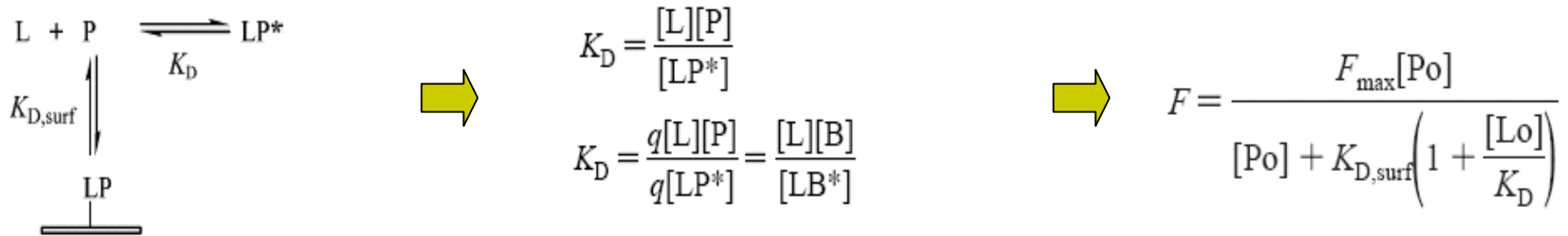
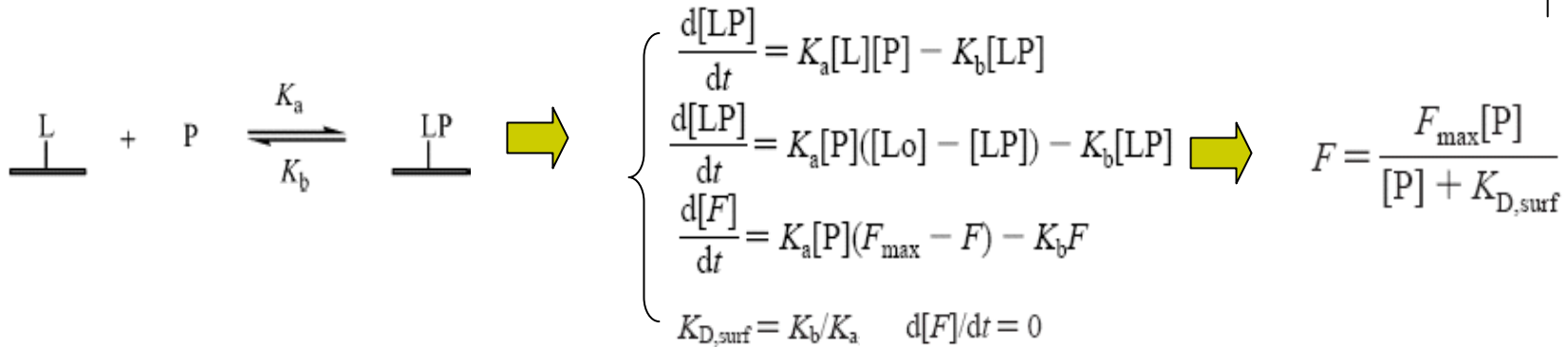
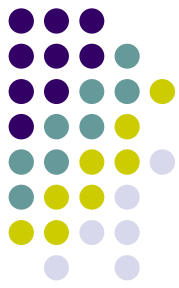
Background:

Glycan Microarray

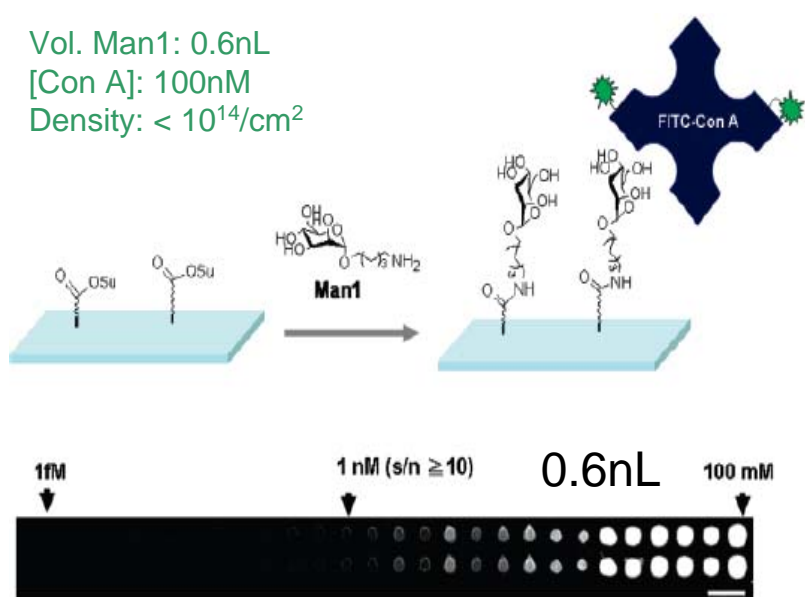


- The most common application of glycan array technology is the analysis of binding specificity of lectins and antibodies.
- Arrays are used to determine if a protein recognizes carbohydrates, identify natural ligands, and develop probes/inhibitors to modulate the activity of lectins.
- The assays are qualitative rather than quantitative. There are no standard computation tools to analyze the data from glycan array assays.
- At present, only minimal technical validation of glycan microarray reproducibility and reliability has been published.

Methods: The Theory of K_D



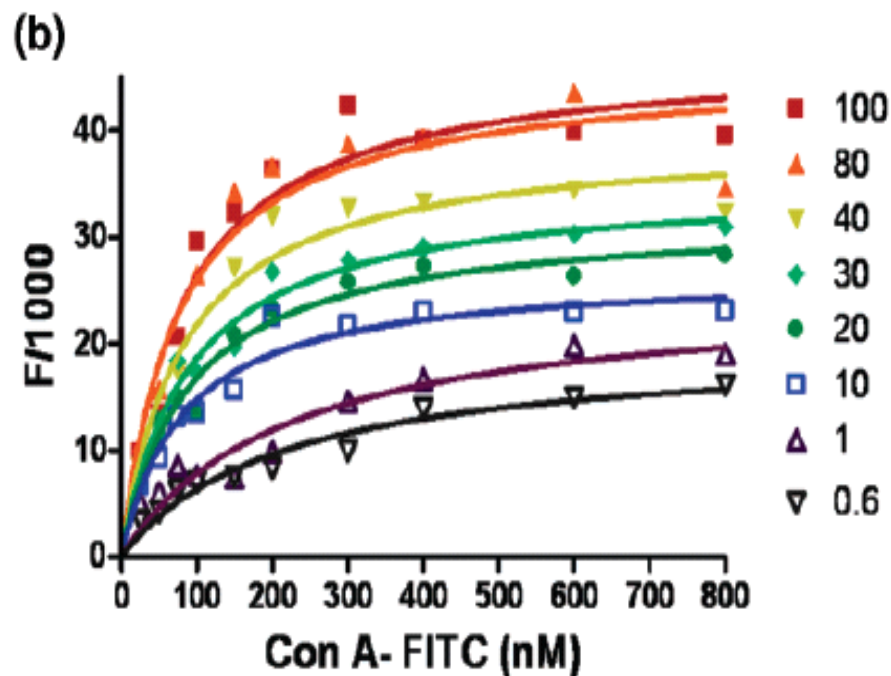
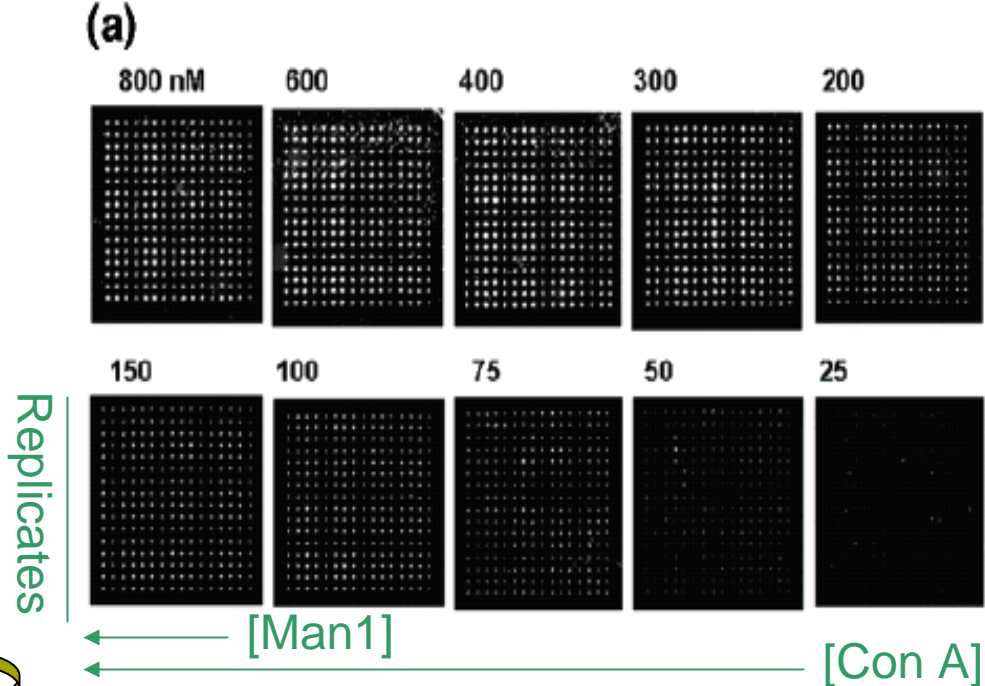
Vol. Man1: 0.6nL
 [Con A]: 100nM
 Density: $< 10^{14}/\text{cm}^2$



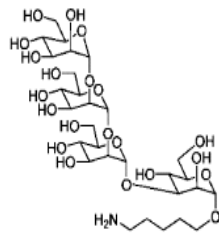
$$F = \frac{F_{\max}[P]}{[P] + K_{D,\text{surf}}}$$

Table 1. Functions of Different Printing Concentrations and the Corresponding Fluorescence Intensities (F_{\max}) and the Dissociate Constants on the Surface ($K_{D,\text{surf}}$)

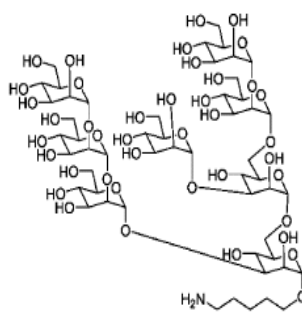
printing concn μM	F_{\max}	$K_{D,\text{surf}}$ nM
100	40950	80.4
80	40030	76.8
40	34050	81.7
30	29490	88.7
20	26910	90.6
10	22670	81.8
1	18250	221
0.6	14250	214



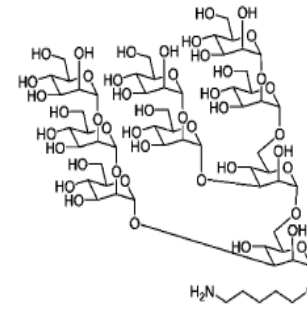
(a)



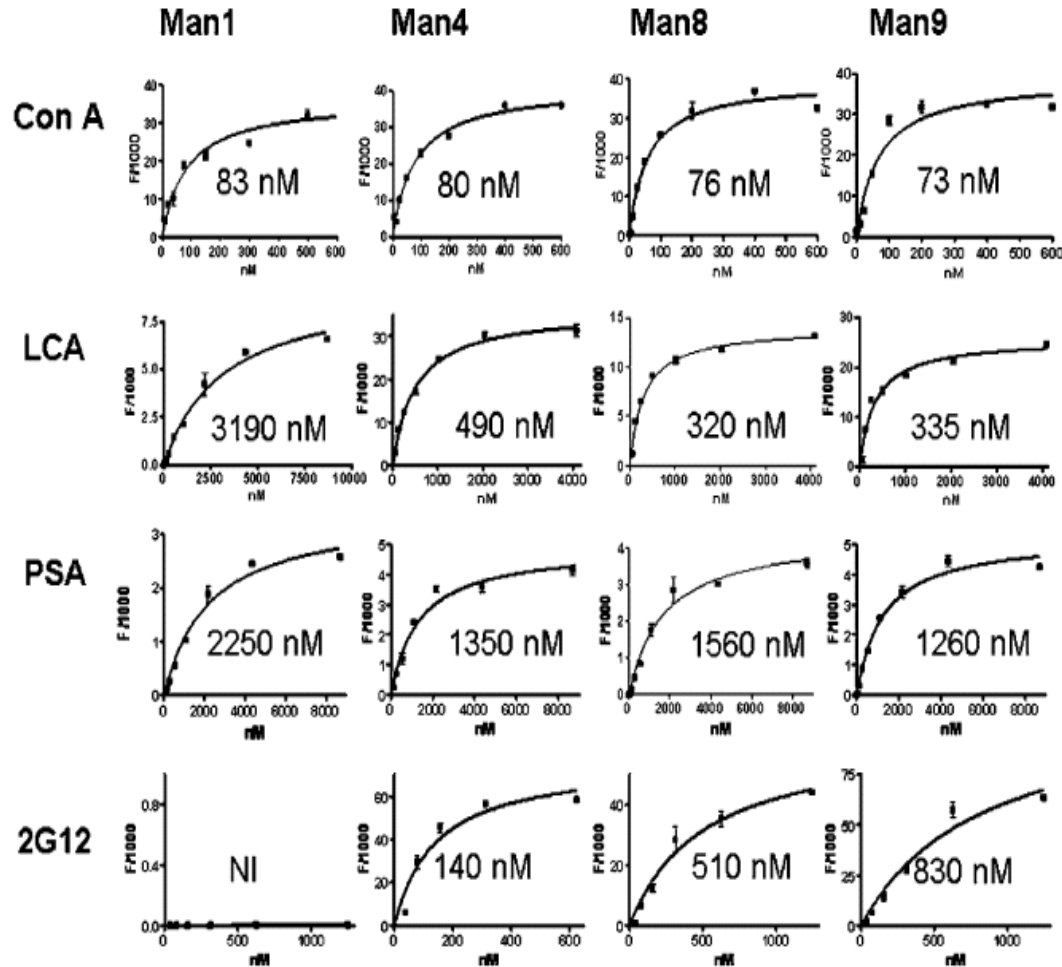
Man4

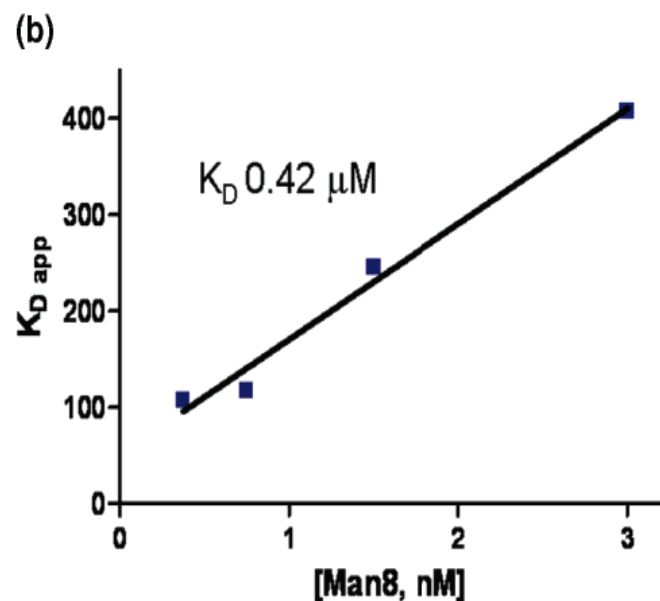
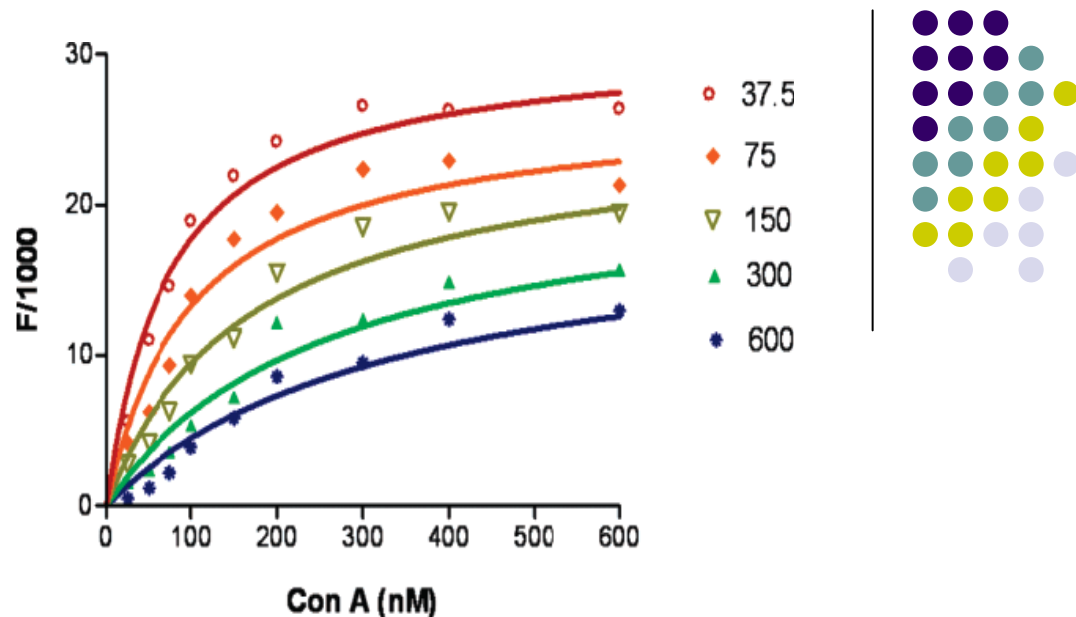
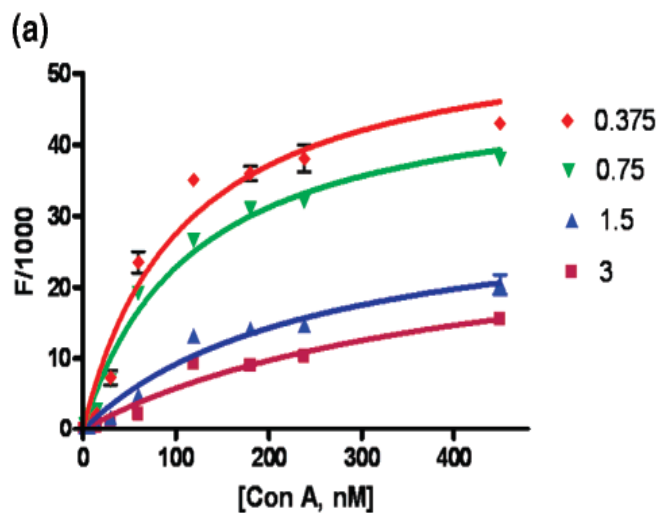


Man8



Man9





$$F = \frac{F_{\max}[Po]}{[Po] + K_{D,\text{surf}}\left(1 + \frac{[Lo]}{K_D}\right)}$$

$$1 - \frac{F}{F_{\max}} = f = \frac{[I]}{[I] + K_i\left(1 + \frac{[Po]}{K_D}\right)}$$

Table 2. Competitors and Solution K_i Values for the Interaction with Con A

competitors	array (mM)	ITC (mM) ^a	SPR (mM) ^b
α -MeMan	0.16	0.12	0.09
α -MeGlc	0.69	0.52	0.29
α -MeGal	— ^c	—	—
mannose	1.2	—	—
glucose	25	—	—
galactose	80	—	—

^a Ref 41. ^b Ref 12. ^c 20% inhibition at 100 mM. “—” not determined.

Summary:

Features and Applications



- Requires small quantities of materials (10^{-18} mol)
- High-throughput
- Quantitative
- Determine both $K_{D,S}$ and K_D
 - $K_{D,S}$ models the multivalent interactions, mimicking the interactions of proteins with cell-surface carbohydrates
 - K_D models the monovalent interactions



Problems and Solutions

- Within plate repetitions
 - Problem: Lower throughput
 - Solution: Improve of intra plate reproducibility
- Multiple plates for one experiment
 - Problem: Higher cost
 - Solution: Improve of inter plate reproducibility
 - Solution: Reuse the plates
- As a result, the assay has not been applied in large scale

THANKS

Questions are welcome!

