

# The study of interactions between lectins and immobilized glycoproteins using Biacore's SPR technology



## Introduction

The carbohydrate moieties of glycoconjugates, such as glycoproteins and glycolipids, play a key role in the recognition of determinants in protein targeting, cell-cell interaction and as cell surface receptors. The increasing use of glycoproteins for therapeutic purposes has led glycobiologists to develop techniques to characterize the sugar moieties of the glycan chains, including the use of lectins to characterize glycan structure on glycoconjugates [1-5]. Several different methods for visualizing the bound lectins have been described [6,7] in which lectins labelled with biotin or digoxigenin have been used. This short communication reports how Biacore® systems may be used in the study of lectin glycoprotein interactions.

The lectins and glycoproteins studied are listed in Table 1.

Lectins and Glycoproteins		
	Abbreviation	MW (kDa)
<b>Lectins</b>		
Canavalia ensiformis	Con A	104
Lens culinaris	LCA	52
Vicia villosa	VVL	110
Ricinus communis	RCA	120
Triticum vulgare	WGA	36
Griffonia (Bandeiraea) simplicifolia	BSII	113
Lotus tetragonolobus	TPL	120
<b>Glycoproteins</b>		
Thyroglobulin		669
Fetuin		50
IgG		150
GP 120		120

Table 1  
Lectins and glycoproteins used in the study.

## Results

Figure 1

An overlay plot of binding curves showing the interaction between lectins and immobilized thyroglobulin.

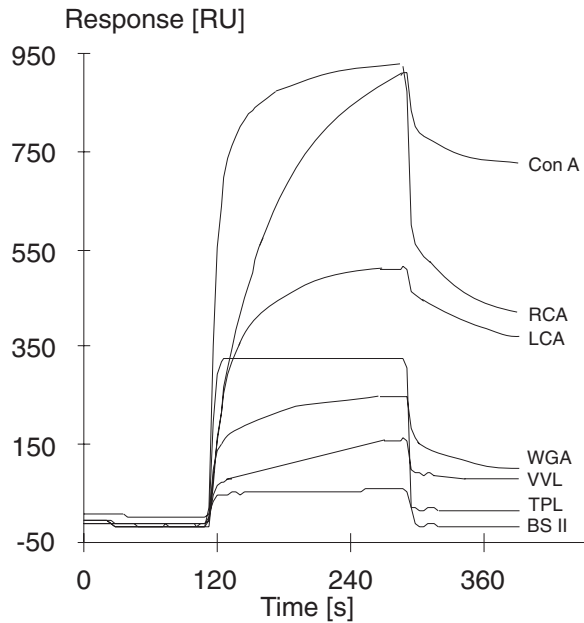
Lectin solutions (50 µg/ml in 10 mM HEPES, 0.5 mM MnCl<sub>2</sub>, 0.5 M CaCl<sub>2</sub> and 0.05% surfactant, pH 7.4) were injected. Bound lectin was dissociated by 100 mM HCl (15 µl, 5 µl/min).

Table 2

Summary of the interaction of seven lectins of different nominal specificities with immobilized glycoproteins. Each glycoprotein was immobilized to a Sensor Chip CM5 by amine coupling. Lectin solutions (15 µl, 50 µg/ml, 5 µl/min) were injected over the immobilized glycoprotein. The amount of bound lectin was recorded as the difference in RU before and after the injection. Binding of lectin to the glycoprotein is indicated by "+" and lack of binding by "-" in Table 2. As control experiments, the lectins were injected over (i) an immobilized non-glycosylated protein (recombinant HIV-1 reverse transcriptase expressed in *E. coli*) and (ii) a blank surface which was subjected to immobilization chemistry in absence of a protein. The lectins did not show any binding in the control experiments.

### References

1. The Lectins - Properties, Functions and Applications in Biology and Medicine, edited by I. E. Leiner, N. Sharon and I. J. Goldstein. (1986) Academic Press inc.
2. Glycoproteins by J. Montreuil, S. Bouquelet, H. Debray, B. Fournet, G. Spik and G. Strecker, Carbohydrate analysis - a practical approach, edited by M. F. Chaplin and J. F. Kennedy, IRL PRESS, (1986), Ch 5, 143-203
3. M. Liljebld, I. Ryden, S. Ohlson, A. Lundblad, P. Pahlsson A Lectin Immunosensor Technique for Determination of alpha(1)-Acid Glycoprotein Fucosylation Anal Biochem 288: 216-224 (2001)
4. M. C. Montalto, C. D. Collard, J. A. Buras, W. R. Reenstra, R. McClaine, D. R. Gies, R. P. Rother, G. L. Stahl A keratin peptide inhibits mannose-binding lectin J Immunol 166: 4148-53 (2001)
5. N. M. Thielens, S. Cseh, S. Thiel, T. Vorup-Jensen, V. Rossi, J. C. Jensenius, G. J. Arlaud Interaction properties of human mannan-binding lectin (MBL)-associated serine proteases-1 and -2, MBL-associated protein 19, and MBL J Immunol 166: 5068-77 (2001)
6. Analysis of glycoproteins - Lectin binding of HIV glycoproteins by S. Eriksson, R. Bhikhabhai and L. Hammar (1989), PhastSystem Application File No 301, Pharmacia LKB Biotechnology, Uppsala, Sweden.
7. Structural Characterization of Glycoprotein Carbohydrate chains by Using Digoxigenin-labeled lectins on blots by A. Haselbeck, E. Schickaneder, H. von der Eltz and W. Hüssel, Analytical Chemistry (1990) 191, 25-30.



### Interaction Matrix and Lectin Specificity

Lectin	Thyroglobulin	Fetuin	IgC	GP 120	Lectin Specificity
Con A	+	+	+	+	aMan>aGlc>GlcNAc
LCA	+	+	+	+	aMan>aGlc>GlcNAc
VVL	+	-	+	+	GalNAca1,3GalNAc=aGalNAcaGalNAc
RCA	+	+	-	-	bGal>aGal>GalNAc
WGA	+	+	-	+	GlcNAc(b1,4GlcNAc)1-2>bGlcNAc>Neu5Ac
BSII	-	-	-	+	a and b GlcNAc
TPL	+	-	-	-	aL-Fuc>L-Fuca1,2 Galb1, 4GlcNAc...

## Conclusion

The lectin specificity of different glycoproteins can be qualitatively and quantitatively compared without the need for labelling the lectins. The comparison

shown in Figure 1, including the immobilization of glycoprotein on the sensor surface, was performed in less than 90 minutes.

*First published in 1996.*