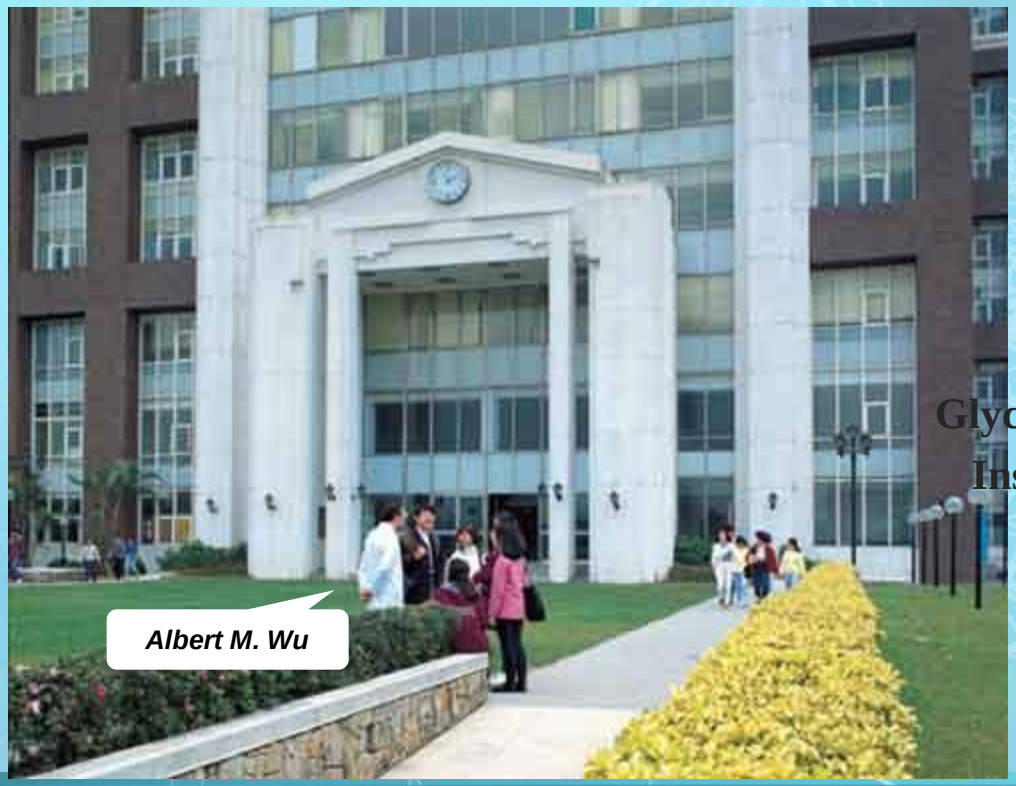
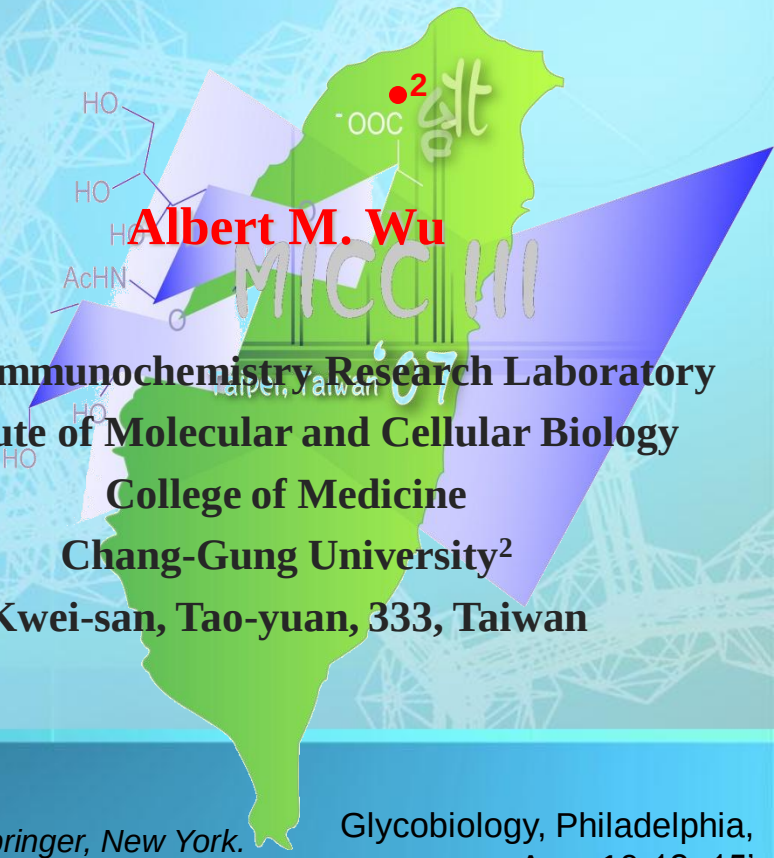
Roles of Polyvalency in the Mechanism of Glyco Recognition.

An Important Direction toward the Future Glycosciences[1]





Albert M. Wu

Glyco-Immunochemistry Research Laboratory
Institute of Molecular and Cellular Biology
College of Medicine
Chang-Gung University[2]
Kwei-san, Tao-yuan, 333, Taiwan

[1] *The Molecular Immunology of Complex Carbohydrates – 3 , 2011, 99-116, Springer, New York.*

[2] Located at 20 km south-west of Taipei, Midpoint, between Taipei-Taoyuan Airport

Glycobiology, Philadelphia, Aug. 10-12, 15'

Introduction

A milestone of Glycans and Carbohydrate Binding Proteins for Half a Century (1/2)

A. Before 1960 – It is a Pre Complex Carbohydrates (C C) Era - An **Unknown** world of Glycans and Recognitions of Carbohydrate-binding Proteins (Abs and Lectins) - it is also at an age of their incubation period.

B. Glycoworld between 1960-1990 – It is an Era of **Complex Carbohydrates** and Lectin / Ab - Carbohydrate interactions for 30 years

C. Glycoworld after 1990 – An Era of **Glycobiology** Combination of glycoimmunochemistry recognition and biology

D. Glycoworld from structural complex carbohydrate to functional roles after 2015 - An Era of Glyco/Recognition-universe

Roles of polyvalency of glycotopes and cryptopes in recognition processes, especially the biological roles of their resulting conformations.

Introduction

The Milestone of Glycans and Carbohydrate Binding Proteins after Half a Century (2/2)

Glycoworld after 2015 - An Era of Glycan and Lectin - universe

The unknown areas of glycan have to be continuously explored and/or these projects that have to be a long term study. These are :

① Roles of polyvalency of glycotopes and cryptopes in recognition processes, especially the biological roles of their resulting conformations.
② Role of **sulfate** in dynamic Glycobiology;
③ Mechanisms of microbial infections;
④ Bacterial fimbriae lectin-recognition processes;
⑤ Glyco factors involved in the mechanisms of inflammation;
⑥ Role of glycans in aging;
⑦ Glycobiology of stem cells;
⑧ Carbohydrate-carbohydrate interactions, etc.

Lectins are carbohydrate binding proteins or glycoproteins of non-immune origin, present in plants, microbes, animals and humans which specifically bind defined monosugars or oligosaccharide structures

Toxic plant seeds

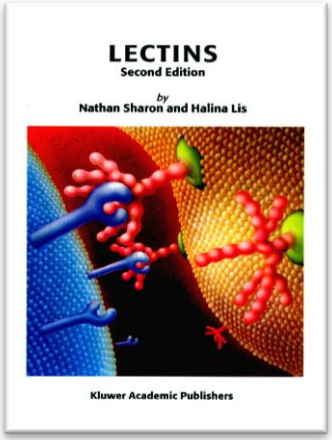
GSL is not illustrated

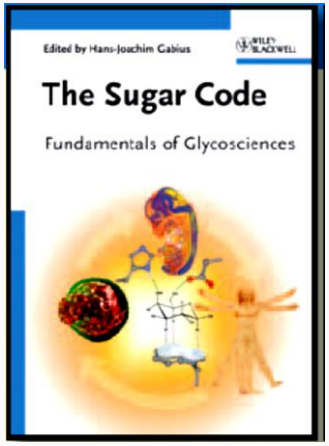
Sharon N., Lis H. : Carbohydrates in Cell Recognition; *SCIENTIFIC AMERICAN Jan. 1993*

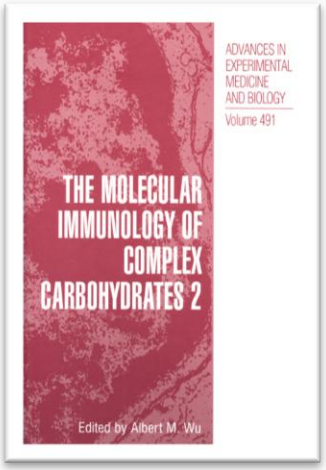
During the past two decades, great progress has been made in understanding crucial roles played by lectins in many biological processes

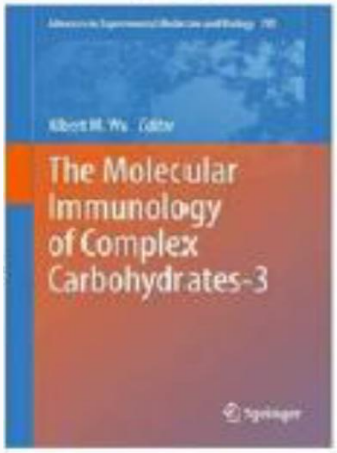
Suggested Articles

1. *Peumans, W.J., Van Damme, E.J., Barre, A., Rouge, P.: Classification of plant lectins in families of structurally and evolutionary related proteins. Adv. Exp. Med. Biol. 491, 27-54 (Wu, ed, 2001)*
2. *Van Damme, E.J., etal.: Novel Concepts about the Role of Lectins in the Plant Cell. Adv. Exp. Med. Biol. (Wu, ed, 2009)*
3. *Sharon, N., Lis, H.: Lectins, 2nd Ed. (Kluwer Academic Publisher, Dordrecht, 2003) p.454*
4. *Kaltner, H., Gabius, H.J.: Animal lectins: from initial description to elaborated structural and function classification. Adv. Exp. Med. Biol. 491, 79-94 (Wu, ed, 2001)*
5. *Crocker, P.R., Paulson, J.C., Varki: Siglecs and their roles in the immune system. Nat. Rev. Immunol.7, 255-66. (2007).*

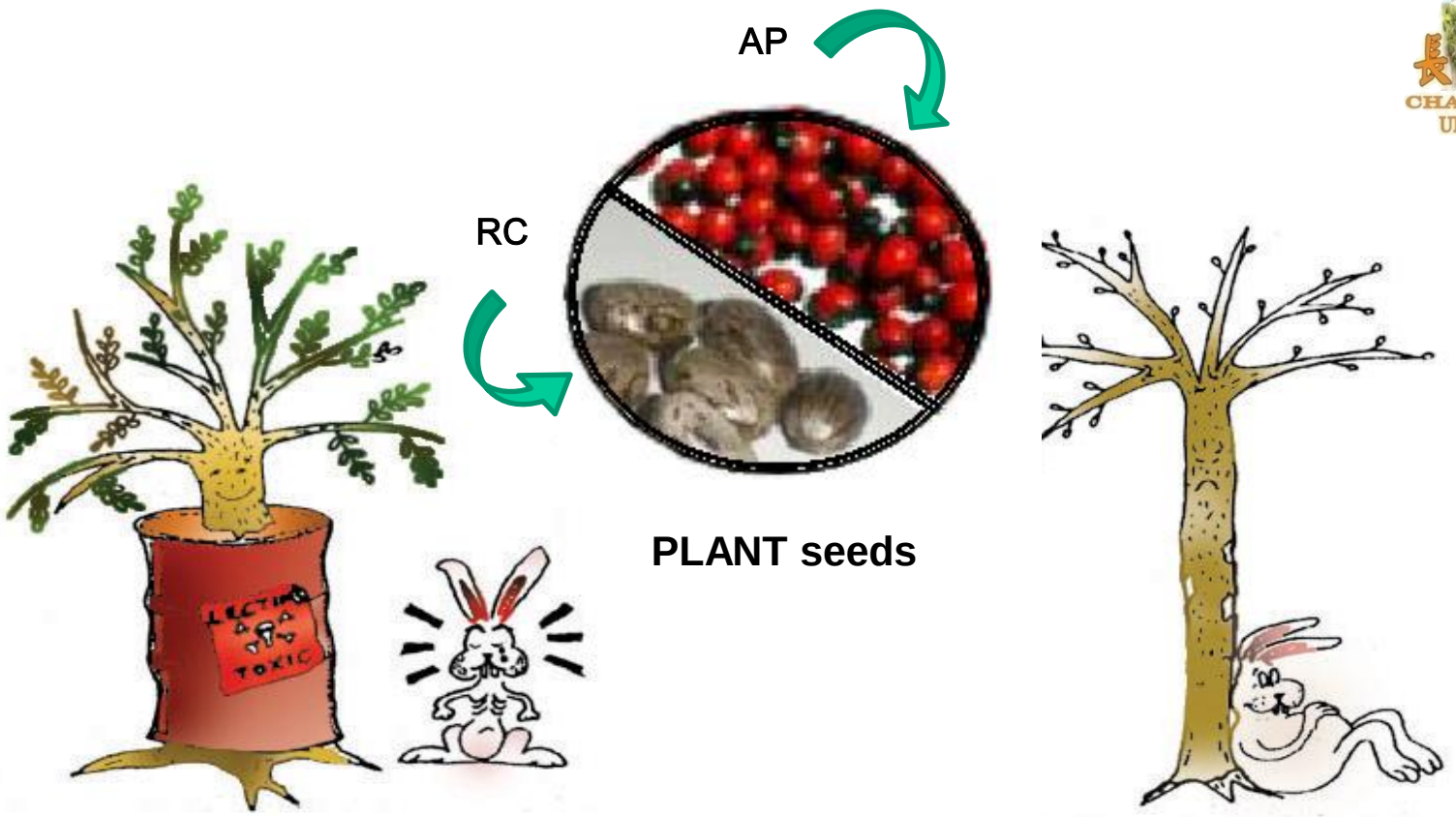








Functional Roles of Lectins (1)



Lectin protects plants against herbivorous, chewing animals and also prevent microbial invasion

Reproduced with permission from Peumans & Van Damme, 1995; copyright 1995, American Society of Plant Biologists

Abrin and Abrin-related toxins, Ricin and ricin-related toxins have been reported to protect plant s against herbivorous animals, and/or phytophagous invertebrate (Peumans, W. J. *et al.,* 2001)

Functional Roles of Lectins (2)

Lectins as Cell Recognition Molecules*

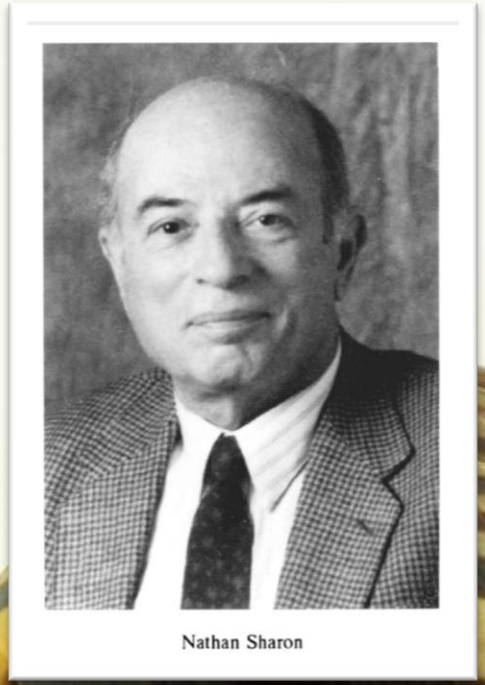
NATHAN SHARON AND HALINA LIS

Lectins on cell surfaces mediate cell-cell interactions by combining with complementary carbohydrates on apposing cells. They play a key role in the control of various normal and pathological processes in living organisms.

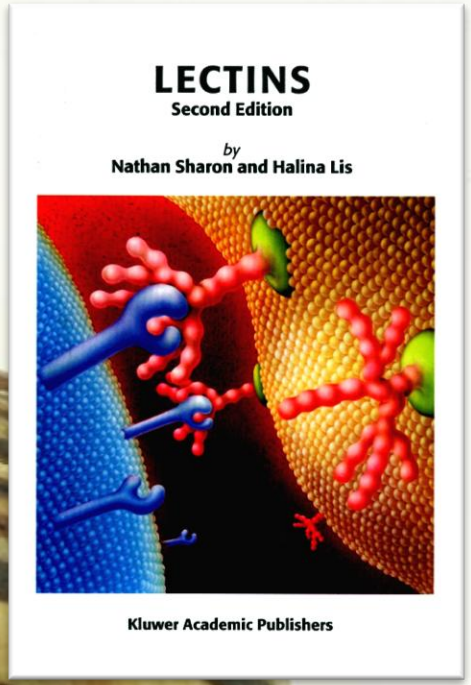
such as fertilization, embryogenesis, cell migration, organ formation, immune defense, and microbial infection. Improper function of cell recognition may cause disease.

*Science, 1989 246,227

The hallmark of lectins is the ability to bind carbohydrates specially and reversibly. Understanding the properties and functions of lectins, as well as using them for diverse purposes, requires knowledge of this specificity.

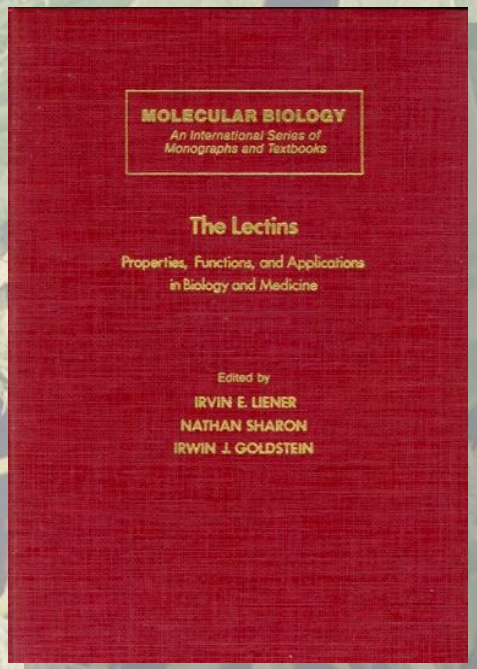


Nathan Sharon



1. Sharon, N., Lis, H.: *Lectins*, 2nd Ed. Kluwer Publisher, Dordrecht, 03', p.454
2. Wu, A.M., ed: The Molecular Immunology of Complex Carbohydrates-2, *Adv Exp Med Biol*. 491,Kluwer /Plenum, New York and London, 01'.

In early seventies many plant lectins were identified, characterized and applied to study complex carbohydrates

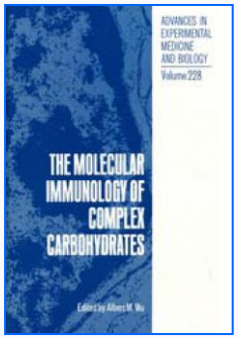


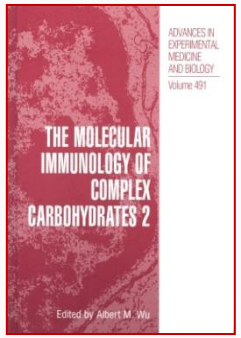
1. Liener, I.E., Sharon, N., Goldstein, I.J., eds. :*The lectins: Properties, functions and applications in biology and medicine.* Orlando, FL: Academic Press. 1986
2. Wu, A.M., ed: The Molecular Immunology of Complex Carbohydrates-2, *Adv Exp Med Biol*. 491, Kluwer/ Plenum, New York and London, 01'.

Lectinology being studied in our laboratory can be divided in two major directions

I. Studies on the intensities of recognition factors of lectins

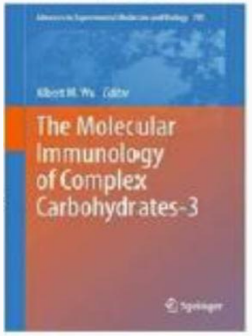
To characterize the structure of lectin molecules, their carbohydrate-binding specificity, conformational/functional properties of their carbohydrate combining site (domain), and their functional roles

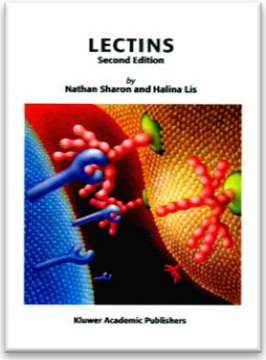




II. Application of lectins as tools

The application of lectins with known specificity as tools to study the function of glycoconjugates, both in solution and on cell surfaces





To obtain a comprehensive recognition profile of a lectin the following aspects have to be considered:

I. From the glycan structural aspects :

(i) Epimers and anomers of a sugar

(ii) Monosaccharide specificity

(Gal, GalNAc, GlcNAc, Man, ʟFuc, and Sialic acid)

(iii) Reactivities toward mammalian disaccharides and **Tn** structural units (in decreasing order)

(iv) The most active ligand

(v) Simple multivalent or cluster effect of carbohydrate structured unit such as

Tn glycopeptides and multi-antennary glycotopes to inhibit binding

(vi) Complex polyvalent or multi- effects present in macromolecules with known glycotopes

Glyco-macromolecules, such as THGP (polyvalent GalNAcβ1→4) and Asialo PSM (polyvalent **T**/**Tn**)

II. From the methods used to study can provide different angles and views to look into carbohydrate-lectin interactions.

Methodology

The methods of measuring lectin-carbohydrate interactions which are used for determination of carbohydrate specificity of lectins are

A. Traditional (classical) methods

a. Hemagglutination-inhibition assay

At Kabat's lab and worldwide for hundred years.

One of the natural and inexpensive ways to study polyvalency

b. Quantitative precipitation/precipitin-inhibition assays (QPA/QQPIA) 1960-1995 (Kabat and Wu's lab)

B. Current Approach

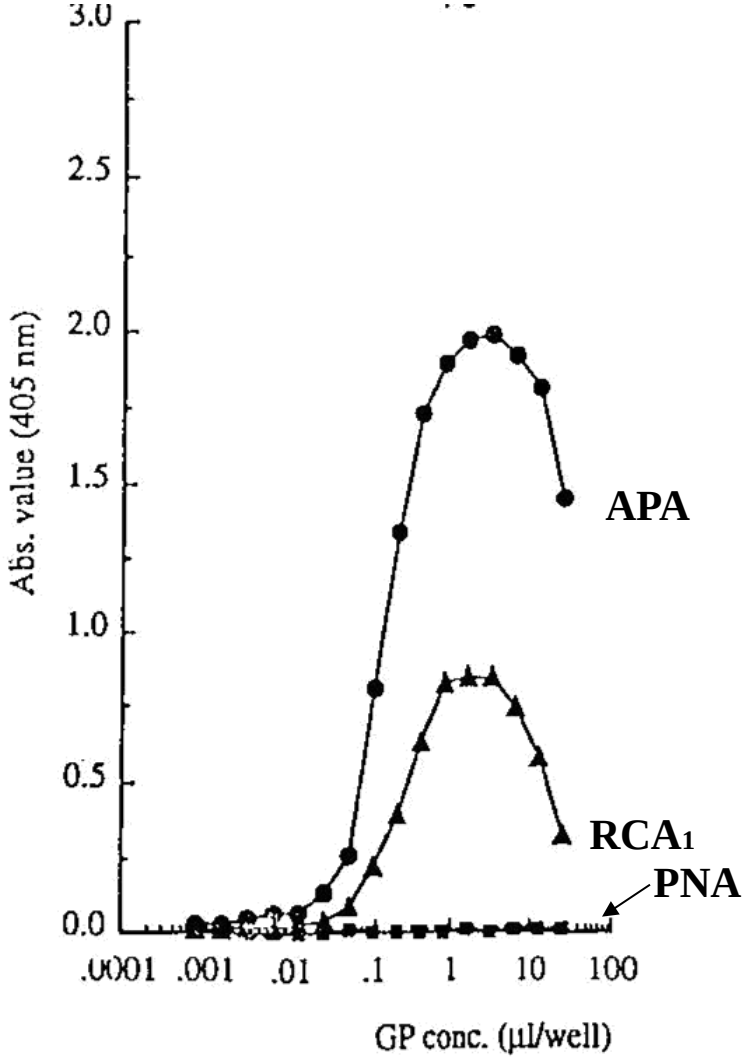
Enzyme-Linked LectinoSorbent Assay (ELLSA)

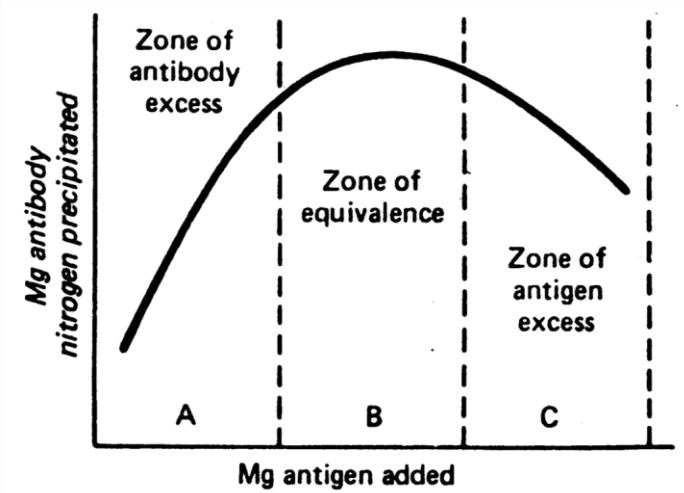
From 1994 to present at Wu's lab

One of the easy and budget ways to study polyvalency

The interaction profile of ELLSA is similar to that of QPA

Interaction of lectins with THGP* analyzed by ELLSA Read at 30 min, Lectin conc.=0.05 μg/well





The main zones of antigen-antibody reaction in QPA

* Tamm–Horsfall urinary glycoprotein

However, different lectins were found to recognize different saccharide sequences as their own receptor sites, although they are considered to be identical in terms of monosaccharide specificity

For example, **T (Galβ1→3GalNAc) specific lectins**

		Binding Contributions	
	Monosaccharide Specificities	GalNAc	Gal
(1) *Agaricus bisporus* (ABA)	GalNAc > Gal	+++++	-
(2) *Maclura pomifera* (MPA)	GalNAc >> Gal	++++	+
(3) *Arachis hypogaea* (PNA)	Gal >>> GalNAc inactive	-	+++++
(4) ***Abrus precatorius* (APA)**	**Gal > GalNAc**	**+**	**++++**

Expression of the Recognition profile of Gal and GalNAc Reactive Lectins — Historical Aspect

Until the early seventies, the carbohydrate specificity of lectins were mainly determined by the ability of

Monosaccharides: Gal, GalNAc, GlcNAc, Man and/or

Their glycosides: Me-αGal, Me-βGal, *p*-NO_2Ph-αGal,.

***p*-NO_2Ph-βGal**

to inhibit lectin-induced hemagglutination

Effect of polyvalency of glycotopes in glycan on lectin-glycoform interaction

During the past three decades, it has been observed that many multi-branched oligosaccharides exhibit a significant increment in lectin binding reactivity as compared to their linear counterparts. Based on the results of previous studies, the concept of glycoside cluster effect can be classified into two groups:

(a) The ‘multi-antennary or simple glycoside cluster effect’ as in reaction of galactosides with hepatic lectin and tri-antennary **II** sequences reactive with a galectin from chicken liver (CG-16), or **Tn** glycopeptides The molecular sizes of these ligands are usually less than 1.5×10^4.

(b) The ‘high-density polyvalent or complex glycoside cluster effect’, such as polyvalent **Tn** in asialo OSM which generates an enhancement in affinity with VVL-B_4 by 3.3×10^5 and 4.5×10^3 times over Gal and GalNAc, respectively, and is about 1000 times more active than monomeric **Tn**. The much stronger inhibition of RCA_1 by a panel of cyst glycoproteins than by disaccharides and galactose is shown.

Glycan structural units and recognition codes

Abbreviation and Structure of Disaccharide Structural Units and Tn Epitope Used to Express Carbohydrate Specificity of Gal and GalNAc Reactive Lectins

(1) Human Blood Groups: **A** (GalNAcα1→3Gal), **B** (Galα1→3Gal)

(2) Tumor Markers: **F** (GalNAcα1→3GalNAc), **T** (Galβ1→3GalNAc)

Tn (GalNAcα1→Ser/Thr)

(3) Toxic and Microbial Agglutinin Receptors: **E** (Galα1→4Gal)

P/S (GalNAcβ1→3/4Gal)

(4) Other structural units: **I/II** (Galβ1→3/4GlcNAc), **L** (Galβ1→4Glc)

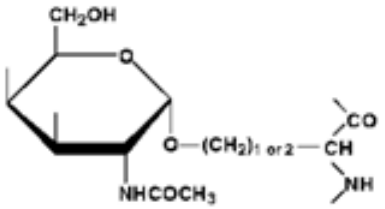
All, expect $\mathbf{L}_{\beta}$, $\mathbf{P}_{\alpha}$, $\mathbf{F}_{\beta}$ and $\mathbf{T}_{\beta}$, are present in **Glycoproteins**.

All, except **Tn** determinant, $\mathbf{T}_{\alpha}$ and $\mathbf{F}_{\alpha}$, provide essential ligands for lectin binding and are found in the carbohydrate moieties of **Glycosphingolipids**.

Figure 1a. Mammalian glycoconjugates structural units used to express and classify the carbohydrate specificity of lectins

I. Group of GalNAcα1→ structural units (Tn, A, F_α and F_β)

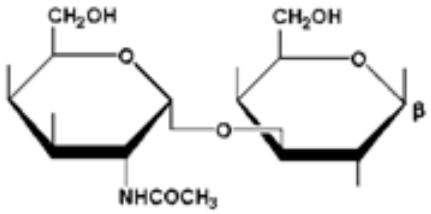
Tn.



Tn, GalNAcα1→Ser/Thr

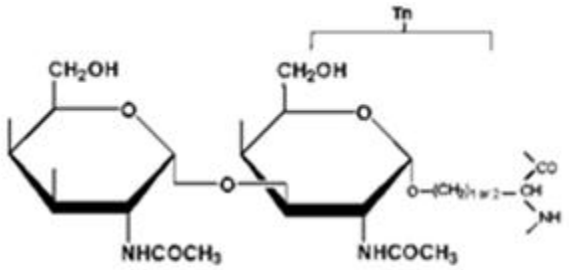
(**Tn**, in *O*-linked glycoprotein)

A.



A_β, GalNAcα1→3Galβ1→

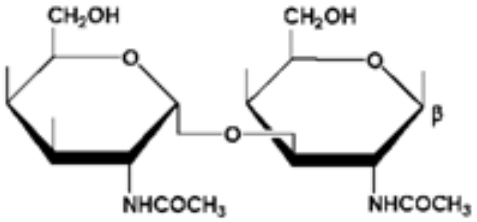
F.



F_α, GalNAcα1→3GalNAcα1→

(Core 5, GalNAcα1→ Tn at the reducing end of *O*-glycan)

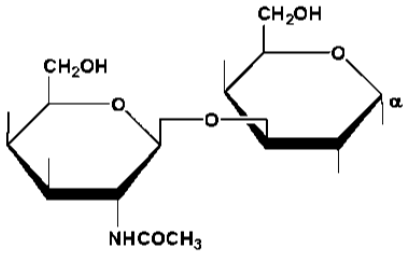
II. Group of GalNAcβ1→ structural units (P_α and S_β)



F_β, GalNAcα1→3GalNAcβ1→

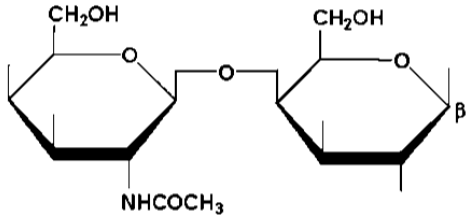
(Terminal disaccharide at the nonreducing end of Forssman glycotope)

P.



Pα, GalNAcβ1→3Galα1→

S.

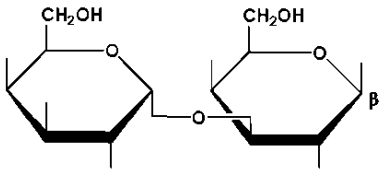


S_β, GalNAcβ1→4Galβ1→

Figure 1b. Mammalian glycoconjugates structural units used to express and classify the carbohydrate specificity of lectins

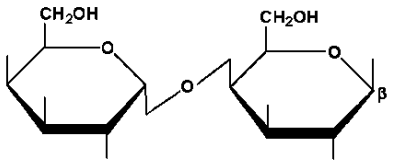
III. Group of Galα1→ structural units (B and E)

B.



B_β, Galα1→3Galβ1→

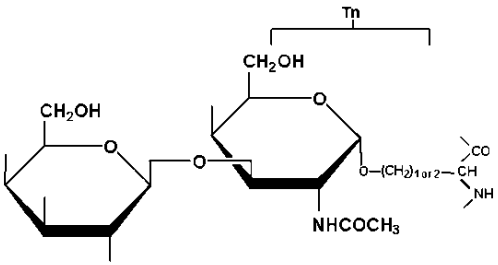
E.



E_β, Galα1→4Galβ1→

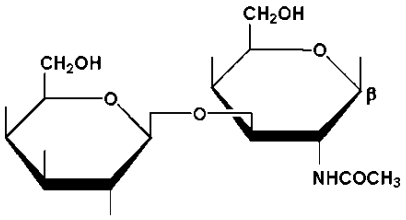
IV. Group of Galβ1→ structural units (T_α, T_β, L, I and II)

T.



Tα, Galβ1→3GalNAcα1→Ser/Thr

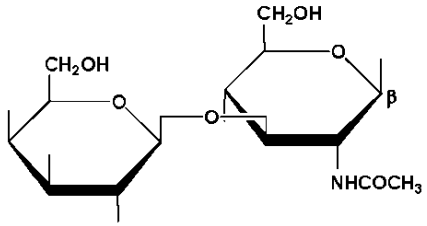
(Tα, GalNAcβ1→3Tn at the reducing end of *O*-glycan)



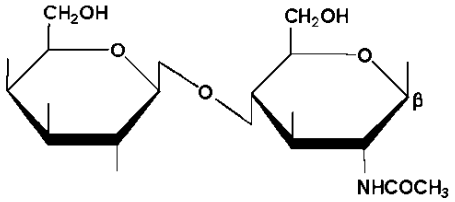
T_β, Galβ1→3GalNAcβ1→

(Terminal disaccharide at the asialo GM1; GSL)

I.

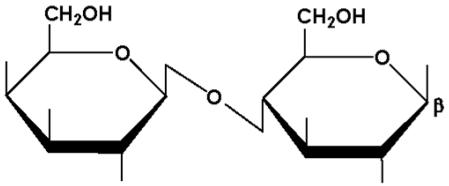


I_β, Galβ1→3GlcNAcβ1→



II_β, Galβ1→4GlcNAcβ1→

L.



L_β, Galβ1→4Glcβ1→ (GSL)

Table 1a. Carbohydrate structural units in mammalian glycoproteins and glycosphingolipids

	Codes[a]	Structural units	Sources
I.. GalNAcα1→series			
1	**F** **F**$_{penta-}$	GalNAcα1→3GalNAc GalNAcα1→3GalNAcβ1→3Galα1→4Galβ1→4Glc	Forssman pentasaccharide. Animal tissue antigens and human oncofetal glycotopes, mainly in glycosphingolipids.
	F$_{\alpha}$	GalNAcα1→3GalNAcα1→Ser/Thr of protein core	In *O*-linked glycoproteins core.
	F$_{\beta}$	GalNAcα1→3GalNAcβ1→	Glycotope at the nonreducing end of **F**$_{penta-}$.
2	**A**	GalNAcα1→3Gal	Human blood group A related **di**-saccharide.
	A$_{h}$	GalNAcα1→3[LFucα1→2]Gal	Human blood group A related **tri**-saccharide.
3	**Tn**	GalNAcα1→Ser/Thr of protein core	Tn antigen, only in *O*-linked glycoproteins.
II. GalNAcβ1→series			
4	**P** **P**$_{\alpha}$	GalNAcβ1→3Gal GalNAcβ1→3Galα1→	Blood group P related disaccharide; glycotope at the nonreducing end of globoside.
5	**S** **S**$_{\beta}$	GalNAcβ1→4Gal GalNAcβ1→4Galβ1→	Brain and asialo-GM$_2$ disaccharide; human blood group Sd(a+) related disaccharide in most human urine secretions, Tamm-Horsfall glycoprotein.

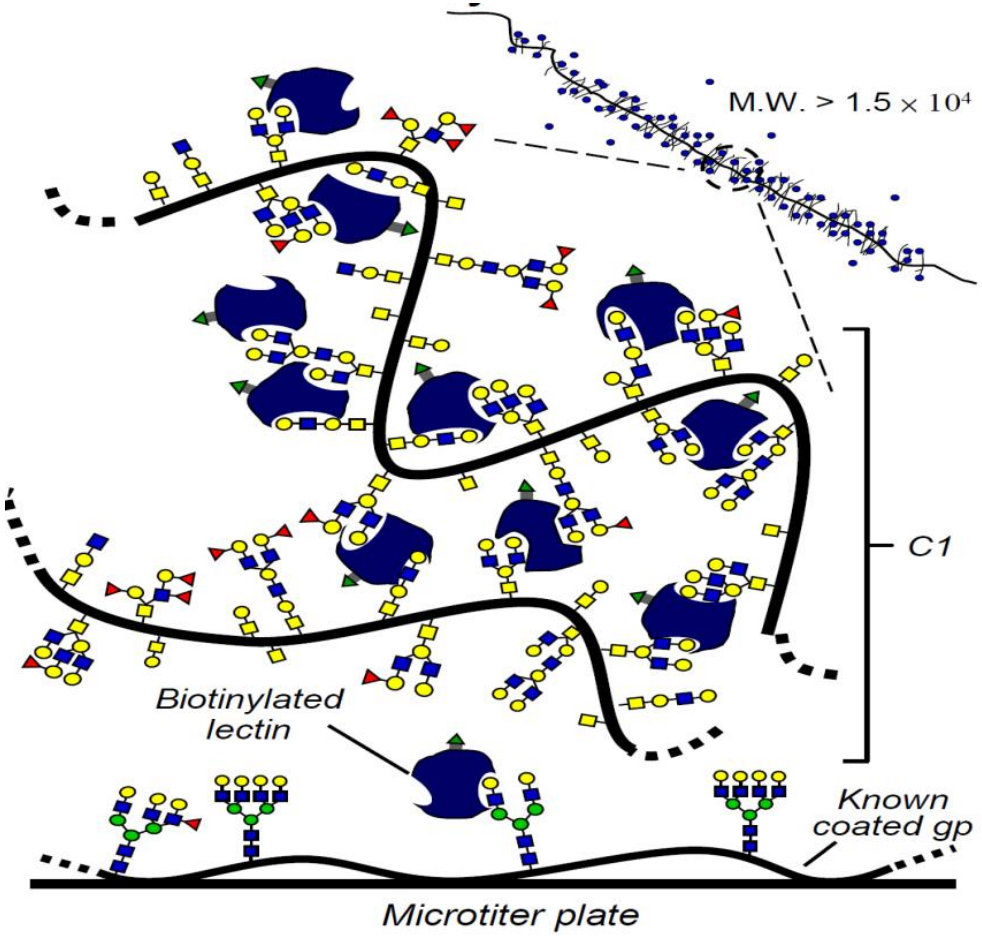
[a]α, β: anomer of sugars; m: multivalent, and Tri: tri-antennary.

Table 1b. Carbohydrate structural units in mammalian glycoproteins and glycosphingolipids

Codes[a]		Structural units	Sources
III. Galβ1→series			
6	**T** $_{\alpha}$	Galβ1→3GalNAcα1→Ser/Thr of protein core	The mucin-type sugar sequence on the human erythrocyte membrane.
7	**I** **I**$_{\beta}$	Galβ1→3GlcNAc Galβ1→3GlcNAcβ1→	Human blood group precursor type I and II carbohydrate sequences. Branched or linear repeated **II** sequence is part of blood group I and i epitopes. **I** and **II** are precursors of ABH and Le^{a}, Le^{b}, Le^{x}, Le^{y} blood group active antigens. Most of the lectins reactive with **II** are also reactive with **I**. Lectin Tri-**II** and m**II** determinants are present at the nonreducing end of the carbohydrate chains derived from *N*- and *O*-glycans.
8	**II** **II**$_{\beta}$ Tri-**II** m**II**	Galβ1→4GlcNAc Galβ1→4GlcNAcβ1→ Triantennary Galβ1→4GlcNAc Multivalent Galβ1→4GlcNAc	
9	**L** **L**$_{\beta}$	Galβ1→4Glc Galβ1→4Glcβ1→	Constituent of mammalian milk. Lactosyl ceramides in brain and part of carbohydrate structures in gangliosides.
IV. Galα1→series			
10	**B** **B**$_{h}$	Galα1→3Gal Galα1→3[LFucα1→2]Gal	Human blood group B related **di**-saccharide. Human blood group B related **tri**-saccharide.
11	**E**	Galα1→4Gal	Blood group p^{k} and P$_{1}$ active disaccharide. Sheep hydatid cyst glycoproteins, salivary glycoproteins of the Chinese swiftlet, glycosphingolipids in human erythrocytes, and small intestine.

[a]α, β: anomer of sugars; m: multivalent, and Tri: tri-antennary.

Recognition Intensities of Mammalian Structural Units, Ligand Cluster and Polyvalency in the *Abrus precatorius* agglutinin, *Ricinus communis* agglutinins and Glycoprotein Interaction



Abrus precatorius agglutinins (APA) / *Ricinus communis* agglutinins (RCA)

Abrus precatorius (Jequirity bean) seeds

Ricinus communis (castor bean) seeds

Separated by Sepharose 4B and DEAD-cellulose column chromatography

Abrin-a Abrin-b Abrin-c Abrin-d

The higher toxic and less agglutinin fractions

The less agglutinin activity

Abrus precatorius **agglutinin** (**APA**)

Less toxicity and high agglutinin fraction

Ricin, RCA_{60}

Highly toxic

RCA_1, RCA_{120}

Less toxic

[1] Lin J.Y. *et al*., Toxicon 19 (1981) 41–45

Abrus precatorius agglutinin (APA)

- Type 2 RIP (ribosome inactivating protein).[1]
- Molecular mass 130 kDa: consists of two sets of toxic subunit chain A (30 kDa) and lectin active subunit chain B (31 kDa).[2]
- Lectin active B chain is crucial for determining the target cells of APA.
- Following B chain binding to cell-surface glyco-receptors, the A chain enters cells through lectin mediated endocytosis and subsequently inhibits protein synthesis by catalytically inactivating the ribosomes.[3]

APA crystal structure[2]
(Resolution 2.6 Å)

[1] Liu C.L. *et al*., J. Biol. Chem. 275 (2000) 1897–2001
[2] Cheng J. *et al*., DOI:10.2210/pdb2zr1/pdb
[3] Peumans W.J. *et al*.,, FASEB J. 15 (2001) 1493–1506

Forty Years of Progress in the Carbohydrate Recognition Profiles of *Abrus precatorius* agglutinin (APA)

	Year	Recognition factors	Recognition activity
1.	1975[1]	Monosaccharides	Gal > GalNAc
2.	1981[2]	Anomers	D-Gal*p*β1→
3.	1992[3]	Oligosaccharides and mammalian glyco-structural units	Galβ1-3GalNAc (**T**) > Galβ1-3/4GlcNAc (**I/II**) **T** - Thomsen-Friedenreich antigen **I/II** - blood group precursor type **I** or type **II** disaccharide
4.	Until 2008 (?)	Clustering and polyvalency of mammalian glyco-structural units	☹ **Not available**

[1] Wei C.H. *et al.*, J. Biol. Chem. 250 (1975) 4790–4795; [2] Kahn M.I. *et al.*, Eur. J. Biochem. 115 (1981) 149–152;
[3] Wu A.M. *et al.*, J. Biol. Chem. 267 (1992) 19130–19139

Interaction Intensities of APA and RCA_1 to Different Polyvalent Glycotopes Analyzed by ELLSA

Curve no	Polyvalent glycotopes (Lectin determinants[b]; blood group activity)	Quantity for 1.5 units of A_{405} (ng)	Maximum A_{405} reading[c]	Binding Intensity[c]
1	**Asialo PSM (T_α, Tn, A, A_h)**	**0.8**	**3.1**	**+++++**
2	**Asialo human α_1 acid gp (mII_β)**	**1.0**	**2.9**	**+++++**
3	**Asialo bovine α_1 acid gp (mII_β)**	**1.2**	**3.0**	**+++++**
4	**Asialo birds nest gp (E_β, T_α, F_α, II_β)**	**8.0**	**2.6**	**+++++**
8	**Asialo fetuin (T_α, mII_β)**	**16.0**	**2.7**	**+++++**
16	**Birds nest gp (E_β, sialyl T_α, F_α, II_β)**	**-**	**1.2**	**++**
20	**Bovine α_1 acid gp (sialyl mII_β)**	**-**	**0.6**	**+**
21	**PSM major (sialyl Tn, T_α, A, A_h)**	**-**	**0.5**	**+**
23	**Fetuin (sialyl T_α, mII_β)**	**-**	**0.5**	**+**
25	**Human α_1 acid gp (sialyl mII_β)**	**-**	**0.3**	**±**

[a]4 ng of biotinylated APA was added to various polyvalent glycotopes containing gps/ps, ranging from 0.01 ng to 1 μg /ml.
[b]The bolded symbols in parenthesis indicate the human blood group activity and/or lectin determinants.
[c]The results were interpreted according to the spectrophotometric absorbance value at 405nm (A_{405}) after 2 h incubation as follows: +++++ ($A_{405} \geq 2.5$), ++++ ($2.5 > A_{405} \geq 2.0$), +++ ($2.0 > A_{405} \geq 1.5$), ++ ($1.5 > A_{405} \geq 1.0$), + ($1.0 > A_{405} \geq 0.5$), ± ($0.5 > A_{405} \geq 0.2$), and - ($A_{405} < 0.2$)

Table 3. Summary of overall contribution of recognition factors of Gal/GalNAc specific lectins

Recognition effects	Mass RP [a]					
	ABA (**T**)	Morniga G (**T/Tn**)	ACL (**T**)	PNA (**T/II**)	VVL-B_4 (**Tn**)	ECL (**II**)
a. Monosaccharide specificity	GalNAc >>> Gal, inactive	GalNAc > Gal	GalNAc >>> Gal, inactive	Gal > GalNAc	GalNAc > Gal	GalNAc ≈ Gal
b. Contribution of recognition factors						
T_α						
Polyvalent	4.7×10^6	4.0×10^3	2.5×10^3	3.4×10^3	–	–
Monomer	8.6	7.5	1.6×10^2	10	–	0.2
Tn						
Polyvalent	1.1×10^5	2.9×10^4	60	–	3.3×10^5	–
Cluster [b]	–	4.0×10^2	9.7	–	1.1×10^3	1.1
Monomer	1.0 [d]	4.8	1.0	–	2.1×10^2	1.8
II_β						
Polyvalency	1.5×10^4 [e]	4.0×10^3 [e]	6.7×10^3	2.2×10^3	–	2.1×10^4
Cluster [c]	–	0.6	1.4	–	–	5.5
Monomer	–	–	–	0.5	–	8.9

[a]Mass RP scale from Wu et al, 2003a; Singh et al, 2006; Wu et al, 2008b; Wu, 2004; Wu et al, 2007; [b]**Tn** glycopeptides, – means inactive; [c]Tri-antennary **II_β**; [d]Monomeric **Tn** was replaced by GalNAc; [e]It has to be further confirmed.

Binding Profile of APA-Polyvalent Glycotopes Interaction

1	Reacted best	**Polyvalent T_α, E_β and II_β** containing gps [0.8–8.0 ng of gps were required to reach 1.5 A_{405} unit within 2 h]
2	Reacted strongly	High-density polyvalent **II_β** and **T_α** glycotopes containing gps [10.0–30.0 ng of gps were required to reach 1.5 A_{405} unit within 2 h]
3	Reacted moderately or negligibly	Insufficient amount or masked **I_β / II_β** and **T_α** glycotope containing gps and human blood group **ABH** and **Lewis** antigen containing gps.
4	Reacted poorly	Sialylated **T_α / II_β** glycotopes

Summary of Intensities of Polyvalent Glycotopes in APA Recognition Process Obtained from Inhibition Assay

1	Most effective polyvalent glycotopes	Polyvalent **T_α**, **I_β /II_β** and **E_β** containing gps [< 10 ng to produce 50% inhibition of the APA-plate coated asialo fetuin interaction] 10^3–10^4 times more potent than the monomeric counterparts (**T**, **I**, **II**, **E**) and Gal.
2	Strongly reactive polyvalent glycotopes	High-density polyvalent **II_β** containing gps, human blood group **ABH**(**O**) precursors equivalent gps [20-90 ng to produce 50% inhibition]
3	Other recognizable glycotopes	Partially cryptic **II_β** glycotopes masked by Galα1- (cyst Beach phenol insoluble), GalNAcα1- (Hog gastric mucin#14), GalNAcβ1- (Sheep Hydatid cyst gp; asialo THGP sd(a+) WM)
4	Poorly reactive polyvalent glycotopes	Cryptic **T_α** and **II_β** glycans glycosylated by sialic acid
5	Inactive or weakly reactive polyvalent glycotopes	Polyvalent **Tn** and GlcNAc1-3**Tn** glycotope containing gp

Roles of Active Ligands, Hydrophobicity and Tri-antennary II_β in APA Recognition

Curve no.	Inhibitors	Quantity giving 50% inhibition (nmol)	Molar Relative potency
1	Galβ1-4GlcNAcβ1-2Man | α1-6 Galβ1-4GlcNAcβ1-2Manα1-3Manβ1-4GlcNAcβ1-4GlcNAcβ1-*N*-Asn | β1-4 Galβ1-3/4GlcNAc — **Tri**-antennary *N*-glycopeptide (tri-II_β)	0.14	7.1×10^2
4	Galβ1-GalNAc (T)	18.0	5.5
8	Galβ1-4GlcNAc | β1-6 Gal*β*1-4Glc | β1-3 Galβ1-4GlcNAc — Lacto-*N*-hexose (di-II_β) [LNH; IIβ1-3(IIβ1-6L)]	25.0	4.0
9	Galα1-4Gal (E)	27.0	3.7
10	Galβ1-4GlcNAc | β1-6 Galβ1-4Glc | β1-3 Galβ1-3GlcNAc — Lacto-*N*-neohexose (di-I_β/II_β) [LNnH; Iβ1-3(IIβ1-6L)]	30.0	3.3
12	Galβ1-4GlcNAc (II)	40.0	2.4
13	Galβ1-3GlcNAc (I)	41.0	2.4
18	Gal	100.0	1.0
19	GalNAc	3800.0	0.02

[a]The inhibitory activities were estimated from the inhibition curves and are expressed as the amount of saccharides giving 50% inhibition. Total volume was 50μl.

[b]Molar relative potency =moles of Gal (curve no. 18) for 50% inhibition is taken as unity/mole of saccharide required for 50% inhibition.

[c]The inhibitory potencies of inactive or less active saccharides are expressed as the maximum amount of sugars tested that yield inhibition (in parenthesis) below 50%.

Intensities of Polyvalent Glycotopes in APA Recognition Process Obtained from Inhibition Assay

	Curve no.	Inhibitors	Quantity giving 50% inhibition (ng)	Mass RP*
2009[1] Polyvalent & Tri-II$_\beta$	1	Asialo PSM (T_α, A, A_h, Tn)	0.6	3.0 x 10^4
	2	Asialo birds nest gp (E_β, T_α, F_α, II$_\beta$)	0.8	2.2 x 10^4
	3	Asialo bovine α_1 acid gp (mII$_\beta$)	2.5	7.3 x 10^3
	4	Asialo human α_1 acid gp (mII$_\beta$)	7.0	2.6 x 10^3
	5	Asialo fetuin (T_α, mII$_\beta$)	9.0	2.0 x 10^3
	6	Tri-antennary *N*-glycopeptide (tri-II$_\beta$)	2.9x 10^2	60.6
1992[2] Structural units/mono-saccharides	7	Galβ1-3GalNAc (T)	6.9 x 10^3	2.6
	8	Galα1-4Gal (E)	9.3 x 10^3	2.1
	9	Galβ1-4GlcNAc (II)	1.5 x 10^4	1.1
	10	Galβ1-3GlcNAc (I)	1.6 x 10^4	1.1
	11	Gal	1.8 x 10^4	1.0

[a]The inhibitory activities were estimated from the inhibition curves in and are expressed as the amount of inhibitors (ng) giving 50% inhibition. Total volume was 50 μl.

[b]Mass relative potency =mass of Gal (curve no. 24) for 50% inhibition is taken as unity/mass of inhibitor required for 50% inhibition.

[c]The inhibitory potencies of inactive or less active gps are expressed as the maximum amount of inhibitors tested that yield inhibition (in parenthesis) below 50%.

*Mass RP, Mass Relative Potency

(1) / (11) = **3.0x10^4** ; (1) / (7) = **1.2x10^4**

(7) / (1) = 2.6

[1] Wu A.M. *et al*., *Mol. Immunol*., 46 (2009) 3427-3437

[2] Wu A.M. *et al*., *J. Biol. Chem*. 267 (1992) 19130–19139

Progress in the Carbohydrate Recognition Profile of APA

	Year	Recognition factors	Activity
1.	1975[1]	Monosaccharides	Gal > GalNAc
2.	1981[2]	Anomers	Galβ1→
3.	1992[3]	Oligosaccharides and mammalian glyco-structural units	Galβ1-3GalNAc (**T**) > Galβ1-3/4GlcNAc (**I/II**)[a] **T** - Thomsen-Friedenreich antigen **I/II** - blood group precursor type **I** or type **II** disaccharide
4.	2009[4]	**(a) Monosaccharide (subtope) and submolecule specificity**	**Gal (β anomer at non-reducing end; configuration of C-2) >> GalNAc (less-active)[a]**
		(b) Glyco-structural units	**Galβ1-3GalNAcα/β1- (T_α/T_β) > Galα1-4Gal (E_β) > Galβ1-3/4GlcNAc (I/II)[a]**
		(c) Clustering and polyvalency of mammalian glyco-structural units	**Polyvalent T_α, II_β/I_β and E_β >> tri-antennary II_β > monomeric T, T, I and II > Gal > GalNAc (weak)[b]**

[a] Molar RP; [b] Mass RP

[1] Wei C.H. *et al.*, J. Biol. Chem. 250 (1975) 4790–4795

[2] Kahn M.I. *et al.*, Eur. J. Biochem. 115 (1981) 149–152

[3] Wu A.M. *et al.*, J. Biol. Chem. 267 (1992) 19130–19139

[4] Wu A.M. *et al.*, Mol. Immonol., 46 (2009) 3427-3437

Summary of polyvalency study for *Abrus precatorius* agglutinin (APA)

Poly-**T**$_{\alpha}$ (3.0x10^4) >> Tri-**II**$_{\beta}$ (60.6) > **T** (2.6) > **II/I,** Gal (1.0)*

2009 | by 1992

Mol. Immunol., 46: 3427-3437 | J. Biol. Chem., 267: 19130-19139

i. T / Gal : 2.6 / 1.0 = 2.6 (by 1992)

ii. **PolyTα / Gal : 3.0 x 10^4** (after 2009)

iii. PolyTα / T : 3.0 x 10^4 / 2.6 = 1.2 x 10^4 (after 2009)

Similar approach as can be applied to abrin-a, RCA $_1$ and other lectins

*Recognition intensity expressed by Mass RP

Summary (1/4)

I. More methods used to study recognition factors of a lectin reveal more comprehensive views of the recognition profiles.

a. Hemagglutination-inhibition assay;

b. Quantitative precipitation/precipitin-inhibition assays;

c. Enzyme-linked lectinosorbent –inhibition assay (ELLSA);

etc.

Summary (2/4)

II. In the processes of the mechanism glycan recognition, six recognition factors in glycans were involved : They are :

(i) several epimers and anomers of sugars;

(ii) Monosaccharide specificity (Gal, GalNAc, GlcNAc, Man, LFuc, and Sialic acid) ;

(iii) Reactivities toward mammalian disaccharides and **Tn** structural units (in decreasing order) ;

(iv) The most active ligand ;

(v) Simple multivalent or cluster effect of carbohydrate structured unit such as **Tn** glycopeptides and multi-antennary glycotopes to inhibit binding ;

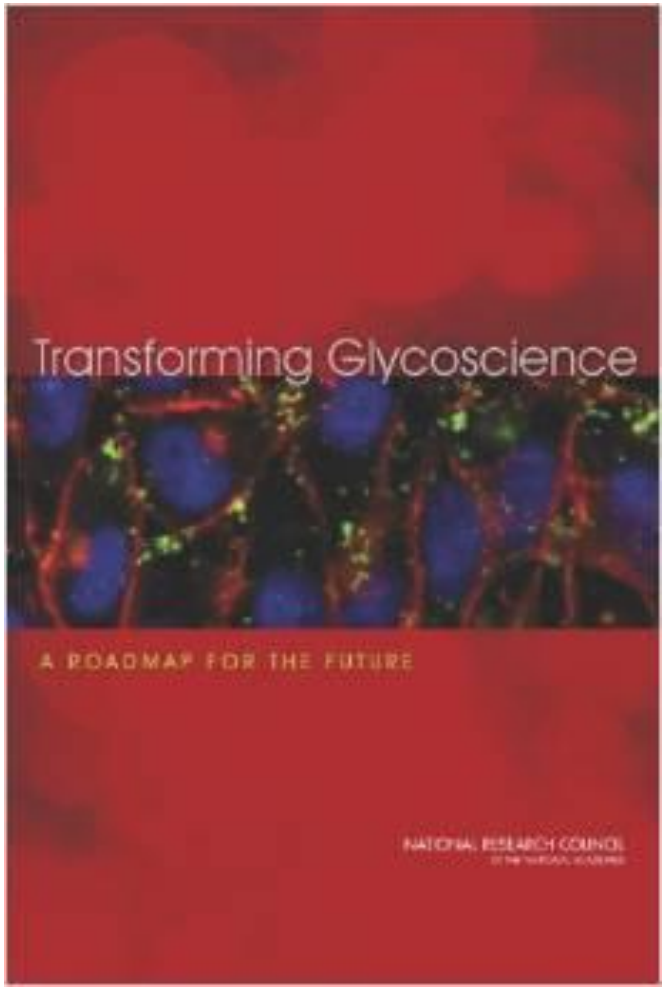
(vi) Complex polyvalent or multi- effects present in macromolecules with known glycotopes, in which

Summary (3/4)

III. Polyvalency of glycotopes and its resulting conformational features as the critical recognition factors in lectin-carbohydrate interactions

IV. This should be one of important directions toward the transforming glycosciences for centuries.

Summary (4/4)



Q : In the book of Transforming Glycoscience (Left):

Does it cover the following subjects ?

- Recognition Codes
- Polyvalency of glycotopes
- Carbohydrate-carbohydrate interactions

A: If it is not very much,

Let's emphasize on the aspects.

Thanks.

Complex Carbohydrates & Medicine Workshop -5
College of Medicine, Chang-Gung University
Tao-Yuan, Taiwan. July 7, 07'

Participants of the Complex Carbohydrates & Medicine Workshop -5
College of Medicine, Chang-Gung University
Tao-Yuan, Taiwan. July 7, 07'

Participants of the INTERNATIONAL SYMPOSIUM on Molecular Immunology of Complex Carbohydrates-2 (MICC-2),
and the Taiwan-Canada Glycobilogy Workshop
國際 醣類 分子免疫學 學術會議-2 台灣・加拿大 醣類生物學 學術研討會
Institute of Biological Chemistry, Academia Sinica, Taipei, Taiwan. Aug. 28-Sep. 2, 1999

Participants of the INTERNATIONAL SYMPOSIUM on
Molecular Immunology of Complex Carbohydrates-3 (MICC-3)
Institute of Biological Chemistry, Academia Sinica,
Taipei, Taiwan. July 8-12, 07'

Gaunylate Cyclase

edited by **Rameshwar K. Sharma**, *The Unit of Regulatory and Mol. Biology, University of Medicine and Dentistry of New Jersey, USA*

From somewhat enigmatic beginnings 40 years ago, guanylate cyclase research has emerged to occupy a position of prominence in the study of signal transduction. Guanylate cyclase has several intriguing features, including existence in two major forms, membrane and soluble, each independently regulated by distinct mechanisms. The membrane form gives rise to a fascinating signal transduction story important to both peptide hormones and sensory neurons. This volume covers the evolution of the field, peptide hormone receptor work, membrane guanylate cycles, related retinal diseases, and the biochemistry and physiology of the soluble form. The 16 chapters are written by leaders in the field.

DEVELOPMENTS IN MOLECULAR AND CELLULAR BIOCHEMISTRY: VOLUME 36
Reprinted from *MOLECULAR AND CELLULAR BIOCHEMISTRY*
Hardbound, ISBN 0-7923-7682-X, March 2002, 200 pp.
EUR 165.00 / USD 150.00 / GBP 105.00

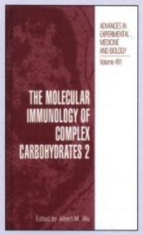
Protein Kinase CK2 - from Structure to Regulation

edited by **Khalil Ahmed, Jorge E. Allende, O. Issinger**

Signal Transduction through phosphorylation and dephosphorylation of proteins in the cell is now a well-recognized mechanism involved in countless physiological and pathological processes. Consequently, the enzymes, known as protein kinases, which catalyze the phosphorylation of proteins, are critical regulators of cellular events.

This volume provides an overview of the state of knowledge concerning this intriguing protein kinase. It brings together contributions from leading investigators engaged in research in this field. Key developments durin the past three years pertain to the elaboration of the crystal structure and definition of novel functions of the kinase, such as its role as an inhibitor of apoptosis. Additionally, the shuttling of the kinase to various compartments in response to physiological and stress stimuli appears to be a key feature of the functional regulation of its activity in the cell.

DEVELOPMENTS IN MOLECULAR AND CELLULAR BIOCHEMISTRY 35
December 2001, Hardbound, ISBN 0-7923-7666-8, Price: TBA



The Molecular Immunology of Complex Carbohydrates -2

An Excellent Textbook!

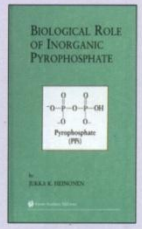
(傑出教科書!)

edited by **Albert M. Wu**, *Institute of Molecular and Cellular Biology, Taiwan, Republic of China*

This volume highlights the informative events of the Symposium on Molecular Immunology of Complex Carbohydrates II, held at the Institute of Biological Chemistry, Academia Sinica, on August 28-September 1, 1999, in Taipei, Taiwan. The Editor intertwined this conference, a satellite meeting of the 15th International Glycoconjugate Conference, with a Glycobiology Workshop, resulting in one of the most comprehensive handbooks on carbohydrate specificities of applied lectins and anti- carbohydrate monoclonal antibodies in the field. The proceedings provide information on glycotopes required for essential basic concepts and applications. It will be a useful tool for researchers and beginners in the fields of immunology, biochemistry, cancer research, and structural biology for years to come.

ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY
Hardbound, ISBN 0-306-46532-9, June 2001, 654 pp.
USD 145.00 / EUR 167.00 / GBP 102.00

Biological Role of Inorganic Pyrophosphate

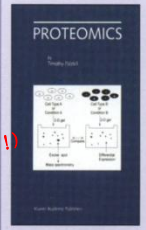


by **Jukka K. Heinonen**, *University of Turku, Finland*

Dr. Heinonen reviews and critically evaluates the scientific literature on the biological role of inorganic pyrophosphate (PPi) published from 1940 to the end of 1999. He describes and classifies all known biochemical reactions that produce Ppi; describes and evaluates all published methods used in biological Ppi; and compiles and critically evaluates information on the concentration of PPi (with the conclusion that, contrary to common belief, PPi exists throughout the living world in rather high concentrations). Many reactions in which PPi is used as a biochemical energy source instead of ATP have been described in recent decades, especially in bacteria, protists, and plants. These reactions are evaluated from the bioenergetic and regulatory points of view. Also considered is the possible role of PPi as a source of biochemical energy in the primitive phases of life, before ATP. Data is presented on the regulatory role of PPi in living systems, such as activities of enzymes, fidelity of syntheses of macromolecules, and proliferation of cells. PPi may also regulate the formation and dissolution of bone as well as pathologic calcification of soft tissues and the formation of urinary stones. The formation of calcium pyrophosphate dihydrate crystals in the extracellular fluids of joints cause the disease called pseudogout.

Biological Role of Inorganic Pyrophosphate book is a unique and invaluable source of references (about 1120) and summarized data for professionals who study or plan to study the role of PPi in living systems.

Hardbound, ISBN 0-7923-7441-X, August 2001, 264 pp.
USD 185.00 / EUR 202.00 / GBP 128.00



Proteomics

by **Timothy Palzkill**, *Baylor College of Medicine, Houston, TX, USA*

Proteomics is an introduction to the exciting new field of proteomics, an interdisciplinary science that includes biology, bioinformatics, and protein chemistry. The purpose of this book is to provide the active researcher with an overview of the types of questions being addressed in proteomics studies and the technologies used to address those questions.

Key subjects covered in this book include:

• an assessment of the limitations of this approach and outlines new developments in mass spectrometry that will advance future research • high-throughput recombinant DNA cloning methods used to systematically clone all of the open reading frames of an organism into plasmid vectors for large scale protein expression and functional studies such as protein-protein interactions with the two-hybrid system • protein structure • an overview of large-scale experimental attempts to determine the three-dimensional structures of representative sets of proteins • computational approaches to determining the three-dimensional structure of proteins.

Proteomics provides a starting point for researchers who would like a theoretical understanding of the new technologies in the field, and obtain a solid grasp of the fundamentals before integrating new tools into their experiments. Written with attention to detail, but without being overwhelmingly technical, *Proteomics* is a user-friendly guide needed by most biologists today.

Hardbound, ISBN 0-7923-7565-3, October 2001, 136 pp.
USD 99.50 / EUR 110.00 / GBP 70.00

MICC-2 *An Excellent Textbook !*

2002 傑出教科書!!

Availability of and results for your eBook

Since its online publication on April 08, 2011, there has been **a total of 28,585 chapter downloads** for your eBook on SpringerLink. The table to the right shows the download figures for the last year(s).

- In addition to the collections, Springer eBooks are available for individual purchase from our web shop. Your book can be ordered directly from its homepage.
- MyCopy: Your book is available as a MyCopy version, which is a unique service that allows library patrons to order a personal, printed-on-demand softcover edition of an eBook for just $/€24.99.
- To further widen the distribution of your book, it has also been made available in the following shop(s):
  Amazon Kindle Shop
  Apple iTunes
  Google play

As you can see, in addition to the print book, the electronic version reaches a broad readership and provides increased visibility for your work. This is especially noticeable in the long run: statistical data show that the usage of electronic publications remains stable for years after publication, so this is what you can expect for your book in the years to

Year	Chapter Downloads
2014	14,090
2013	10,775
2012	1,883
2011	1,837

Total 28,585 *

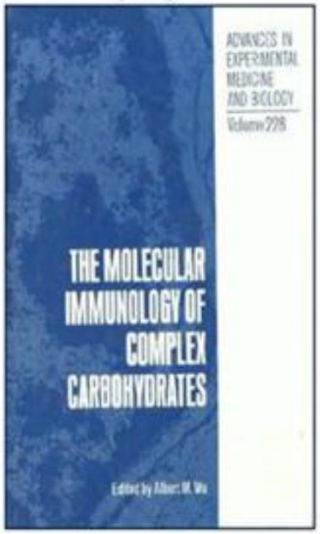
必勝！

Victory !

Near 30 thousands Download

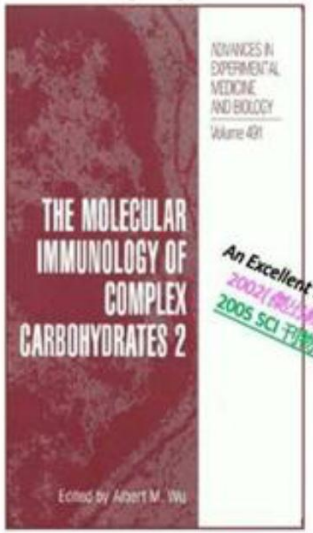
CGU Glyco
長庚大學 CHANG GUNG UNIVERSITY

MICC-1
(1988)



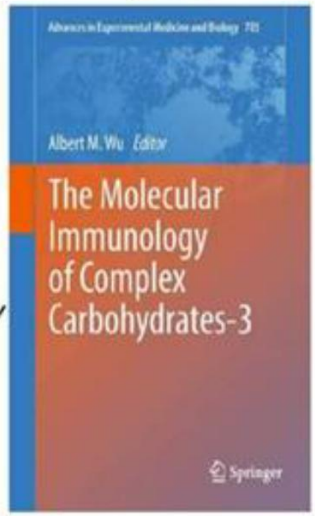
(Advance in Experimental Medicine and Biology, 228)
Albert M. Wu, L. Garry Adams (Editors)
(Hardcover-June 1988)
List Price: $ 416.00
Our Price:$ 416.00

MICC-2
(2001)



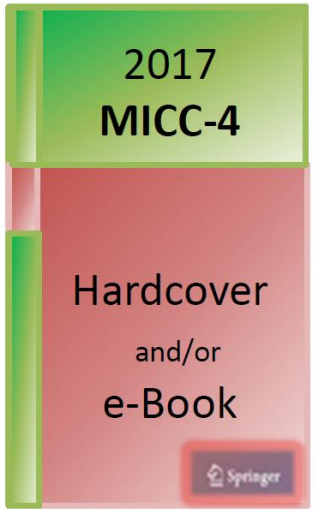
(Adv. Exp. Med. Biol., 491)
Albert M. Wu (Editor)
(Hardcover-June 2001)
List Price: $ 232.00
Our Price:$ 232.00

MICC-3
(2011)



(Adv. Exp. Med. Biol., 705)
Albert M. Wu (Editor)
(Hardcover-July 2011)
List Price: $ 279.00

MICC-4
(2017)



- Glyco recognitions in medicine
- lectin / Mo Ab recognition index
- Polyvalency of Glycotopes/Glycoconjugates
- Glycobiology in diseases

MICC-2 and MICC-3 single editor, Bookseller Rating-five stars ★★★★★
MICC-3 SCI 2.0 and >2.86x10⁴ times download

MICC-4 (Impossible ?,☹ ; After 2016 , Your encouragement and support will make book issued and the glyco science,further advanced,)

Molecular Immunology of Complex Carbohydrates – 4 (MICC-4) will be held in **Taipei, Taiwan** and **Angkor, Cambodia** between **April 11 – 18, 16'**.

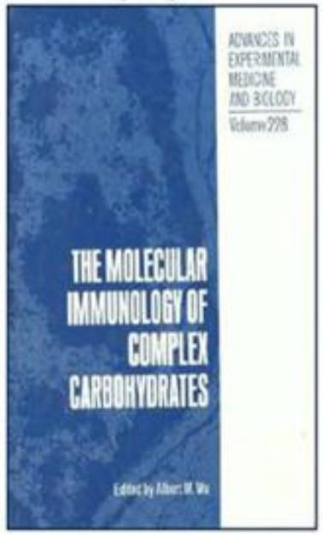
Theme of this symposium : Glyco recognitions in medicine, Lectin/MoAb recognition index, Polyvalency of glycotopes / glycoconjugates, Glycobiology in diseases.

You are cordially welcome to join us.

Molecular Immunology Complex Carbohydrates - 4 (MICC-4) will be held in **Taipei, Taiwan** and **Angkor, Cambodia** between **April 11 – 18, 16'**.

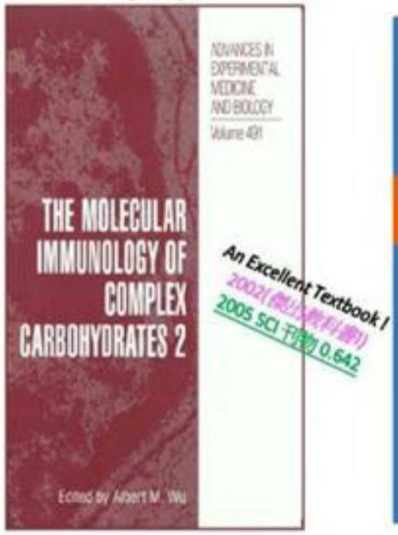
You are cordially welcome to join us.

MICC-1
(1988)



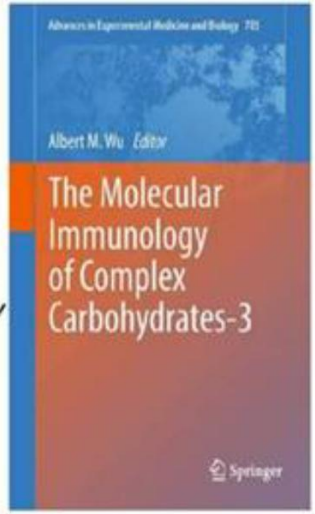
(Advance in Experimental Medicine and Biology, 228)
Albert M. Wu, L. Garry Adams (Editors)
(Hardcover-June 1988)
List Price: $ 416.00

MICC-2
(2001)



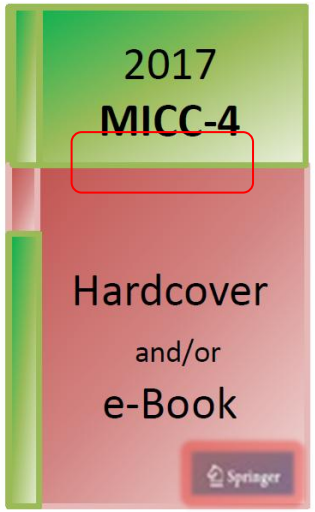
(Adv. Exp. Med. Biol., 491)
Albert M. Wu (Editor)
(Hardcover-June 2001)
List Price: $ 232.00
Our Price:$ 232.00

MICC-3
(2011)



(Adv. Exp. Med. Biol., 705)
Albert M. Wu (Editor)
(Hardcover-July 2011)
List Price: $ 279.00

MICC-4
(2017)



- Glyco recognitions in medicine
- lectin / Mo Ab recognition index
- Polyvalency of Glycotopes/Glycoconjugates
- Glycobiology in diseases

Acknowledgements

This work was supported by MICCs Forever Foundation, Kwei-san, Tao-yuan, 15' and partially by Grants from the Chang-Gung Medical Research Project (CMRPD No. 330225 and 170441), and The National Science Council (NSC 97-2320-B-182-020 and 97-2628-B-182-002), Taipei, Taiwan.


Thank you for your attention !
See you again