Study on Bioactive Substances from Lingzhi (*Ganoderma* Species) Cultivated in China

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Content

- ☑ Background
- ☑ Structure of Polysaccharide from *Ganoderma*
- ☑ Bioactivity of Polysaccharide from *Ganoderma*

Background

Background

The practice of using fungi as Chinese herbal medicines can be traced in the different early records of the 'Materia Medica' of Chinese. For example, the earliest book on medicinal materials in China, the "Shen Nong's Herbal" (Shen Nong Pen Ts'ao Jing) (100-200 AD), recorded the medicinal effects of several fungi such as *Ganoderma lucidum, Poria cocos, Polyporus umbellatus, Polyporus mylittae, Tremella fuciformis* and other unidentified fungi. Subsequently, there were more and more records of fungi used as Chinese herbal medicines.

Background

Ganoderma Ling Zhi

Ancestor of Chinese medicine

—Shennong

Background

Background

Background

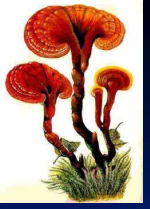
- Overview of *Ganoderma* Research
  - Most Research papers
  - deepest influence to field of medicinal fungi
- Develpoment Status of Ganoderma Product In China
  - production value about 2 billion
  - fastest growth in the function food

Background

- *The bioactive compounds of Ganoderma lucidum*
  - Polysaccharide (over 200)
  - Triterprene (over 130)
  - Steroid
  - Alkaloid
  - ……

Background

- The biological activity of *Ganoderma lucidum*
  - Immunostimulation effect
    - Innate immune system
    - Acquired immune system
  - Inhibition to tumor cells
    - Cell toxicity
    - Induce apoptosis
  - Hepatoprotective activity



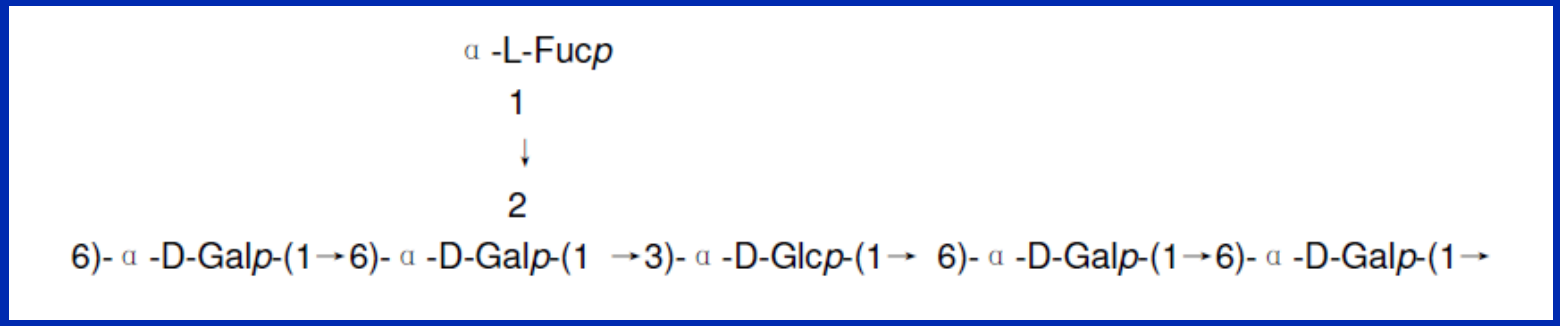
Background

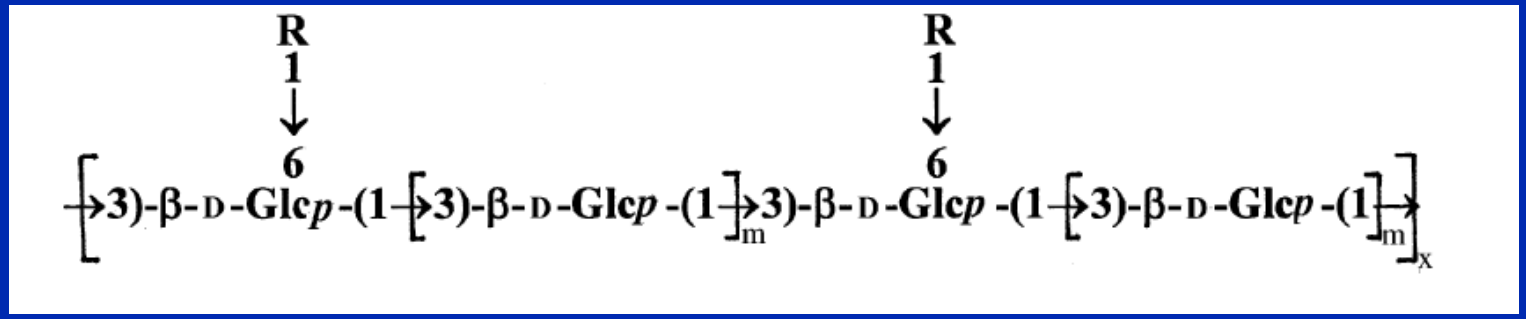
- Hypoglycemic activity
- Anti-angiogenic activity
- Effect on wound healing
- Cholesterol-lowering effect
- Anti-oxidation activity
- Antivirus and antifungal activity

Structure of Polysaccharide From Ganoderma

- ✓ Ganoderma australe
- ✓ Ganoderma applanatum
- ✓ Ganoderma formosanum
- ✓ Ganoderma lucidum
- ✓ Ganoderma neo-japonicum
- ✓ Ganoderma resinaceum
- ✓ Ganoderma sinensis
- ✓ Ganoderma tusge

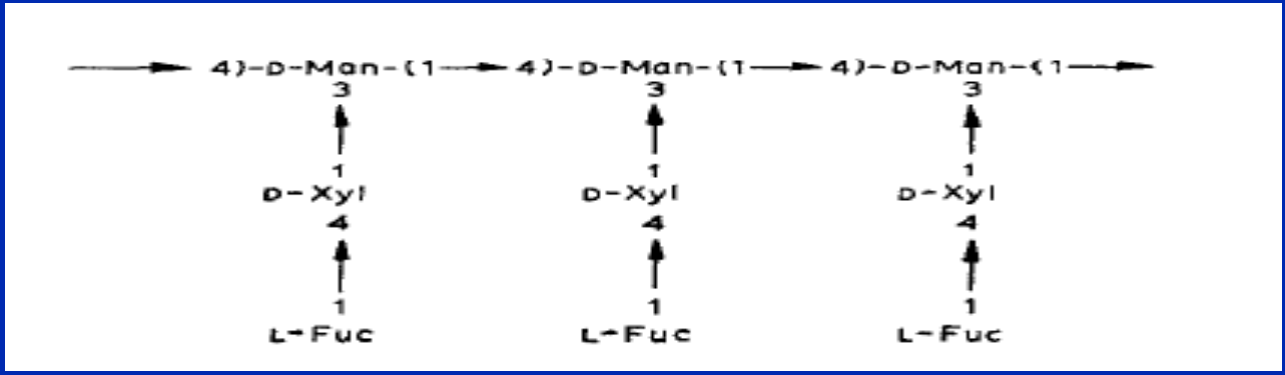
Heterogeneous Glycan

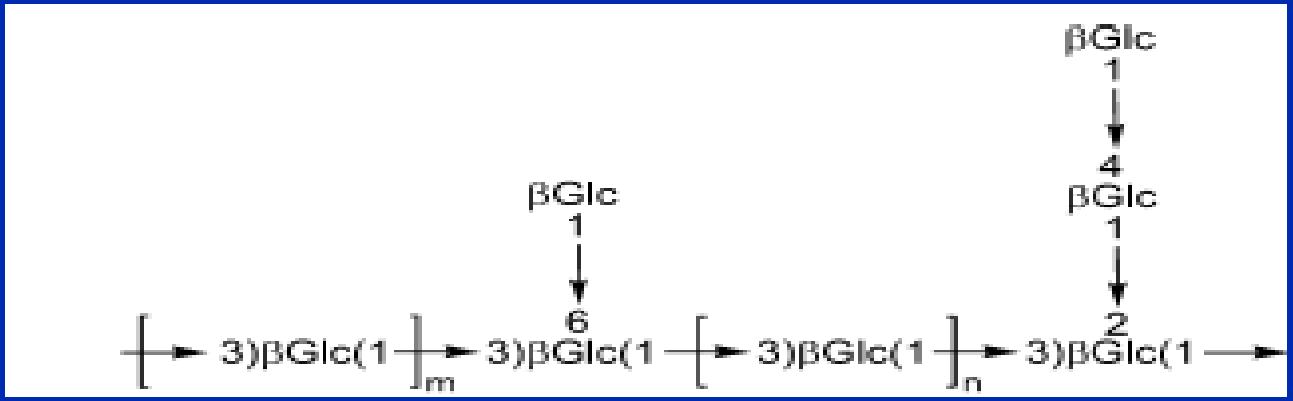




Friuting body of Ganoderma lucidum
YE et al 2008, 2009

Heterogeneous Glycan

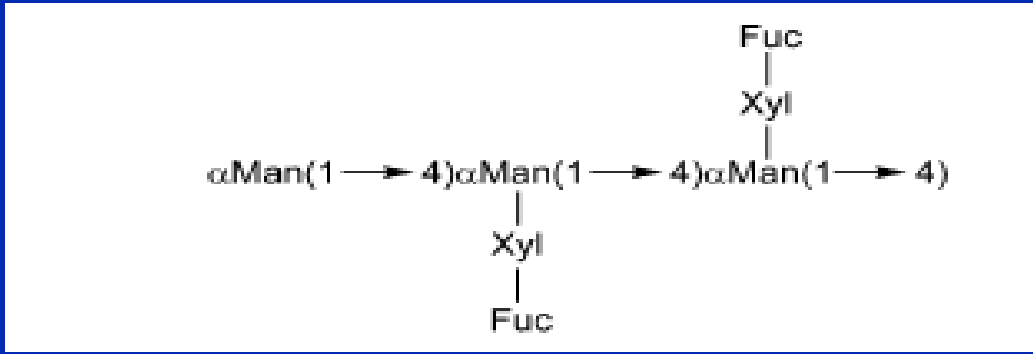




Friuting body of Ganoderma lucidum

Russell et al, 2006

Heterogeneous Glycan



Friuting body of Ganoderma lucidum

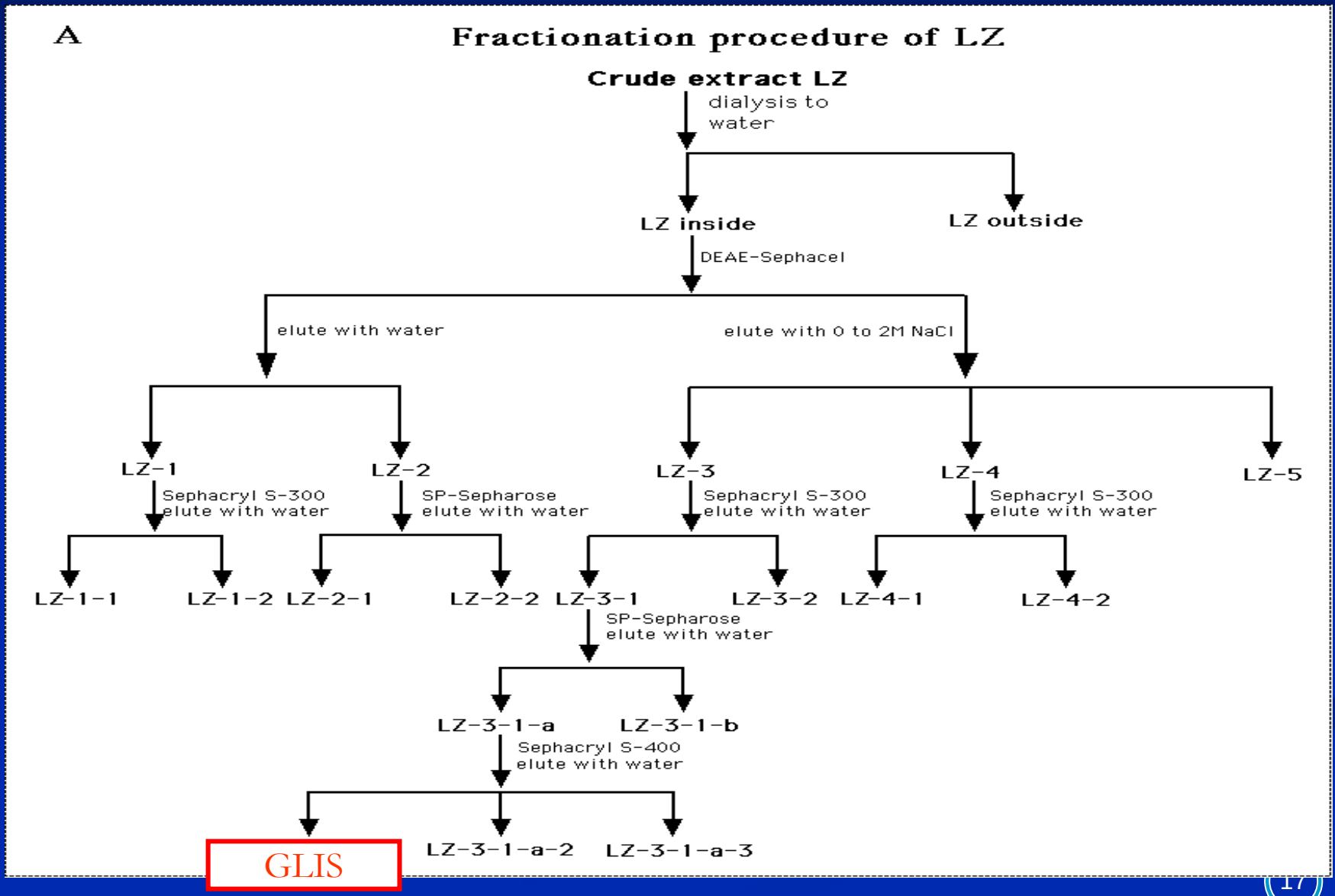
α – (1 – 3) – D-glucan

Spore of Ganoderma lucidum

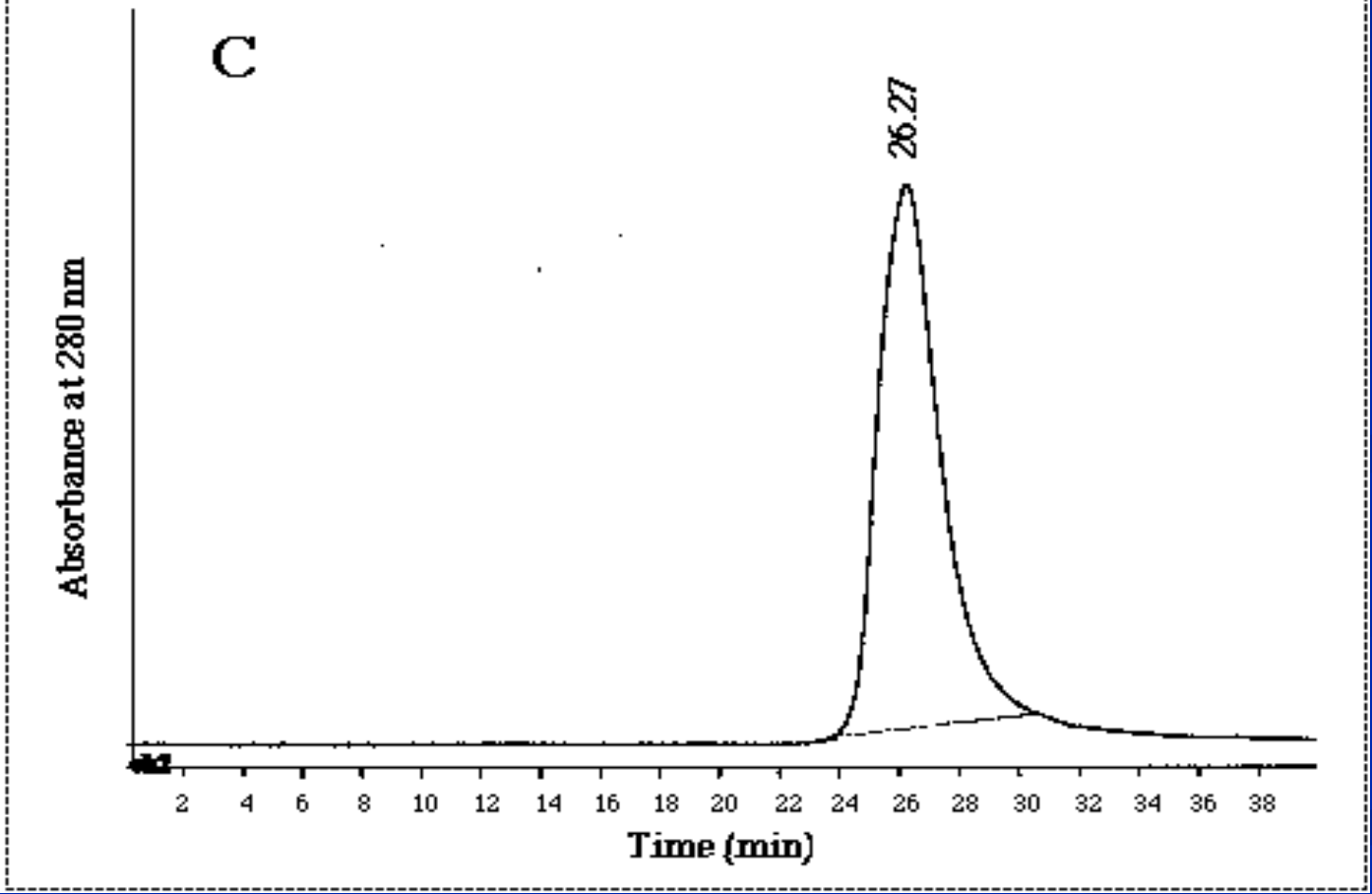
Bao， 2001

STRUCTURE OF POLYSACCHARIDES FROM GANODERMA?

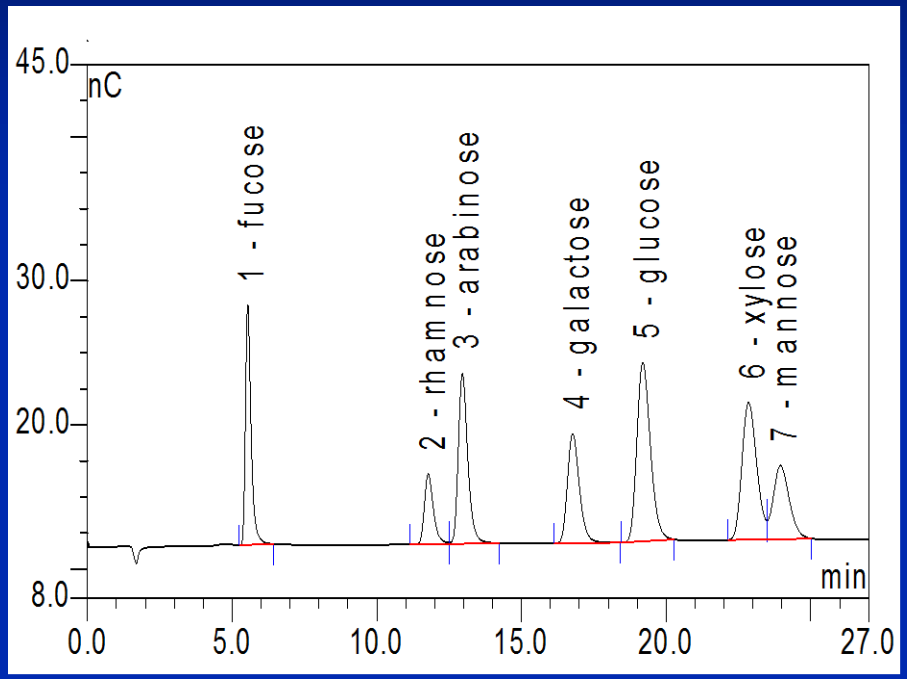
Fractionation of Crude Extract by Different Chromatographic Methodes



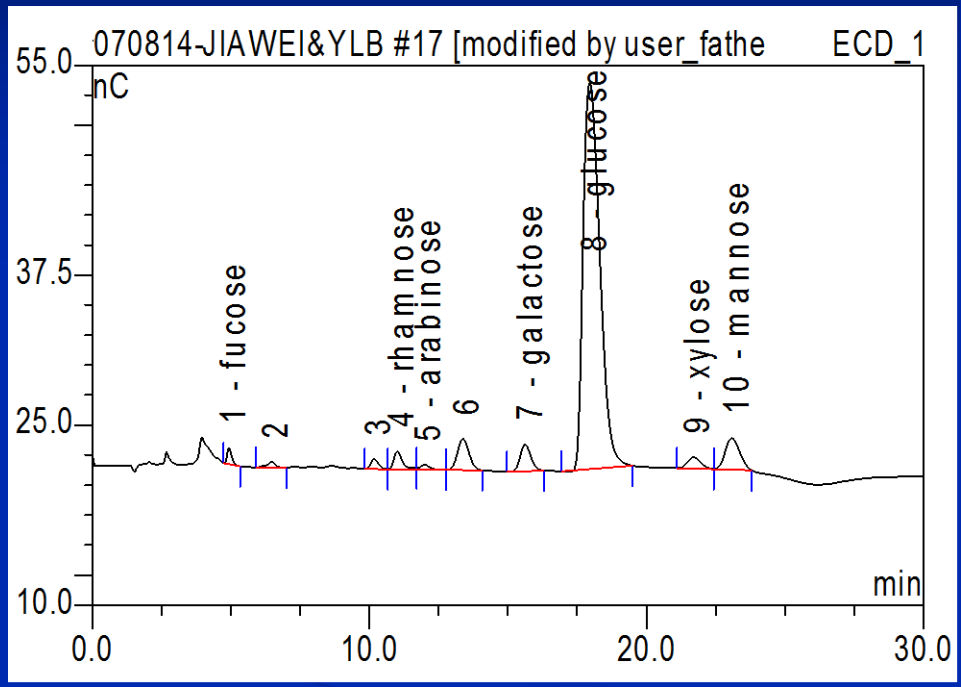
Determination of purity of GLIS by HPLC



Monosaccharide Analysis

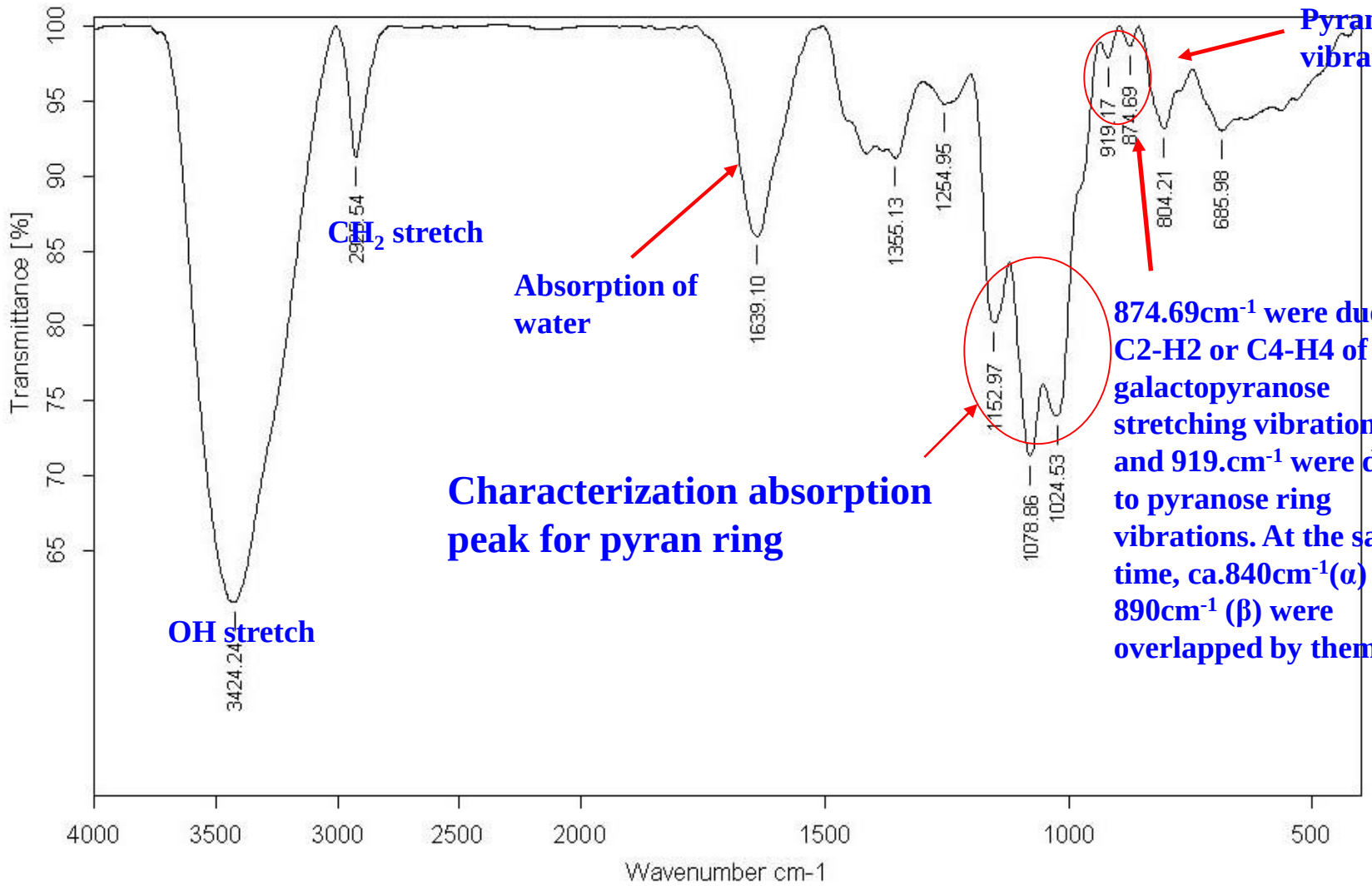


Standard



GLIS

IR spectroscopy



Methylation

Methylated sugar	linkages	Molar ratios
2,3,4,5-Me_4-Fuc*p*	1-linked Fuc*p*	0.93
3,4-Me_2-Gal*p*	1,2,6-linked-Gal*p*	1.00
2,3,4-Me_3-Man*p*	1,6-linked-Man*p*	0.32
2,3,4,6-Me_4-Glc*p*	1-linked-Glc*p*	1.91
2,3,4-Me_3-Gal*p*	1,6-linked-Gal*p*	3.89
2,4,6-Me_3-Glc*p*	1,3-linked-Glc*p*	2.23
2,3-Me_2-Glc*p*	1,4,6-linked-Glc*p*	2.03
Other	Terminal	0.57

^{1}H NMR

anomeric hydrogen

Non-anomeric hydrogen

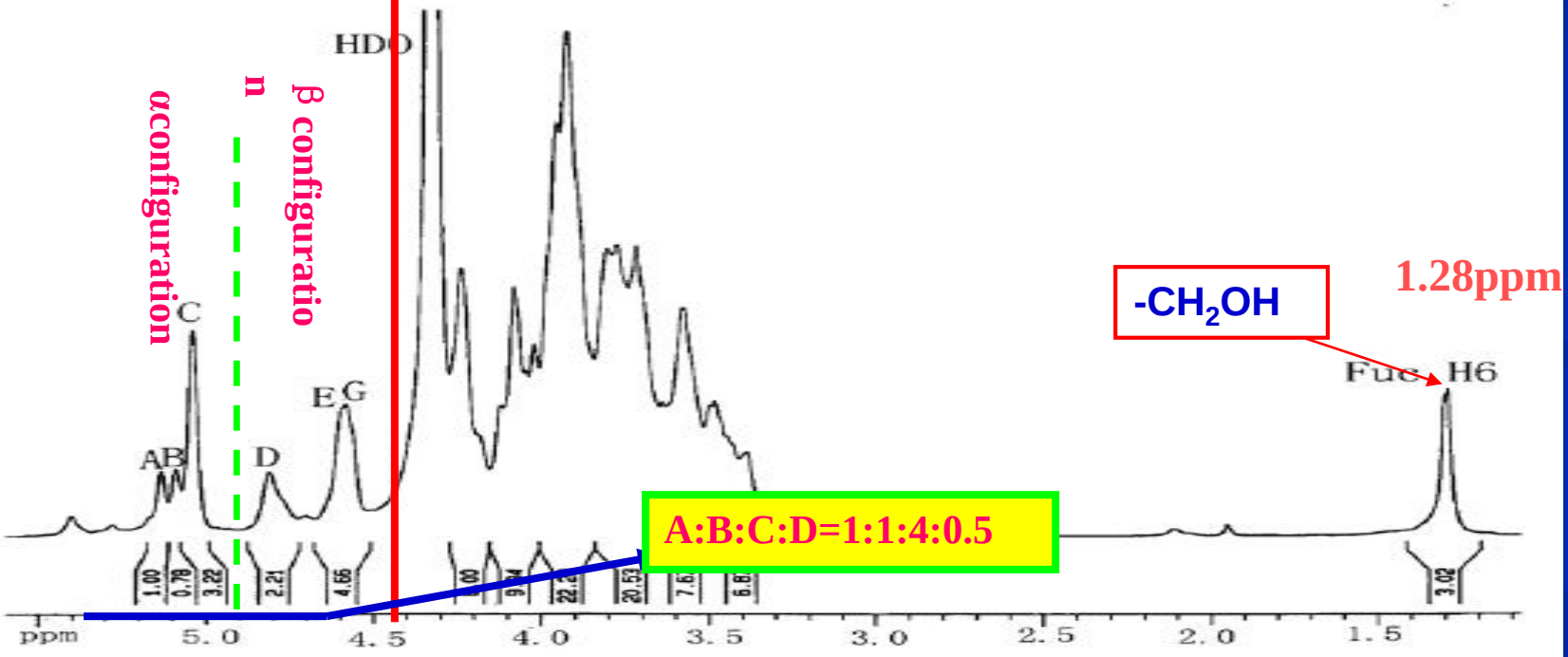
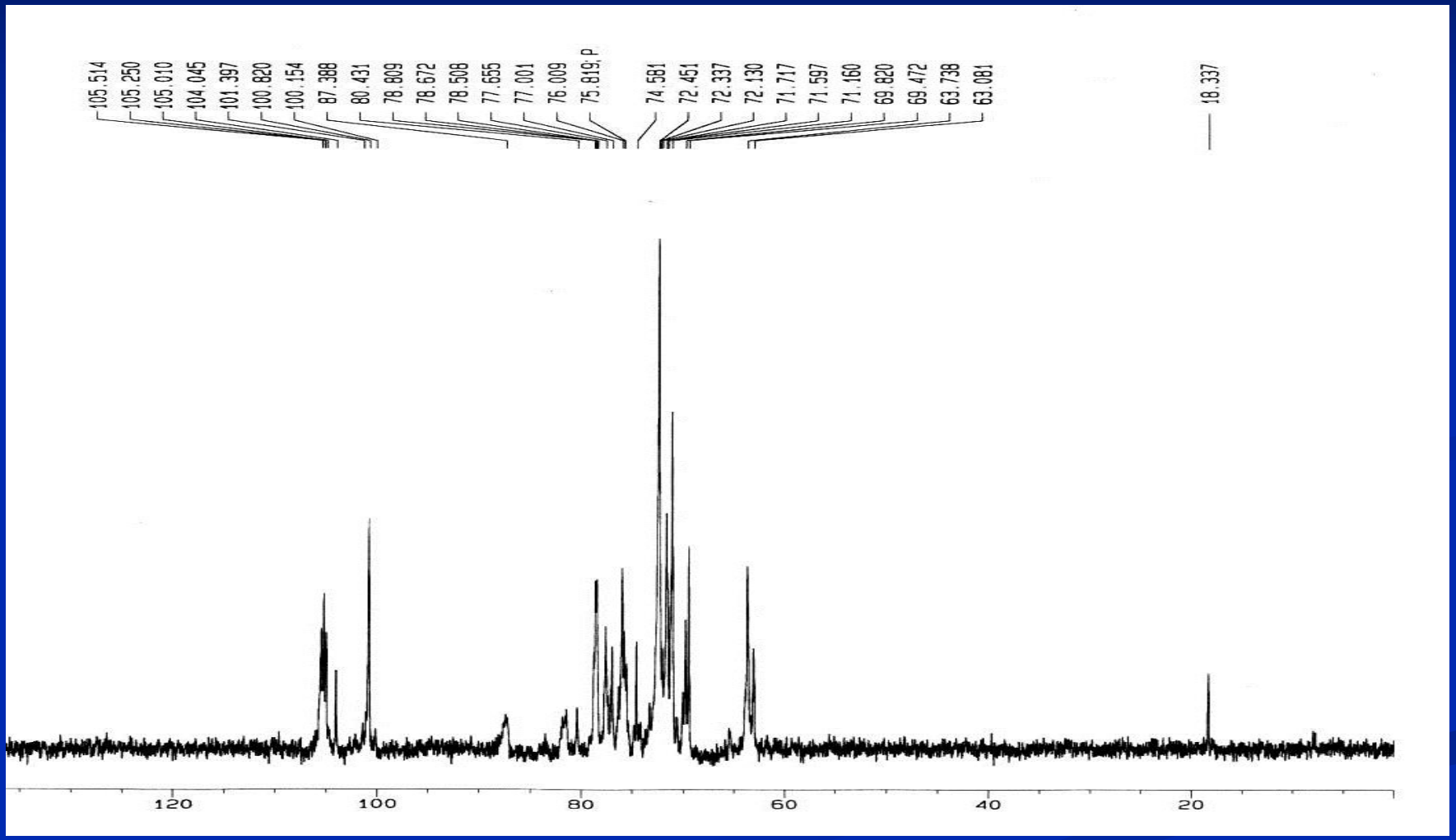
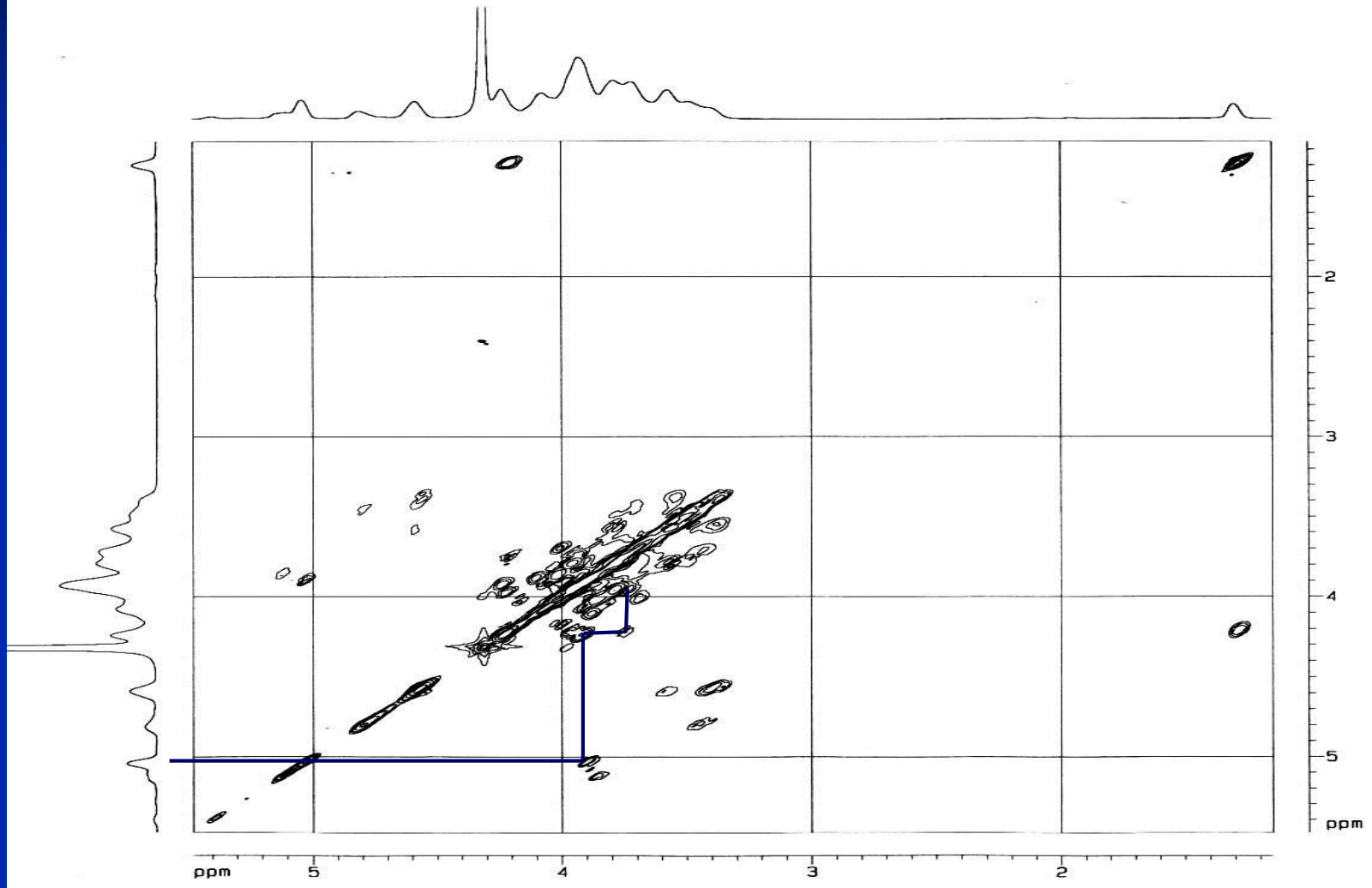


Fig. 1. ^{1}H NMR spectrum of *G. lucidum* polysaccharide LZ C 1.

^{13}C NMR

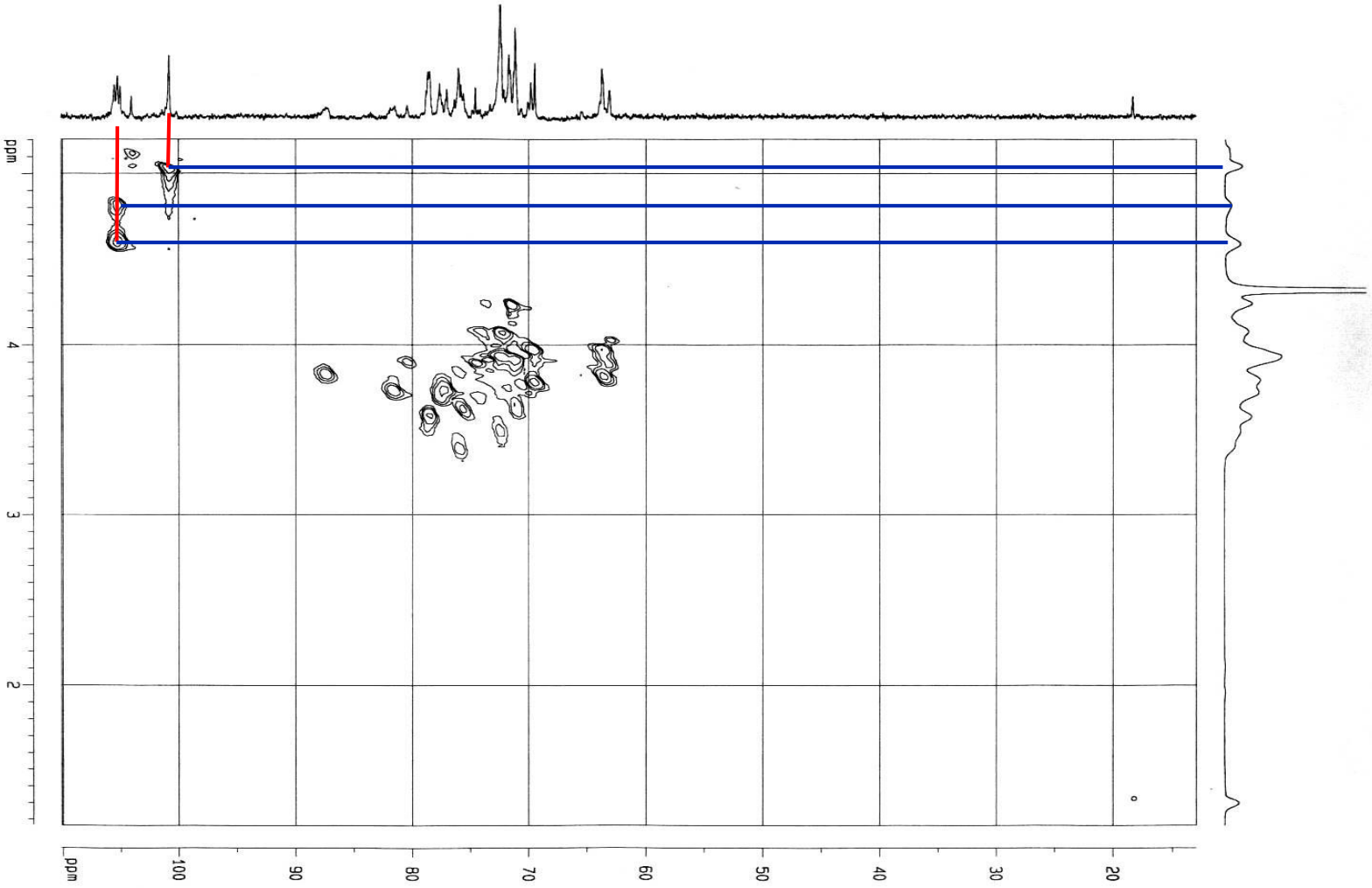

105.514
105.250
105.010
104.045
101.397
100.820
100.154
87.388
80.431
78.809
78.672
78.508
77.655
77.001
76.009
75.819; P
74.581
72.451
72.337
72.130
71.717
71.597
71.160
69.820
69.472
63.738
63.081
18.337
120
100
80
60
40
20

2D NMR



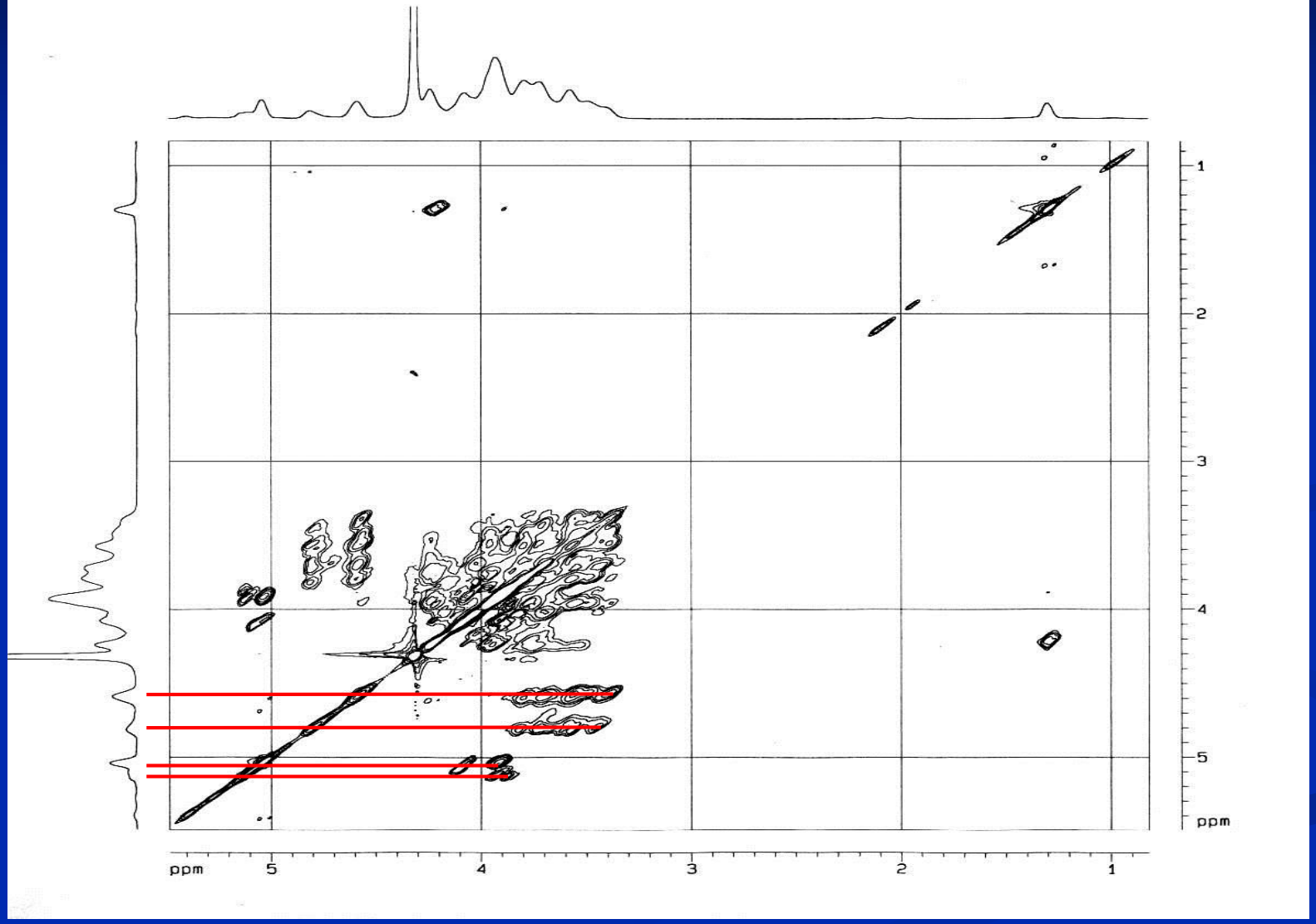
^{1}H— ^{1}H COSY

2D NMR



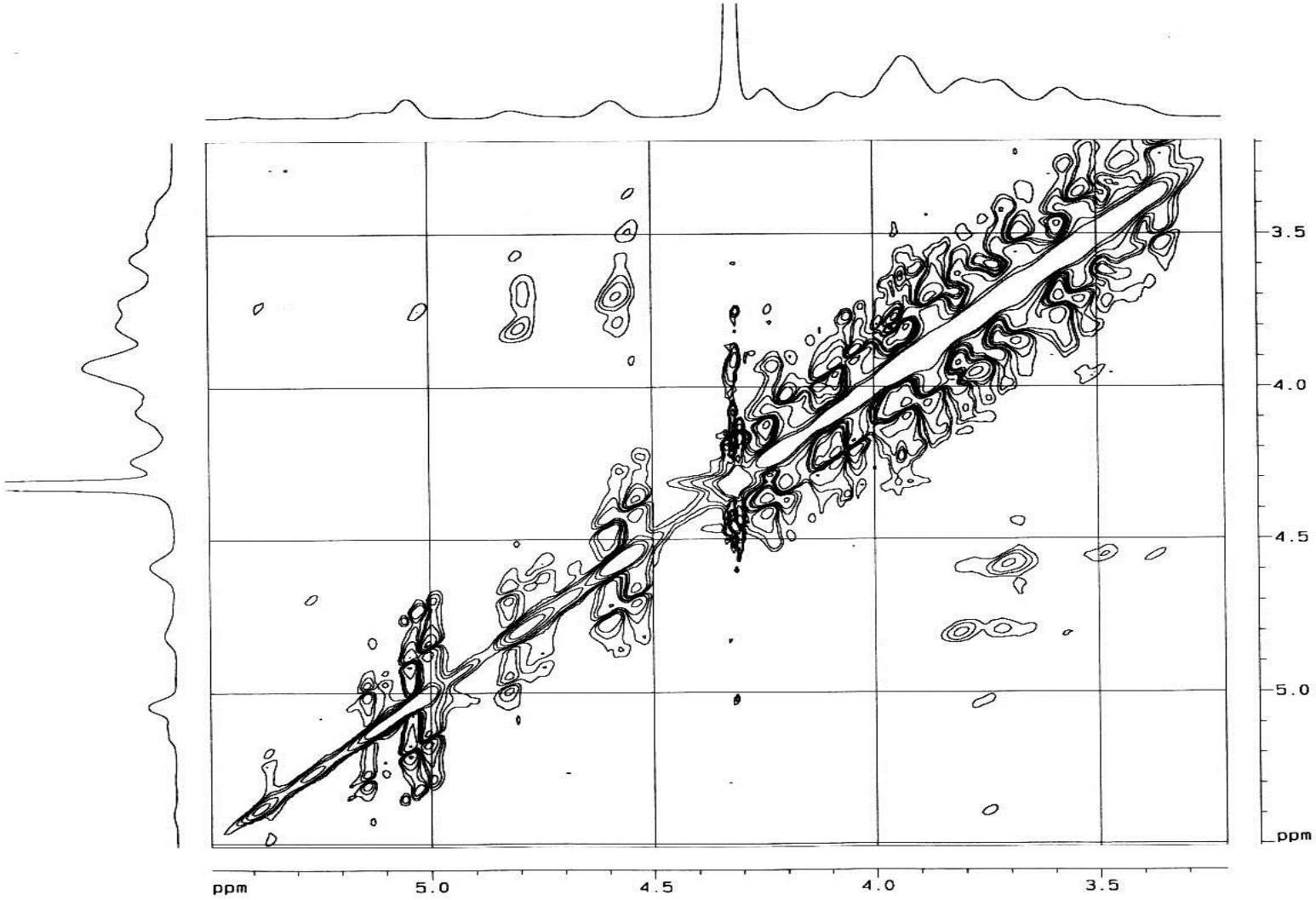
HMQC

2D NMR



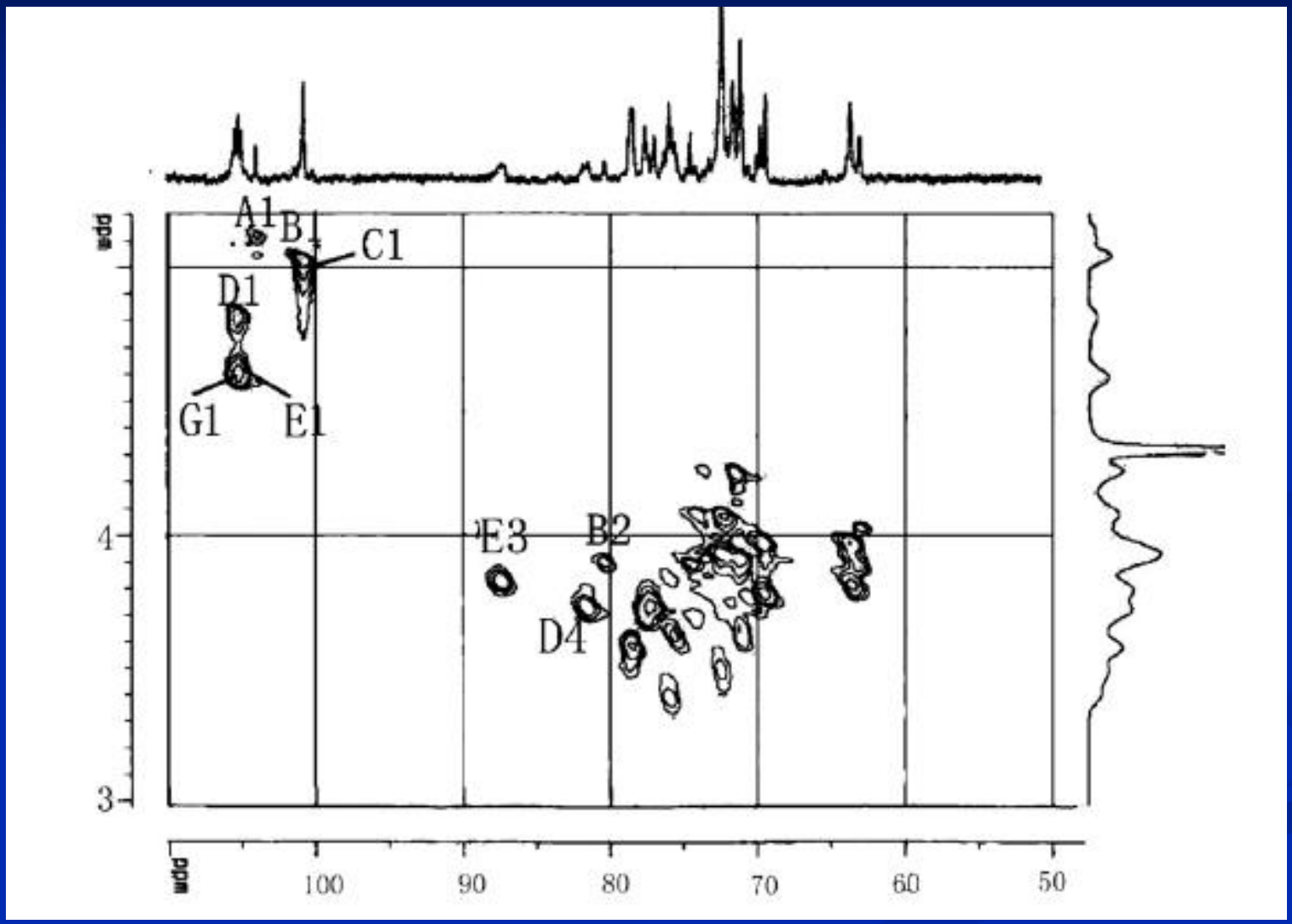
TOCSY

2D NMR



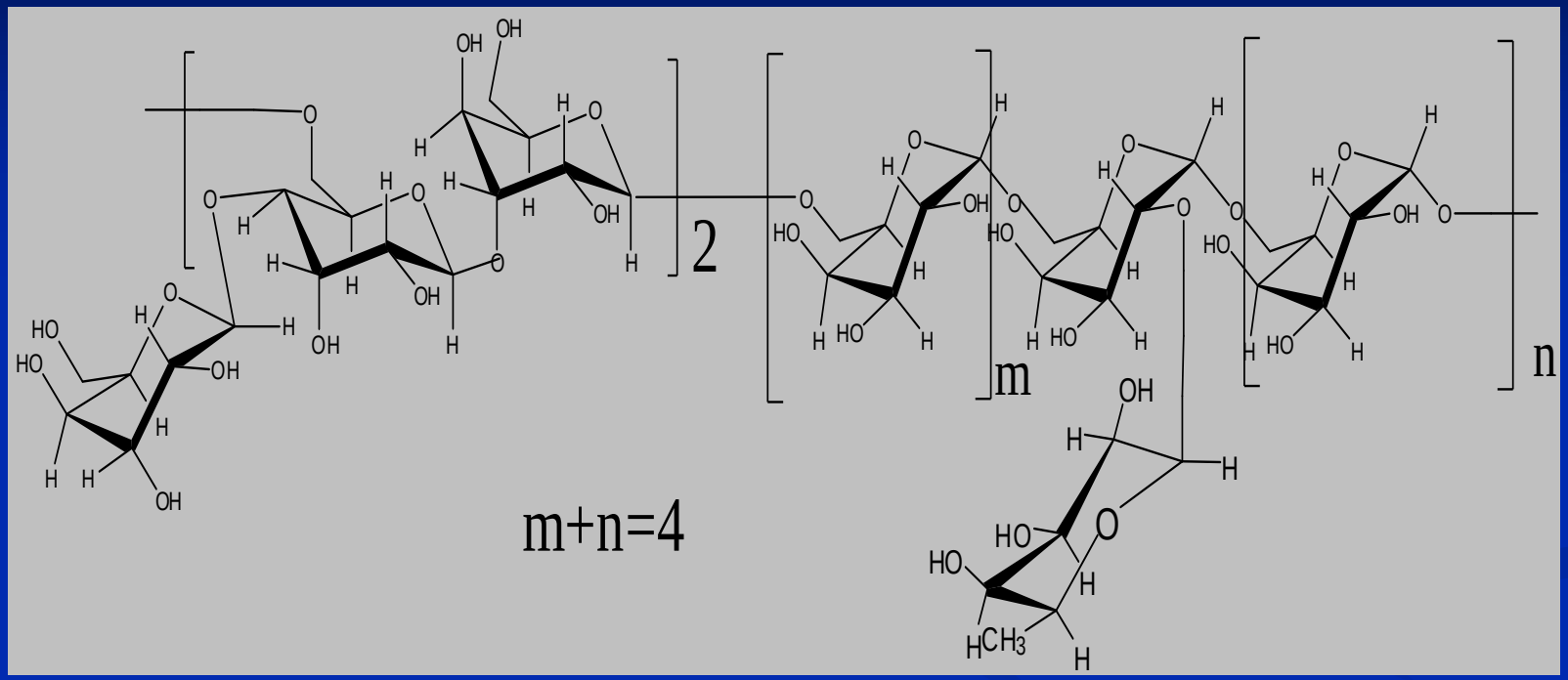
NOESY

2D NMR



HMBC

Structure of GLIS



1-Fuc	1,2,6-Gal	1-Glc	1.6-Glc	1,4,6-Glc
1	1	2.4	2	2

Structural characterization of a heteropolysaccharide by NMR spectra. *Food Chemistry*, 2009, 112:962-966

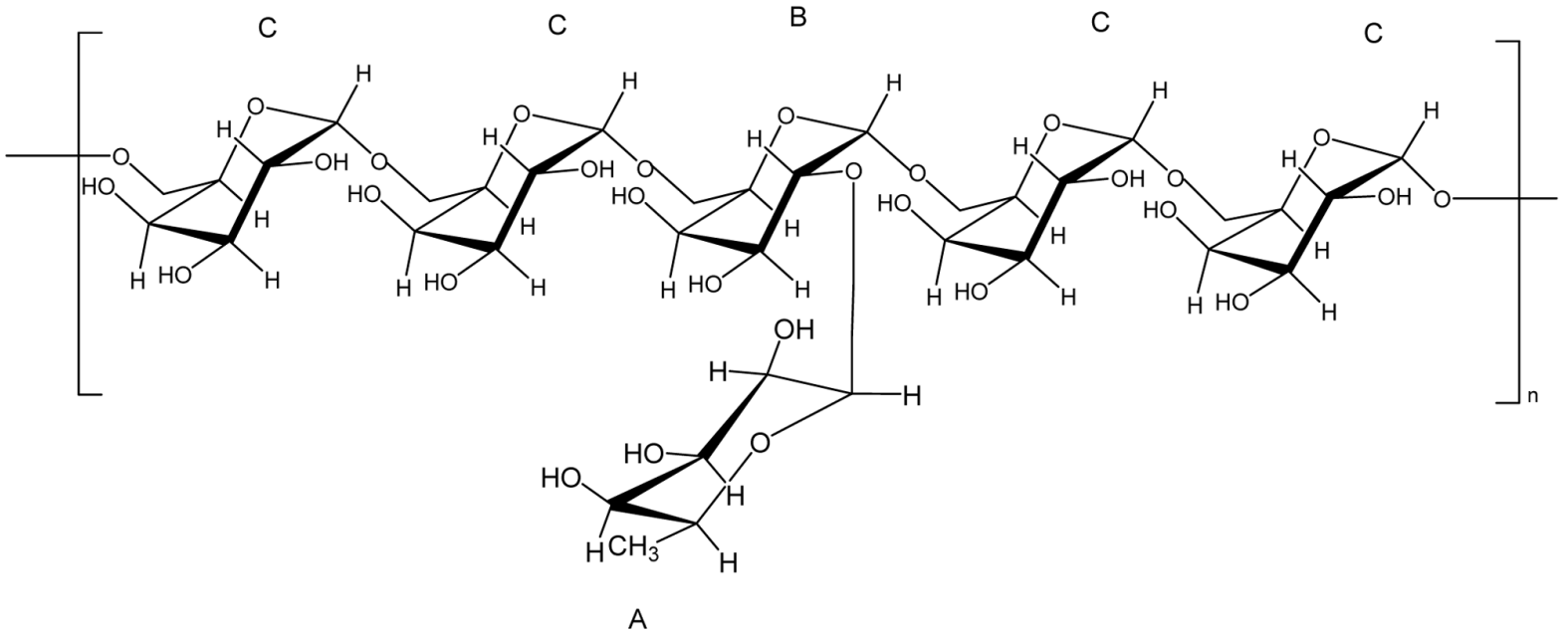
Structure of GLIS

Ye LB et al. Purification, NMR Study … Planta Med 2008; 74: 1730 – 1734

Purification, NMR Study and Immunostimulating Property of a Fucogalactan from the Fruiting Bodies of *Ganoderma lucidum*

Author: LiBin Ye[1, 2], JingSong Zhang[2], Kan Zhou[3], Yan Yang[2], Shuai Zhou[2], Wei Jia[2], RuiXia Hao[2], YingJie Pan[4]

Affiliation: [1] College of Life Sciences, Nanjing Agricultural University, Nanjing, P. R. China
[2] Institute of Edible Fungi, Shanghai Academy of Agricultural Sciences, Shanghai, P. R. China



Structure of GLIS

Fitoterapia 81 (2010) 93–96

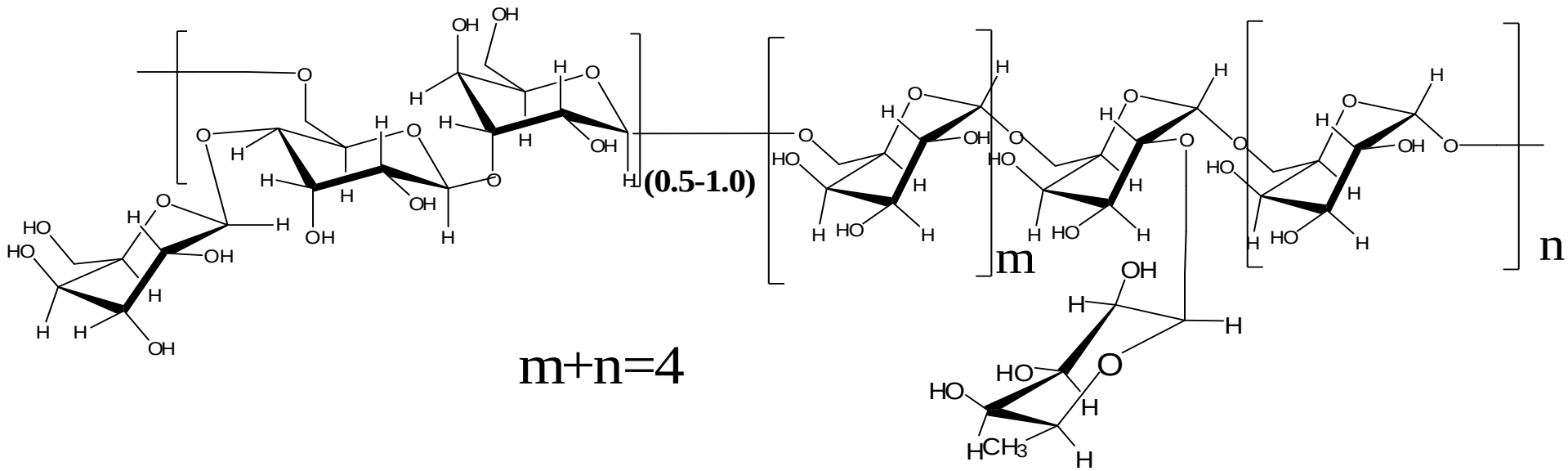
Contents lists available at ScienceDirect

Fitoterapia

journal homepage: www.elsevier.com/locate/fitote

NMR characterization for polysaccharide moiety of a glycopeptide

Libin Ye [a], Jianrong Li [a], Jingsong Zhang [b,*], Yingjie Pan [c,*]



Structure of GLIS

Available online at www.sciencedirect.com
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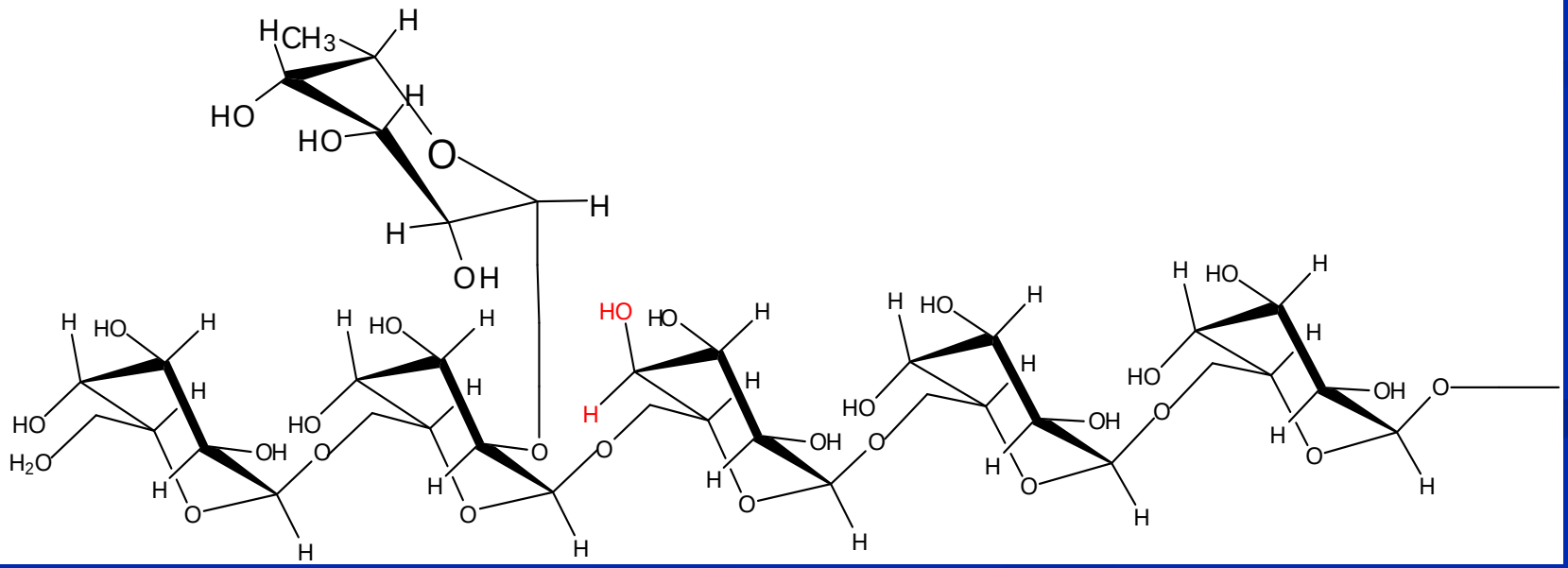
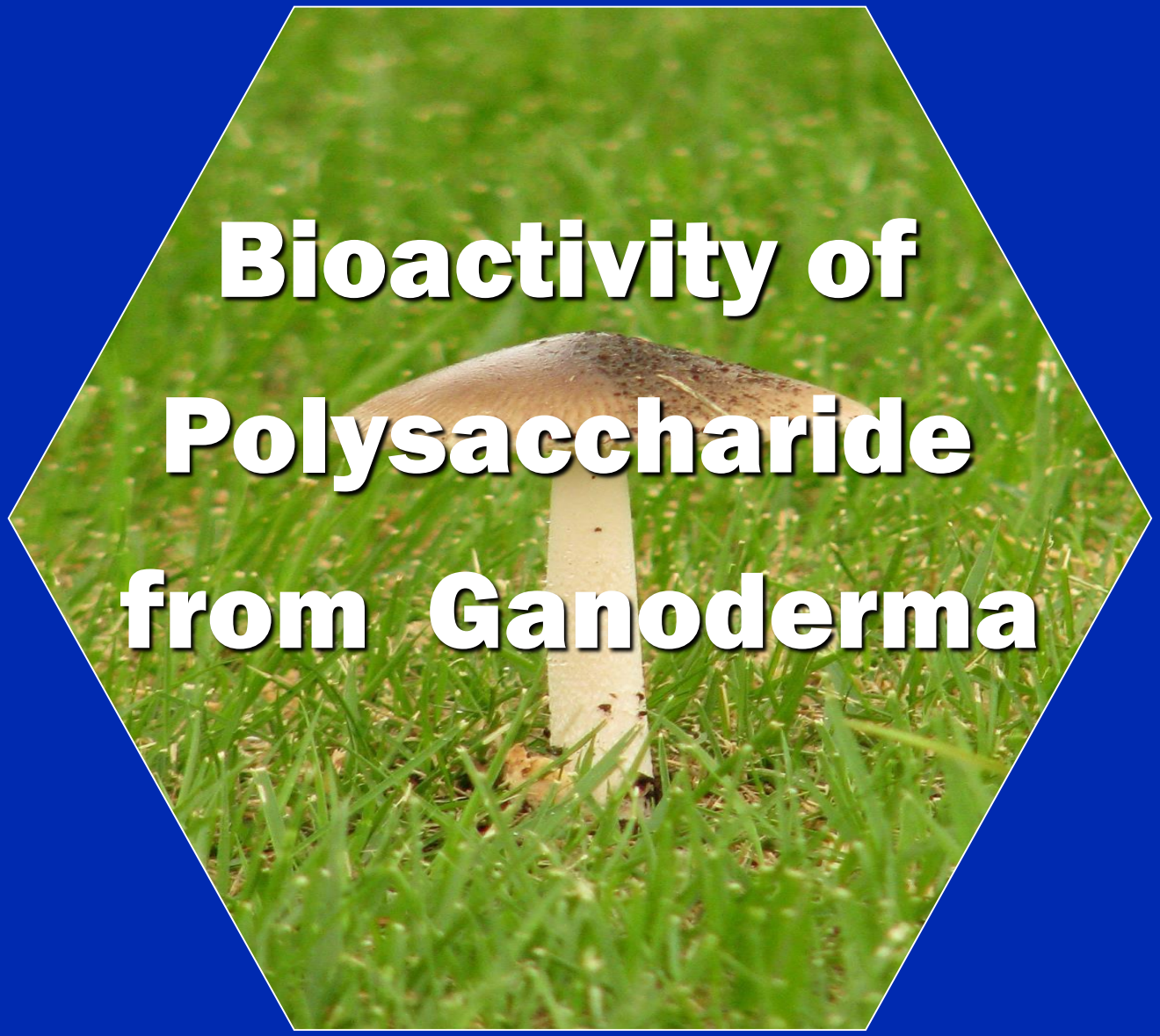
Carbohydrate Research 343 (2008) 746–752
Carbohydrate RESEARCH

Structural elucidation of the polysaccharide moiety of a glycopeptide (GLPCW-II) from *Ganoderma lucidum* fruiting bodies

LiBin Ye,[a,b] JingSong Zhang,[b,*] XiJun Ye,[c] QingJiu Tang,[b] YanFang Liu,[b] ChunYu Gong,[d] XiuJui Du[a] and YingJie Pan[d,*]

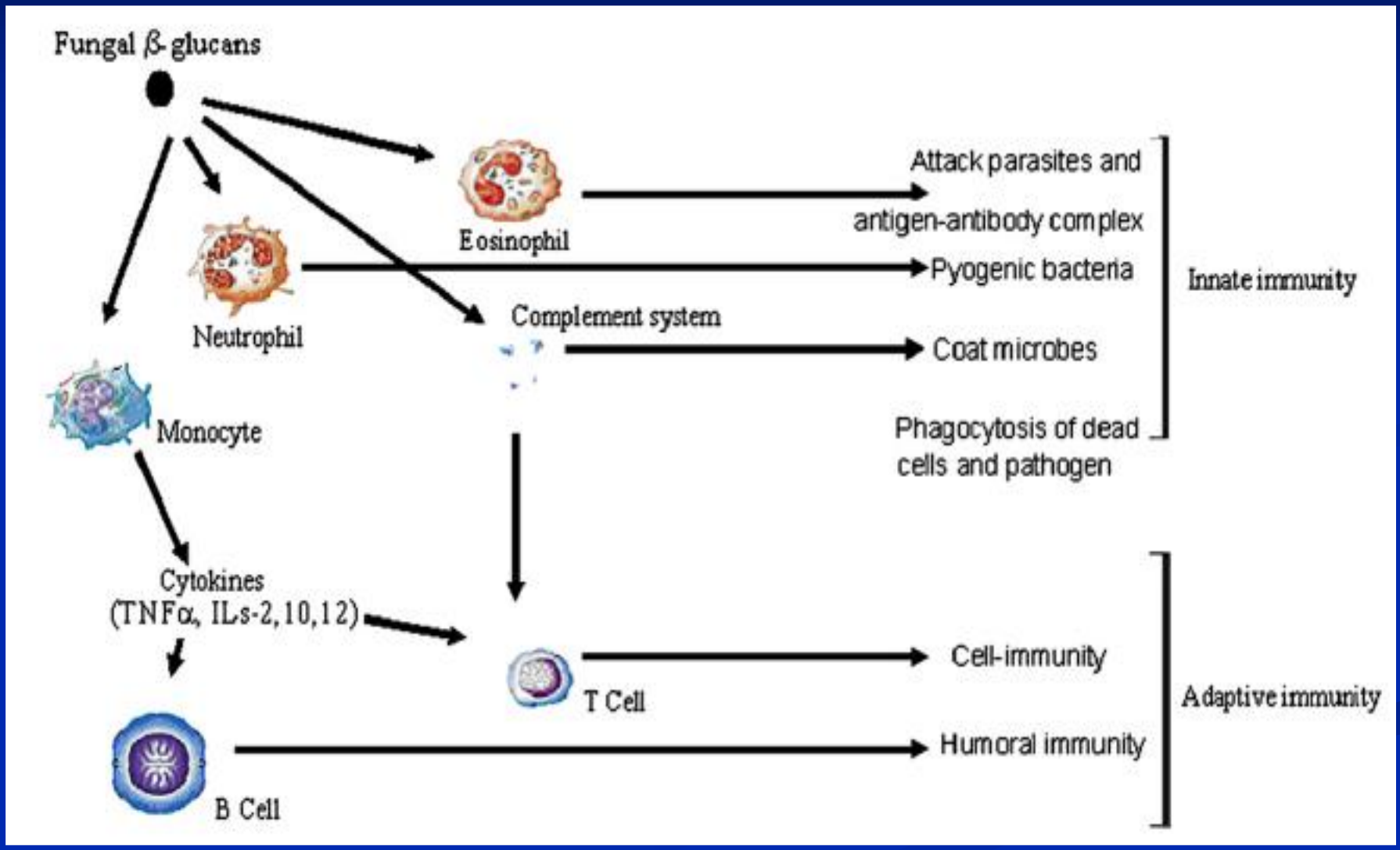
[a]College of Life Sciences, Nanjing Agricultural University, Nanjing, Jiangsu 210095, China





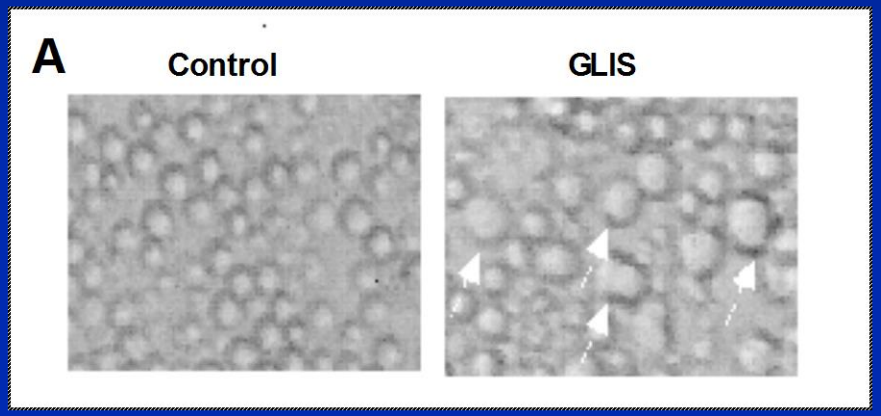
Bioactivity of Polysaccharide from Ganoderma

Immunostimulation by fungal β-Glucans



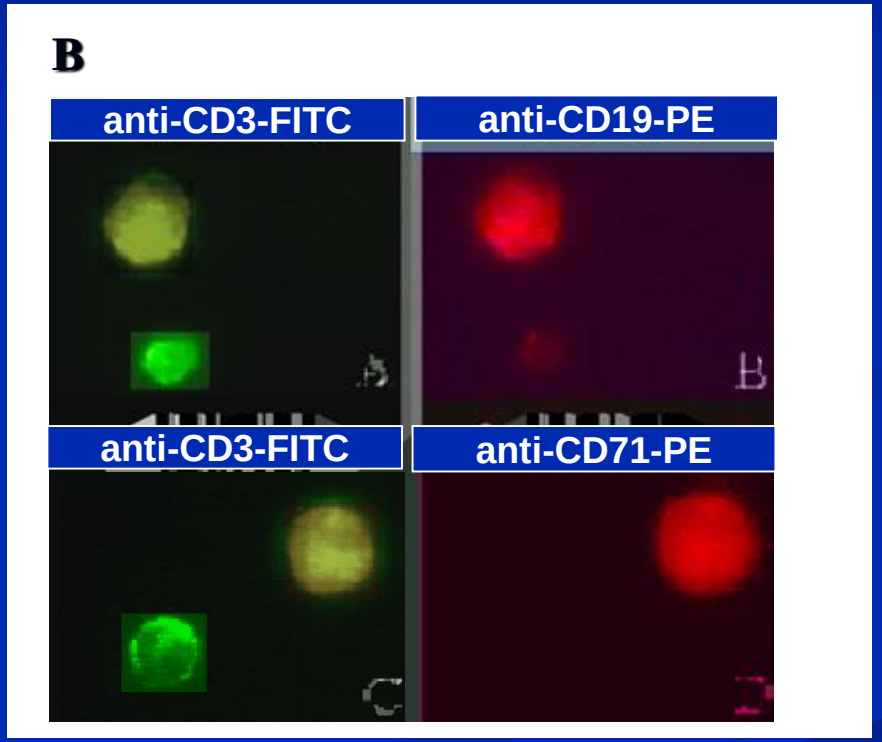
CHEN et al, 2007

Determination of the activated B-cells in MSLs by Immunomicroscopy

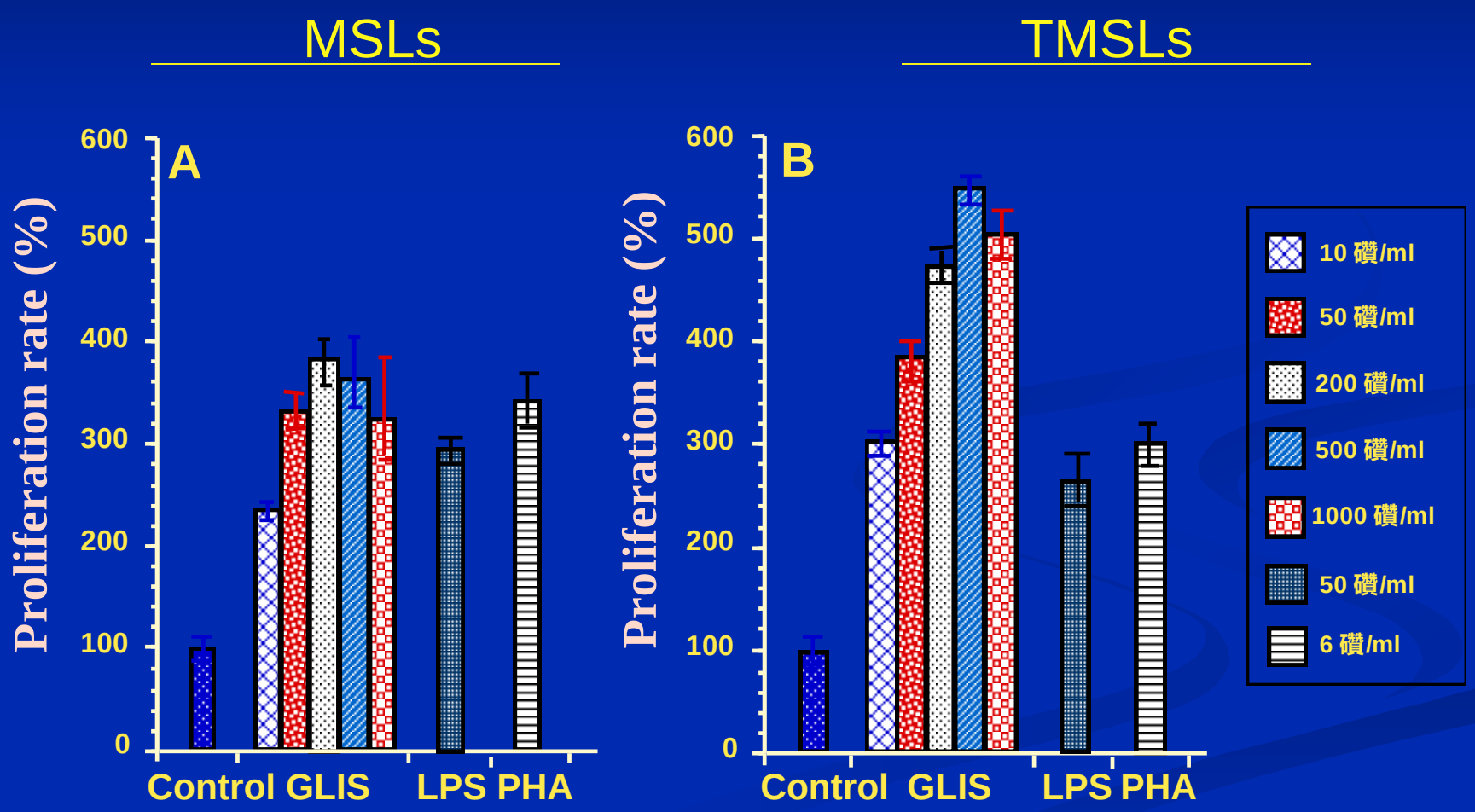


A. Morphological changes of MSLs
In situ after stimulation with GLIS

B. After stimulation with GLIS, the cells
Were stained by anti-CD3-FITC,
anti-CD19-PE or anti-CD71-PE mAbs.

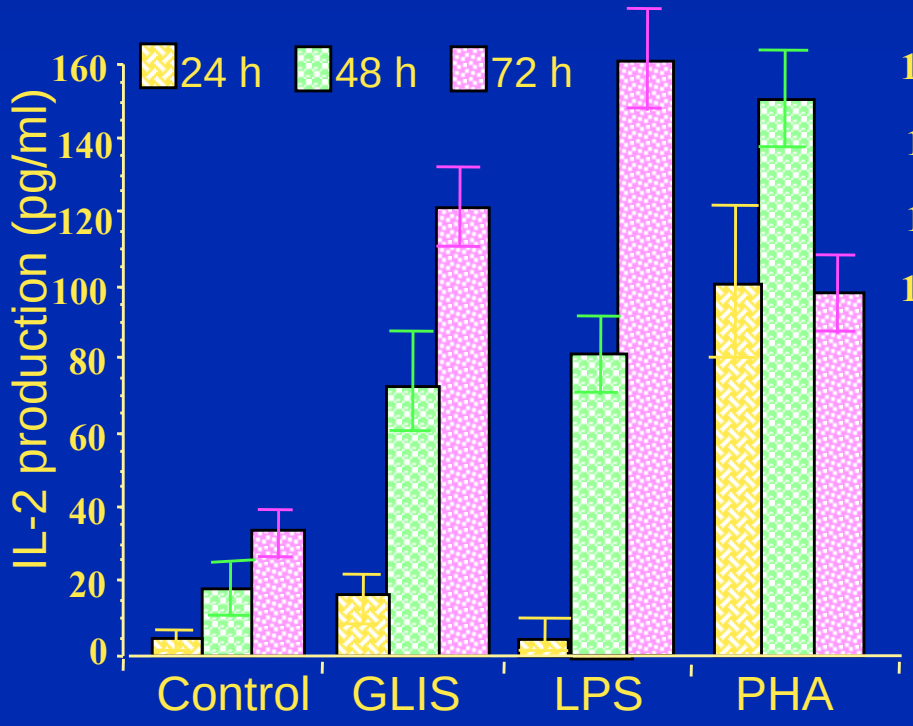


Proliferation of Lymphocytes stimulated by GLIS

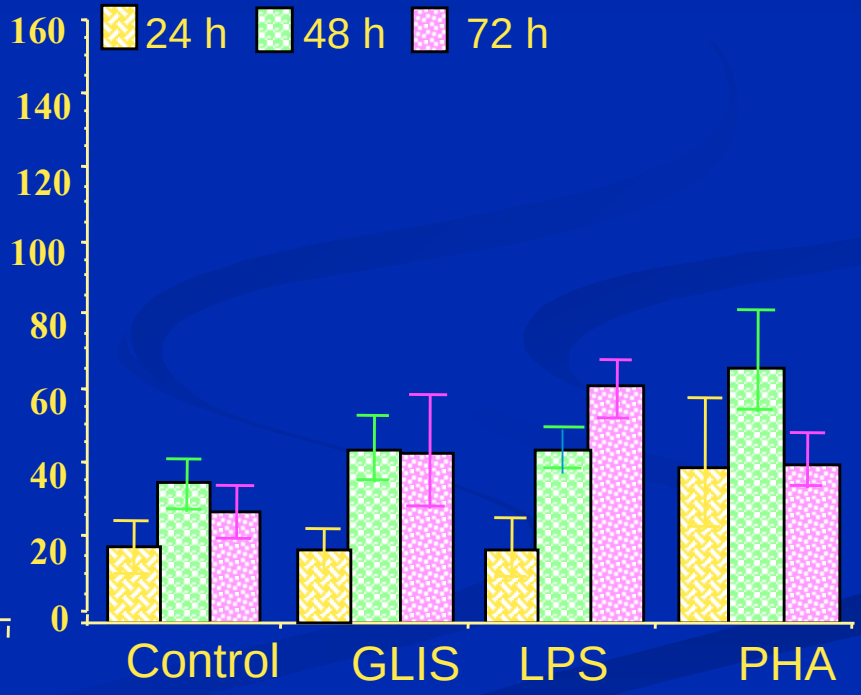


IL-2 Production of Lymphocytes stimulated by GLIS

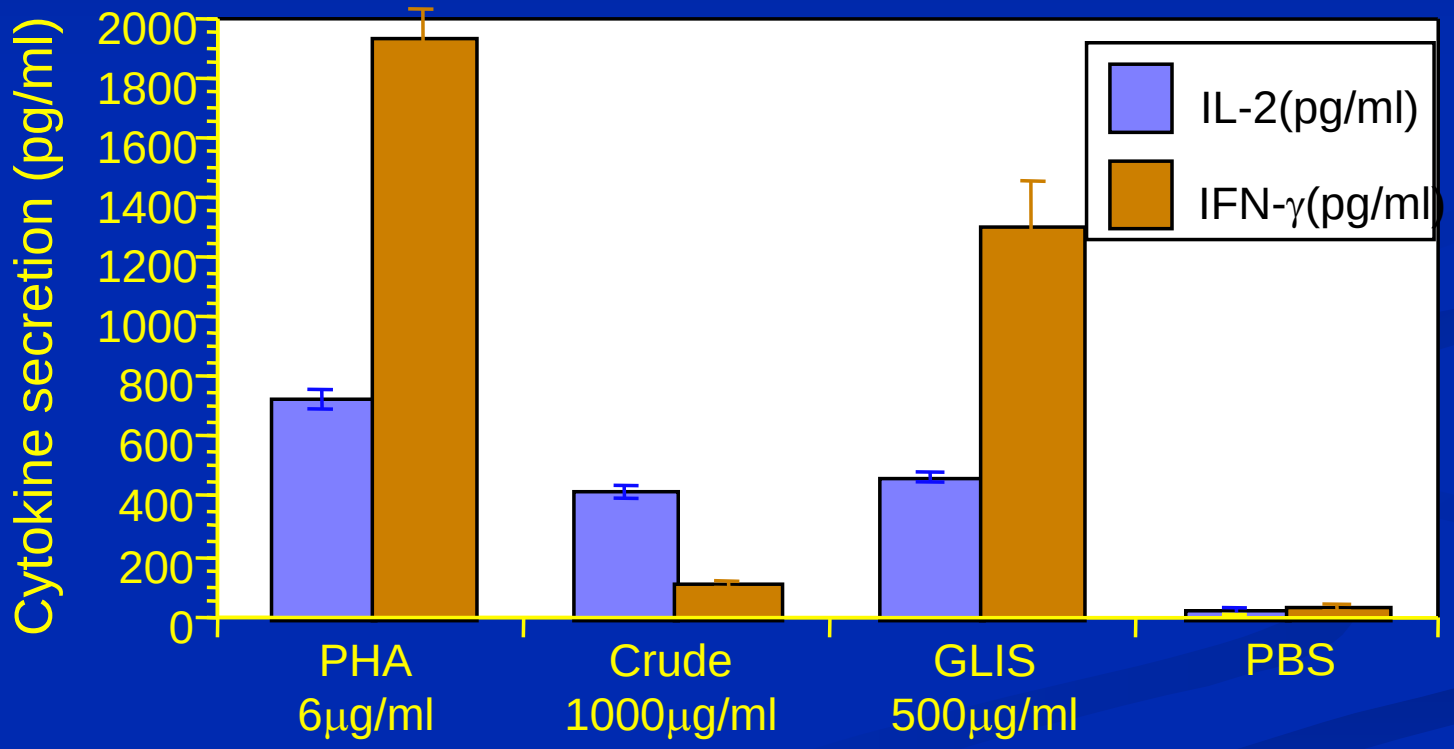
MSLs



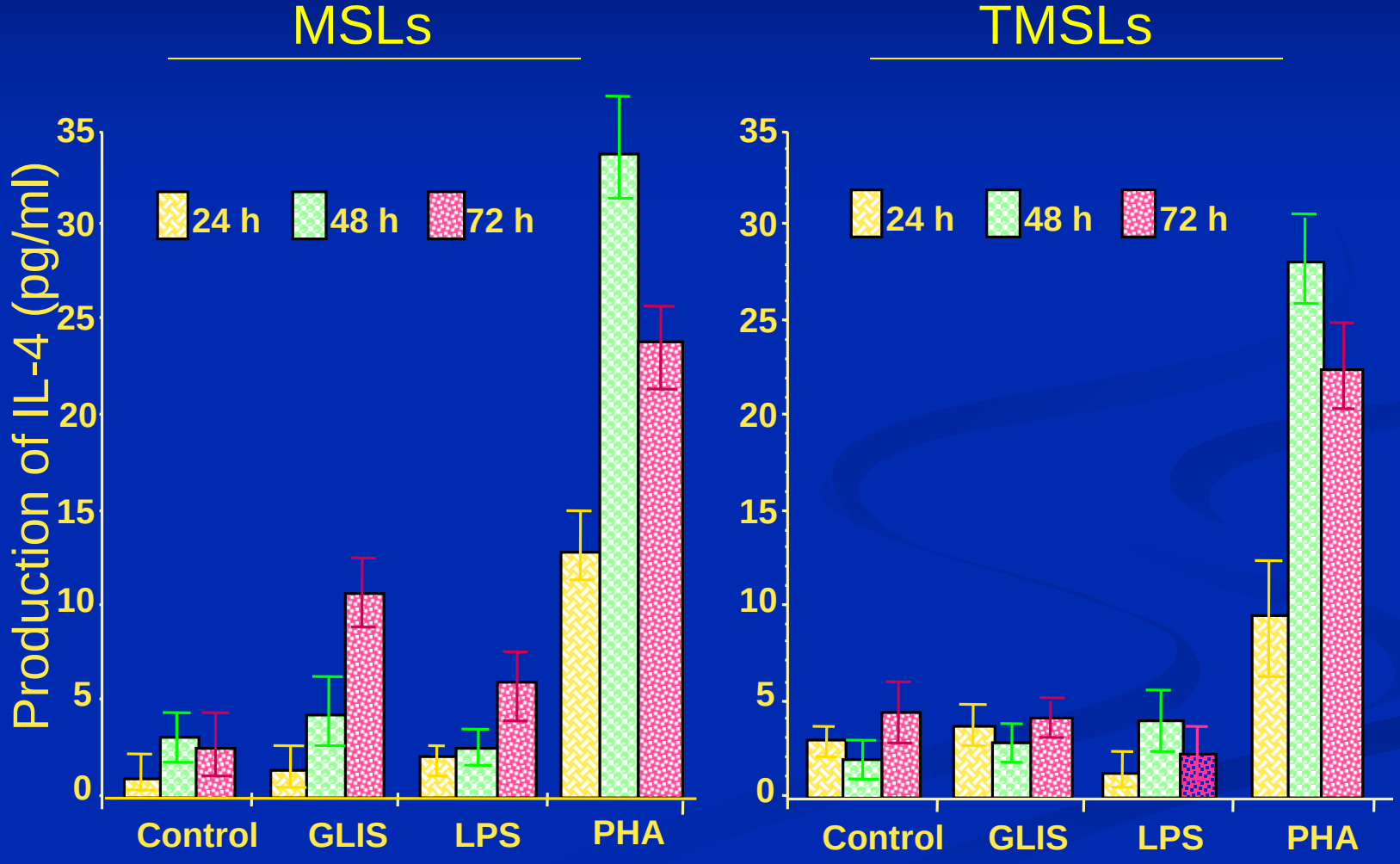
TMSLs



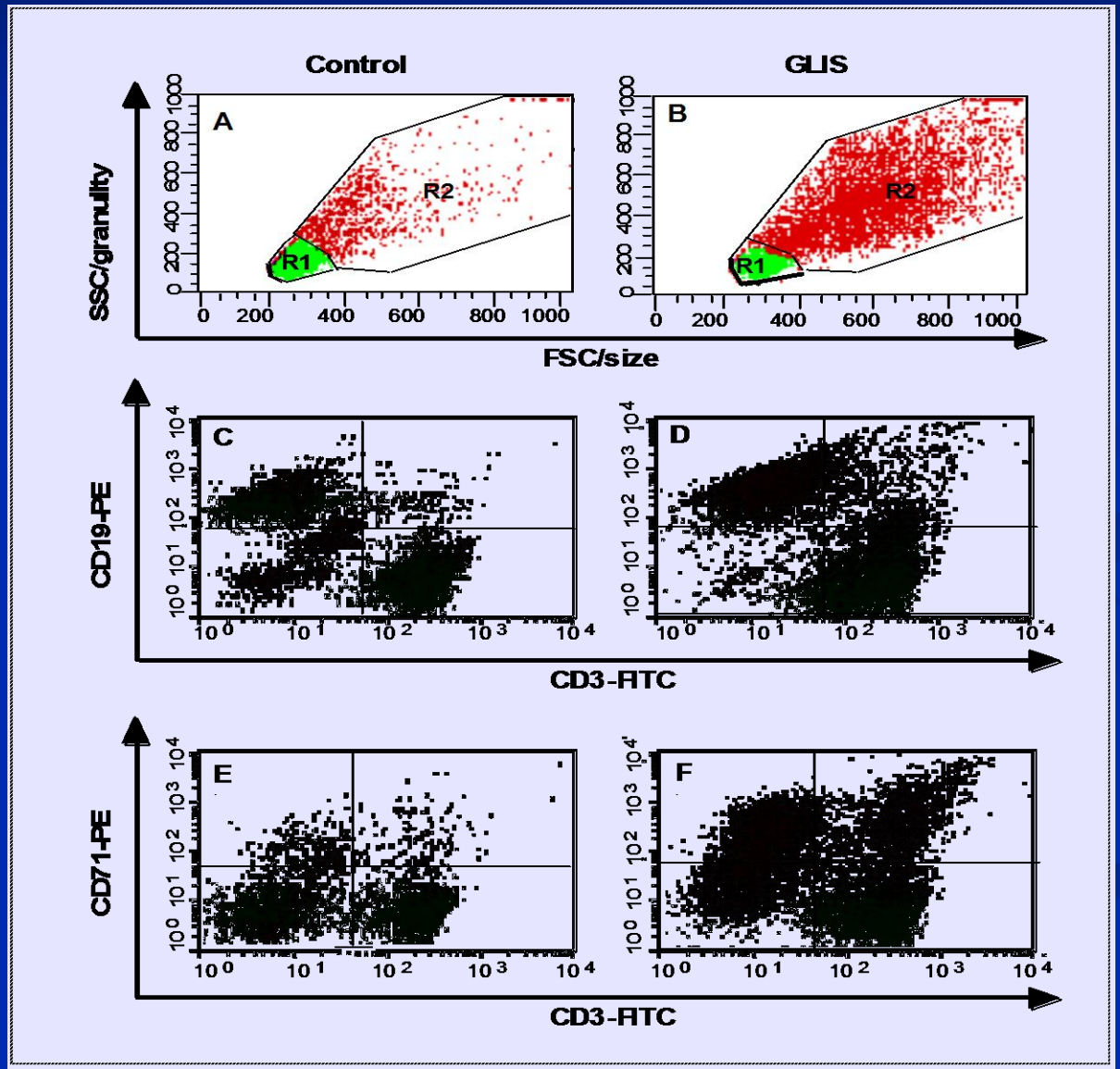
IL-2 and IFN-γ Production of HPBLs after Stimulation by GLIS in vitro



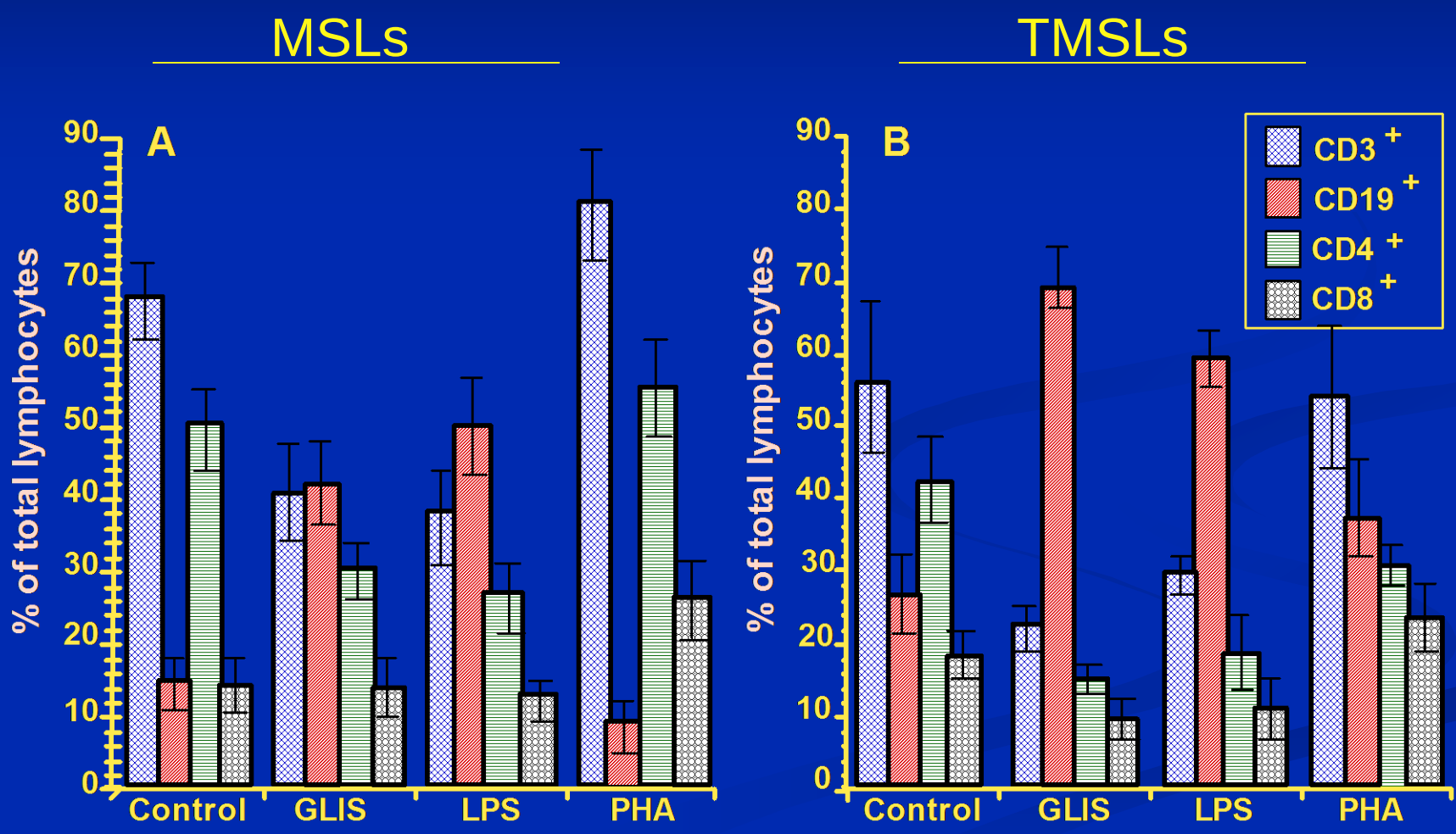
IL-4 Production of Lymphocytes stimulated by GLIS



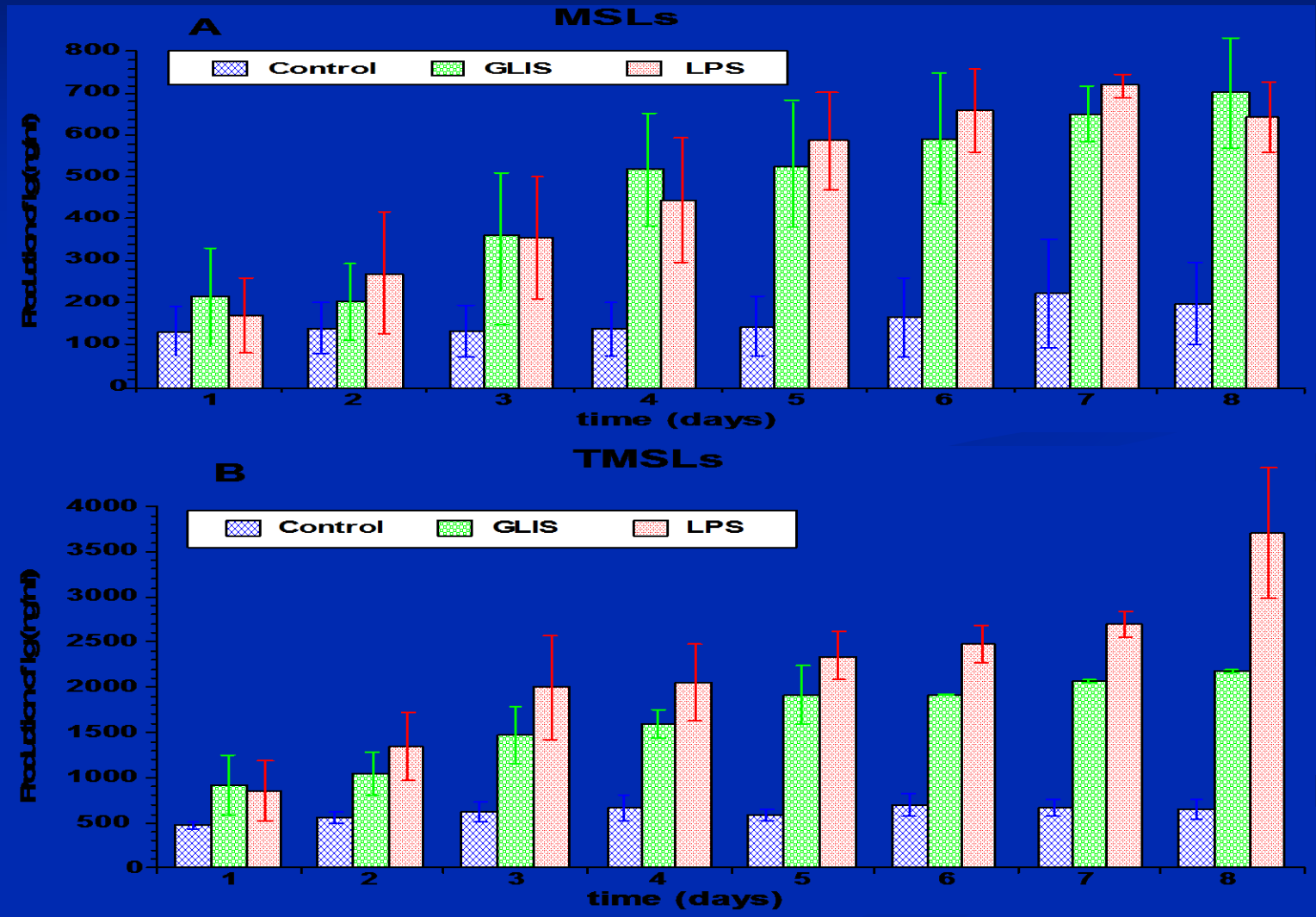
Analysis of Lymphocytes by FACScan after Stimulation by GLIS



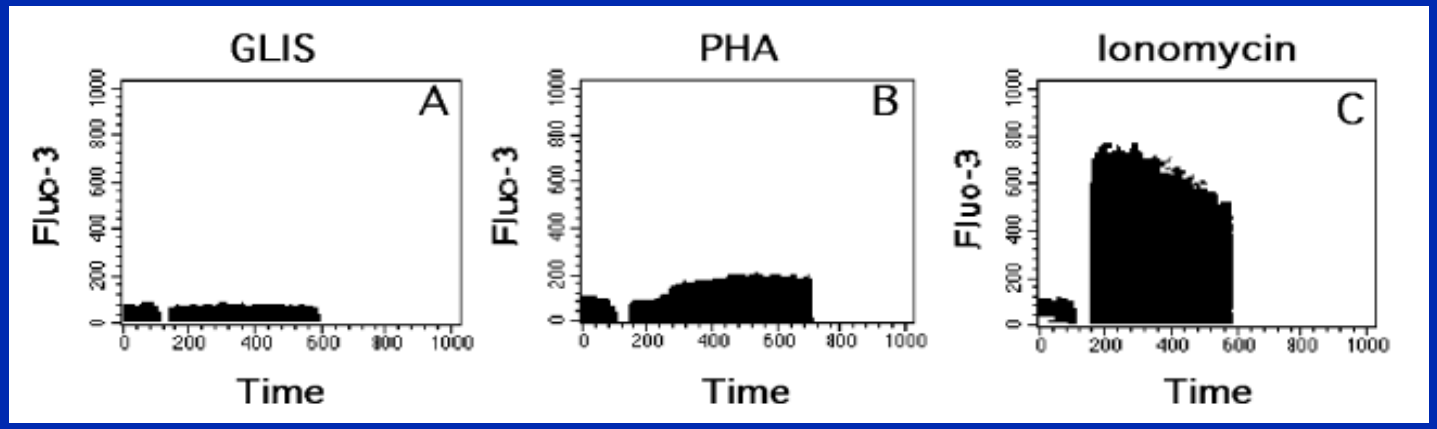
Change of Percentage of Lymohicyte Subpopulation after Stimulation by GLIS



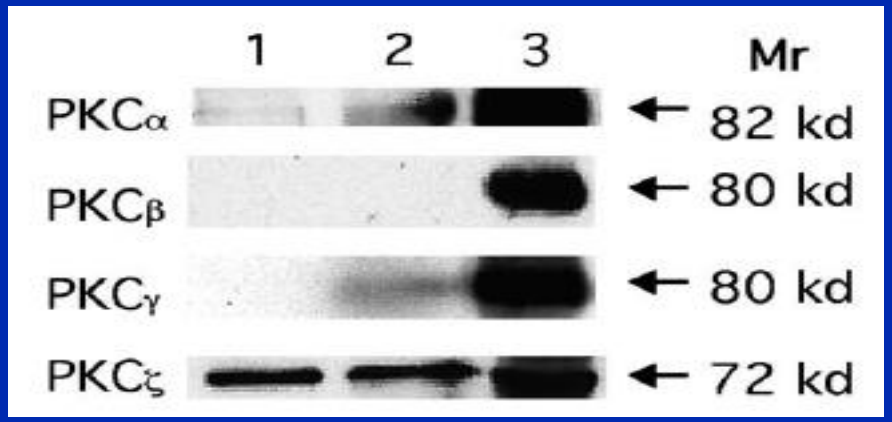
Production of Immunoglobulins of MSLs and TMSLs after Stimulation by GLIS



Influence of GLIS on the Concentration of Ca2+ and Expression of Protein Kinases

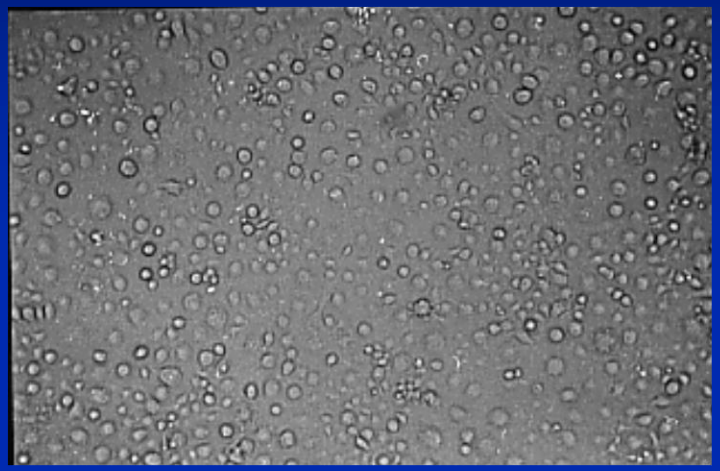


1. Untreated control; 2. GLIS 500 μg/ml for 54 h; 3. PKC in Rat brain

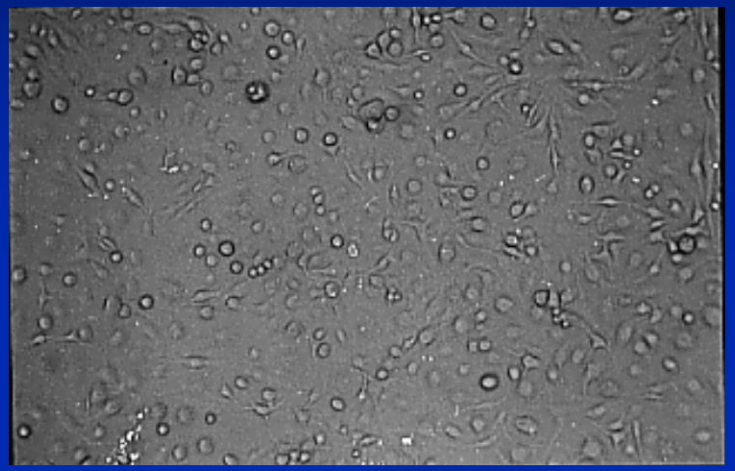


Activation of Macrophages by GLIS

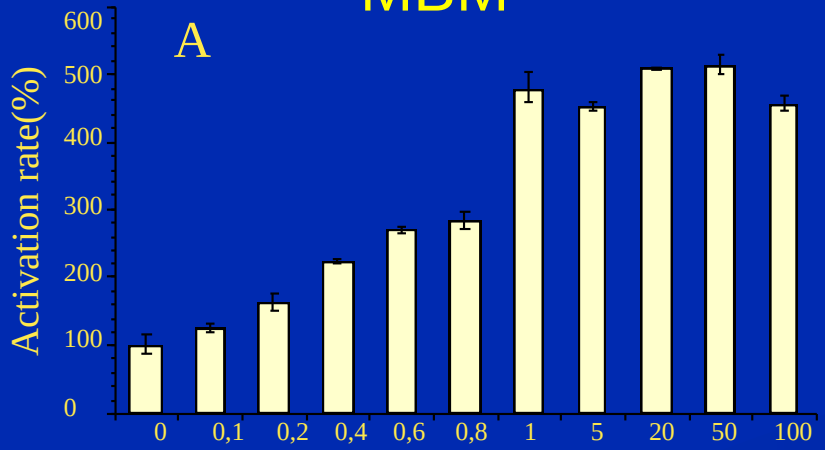
Control



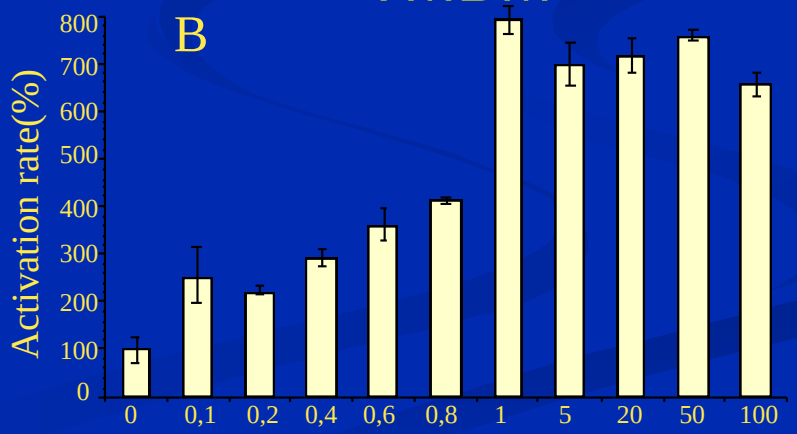
GLIS



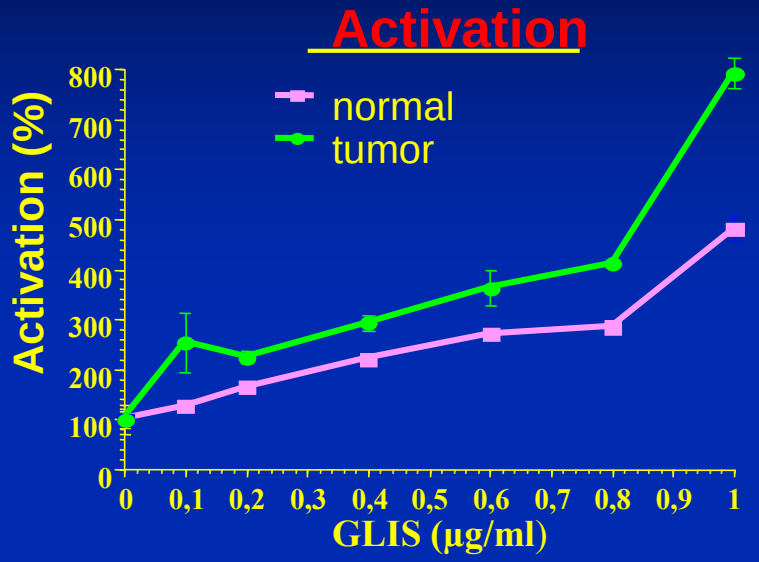
MBM

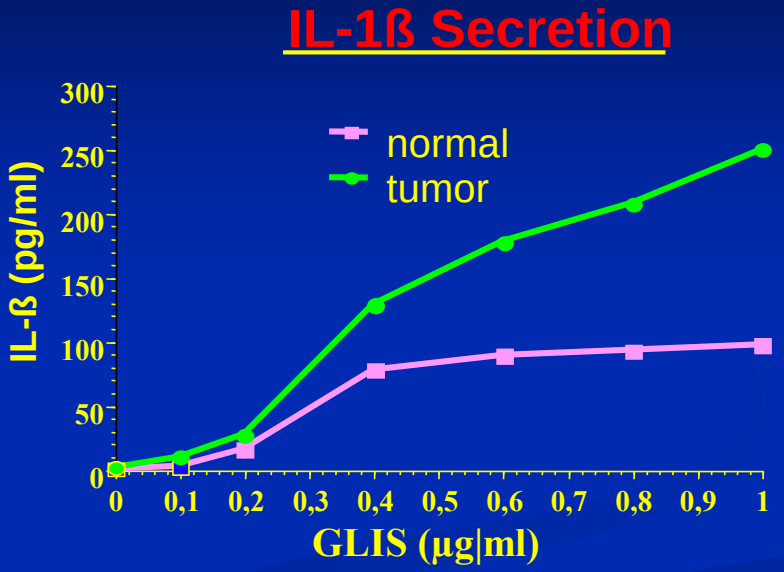


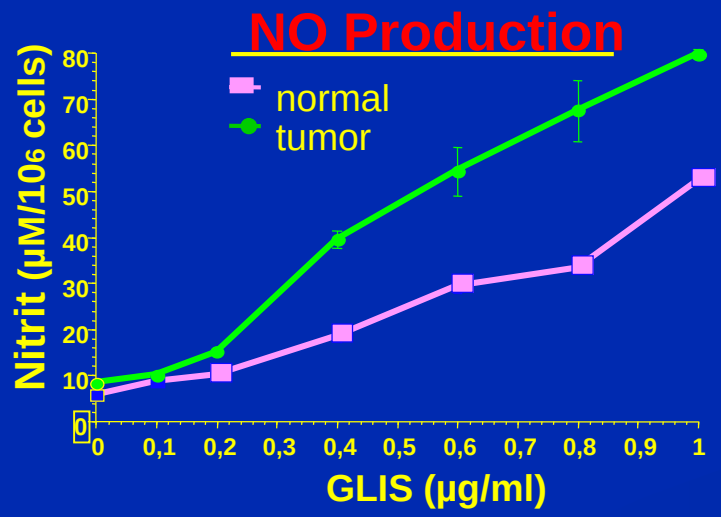
TMBM

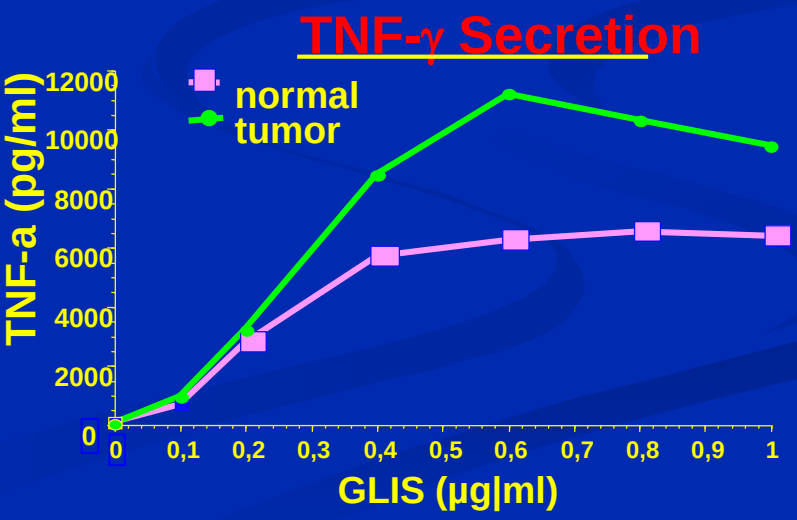


Activation of Macrophages by GLIS



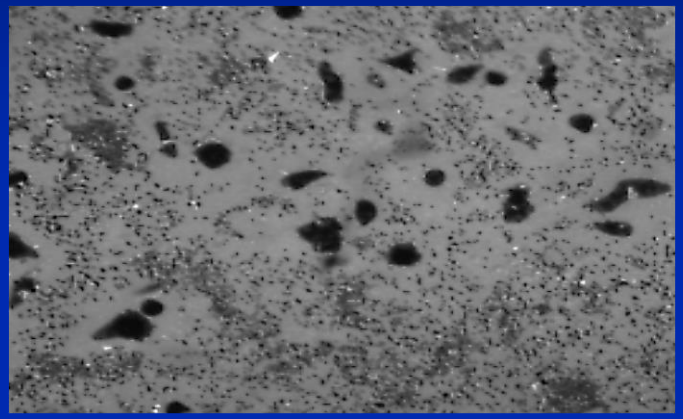




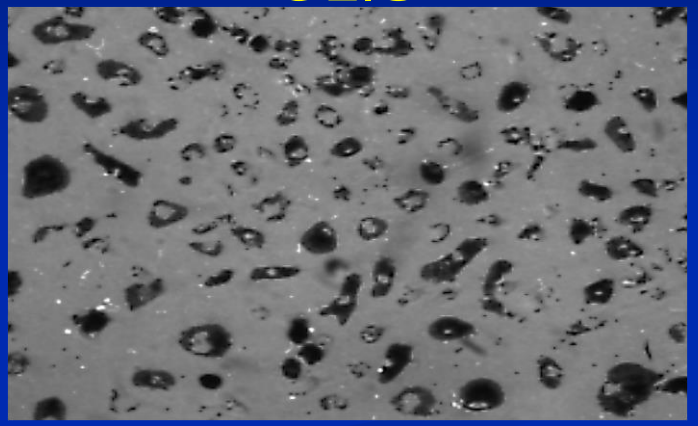


Phagocytosis of Macrophages stimulated by GLIS

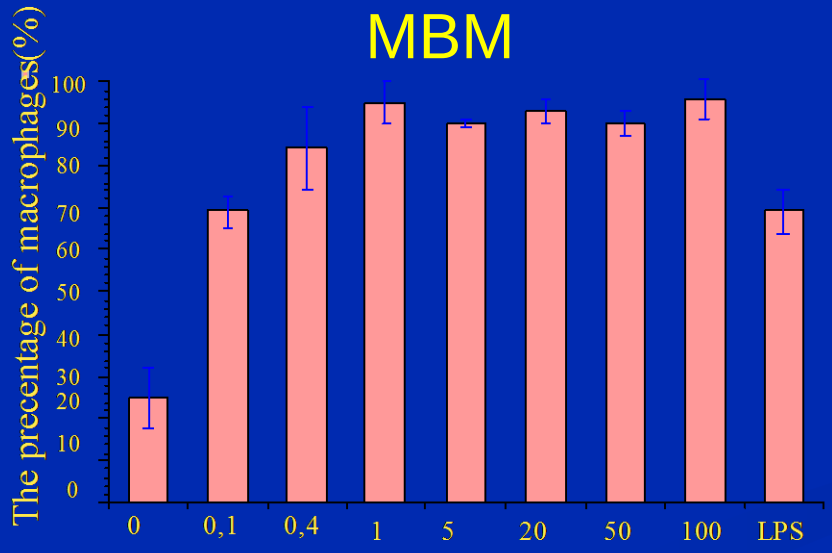
Control



GLIS



MBM



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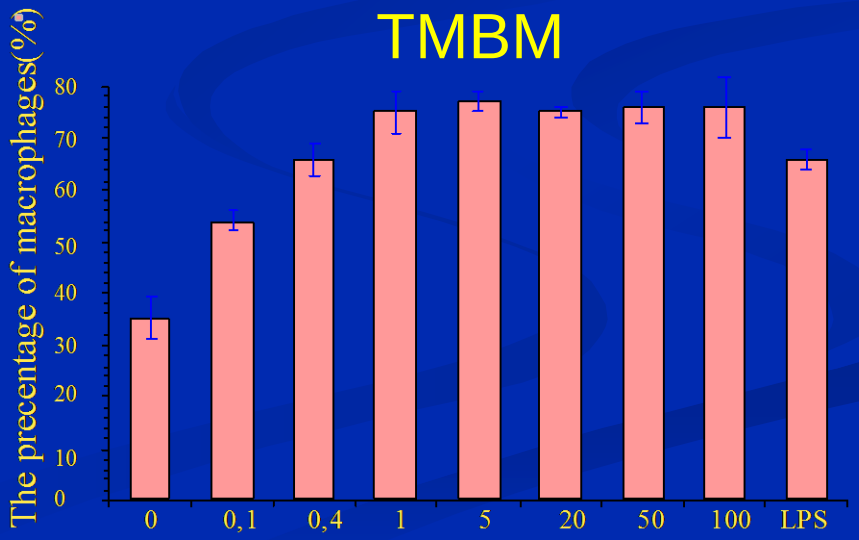
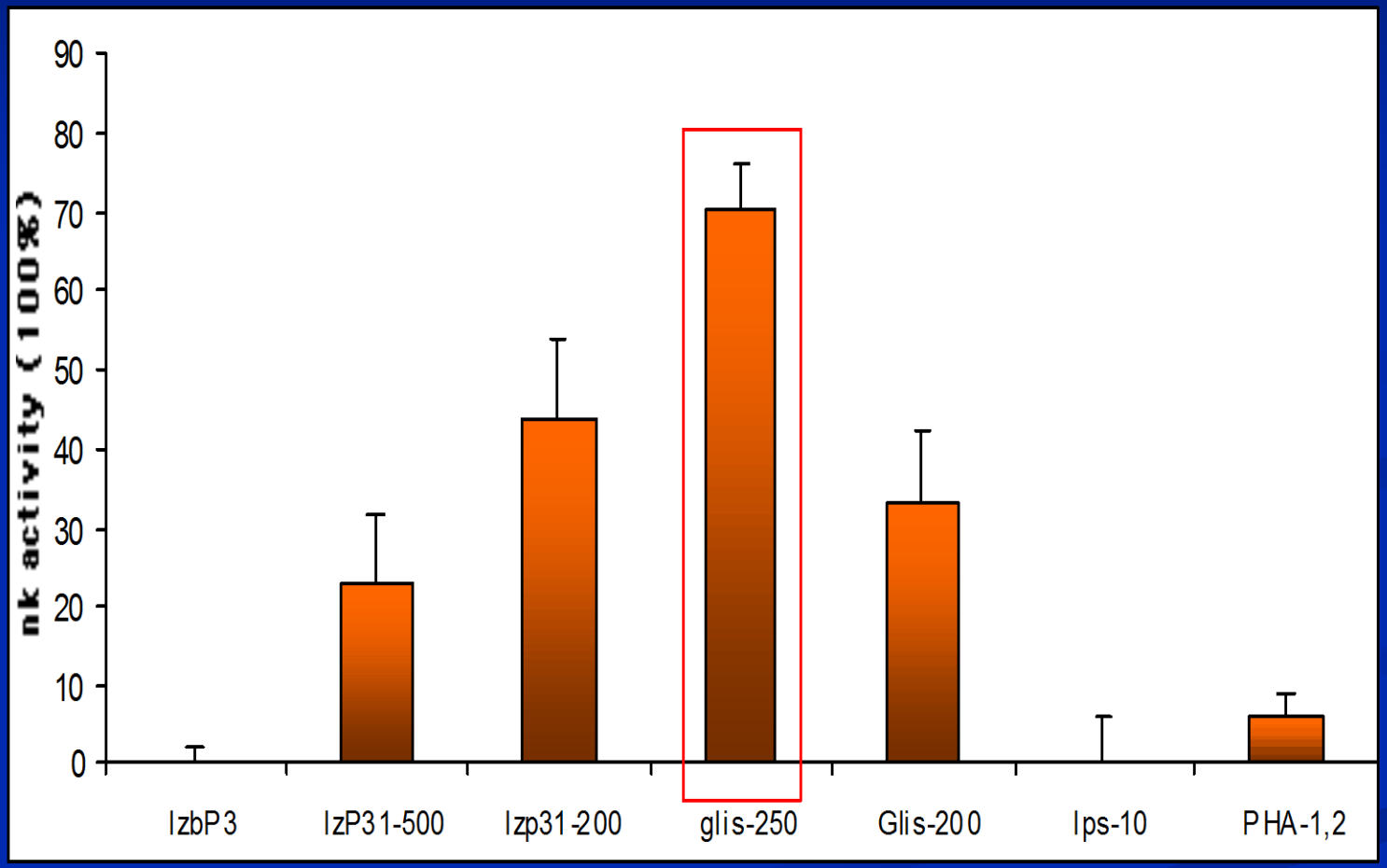
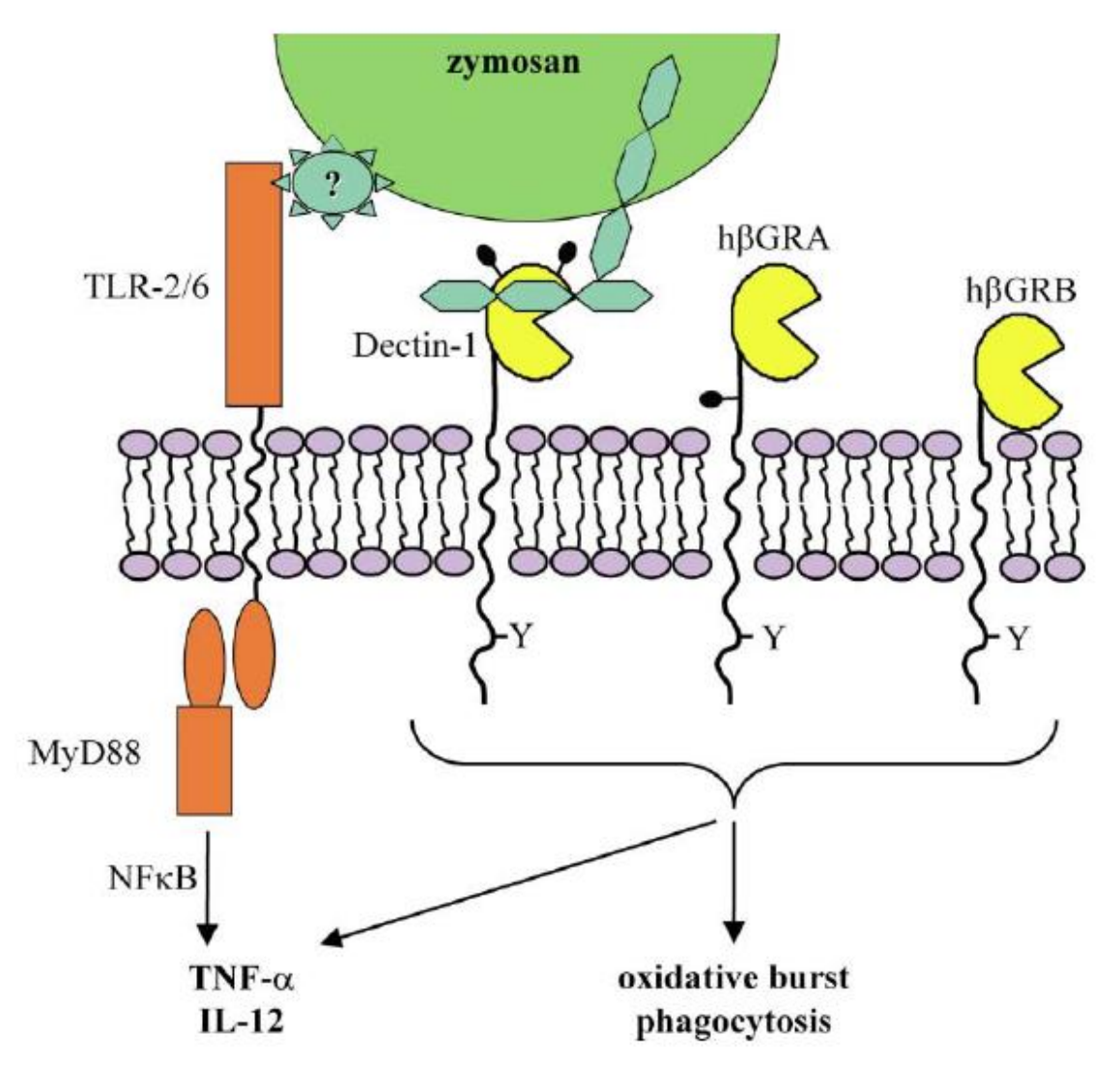


Tumor Killing Activity of HPB-NK Cells stimulated by GLIS



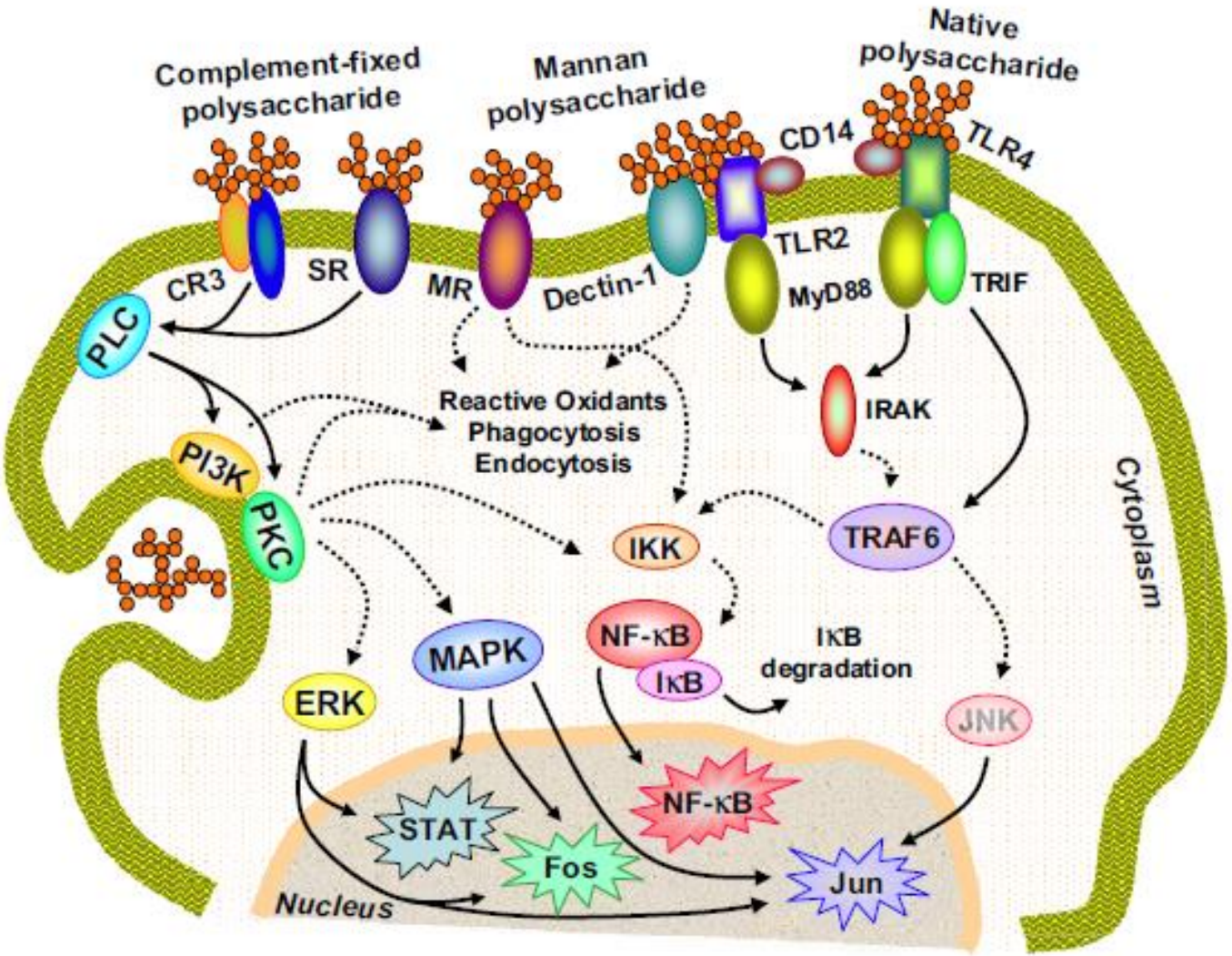
Are there receptors for GLIS

Receptors for Zymocan



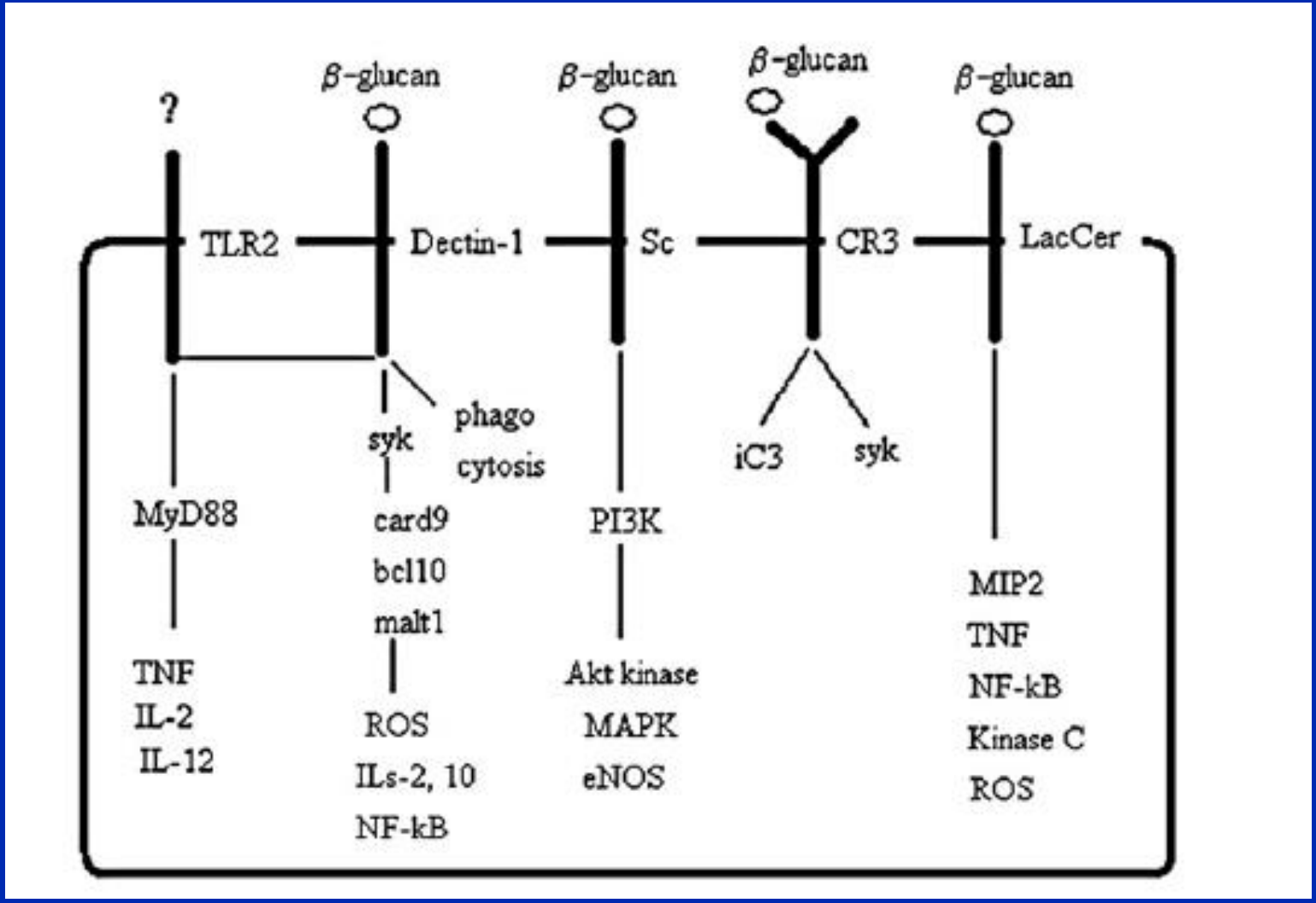
Brown 2003

Receptors of Glucan on macrophsge



Schepetkin 2006

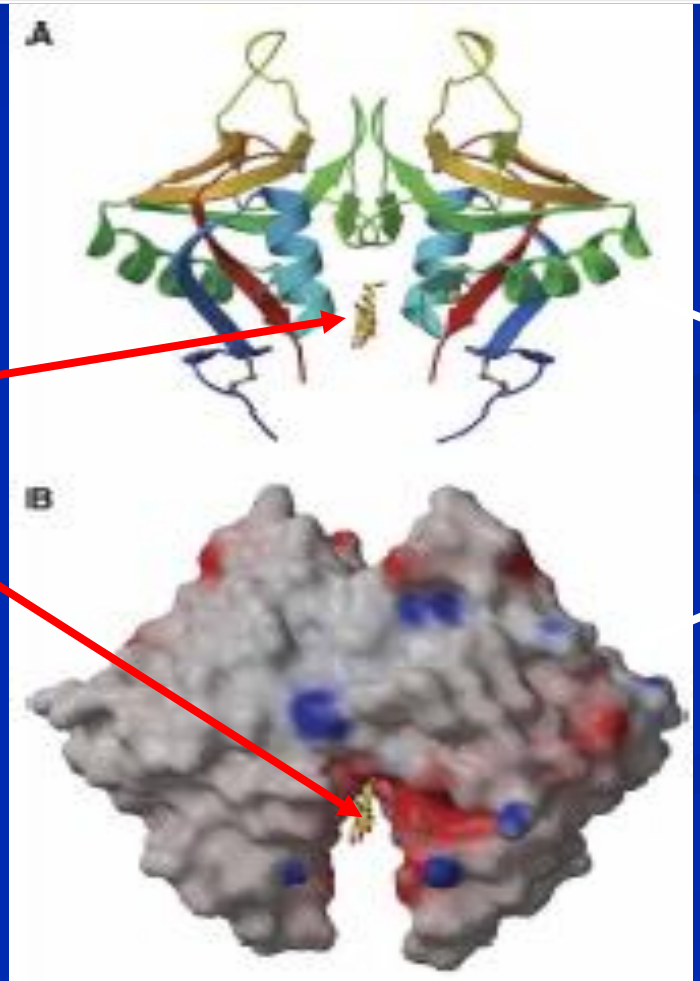
Singnal pathway of Glucan on immne cells



Brown 2005

Binding Mechanism of β-glucan & Dectin-1?

We still don't know how the binding of the β-glucan to its receptor, dectin-1 occurs.
How does the side chain affect the binding to the receptor?



b-glucan

Dectin-1 dimer

Brown *et al.*, *Protein Sci.* (2007)

Receptors of Glucan

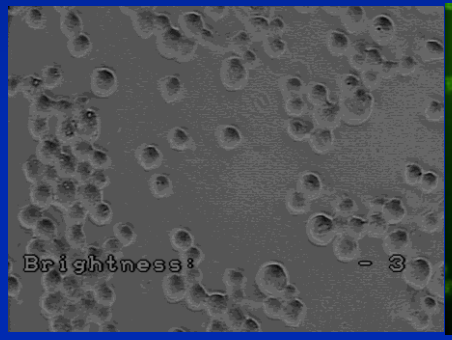
Receptor type	Chemical nature	Affected cells	Effects on immune response	Other ligands
Dectin-1	Glycoprotein	Monocytes Macrophages Neutrophils DC cells T-cells	Phagocytosis Endocytosis ROS production Cytokines (TNF-α, IL-2, 10, 12) MIP-2,	Intact fungi, T-cells
CR3	A heterodimer Consist of CD11b and CD18 chains	Myeloid NK Neutrophils Lymphocytes	Tumour cytotoxicity	A variety of pathogens
Scavenger	A heterogeneous group of molecules	Myeloid Endothelial	Immunity	LDL HDL Polyanionic Microbes
LacCer	Glycosphingolipid containing a hydrophobic ceramide lipid and a hydrophilic sugar	Neutrophils Epithelial	ROS Anti-microbial Cytokines (NF-κB) Lyn kinase	A variety of microbes
TLR	A novel protein family with associated protein MyD88	Macrophages DC Lymphocytes Epithelial	Cytokines (NF-κB, TNF-a, IL12)	A wide spectrum of microorganisms

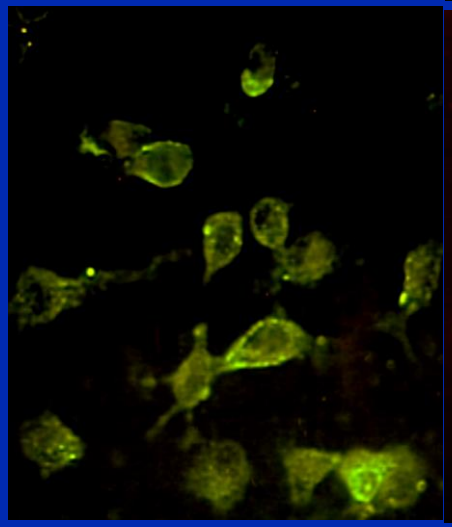
CR3, complement receptor 3; CRD, carbohydrate-recognition domain; DC, dendritic cell; eNOS, endothelial nitric oxide synthase; LacCer, lactosylceramide; IL, interleukine; MIP, macrophage inflammatory protein-2; NK, natural killer cell; NFκB, nuclear factor-kappaB; ROS, reactive oxygen species; TNF-α, tumour necrosis factor-alpha.

Labelling of GLIS-FITC on Cell Surface

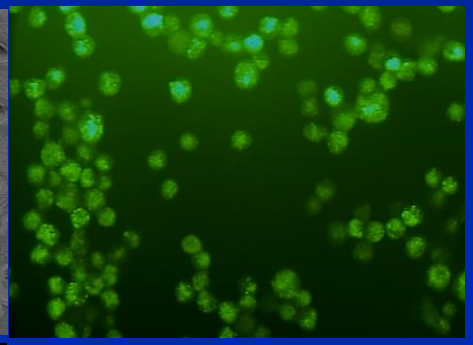
Macrophage

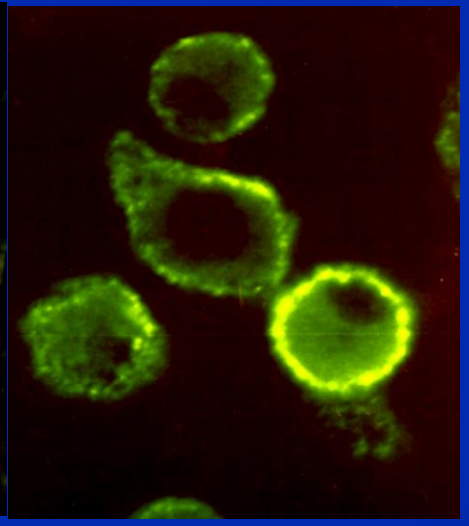
control





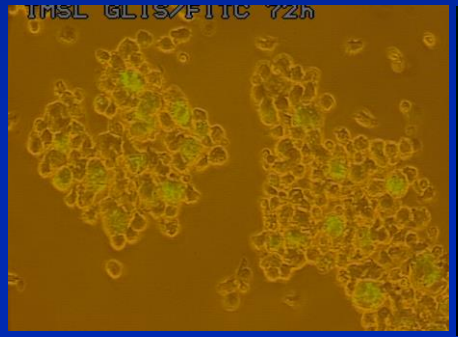
GLIS-FITC

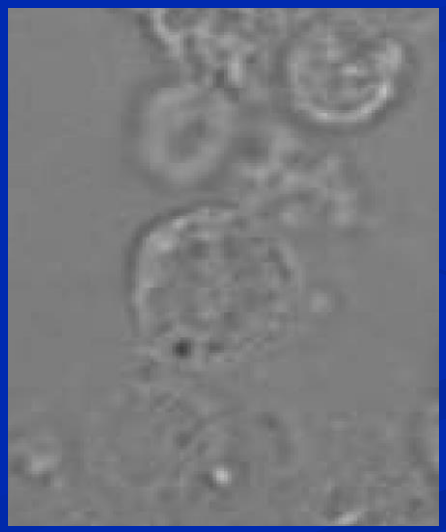




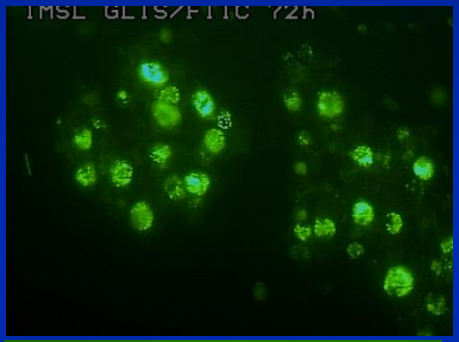
Lymphocytes

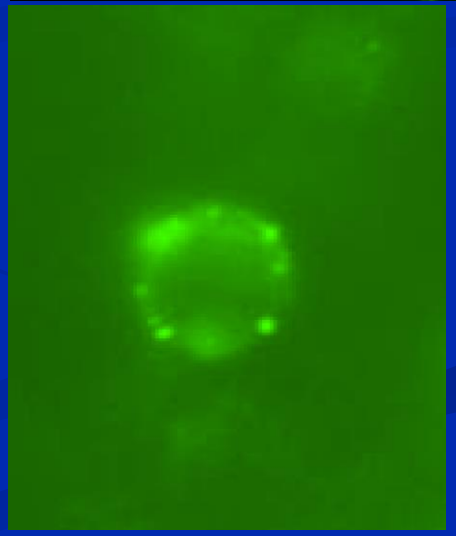
control





GLIS-FITC






Prospect

Our Team


Thank you!