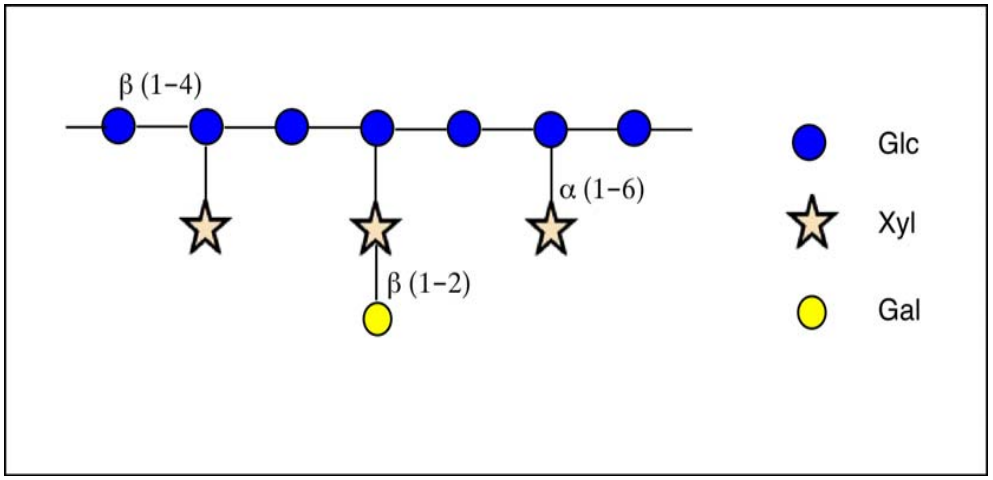
Therapeutic Polysaccharides



Stephen Harding
NCMH, University of Nottingham

Steve Harding (GB)

Berit Smestad Paulsen (N)

Thomas Heinze (D)

Zdenka Hromádková (SK)

Vera Hříbalová (CZ)

Cleanthes Israilides (G)

Anna Ebringerova (SK)

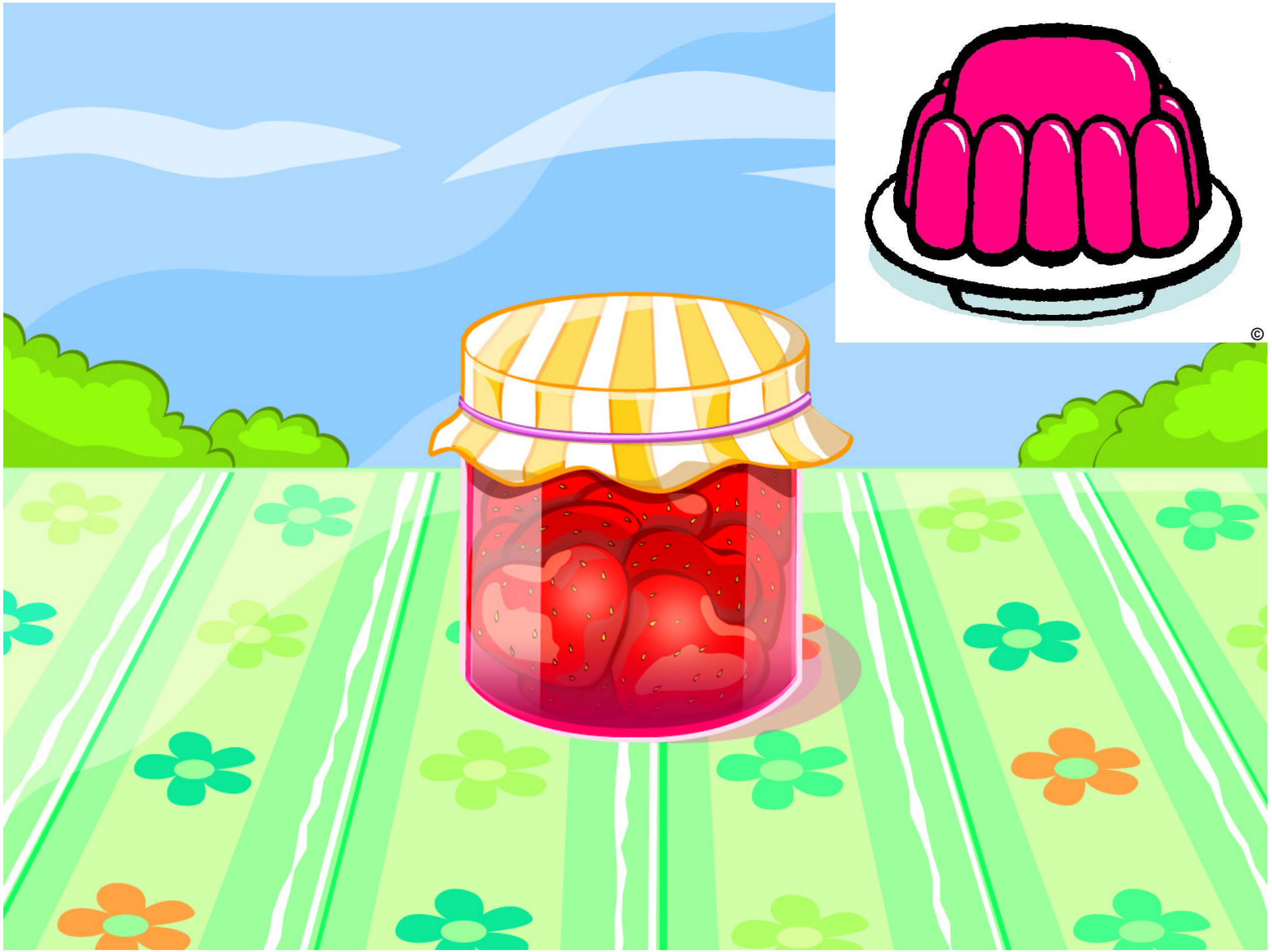
Andreas Koschella (D)

Marcin Deszczynski (P & GB)

Trushar Patel (I & GB)

Kari Inngjerdingen (N)

EPSRC
Engineering and Physical Sciences Research Council




Colgate
Crest
Kolynos
PEPSODENT
CLOSE-UP


Fixodent
Extra Hold


L'ORÉAL
PROFESSIONNEL
OSMO
SPÉCIAL SO


SHANGRI-LA
ALOE VERA GEL
96%
SHANGRI-LA
ALOE VERA GEL
96%


NDC 49281-589-05
Meningococcal (Groups A, C, Y and W-135) Polysaccharide Diphtheria Toxoid Conjugate Vaccine
5 VIALS
1 Dose each
Rx only
Menactra™
Aventis Pasteur
Aventis


QCI NUTRITIONAL
Beta 1,3 glucan
A Dietary Supplement
90 Capsules


RESISTANCE ET VITALITE
SHII-TA-KER
SHII-TA-KER


Natural Product
Linze
120 capsules


now
Guar Gum
700 mg
120 Capsules

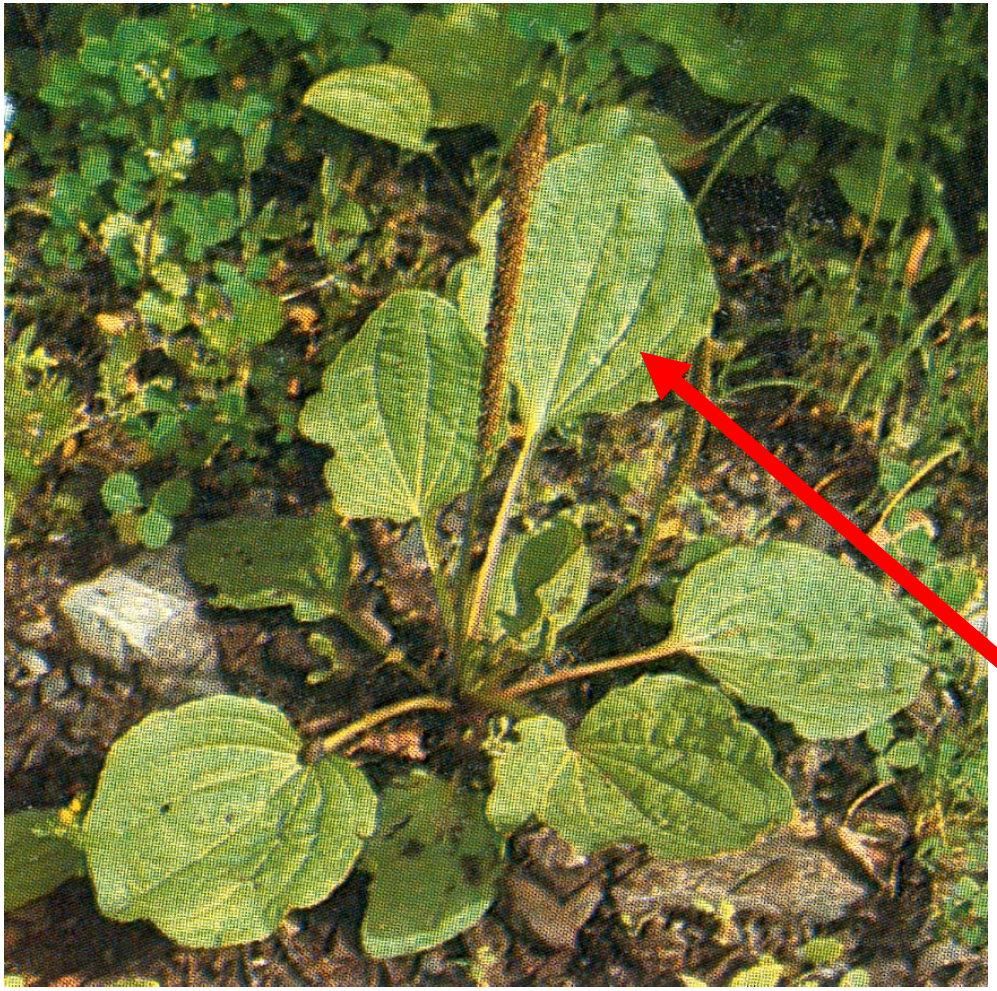

Natural
Natural
ALOE VERA FREEDOM

Plantago major

“Traditional use in Scandinavia:

Wound healing”

Plantago major

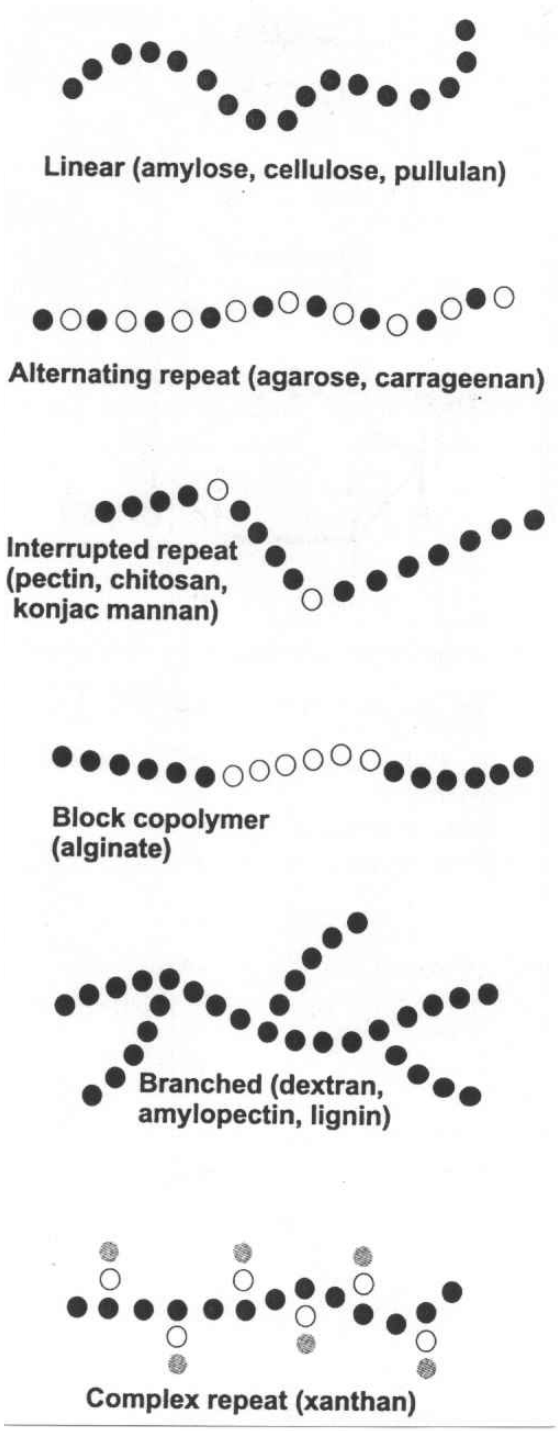


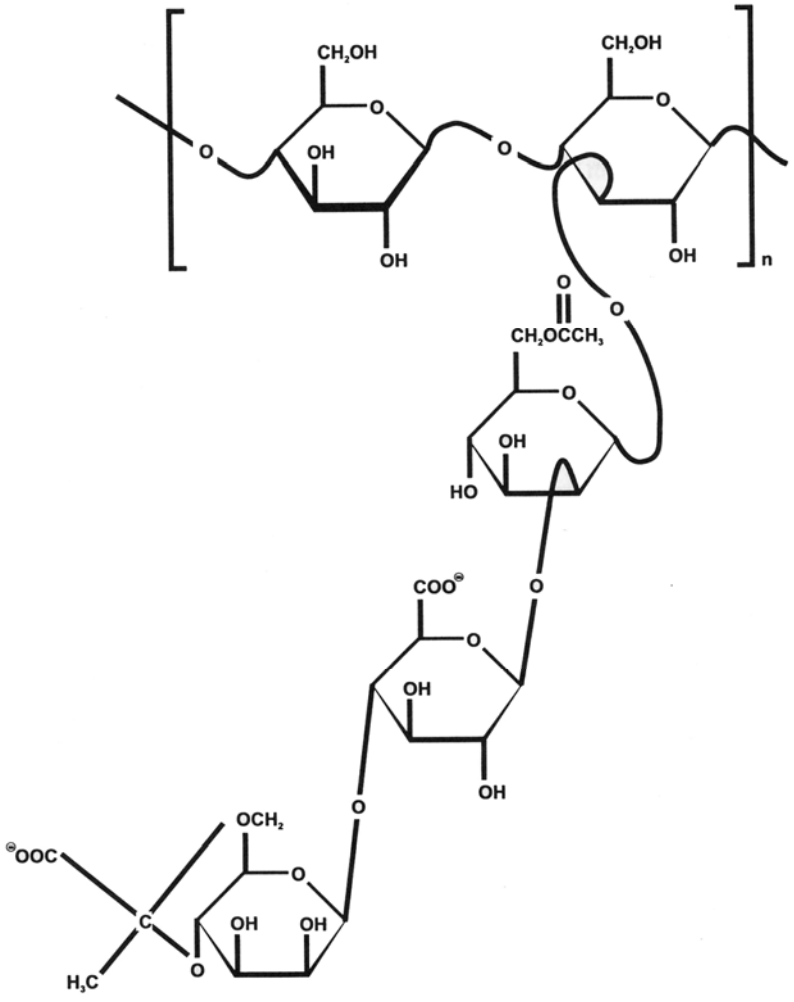
Traditional use in Scandinavia:

Wound healing

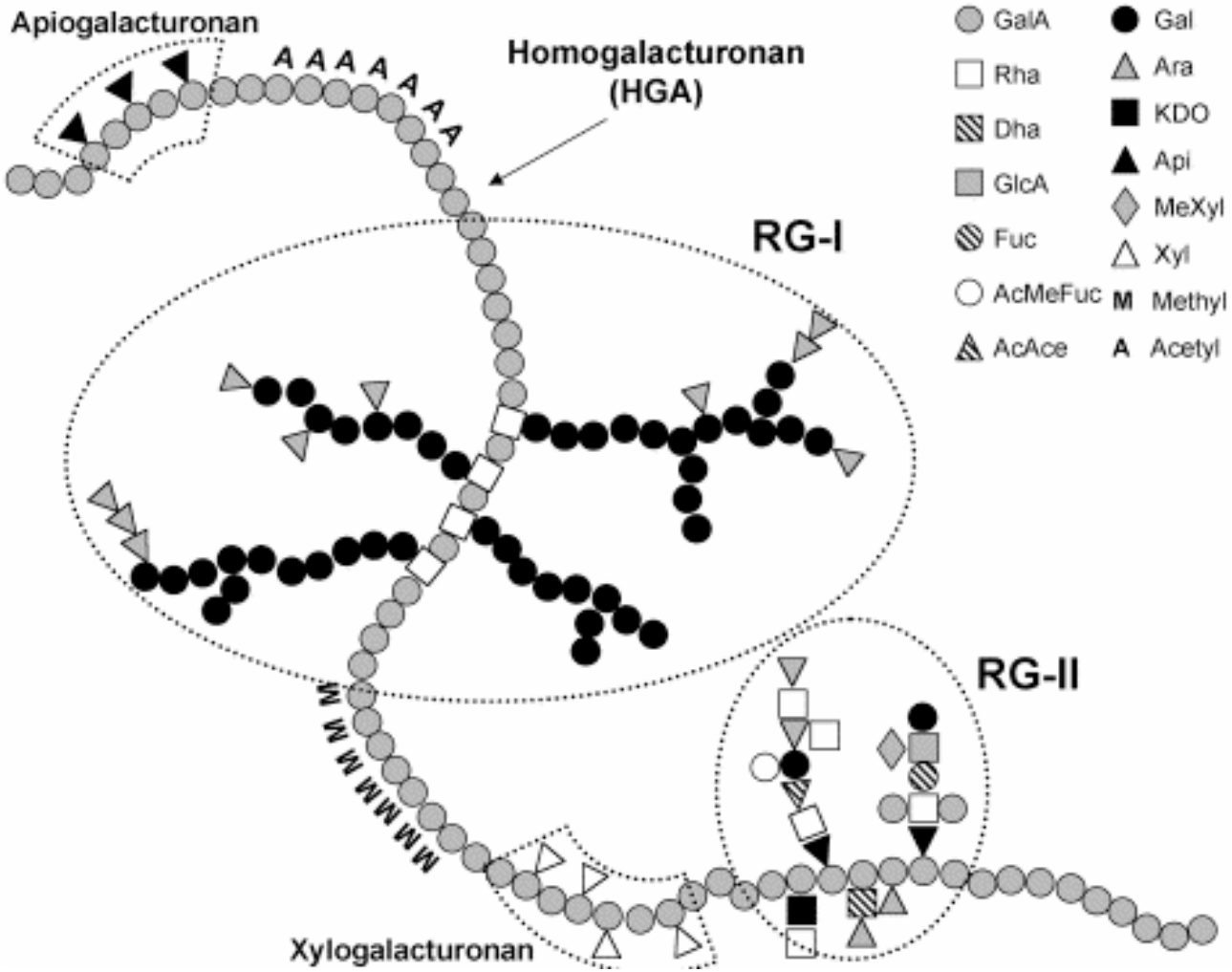
UK: "Doc leaf"

Variety of primary structures




CH2OH
O
OH
OH
O
CH2OH
O
OH
n
O
CH2OCCH3
O
O
OH
HO
COO⊖
O
O
OH
OH
O
OCH2
⊖OOC
O
C
OH
OH
H3C
O

...we often use symbol notation



Perez et al, 2003

Variety of conformations

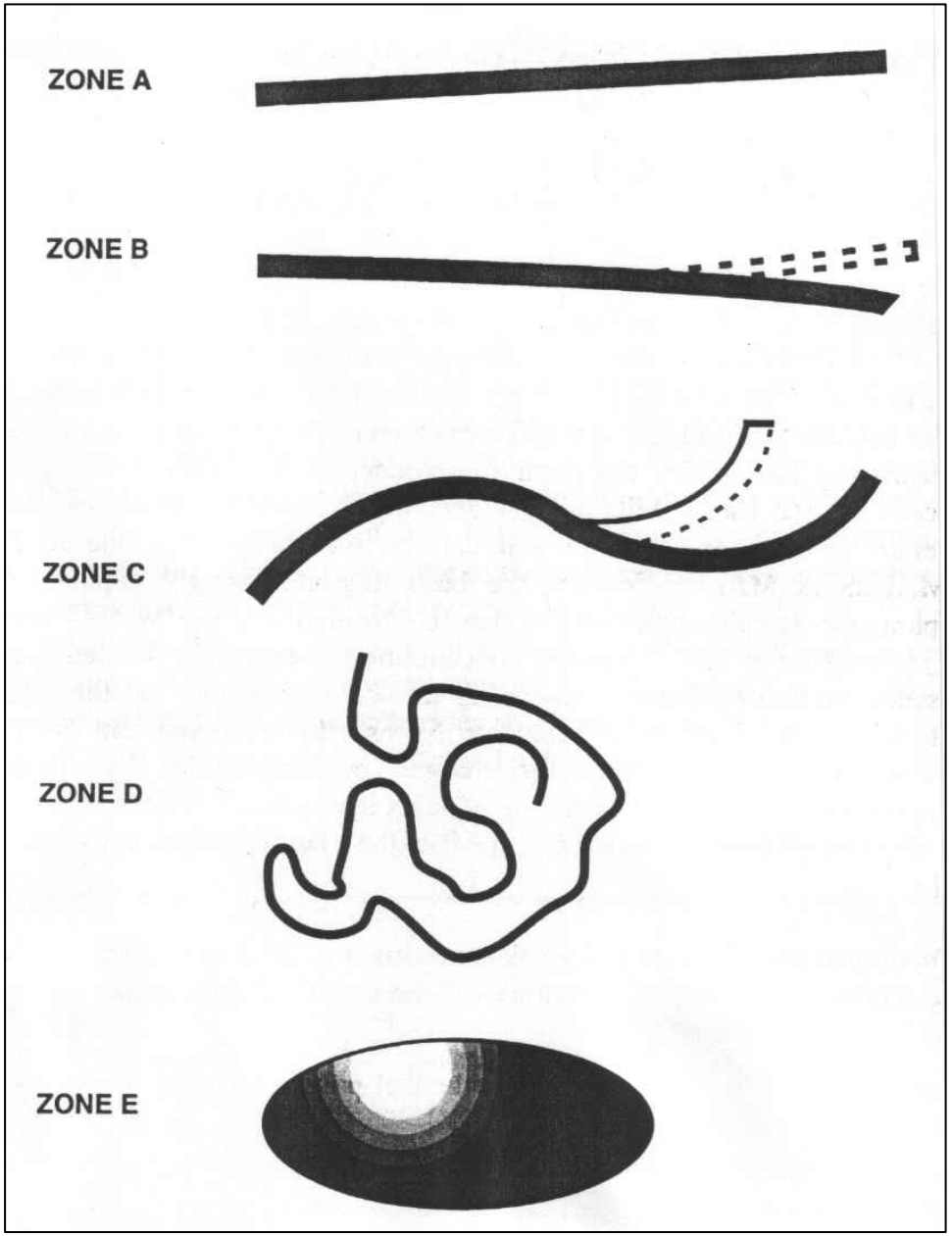
Zone A: Extra-rigid rod: schizophyllan

Zone B: Rigid Rod: xanthan

Zone C: Semi-flexible coil: pectin

Zone D: Random coil: dextran, pullulan

Zone E: Highly branched: amylopectin, glycogen



Some classes of polysaccharide with therapeutic properties

I Pectins

II Galactomannans/ mannoglycans

III Xyloglucans

IV Fungal beta-glucans/ lentinans

Aims

"To establish an understanding of the chemical and physical properties of biologically active polysaccharides of plant and fungal origin in relation to function, and explore possible trends or relations underpinnning their potential in pharmaceutical and cosmetic applications"

I Pectins

II Galactomannans/ mannoglycans

III Xyloglucans

IV Fungal beta-glucans/ lentinans

The polysaccharides:

I Pectins **Immunomodulatory pectic polysaccharides from medicinal plants (e.g. *Plantago m., Acanthus ebracteatus, Vernonia kotschyana, Biophytum*)**

II Galactomannans/mannans **Immuno-modulatory and anti-tussive polysaccharides from e.g. guar and medicinal plants like *Aloe vera***

III Xyloglucans **Immunomodulatory from tamarind seeds - phagocyte enhancement, leucocyte migration inhibition, inhibition of cell proliferation etc.** **Arabinoxylans**

IV Fungal beta-glucans/ lentinans **Anti-mitogenic/cancer activity of *Lentinan edodes***

Relevant underpinning physical information

1 Heterogeneity **size-exclusion chromatography & sedimentation velocity**

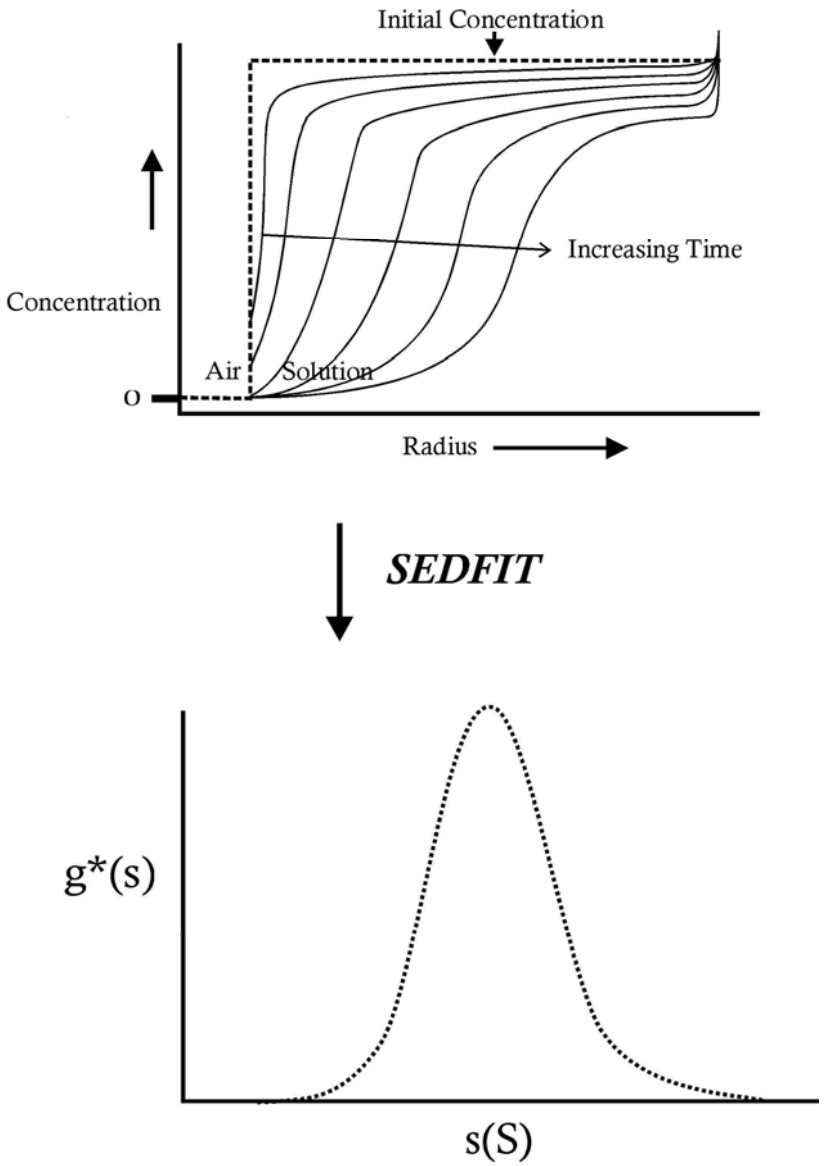
2 Molecular weight **& molecular weight distribution; SEC-MALLs and sedimentation equilibrium**

3 Conformation **Mark-Houwink coefficients and persistence length L_p.**

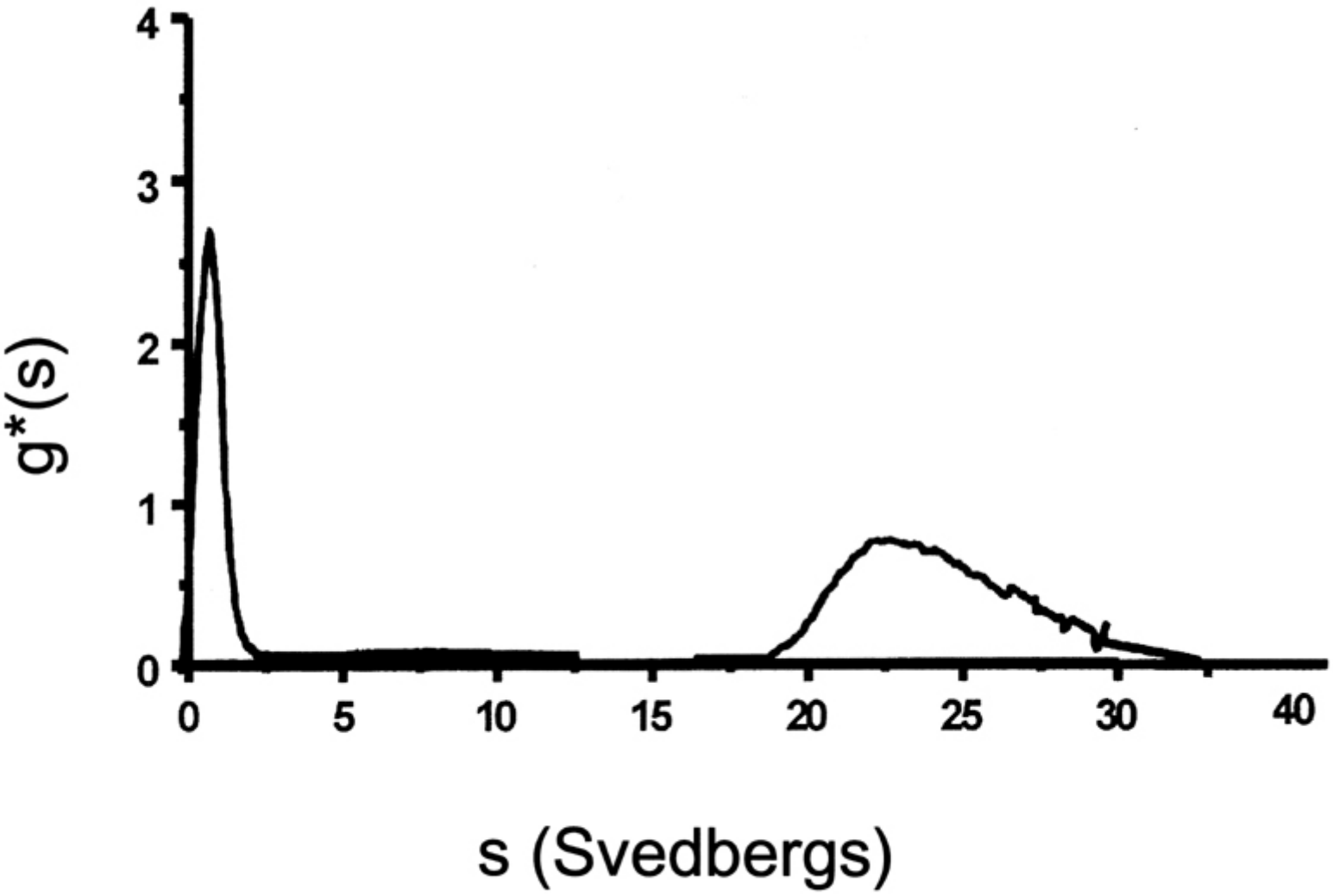
4 Interaction properties **stoichiometry, dissociation constants K_d**

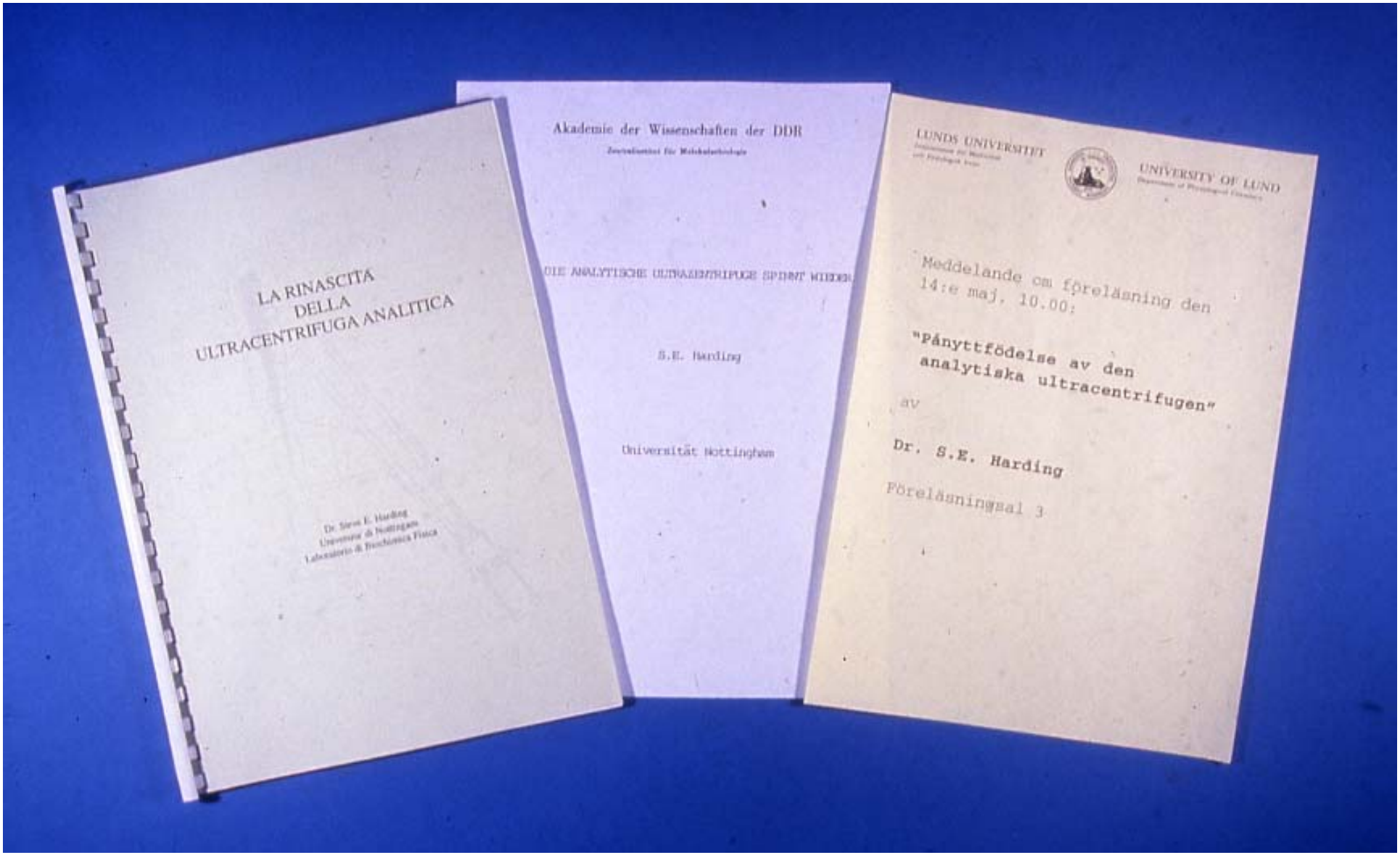
Heterogeneity: sed. coeff. distribution

Heterogeneity: sed. coeff. distribution



Starch





LA RINASCITA
DELLA
ULTRACENTRIFUGA ANALITICA

Dr. Steve E. Harding
Università di Nottingham
Laboratorio di Biochimica Fisica

Akademie der Wissenschaften der DDR

Zentralinstitut für Molekularbiologie

DIE ANALYTISCHE ULTRAZENTRIFUGE SPRICHT WIEDER

S.E. Harding

Universität Nottingham

LUNDS UNIVERSITET

UNIVERSITY OF LUND

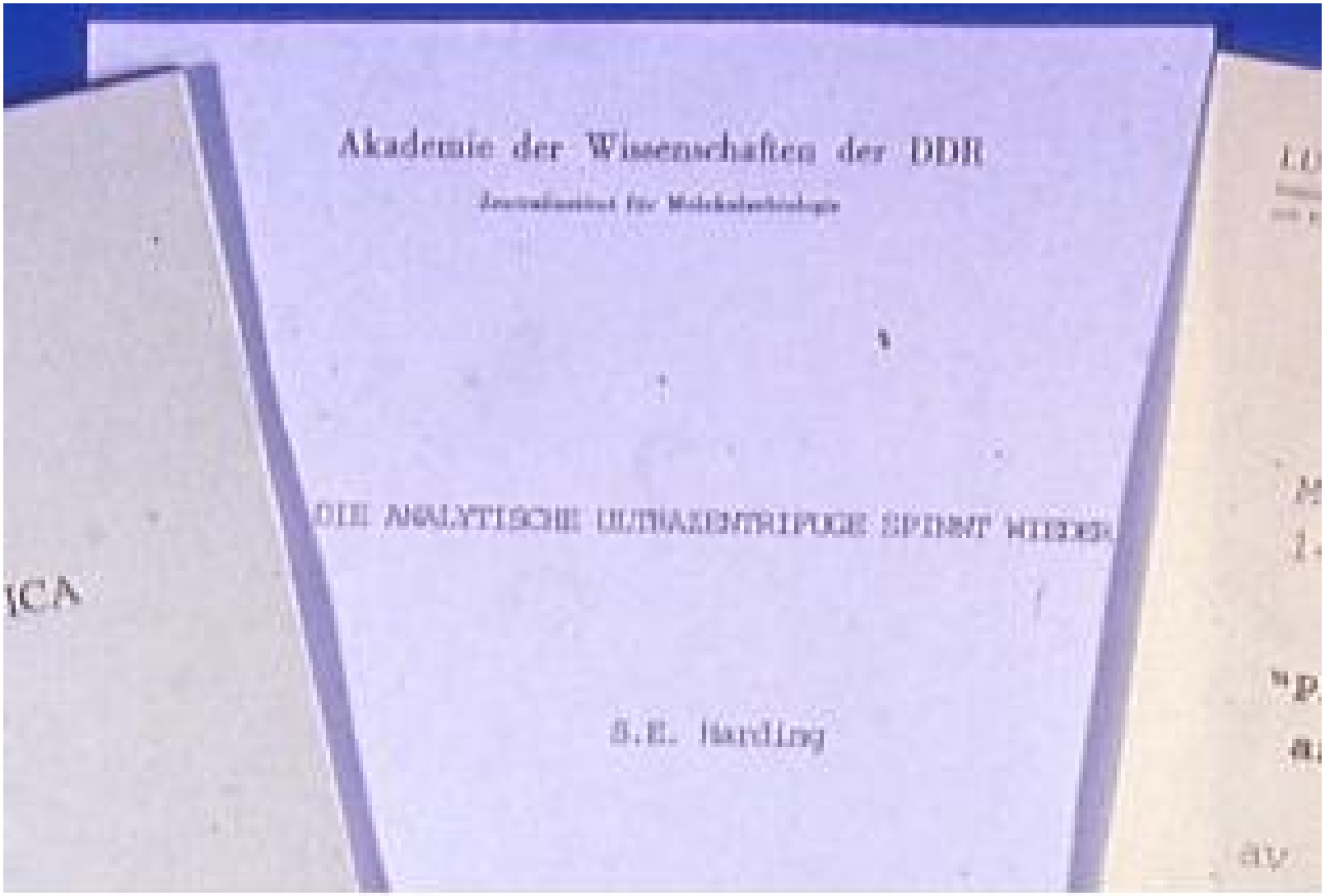
Meddelande om föreläsning den
14:e maj, 10.00:

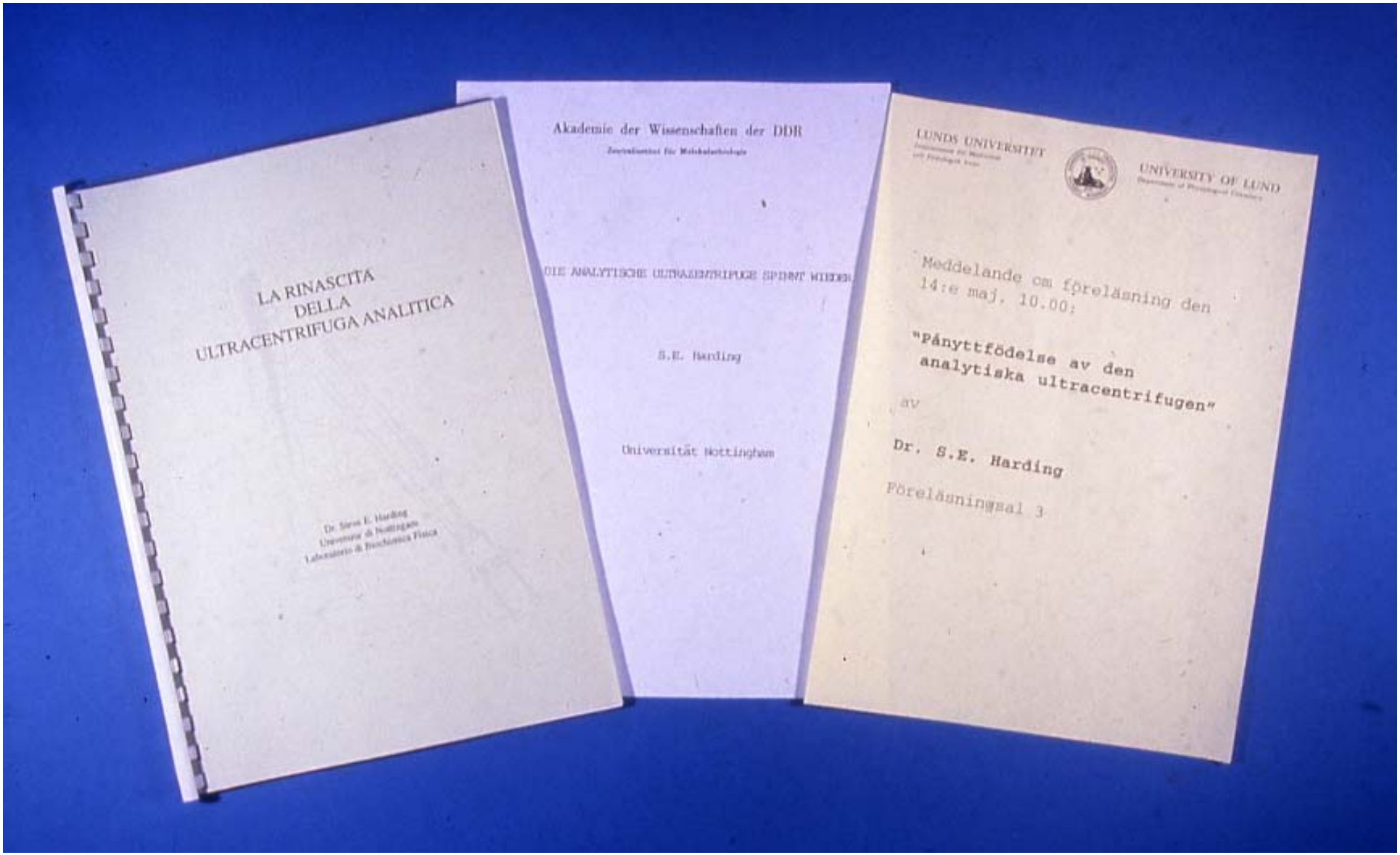
"Pånyttfödelse av den analytiska ultracentrifugen"

av

Dr. S.E. Harding

Föreläsningssal 3


Akademie der Wissenschaften der DDR
Zentralinstitut für Molekularbiologie
S.E. Harding



LA RINASCITA
DELLA
ULTRACENTRIFUGA ANALITICA

Dr. Steve E. Harding
Università di Nottingham
Laboratorio di Biochimica Fisica

Akademie der Wissenschaften der DDR

Zentralinstitut für Molekularbiologie

DIE ANALYTISCHE ULTRAZENTRIFUGE SPRICHT WIEDER

S.E. Harding

Universität Nottingham

LUNDS UNIVERSITET

UNIVERSITY OF LUND

Meddelande om föreläsning den
14:e maj, 10.00:

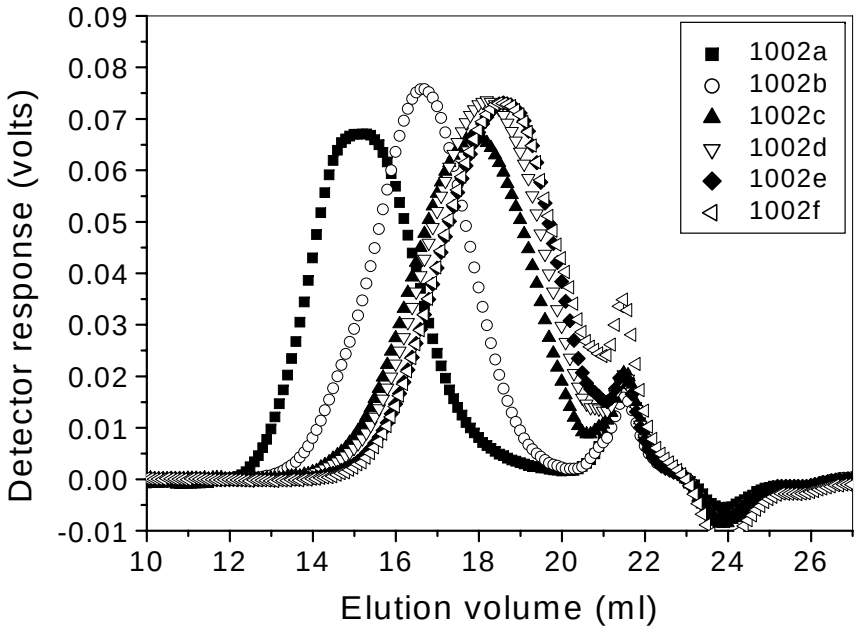
"Pånyttfödelse av den analytiska ultracentrifugen"

av

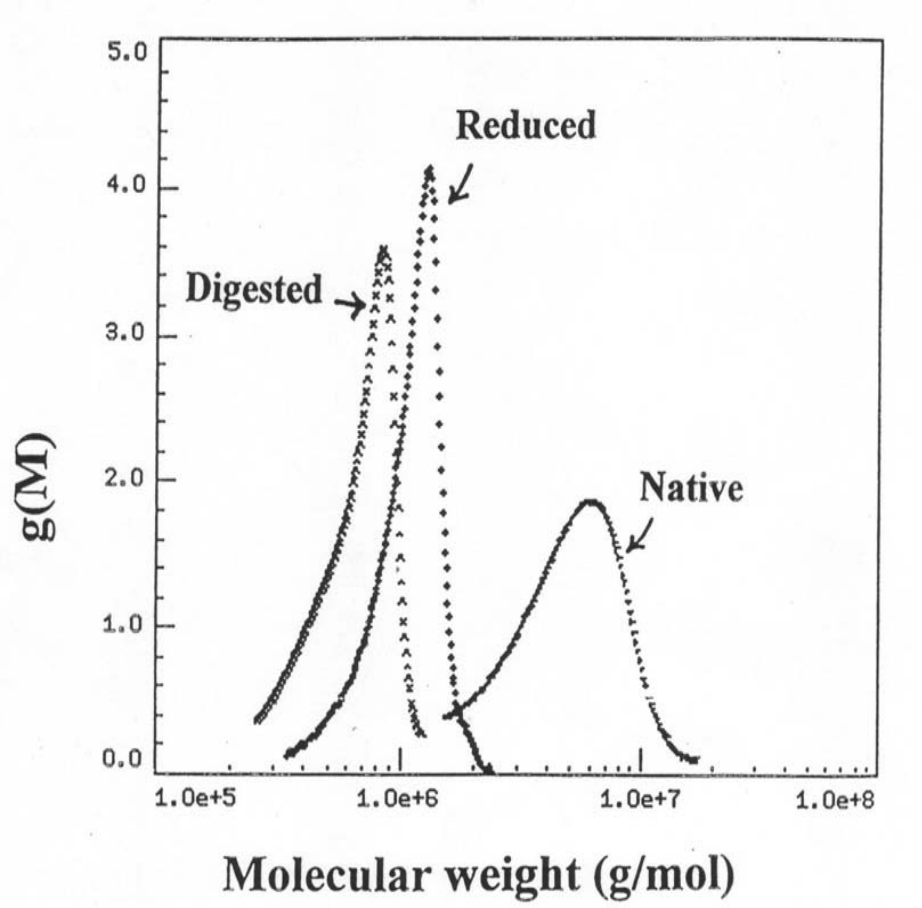
Dr. S.E. Harding

Föreläsningssal 3

Molecular Weight: SEC–MALLS



Molecular Weight: SEC–MALLS

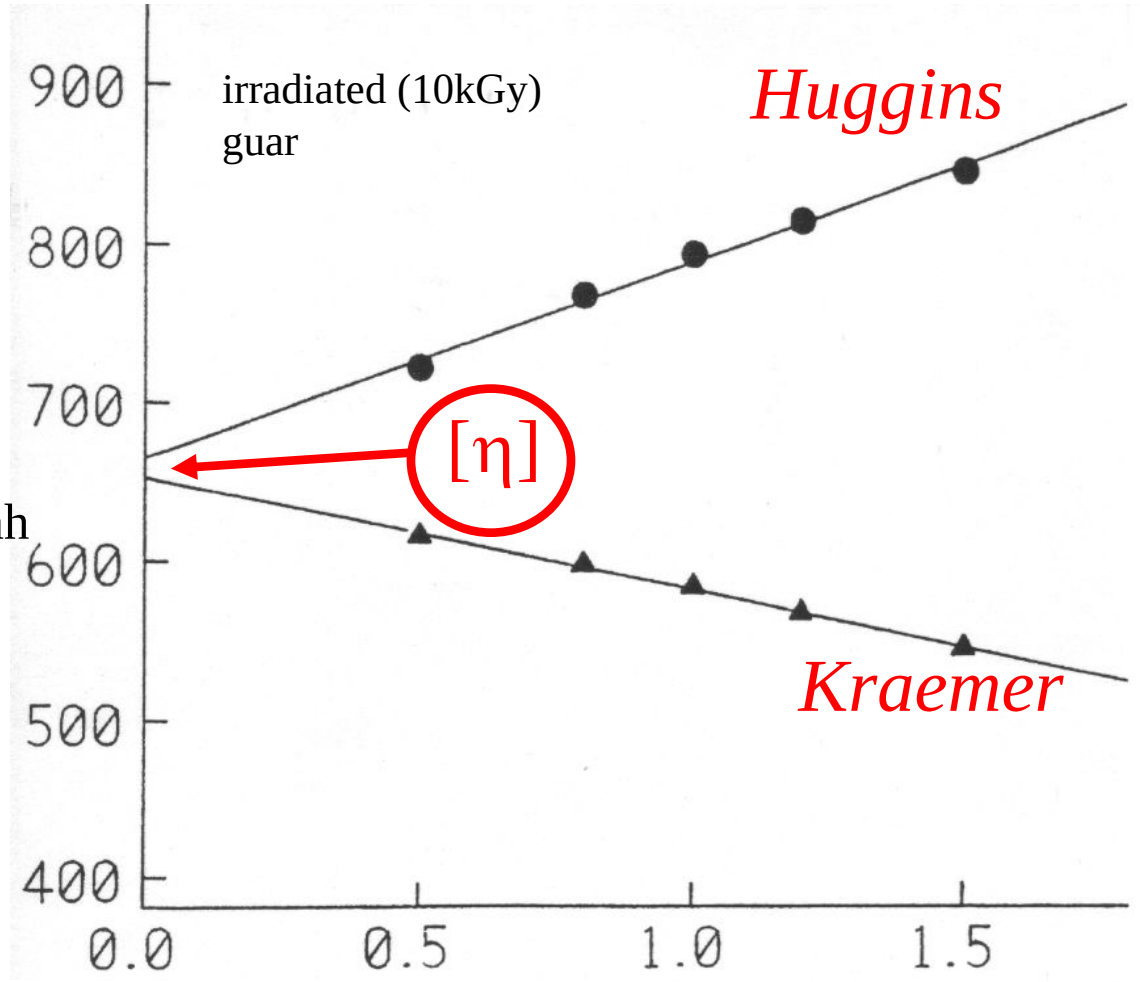


Conformation: Intrinsic viscosity

$\Rightarrow [\eta]$

Intrinsic viscosity, ml/g

Viscometry – Huggins/Kraemer plot



Conformations

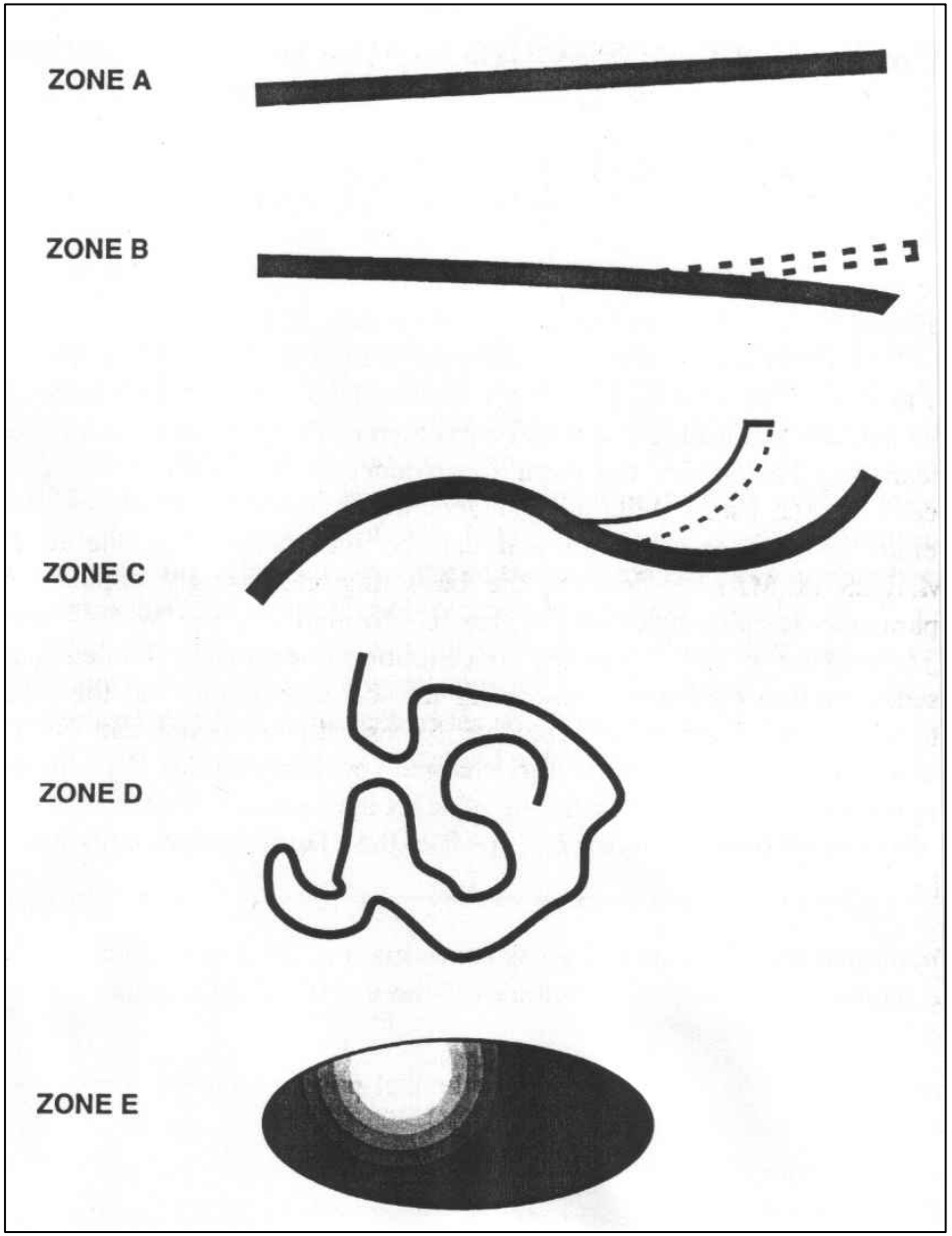
Zone A: Extra-rigid rod: schizophyllan

Zone B: Rigid Rod: xanthan

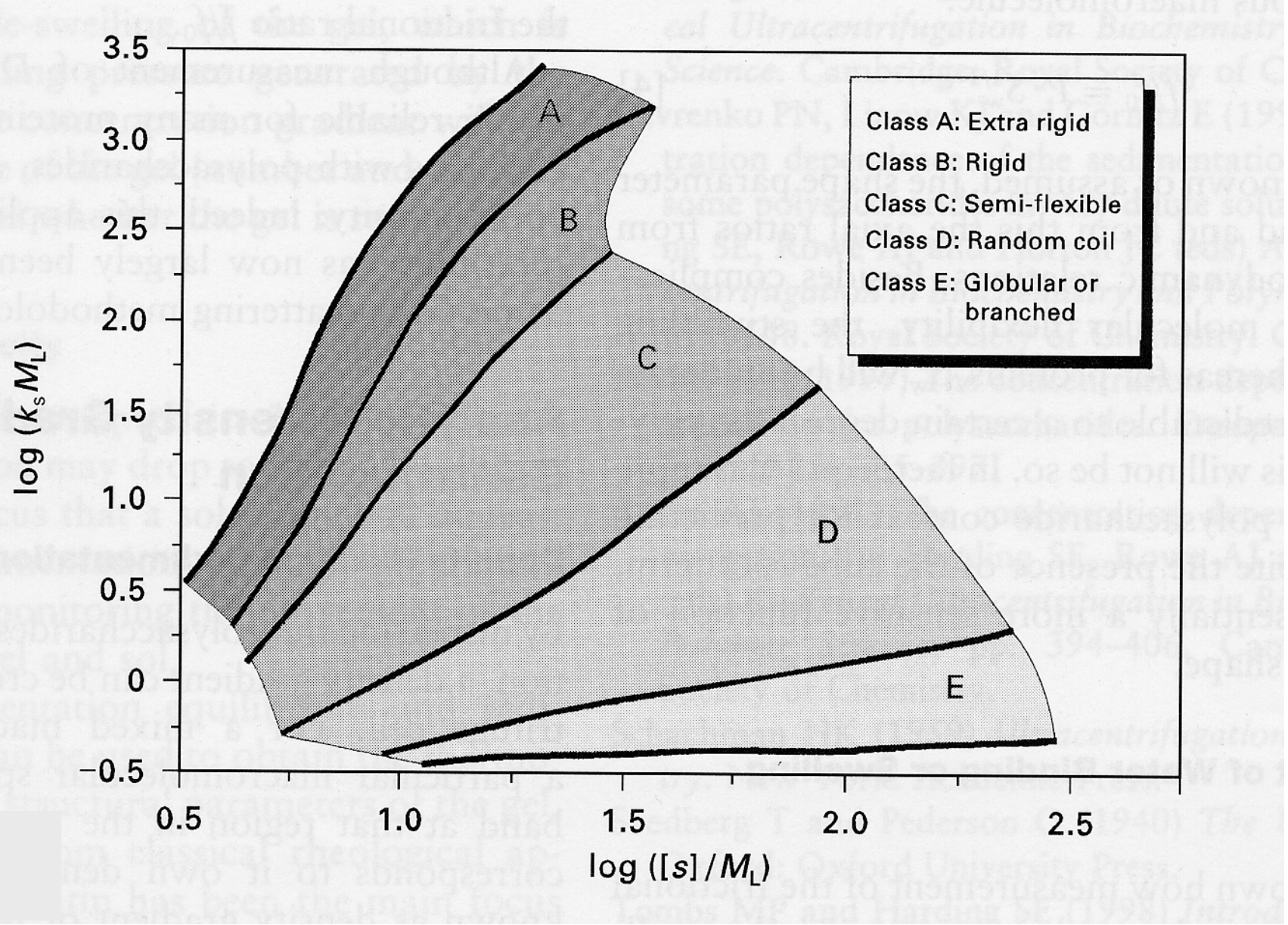
Zone C: Semi-flexible coil: pectin

Zone D: Random coil: dextran, pullulan

Zone E: Highly branched: amylopectin, glycogen

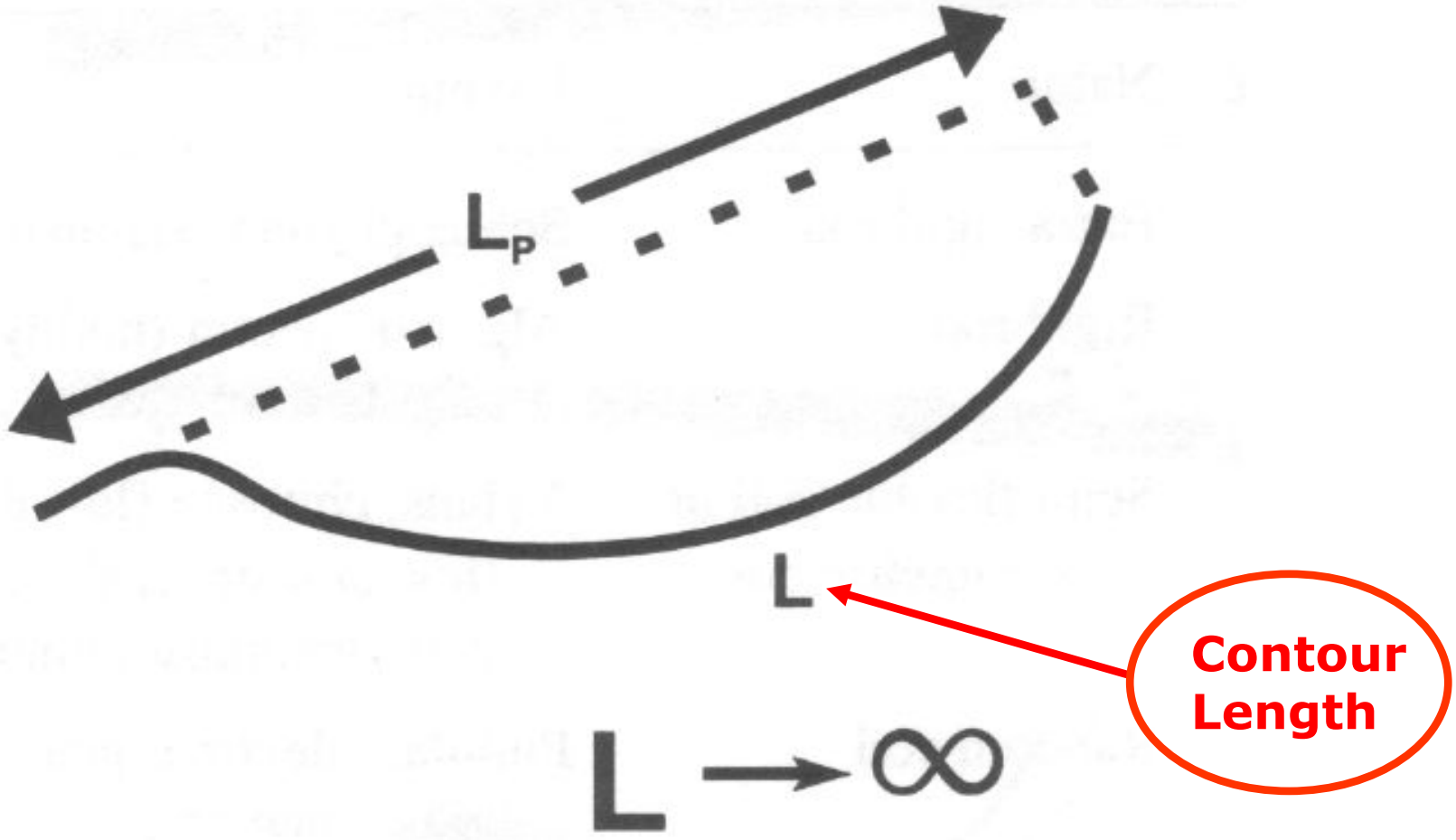


Conformation Zoning:



Pavlov, Harding & Rowe., 1997

Flexibility parameter: Persistence length L_p



Flexibility parameter: Persistence length L_p

Theoretical limits: Random coil $L_p = 0$
Rigid rod L_p = infinity

Practical limits: Random coil $L_p \sim$ 1-2nm
Rigid rod $L_p \sim$ 200nm

Flexibility: Persistence length relations

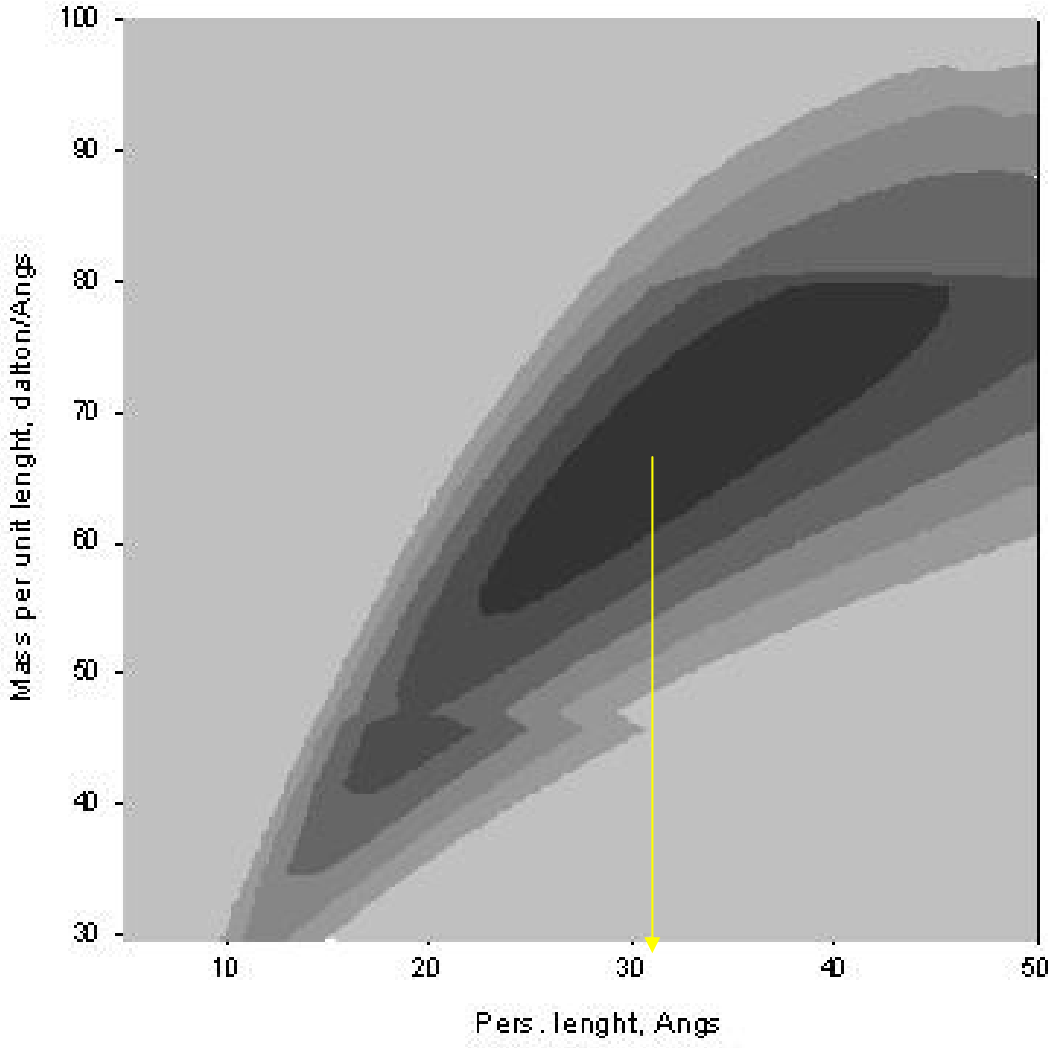
"Bohdanecky" relation

$$\left(\frac{M_w^2}{[\eta]}\right)^{1/3} = A_0 M_L \Phi^{-1/3} + B_0 \Phi^{-1/3} \left(\frac{2L_p}{M_L}\right)^{-1/2} M_w^{1/2}$$

"Yamakawa-Fujii" relation

$$s^0 = \frac{M_L \left(1 - \bar{v}\rho_0\right)}{3\pi\eta_0 N_A} \times \left[1.843 \left(\frac{M_w}{2M_L L_p}\right)^{1/2} + A_2 + A_3 \left(\frac{M_w}{2M_L L_p}\right)^{-1/2} + \ldots.\right]$$

Global analysis of pullulan: $L_p \sim 3nm$



Conformation: Power law coeffs *a*,*b*,*c*

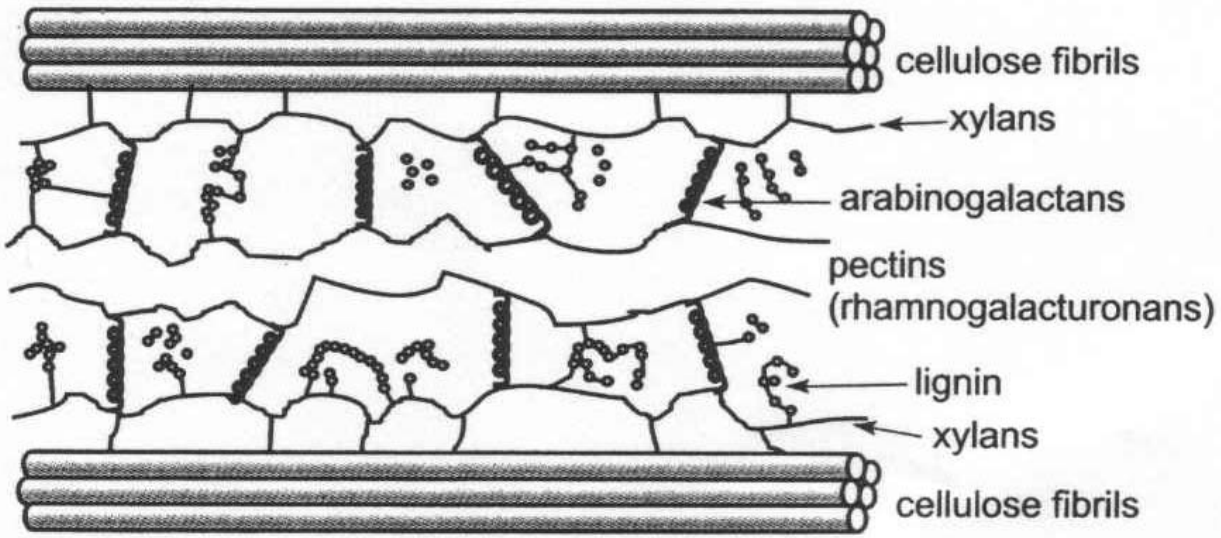
$[\eta] \sim M_w^{a}$

$s \sim M_w^{b}$

$R_g \sim M_w^{c}$

I. Pectin (E440)

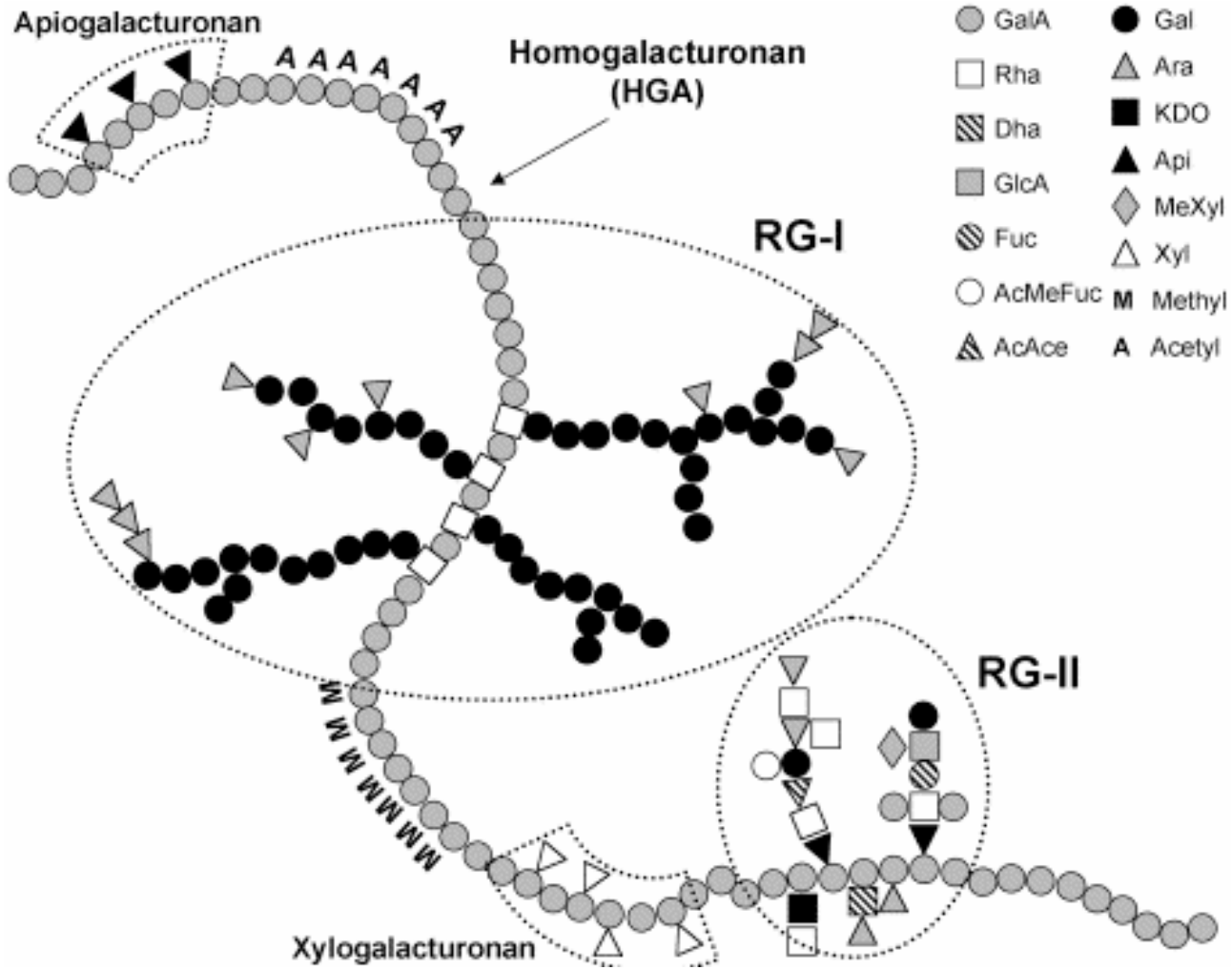
Cell wall polysaccharides:



Flexibilities of pectins

Carbohydrate Polymer	L_p (nm)
Pullulan	2.0
Heparin	2.0-2.1
Amylose	2.8
Cellulose	7.0
Pectin (69% esterified)	**30**
Pectin (0% esterified)	**34**
DNA	45
Schizophyllan	115-200
Scleroglucan	180±30
Xanthan	210

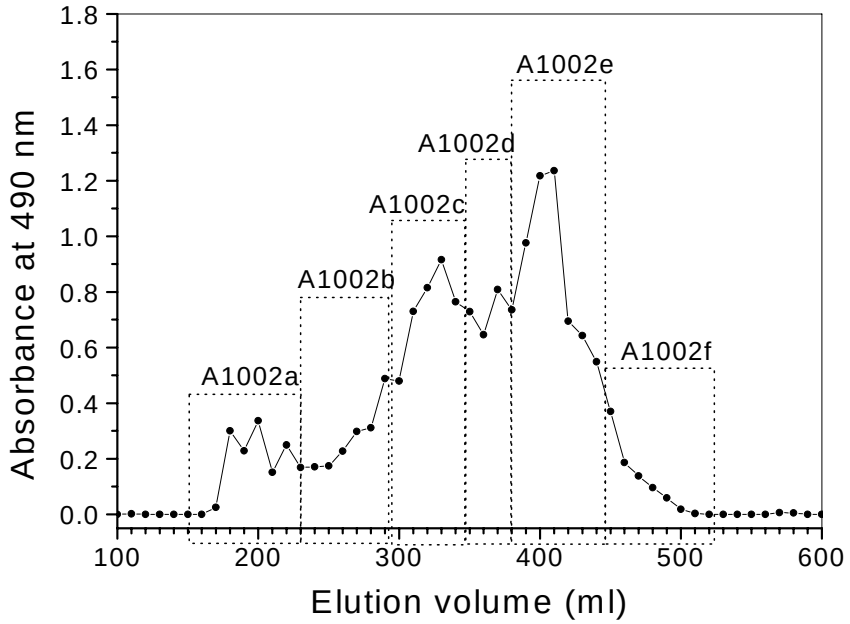
Pectins from medicinal plants



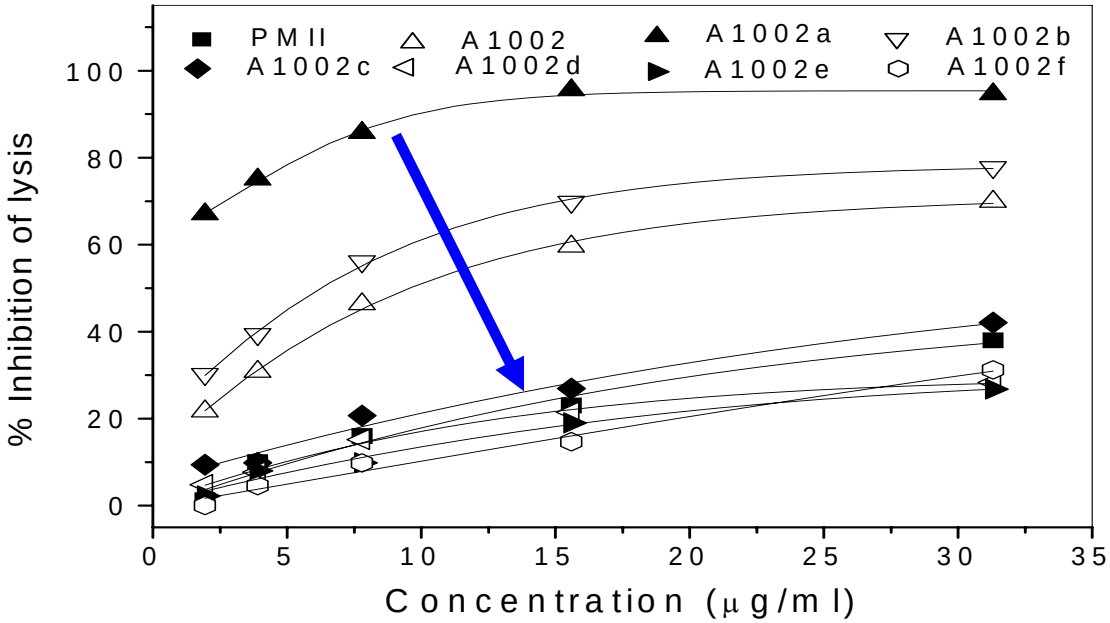
Perez et al, 2003

Acanthus ebracteatus

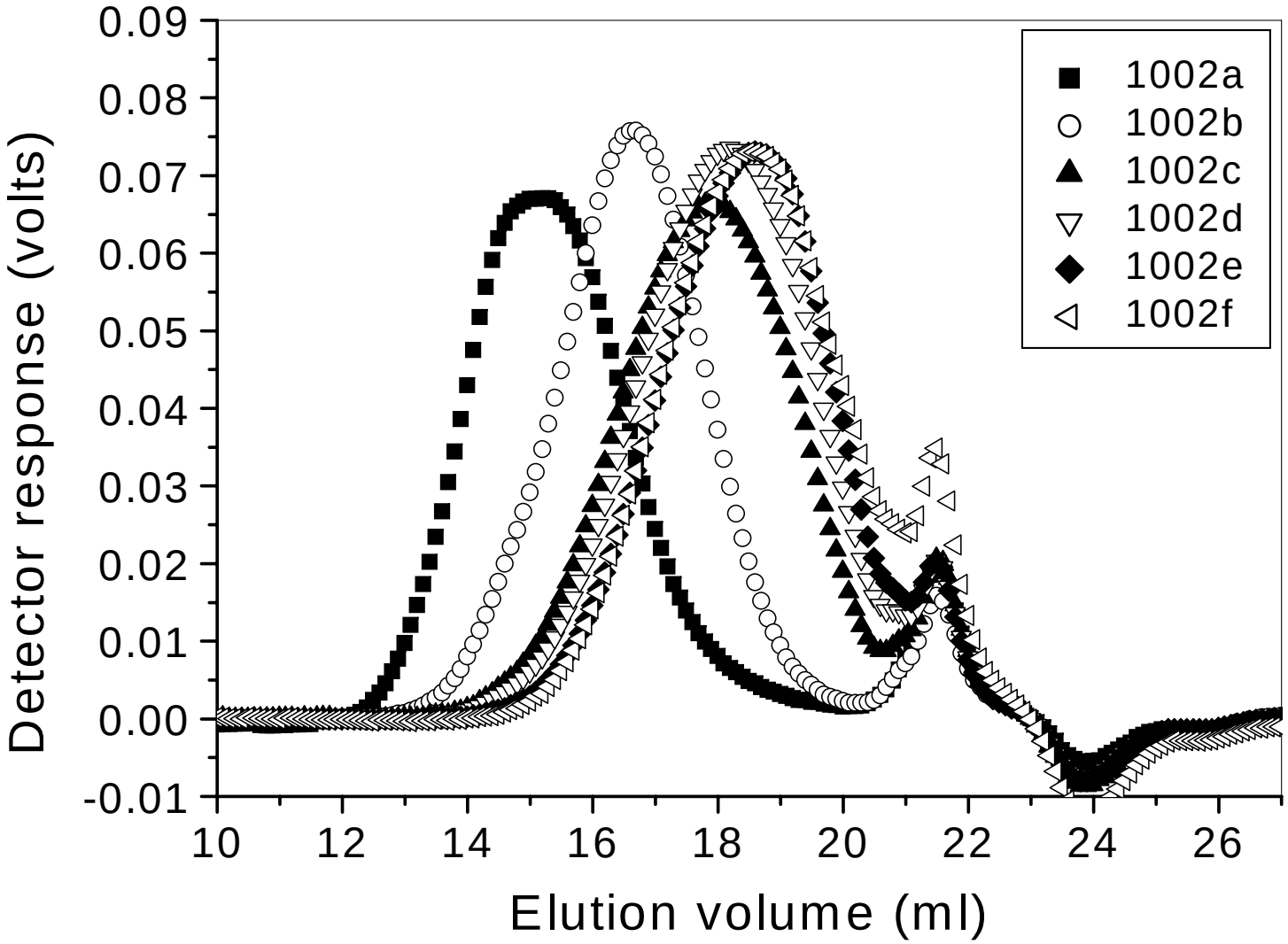
Fractions of pectic polysaccharides from *Acanthus* stem extracts



Effects on the complement system



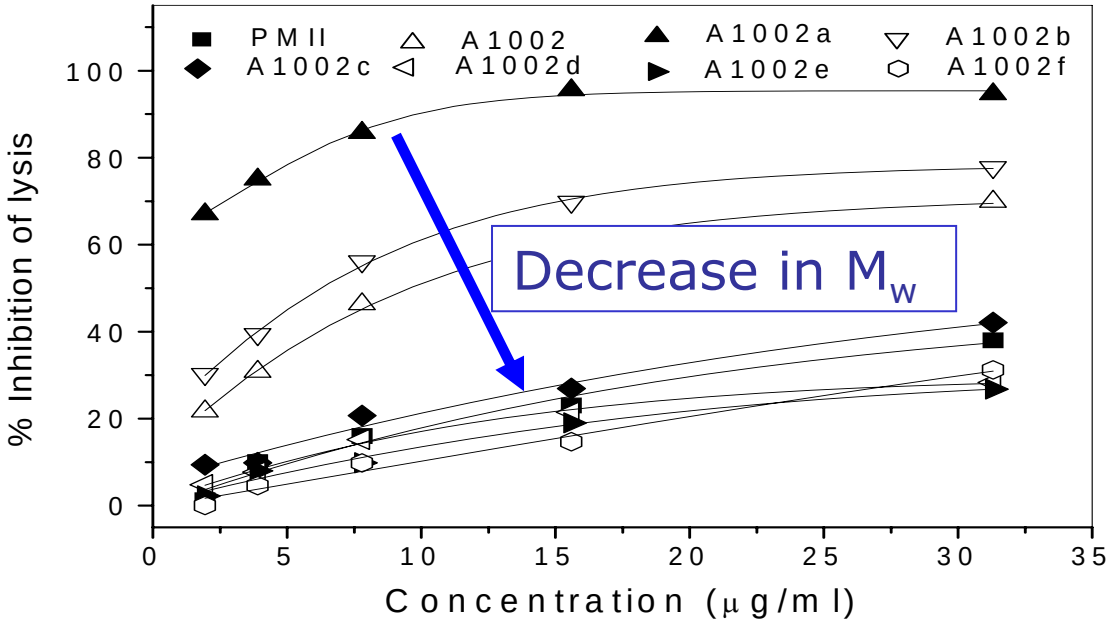
SEC-MALLs data:
subfractions of pectic polysaccharide from *Acanthus*



Yield, M_w and sugar composition
subfractions of pectic polysaccharide from *Acanthus*

	Fraction						
	A1002a	A1002b	A1002c	A1002d	A1002e	A1002f	PM II[a]
Yield[b]	8.6	8.6	24.0	16.0	31.4	3.4	-
Molecular weight (kDa)	**1500±30**	**276±20**	**58±6**	**42±3**	**32±4**	**29±4**	**46-48**
Sugar composition[c] (mol %)							
Ara	7.3	7.1	5.6	4.3	3.9	4.3	8.8
Rha	5.9	5.7	5.6	5.5	5.5	5.7	4.2
Fuc	0.6	0.3	0.3	0.3	trace	0.3	-
Xyl	0.7	0.6	0.6	0.6	0.5	0.6	-
Man	1.4	-	-	-	-	-	-
Glc	5.1	2.1	1.5	1.6	1.5	2.3	7.3
Gal	16.2	13.1	10.0	8.2	6.9	8.1	8.0
3-*O*-Me-Gal	13.9	1.8	0.5	trace	trace	trace	-
Total neutral sugars	51.1	30.7	24.1	20.5	18.3	21.3	28.3
GalA (A)	49.1	69.4	75.9	79.5	81.8	78.7	71.1

Effects on the complement system

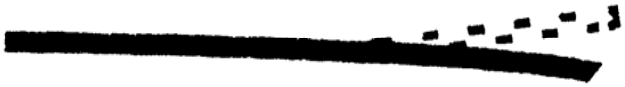


Conformation Zoning:

ZONE A

Extra-rigid Rod: Schizophyllan

ZONE B



Rigid-rod: DNA

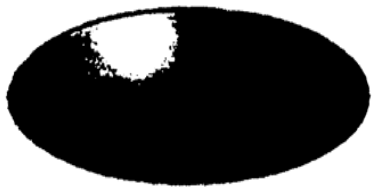
ZONE C

Semi-flexible coil: cellulose derivatives, chitosans

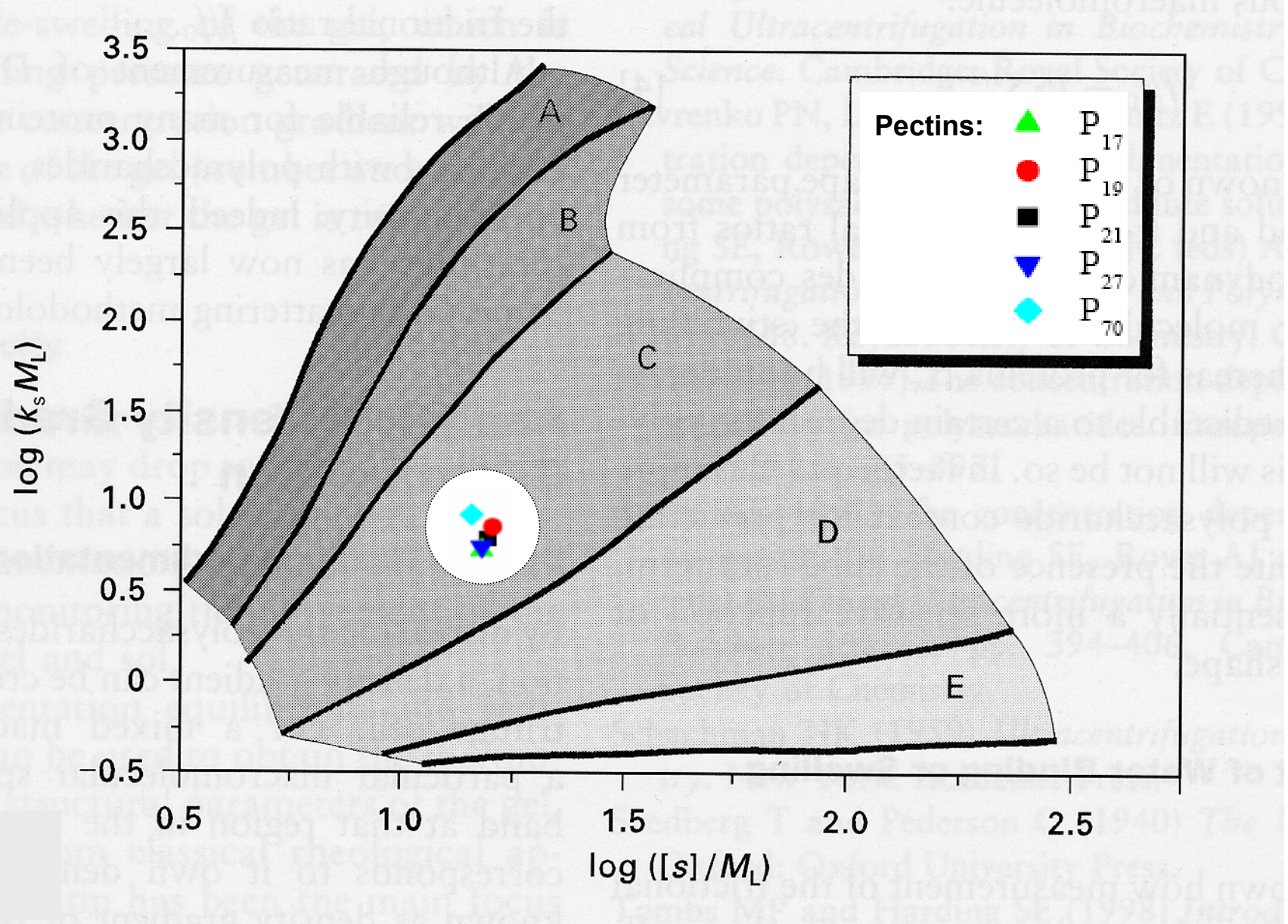
ZONE D

Random coil: Dextran, glycoproteins from mucus

ZONE E

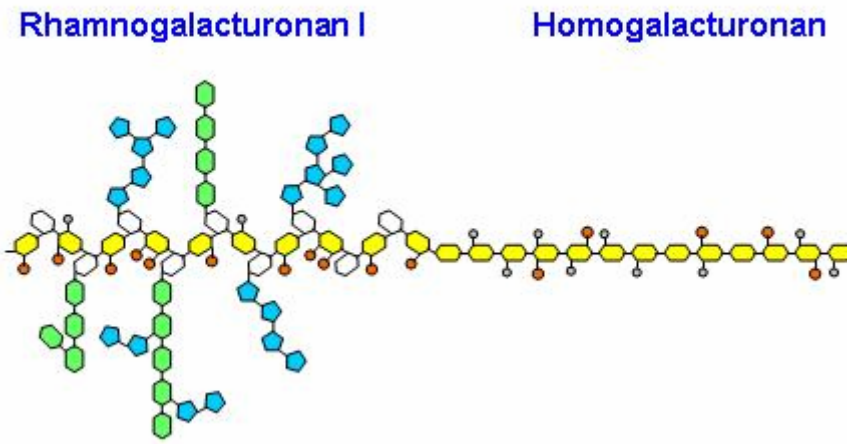


Globular/Branched: Proteins/glycogen

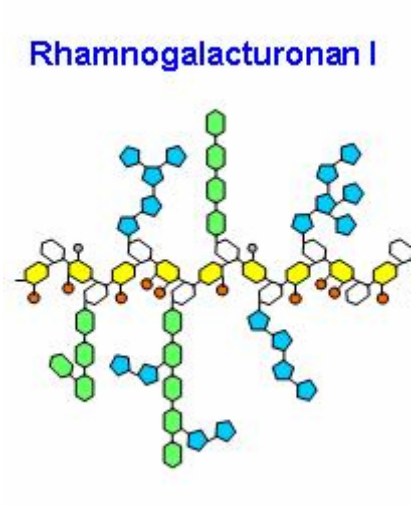


Morris et al., 2007

Enzyme degradation of pectins

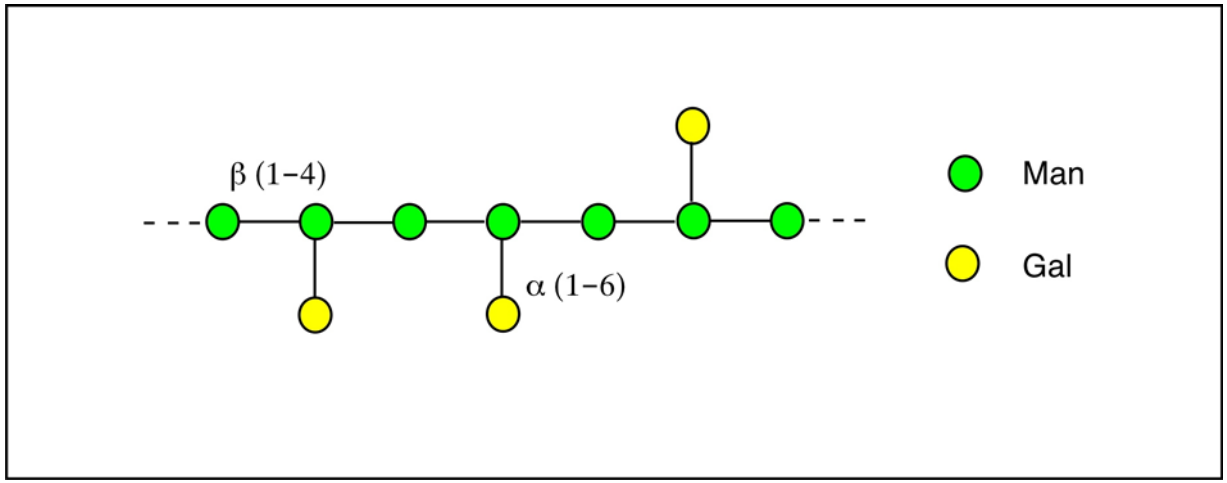


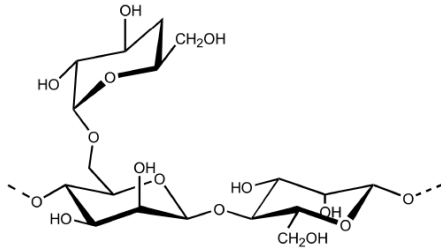
Endo-α-D(1-4)-polygalacturonase

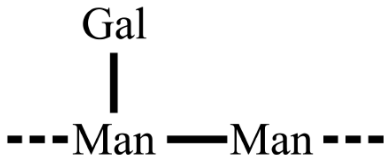


"HAIRY REGION"

II Galactomannans





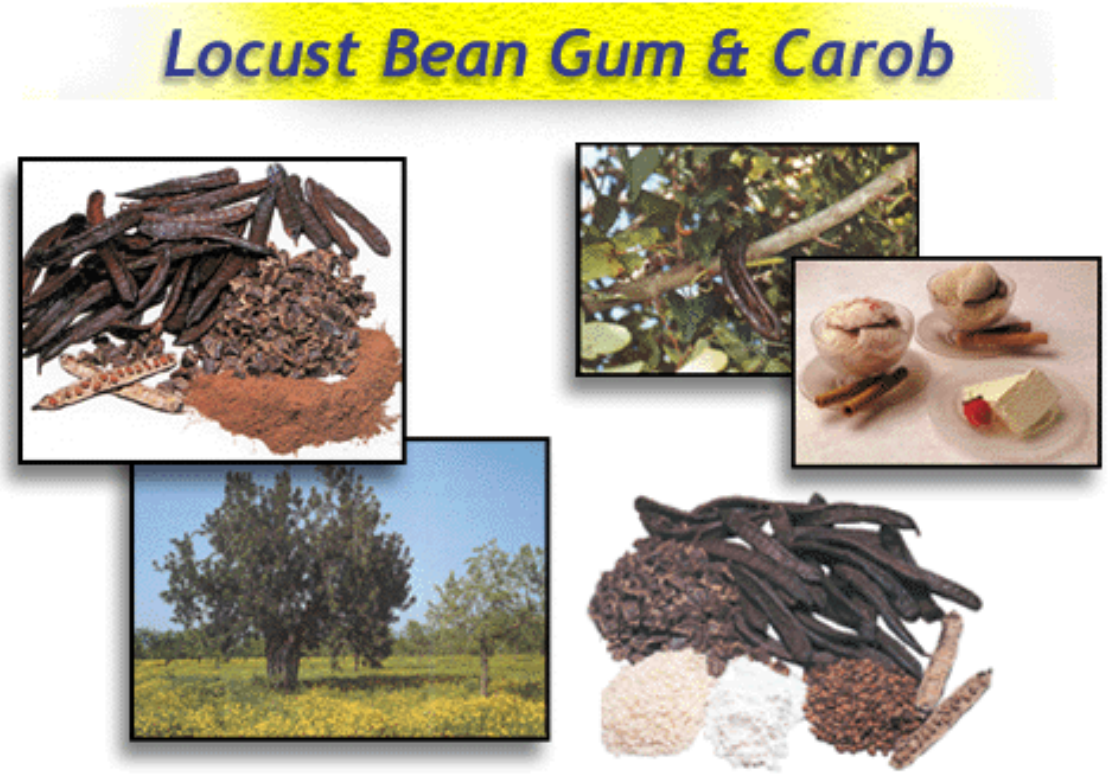


Man/Gal ratio: Guar gum ~2;
Locust bean gum ~4

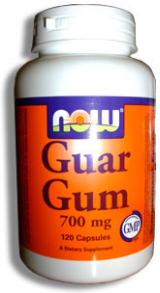
Complex mannans: *Aloe vera* leaf extracts

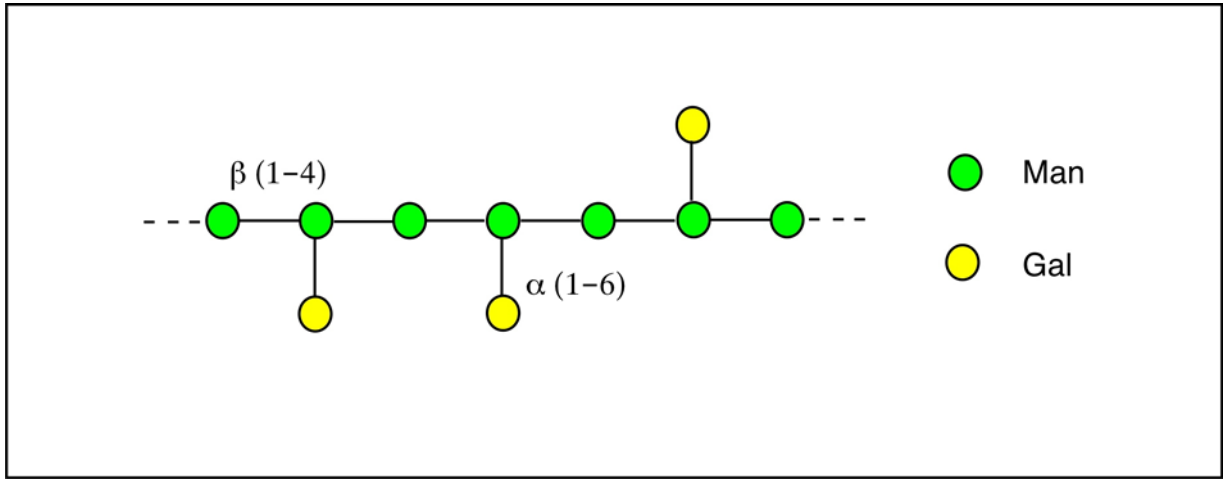
Sample	Sugar components, Mol %				Man/Gal
	Xyl	Man	Glc	Gal	
P3 (gel)	1.6	79.5	11.8	7.1	11.2
P5 (skin)	1.6	31.6	64.0	2.8	0.5

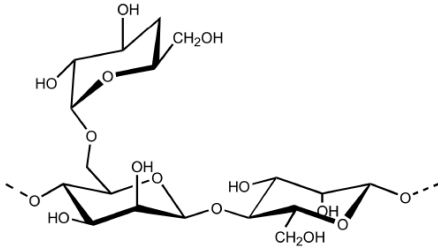
Guar, Locust bean gum, *Aloe vera* polysaccharides

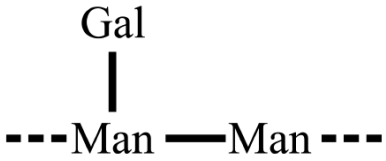


Guar









Man/Gal ratio: Guar gum ~2;

Solubilise by high temperature/pressure treatment

Heterogeneity: sed. coeff. distribution

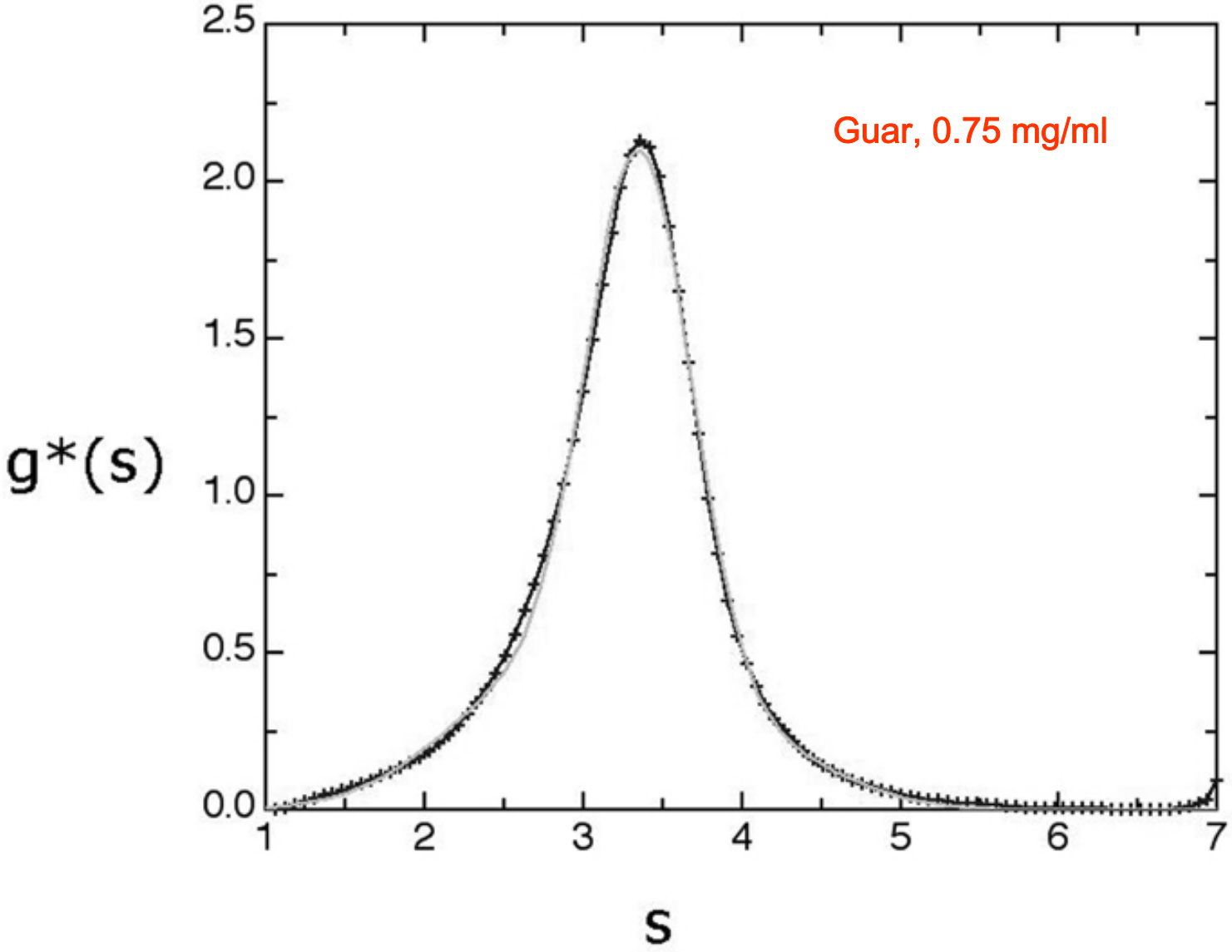


Table 1: Dependence of measured parameters on treatment conditions for guar gum

Treatment	**10^{-5} x M_w** * (g/mol)	**$R_{g,z}$** * (nm)	**$s^0_{20,w}$** (S)
Untreated	18.7	133	8.3 ± 0.3
100 ° C, 10 min	13.9	169	7.7 ± 0.2
130 ° C, 10 min	8.8	105	6.7 ± 0.3
130 ° C, 10 min, 4 bar	10.2	134	6.2 ± 0.3
130 ° C, 10 min, 5 bar	6.3	78	7.8 ± 0.2
160 ° C, 10 min, 3 bar	5.9	80	4.5 ± 0.4
160 ° C, 10 min, 7 bar	7.6	87	5.0 ± 0.3

(* from Picout *et al.*, 2001 [8])

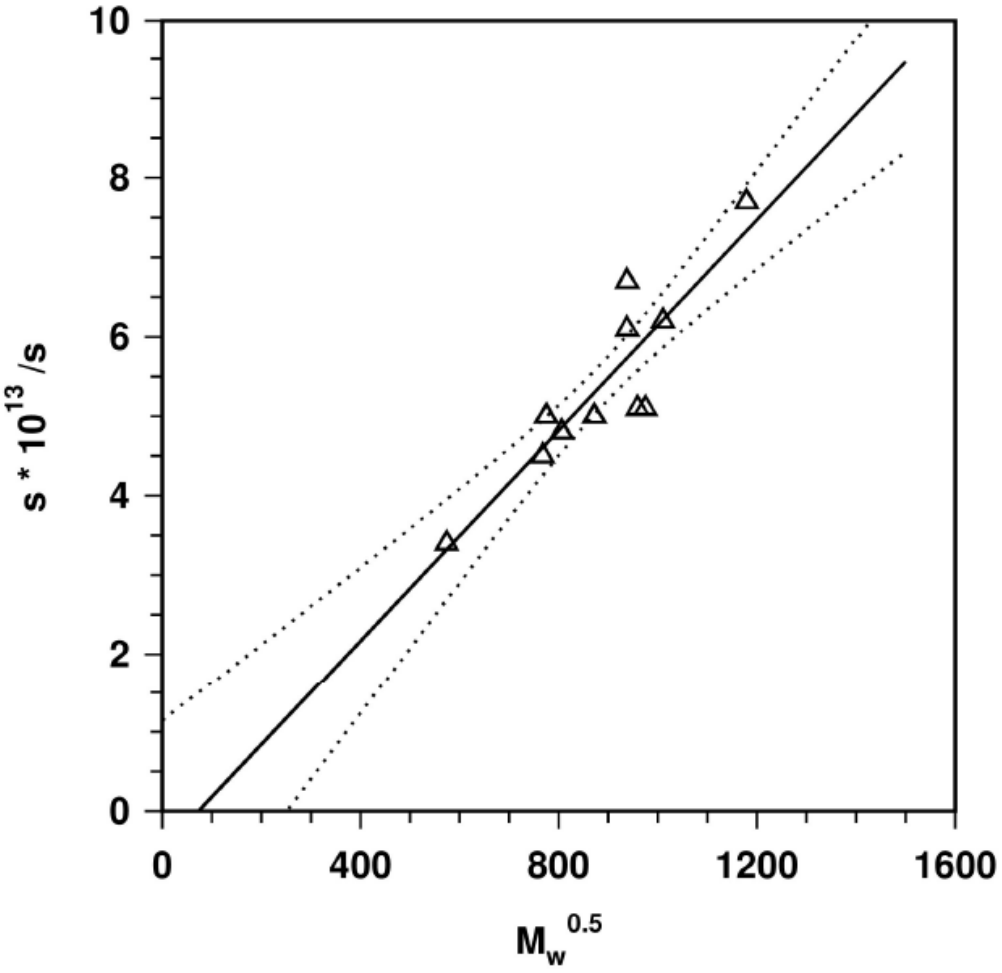
“Bohdanecky” relation

$$\left(\frac{M_w^2}{[\eta]}\right)^{1/3} = A_0 M_L \Phi^{-1/3} + B_0 \Phi^{-1/3}\left(\frac{2L_p}{M_L}\right)^{-1/2} M_w^{1/2}$$

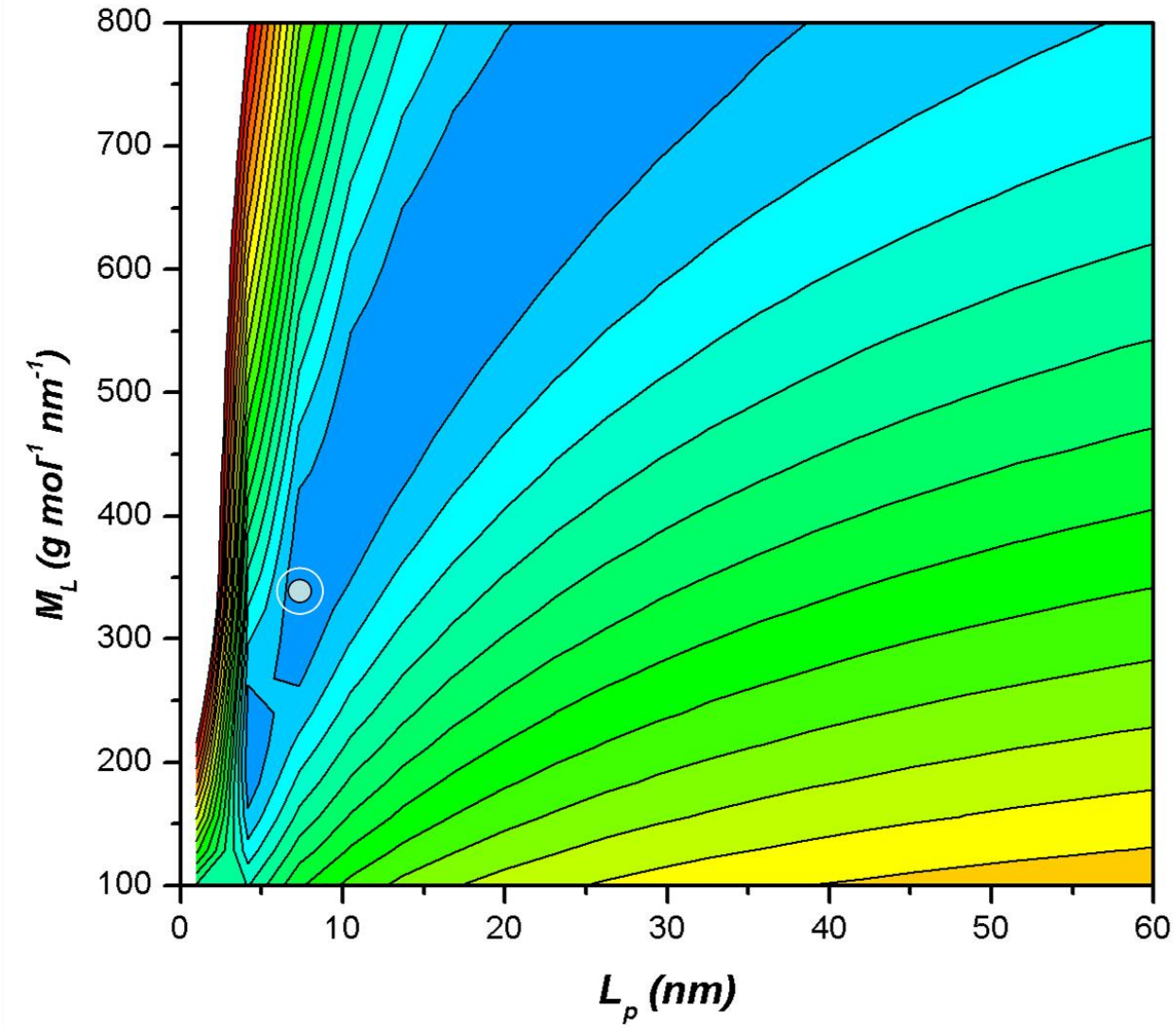
“Yamakawa-Fujii” relation

$$s^0 = \frac{M_L\left(1-\bar{v}\rho_0\right)}{3\pi\eta_0 N_A} \times \left[1.843\left(\frac{M_w}{2M_L L_p}\right)^{1/2} + A_2 + A_3\left(\frac{M_w}{2M_L L_p}\right)^{-1/2} + \ldots.\right]$$

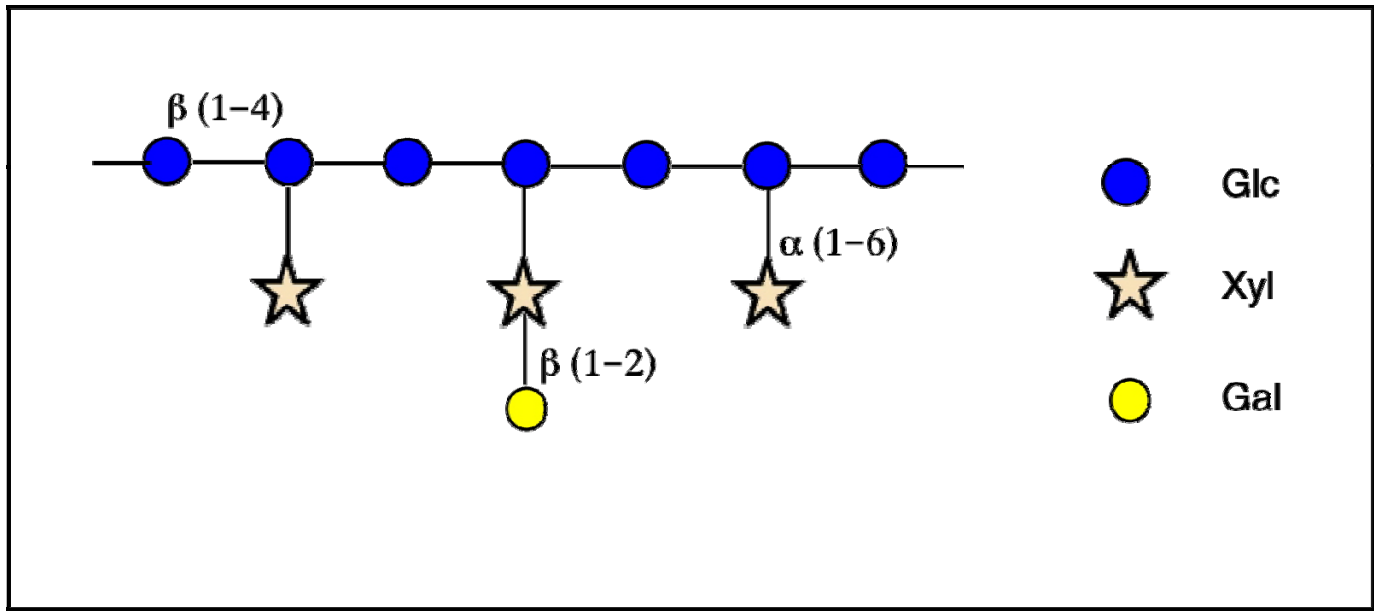
Yamakawa-Fujii analysis: L_p ~ 6-9nm



Global plot: persistence length, L_p ~ 7 nm



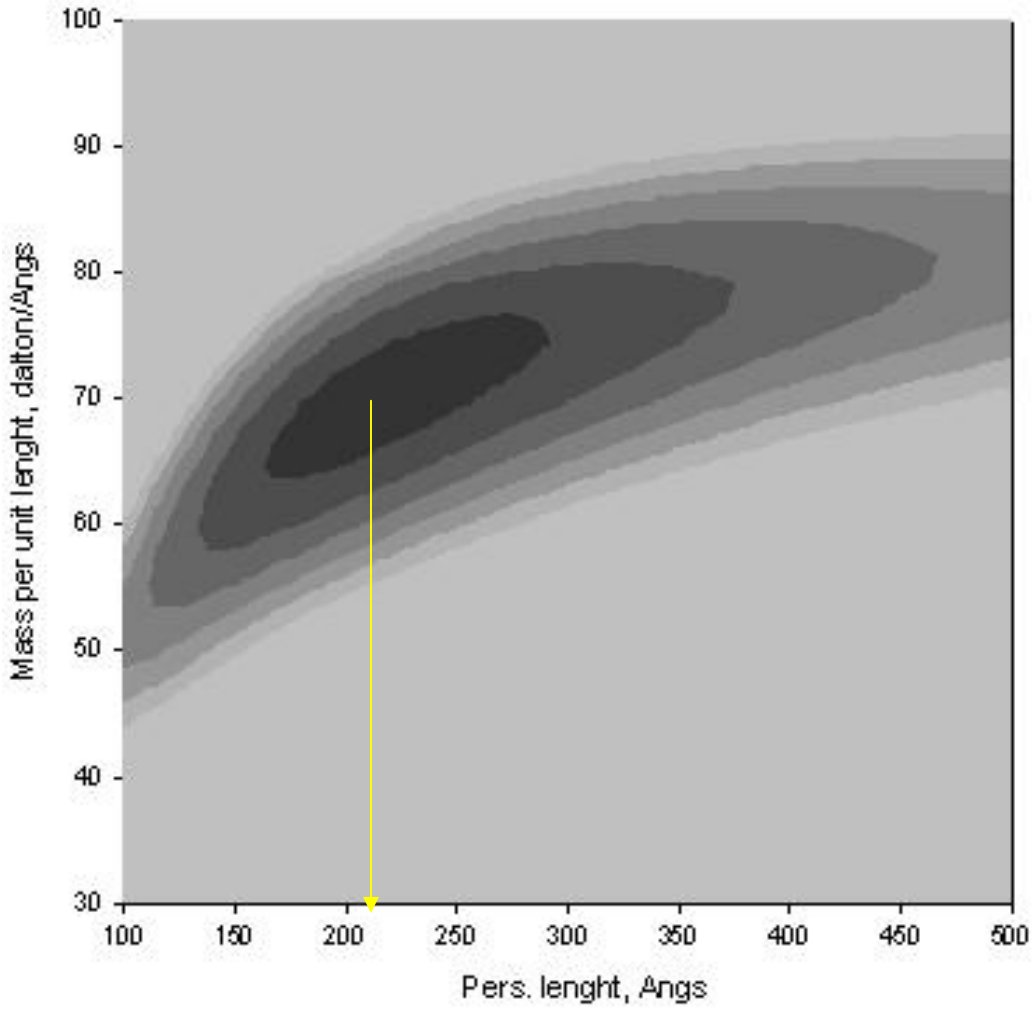
III Xyloglucans



anti-oxidant, anti-microbial, mutagenic
immunomodulatory activities

High mol wt (~10^6 g/mol - ultrasonicate to reduce)

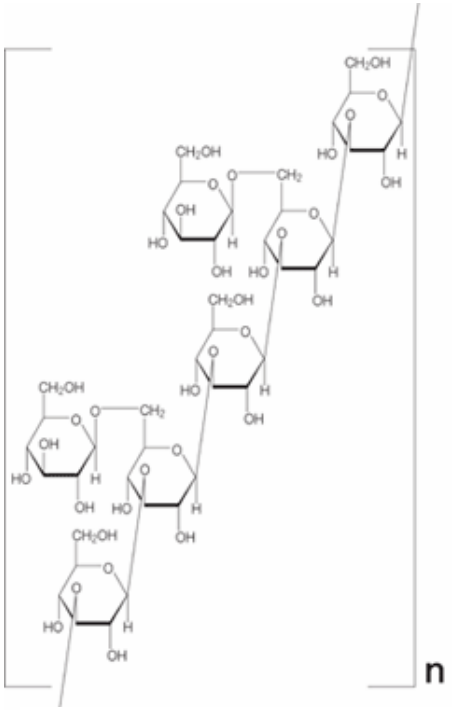
Xyloglucan persistence length from global analysis: $L_p \sim 21nm$



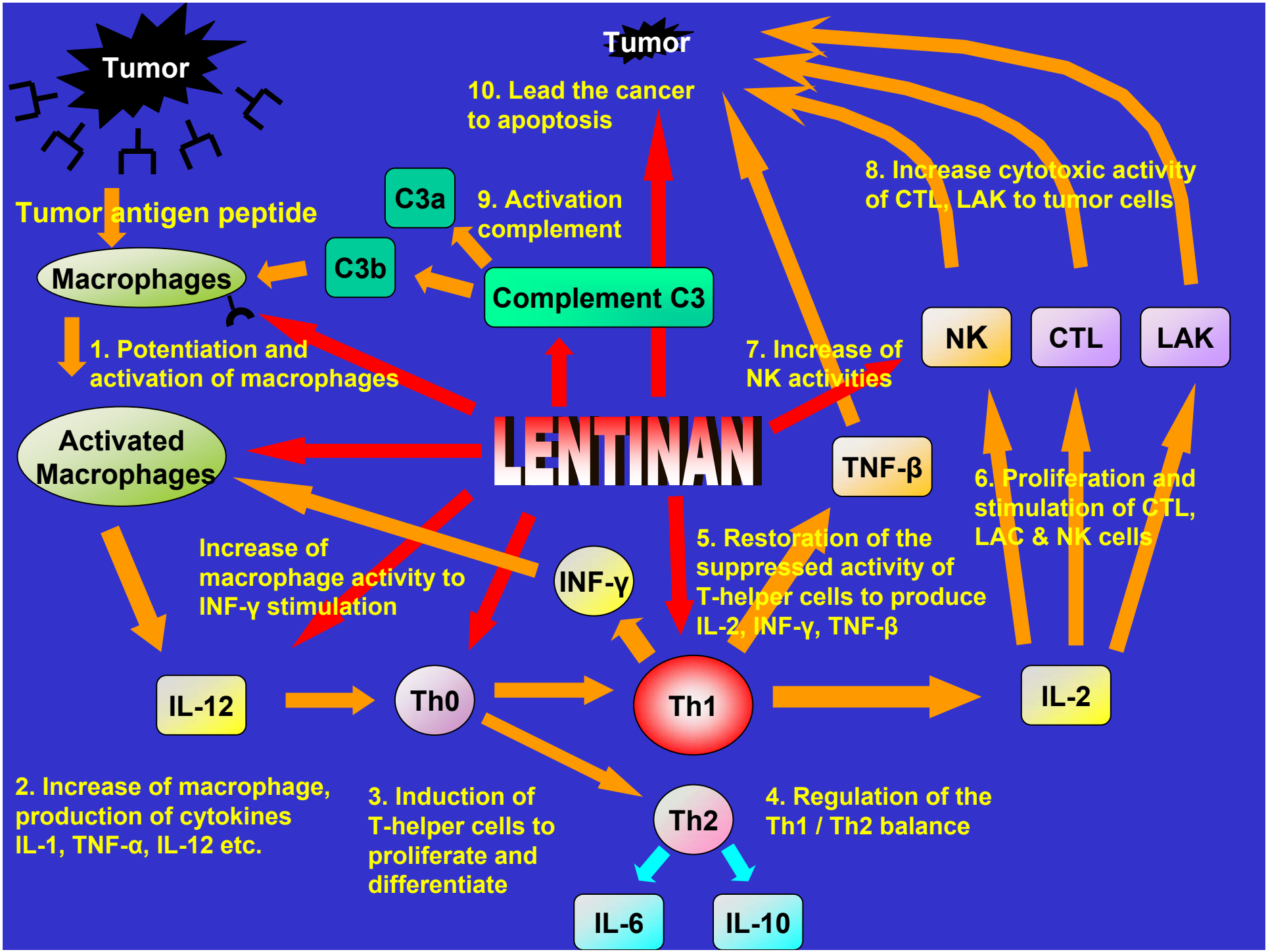
Flexibility of xyloglucan

Carbohydrate Polymer	L_p (nm)
Pullulan	2.0
Heparin	2.0-2.1
Amylose	2.8
Xyloglucan (ultrasonicated)	**21**
Pectin (69% esterified)	30
Pectin (0% esterified)	34
DNA	45
Schizophyllan	115-200
Scleroglucan	180±30
Xanthan	210

IV Fungal beta glucans: lentinans

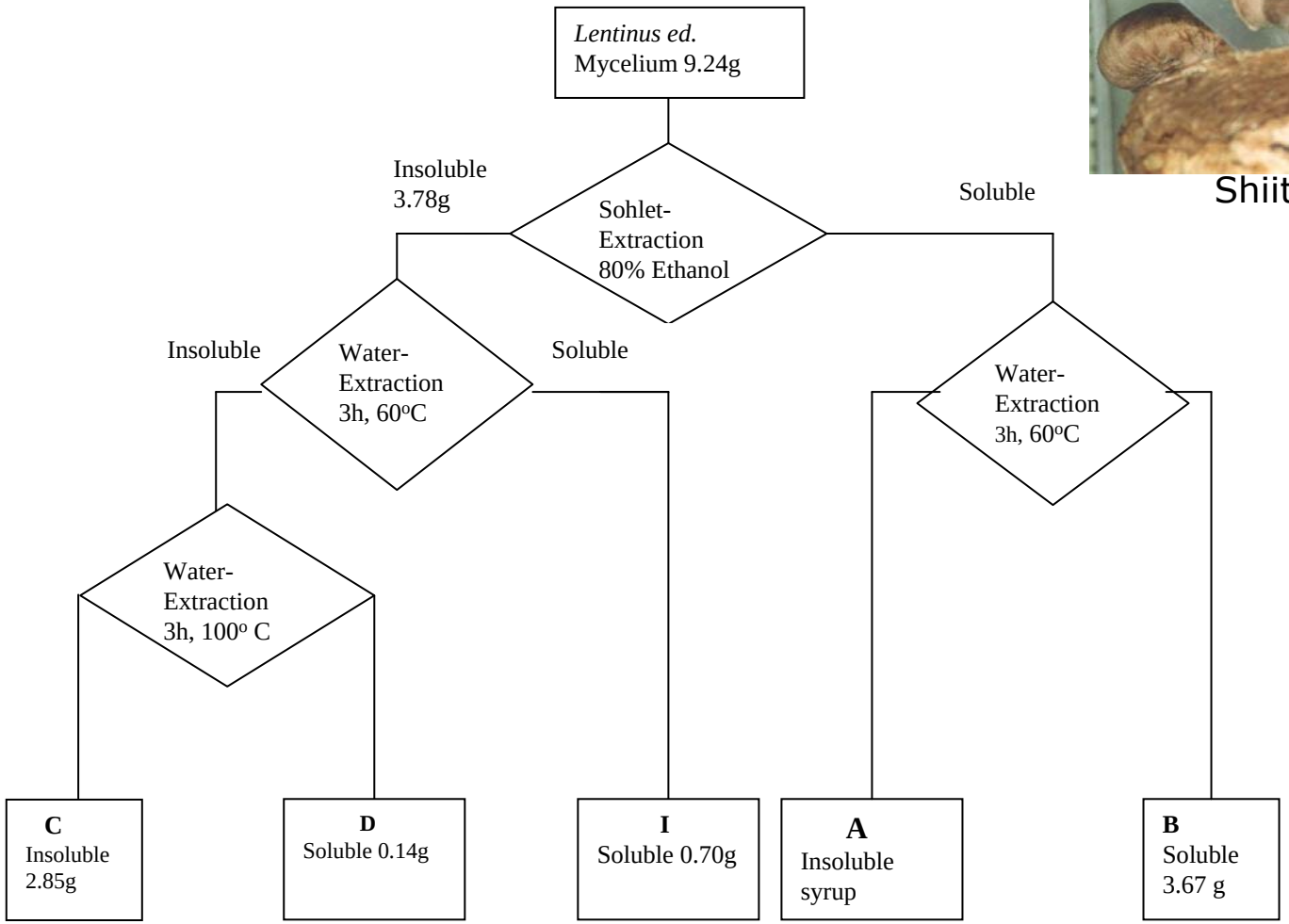


Lentinan-an extract of the shiitake Mushroom is approved as an anti-cancer drug in Japan


Tumor
Tumor
10. Lead the cancer to apoptosis
8. Increase cytotoxic activity of CTL, LAK to tumor cells
Tumor antigen peptide
C3a
9. Activation complement
C3b
Macrophages
Complement C3
1. Potentiation and activation of macrophages
7. Increase of NK activities
NK
CTL
LAK
Activated Macrophages
LENTINAN
TNF-β
6. Proliferation and stimulation of CTL, LAC & NK cells
Increase of macrophage activity to INF-γ stimulation
INF-γ
5. Restoration of the suppressed activity of T-helper cells to produce IL-2, INF-γ, TNF-β
IL-12
Th0
Th1
IL-2
2. Increase of macrophage, production of cytokines IL-1, TNF-α, IL-12 etc.
3. Induction of T-helper cells to proliferate and differentiate
Th2
4. Regulation of the Th1 / Th2 balance
IL-6
IL-10

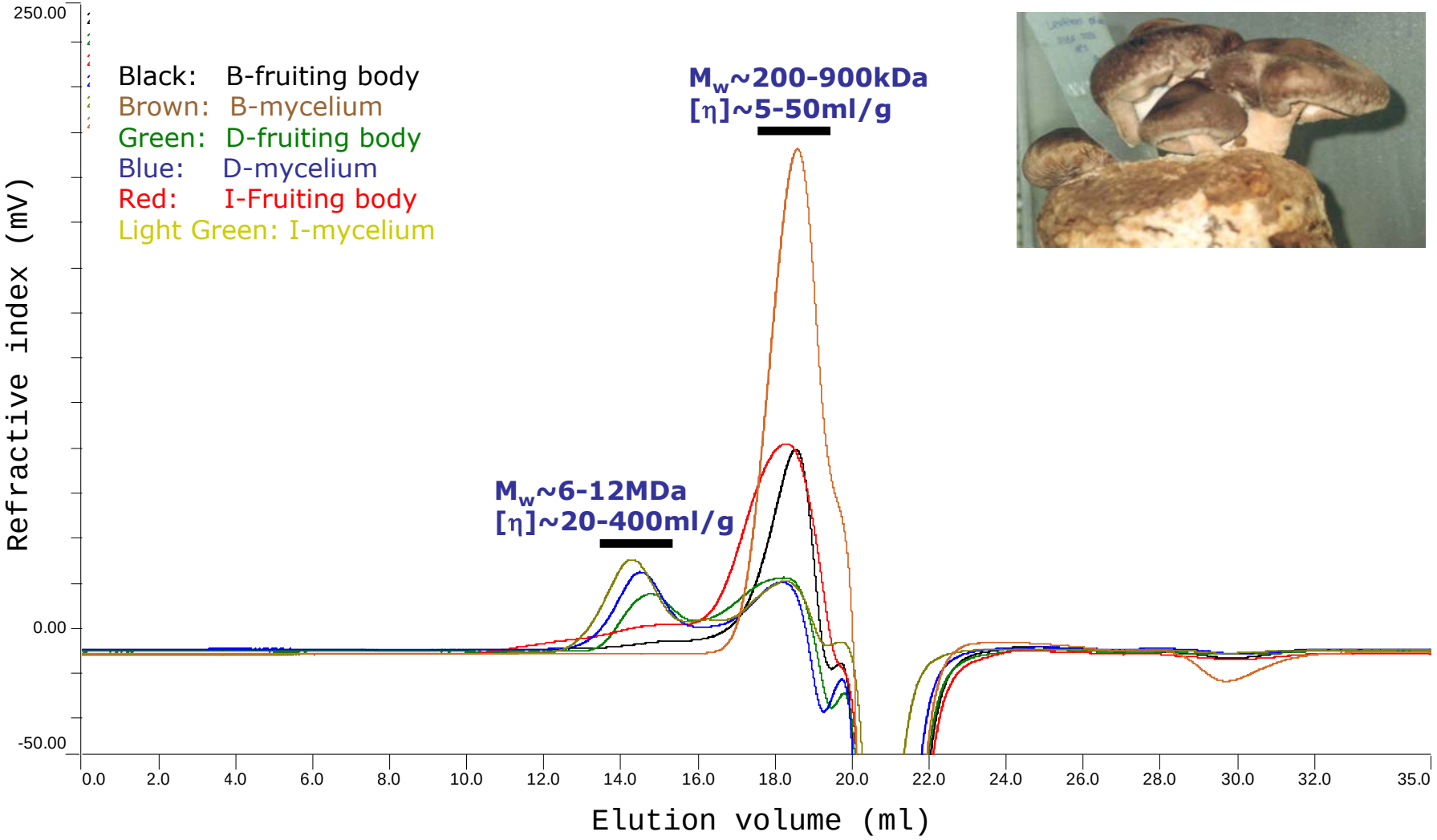
Lentinans: extraction from the mushroom

Shiitake (*Lentinan edodes*)

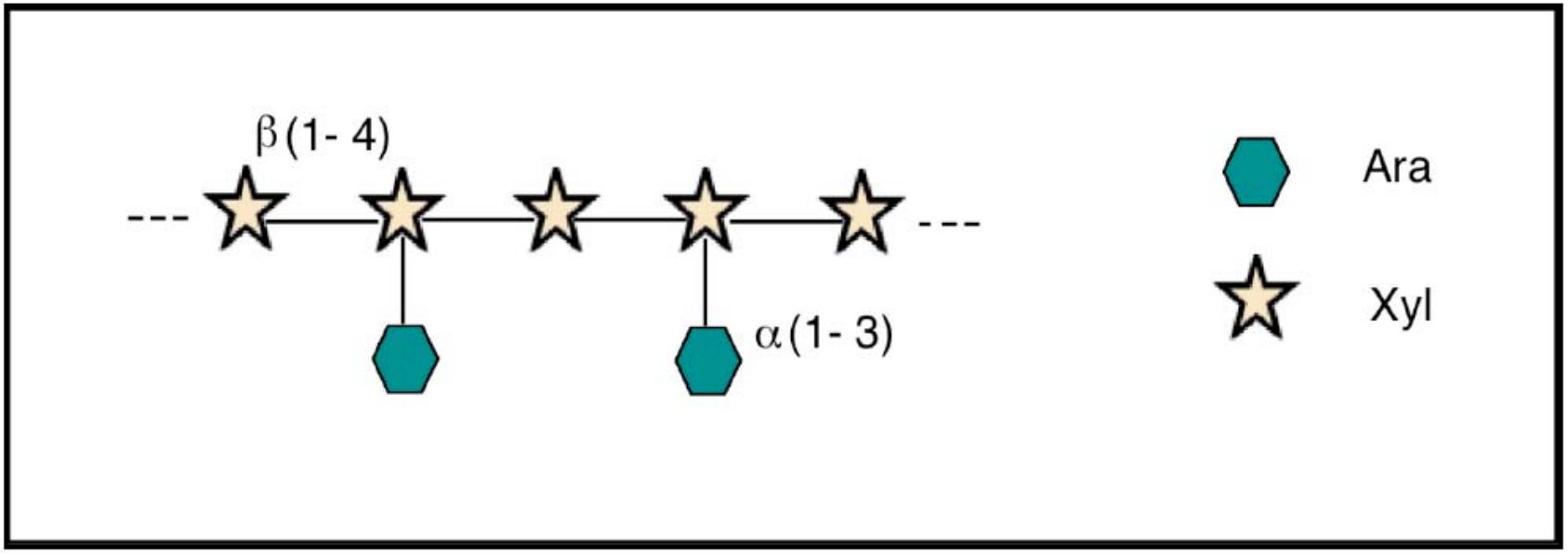


"Triple detection" analysis on *Lentinan edodes extracts*

Refractive index, molecular weight, viscosity

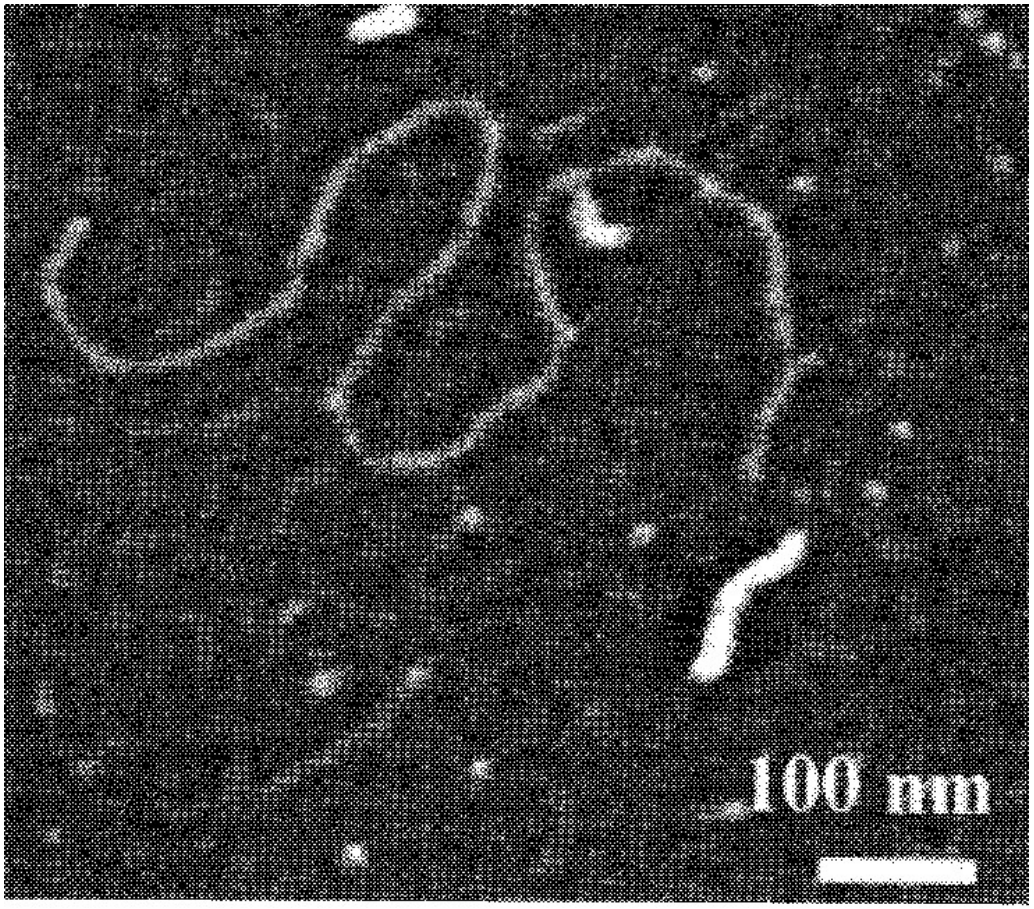


Arabinoxylans – polysaccharides that interact weakly



…Polysaccharides either interact very strongly … or not at all

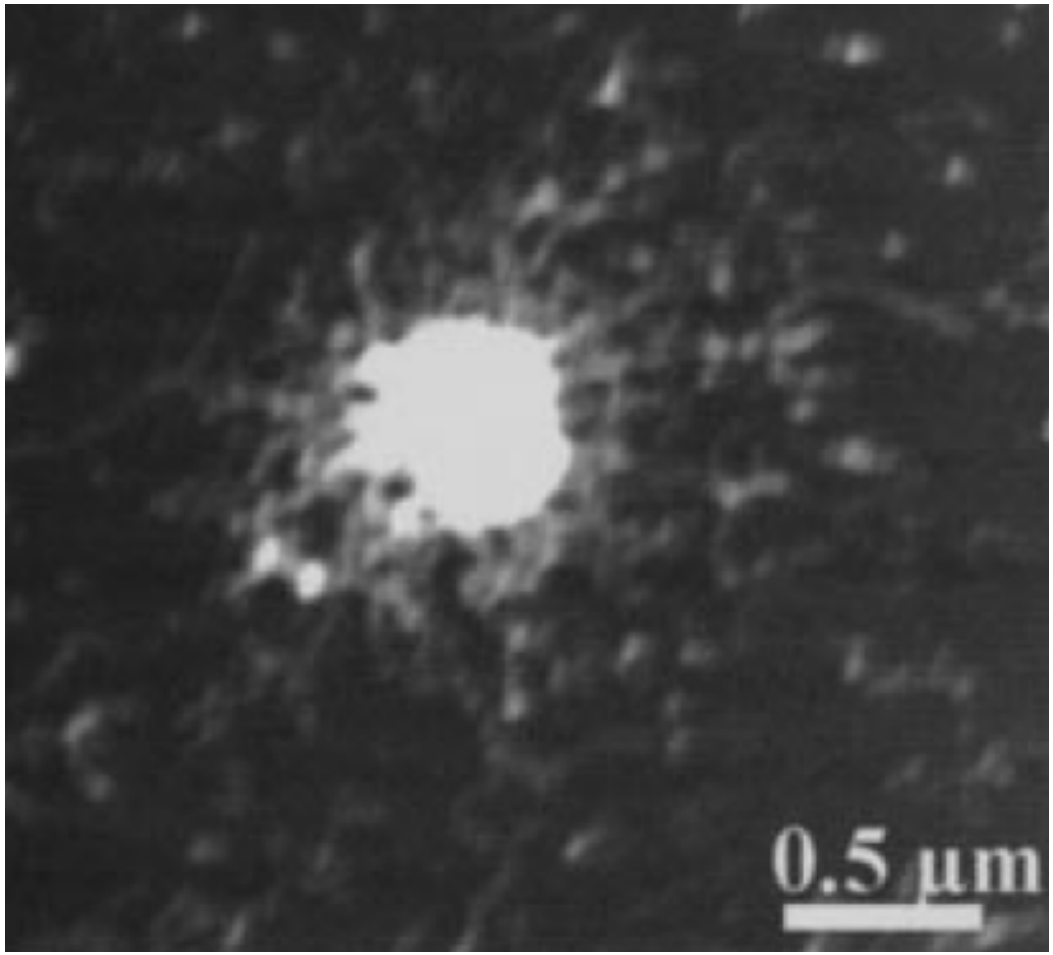
Chitosan



Sedimentation coefficient $s^{o}_{20,w}$ ~ 1S

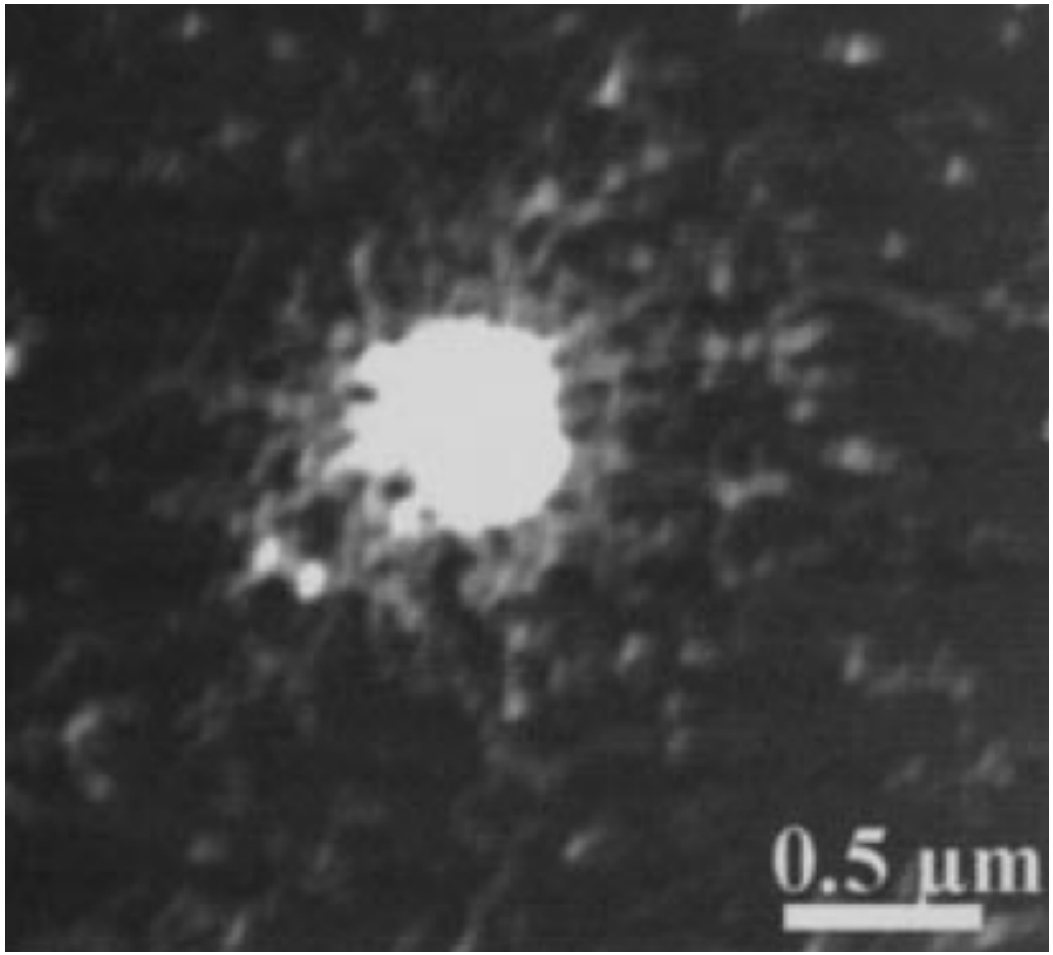
Deacon et al, 2000

chitosan-mucin complex



Sedimentation coefficient $s^{o}_{20,w}$ ~ 2000S

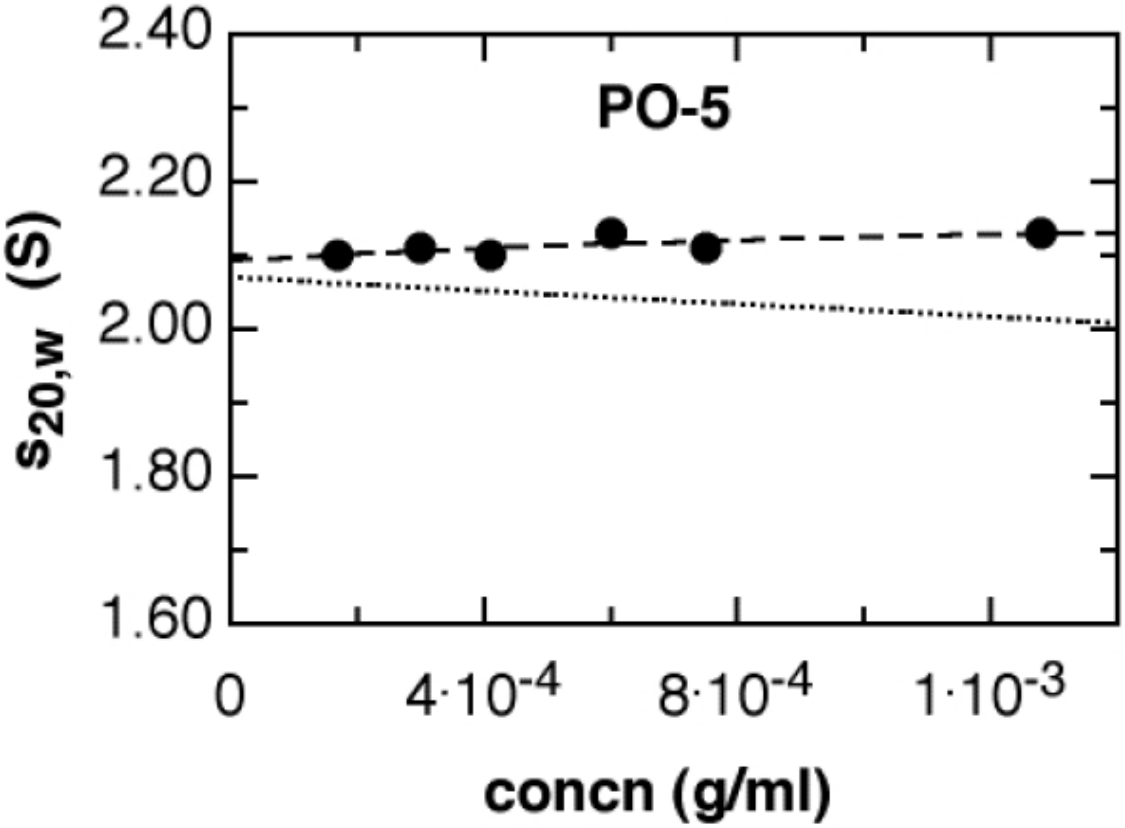
chitosan-mucin complex



Sedimentation coefficient $s^{o}_{20,w}$ ~ 2000S

very strong, irreversible interaction

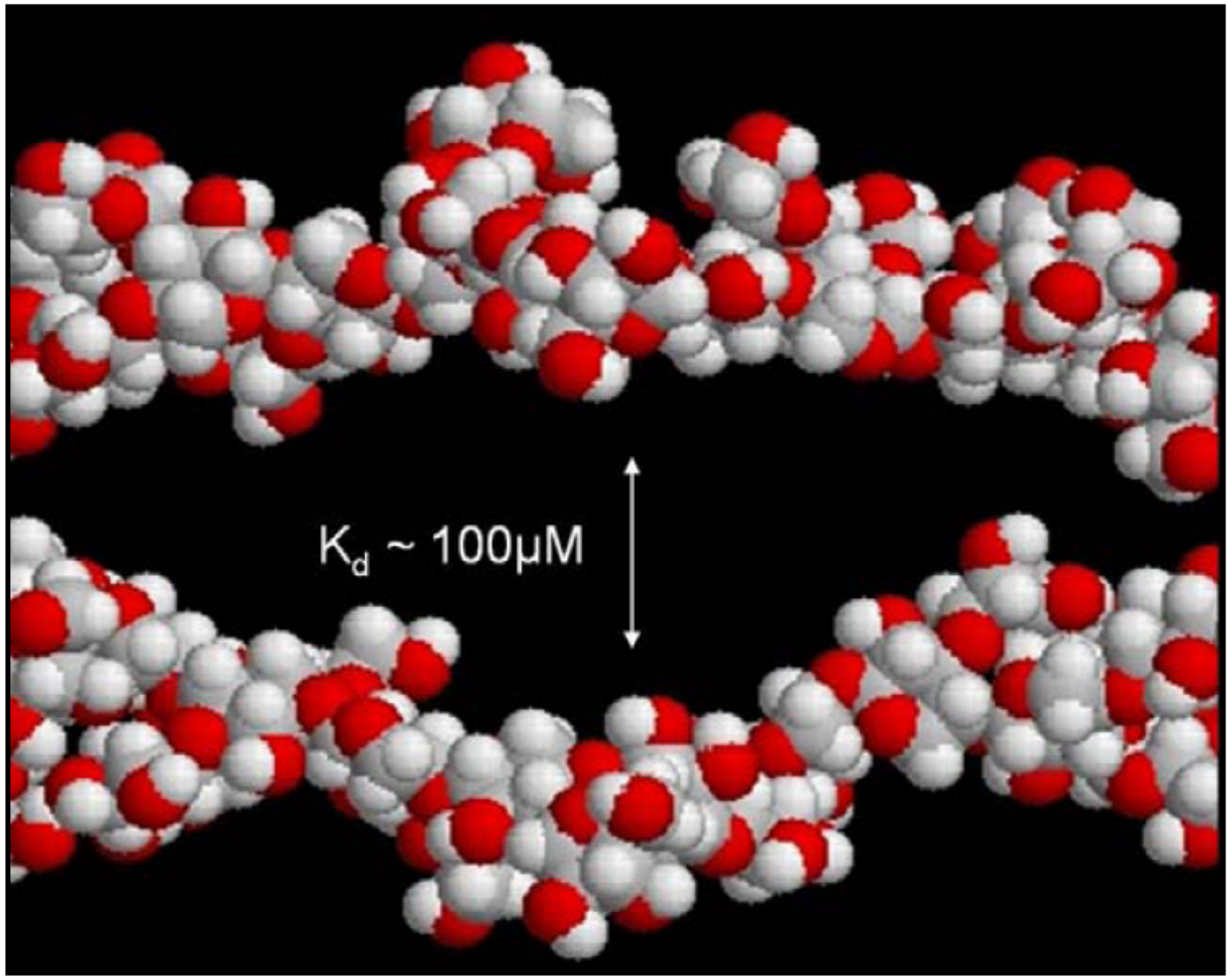
“bioactive” arabinoxylan



very weak, reversible interaction

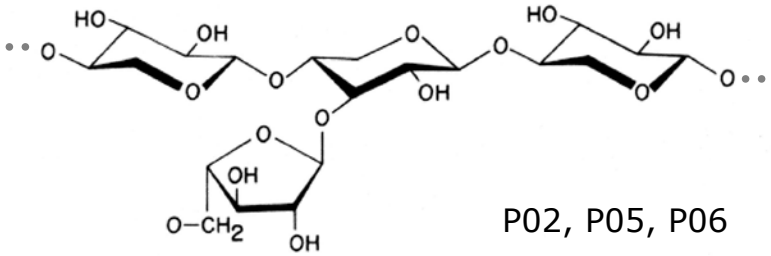
Patel et al, 2007

“bioactive” arabinoxylan



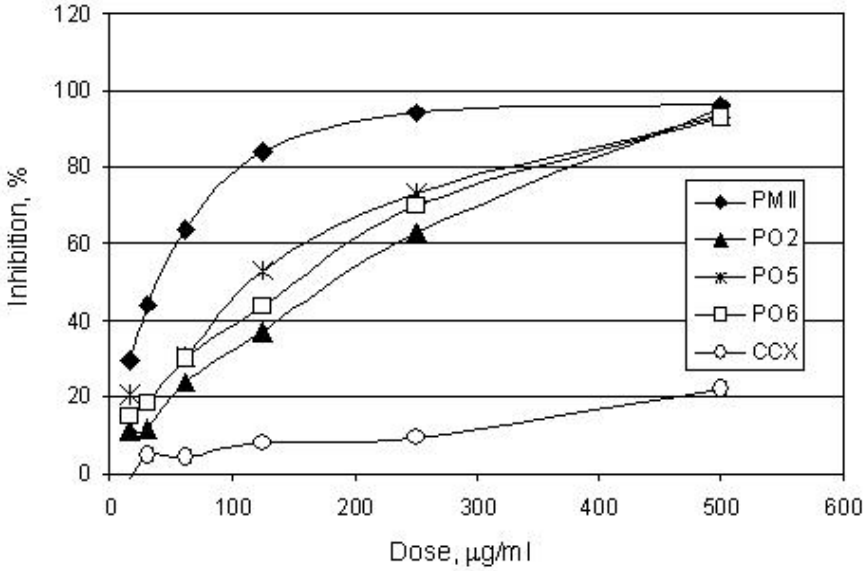
1st reported weak interaction in polysaccharide?

Arabinoxylans

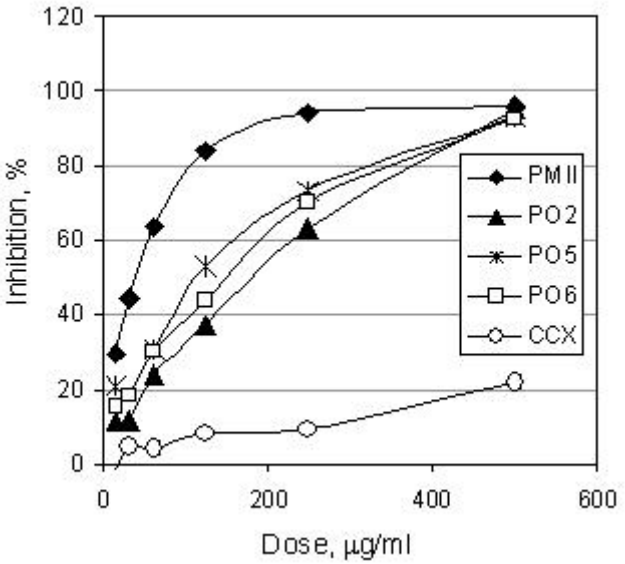


P02, P05, P06

Cell stimulation



Complement activation/ inhibition of hemolysis

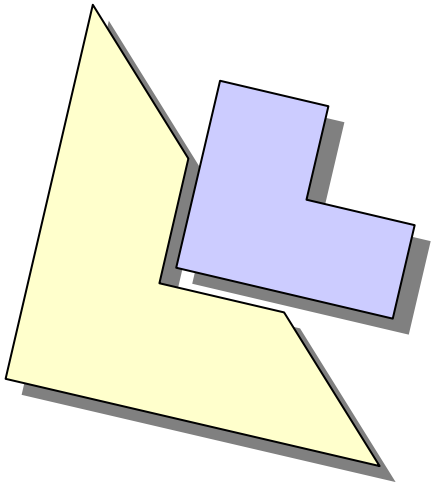


Some interaction strengths in biological systems

Interaction	Strength, K_d
Enzyme inhibitor:Enzyme	~0.01nM
Cytokine:receptor	~1nM
Antibody:antigen	~10nM
Cell-cell recognition molecules	10-200μM
CD2:CD58	~10μM
2B4:CD48	10μM
KIR:MHC I	10μM
CD28:CD86	20μM
CD2:CD48	50μM
CD8:MHC classI	50-200μM
CD4:MHC class II	>200μM

Van der Merwe & Davis, 2003

Immune receptors for polysaccharides



- **TLR4**

  -Bind several low-molecular weight polysaccharides (e.g. inulin)

- **Dectin-1**

  -C-type lectin-like receptor on macrophages that bind β-glucan

Acknowledgements

The team:

Berit Smestad Paulsen (Oslo)

Thomas Heinze and Andreas Koschella (Jena)

Zdenka Hromádková & Anna Ebringerova (Bratislava)

Vera Hříbalová (Prague)

Cleanthes Israilides (Lycovrissi)

Marcin Deszczynski, Arthur Rowe (Nottingham)

Students:

Kari Inngjerdingen, Cecilie Sogn Nergård (Oslo)

Trushar Patel, Sanya Hokputsa (Nottingham)

EPSRC

Engineering and Physical Sciences Research Council

National Centre for Macromolecular Hydrodynamics

http://www.nottingham.ac.uk/ncmh