From Sticky Mucus to Probing our Past: Aspects and problems of the Biotechnological use of Macromolecules

Datum/Zeit	Veranstaltungsort	Thema
Mi, 30.06.2010 12.15-13.45	SR 309 Carl-Zeiss-Str. 3	***Macromolecules as BioPharma mucoadhesives***
Do, 01.07.2010 08.15-09.45	SR 308 Carl-Zeiss-Str. 3	***Macromolecules as vaccines***
Do, 01.07.2010 13.15-14.45	HS Haus 1 August-Bebel-Str. 2	***Stability in response to Bioprocessing I. Thermal Processing, D, z and F values***
Fr, 02.07.2010 08.15-09.45	HS Haus 1 August-Bebel-Str. 2	***Stability in response to Bioprocessing II: Irradiation and freezing***
Fr, 02.07.2010 12.15-13.45	SR 307 Carl-Zeiss-Str. 3	***The use of non-recombining parts of the Y-chromosomal DNA and mitochondrial DNA as a probe into our past***

Macromolecules as Vaccines

Steve Harding

The University of Nottingham

Vaccination

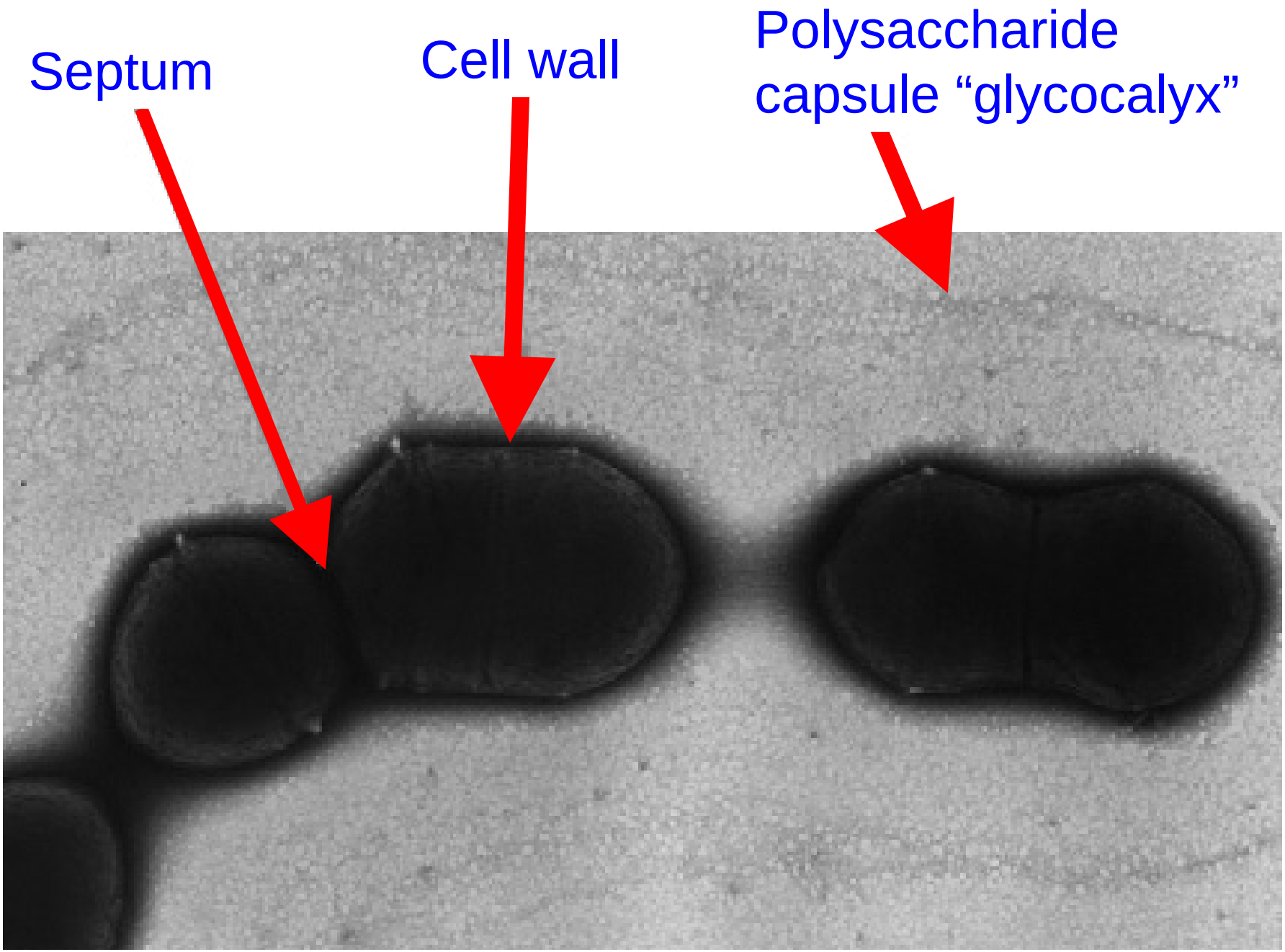
- Vaccine produces immunity
- Response similar to natural infection but without risk of disease
- Certain bacteria with capsular polysaccharide particularly dangerous
- Design vaccines based on capsular polysaccharides

Advantages of polysaccharide vaccines compared to antibiotics

- A vaccine prevents disease rather than cures it, so toxic effects of infection, such as release of endotoxin, do not occur
- Vaccination of infants is less dependent on access to a medical expertise and hospital timelines are are much less critical
- Vaccination can be carried out by partly trained staff – important in developing countries
- For most bacteria, evasion of vaccine-based protection is much more difficult than development of antibiotic resistance
- Reduction of bacterial carriage reduces transmission of disease, so that even unvaccinated children are less likely to be affected.

Disadvantages of polysaccharide vaccines compared to antibiotics

- The vaccine protects against only a single serotype/serogroup, so that multicomponent or “multivalent” vaccines are usually required
- The pattern of disease may change, with novel serotypes or serogroups becoming important. New vaccines are then required
- The duration of protection may be limited, and older children for example may not be protected
- Repeated immunisation with a polysaccharide can lead to reduced responsiveness and lower antibody levels
- Not all polysaccharides can be used to make vaccines – e.g. meningococcal Group B



S. Sorensen et al (1988), *Infect.Immun.* 56, 1890-1896

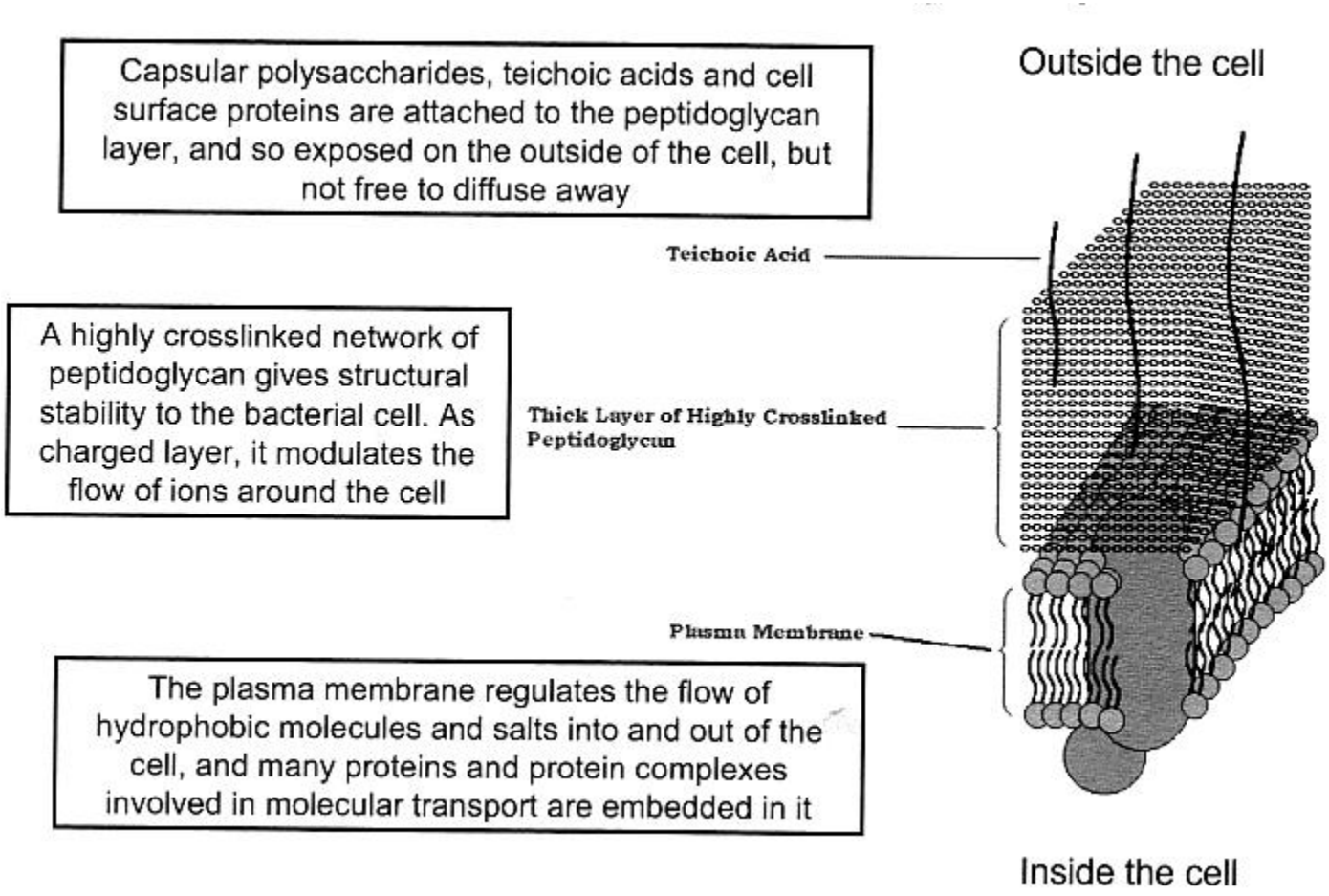
Some dangerous capsular bacteria

- *Streptococcus pneumoniae*
- Group B *Streptococcus*
- *Neisseria meningitidis* “Meningococcus”
- *Haemophilus influenzae*

Capsules consist of high molecular weight polysaccharides

Capsular polysaccharides are attached to the surface of the bacteria and not free to move away.

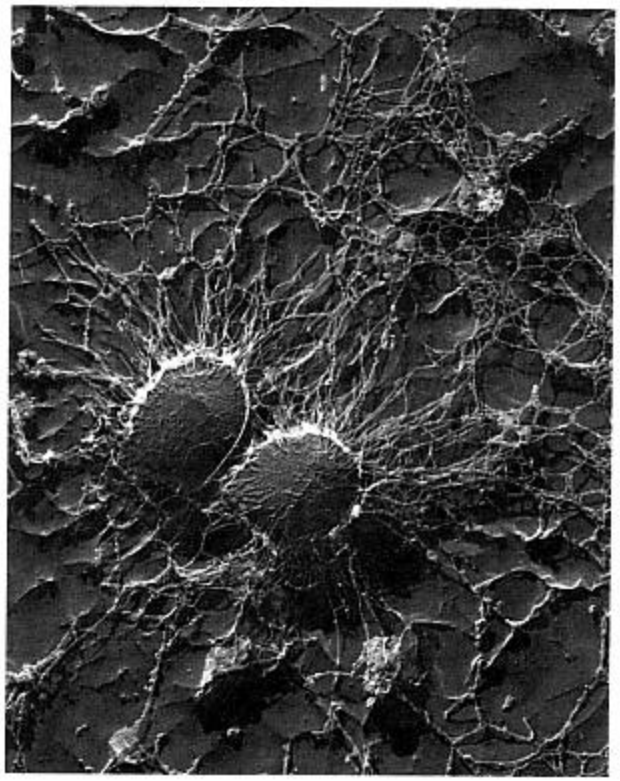
Gram positive bacteria



http://en.wikipedia.org/wiki/Gram-positive

Gram positive bacteria

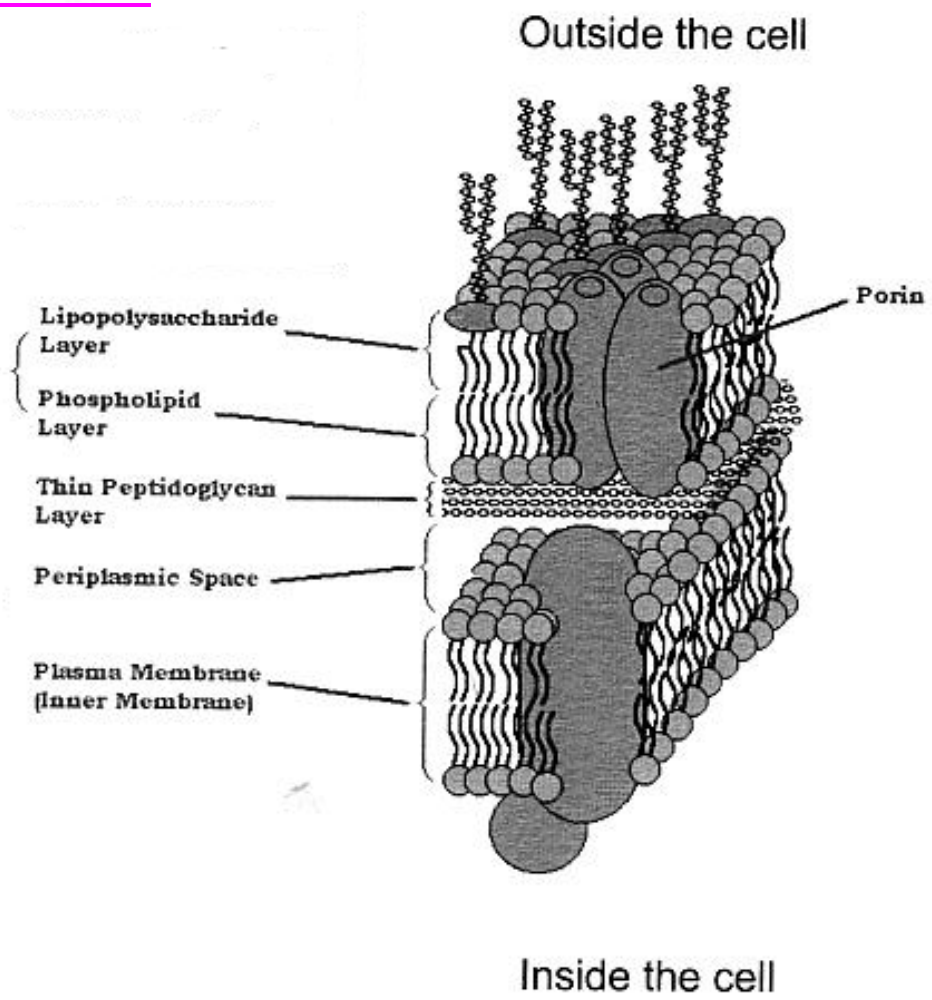
- *Streptococcus pneumoniae*
- Group A Streptococcus
- Group B Streptococcus
- *Staphylococcus aureus*



Staphylococcus aureus: image from Wikopedia article

Capsular polysaccharides are attached to the surface of the bacteria and not free to move away.

Gram negative bacteria

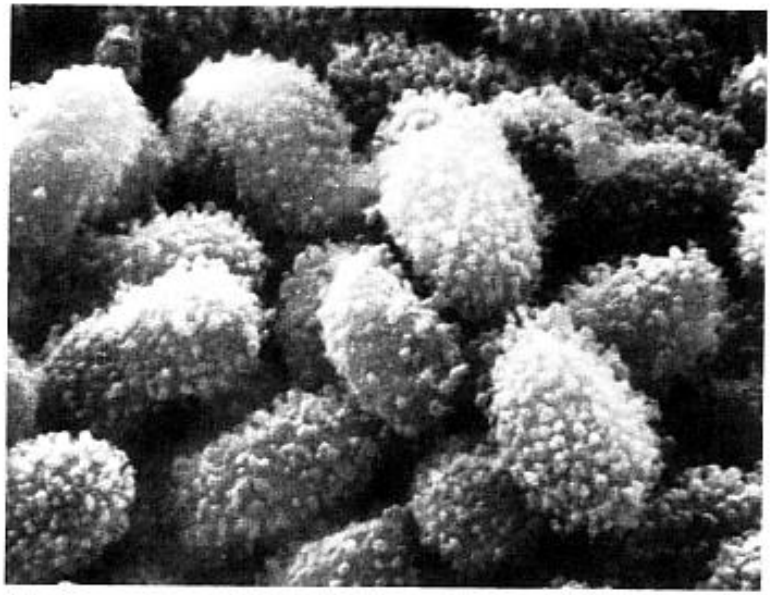


http://en.wikipedia.org/wiki/Gram-negative

Gram negative bacteria

- *Haemophilus influenzae*
- *Neisseria meningitidis*
- *Neisseria gonorrhoae*
- *Salmonella entericus* serovar *Typhi*

- *Shigella flexneri*
- *Shigella*
- *Shigella*
- *Pseudomonas aeruginosa*



Electron micrograph of *Haemophilus influenzae*

Role of capsular polysaccharides in nature

- Modulation of flow of nutrients to bacterial cell surface
- Prevention of dessication by maintaining an easily hydrated layer close to the bacterial surface
- Provides a suitable matrix to allow attachment to surfaces

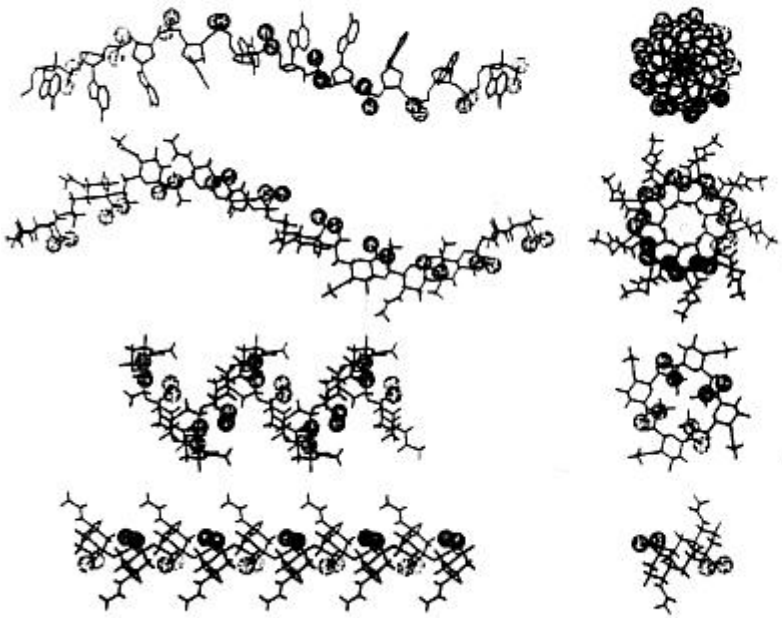
Role of capsular polysaccharides in infection

- Protects cell surface components from host immune responses (inate and acquired)
- Provides a “non-threatening” target for deposition of complenent, which does not lead to cell damage
- When phagocytosis does occur, the capsule helps protect against host cell-mediated killing through activated oxidative species

Structures of capsular polysaccharides:

- High molecular weight (50 000 to >1 000 000Da) with repeating structure
- Repeat unit of up to ~10 sugars – in most cases the repeat unit is pre-assembled and polymerised
- Mostly –vely charged
  - uronic acids
  - sialic acid
  - phosphate groups (in-chain phosphodiester)
  - substituents such as pyruvate ketals
- Some are neutral, some are zwitterionic

Structures of capsular polysaccharides:

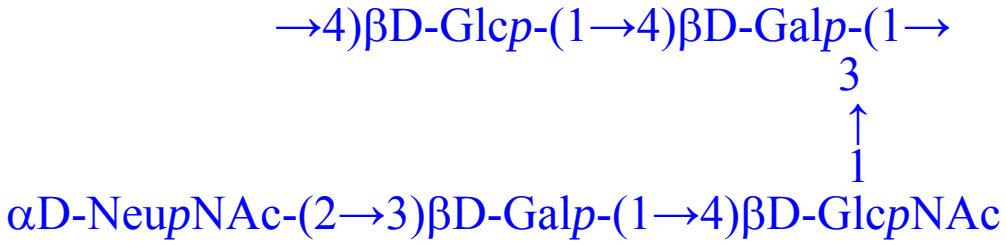


- The only stable repeating structure is a helix
- Evidence is that this helix is ill-defined and flexible.
- Arguments continue about existence of well-defined secondary structure
- Epitopes are essentially "primary" – dependent on primary sequence rather than complex folding

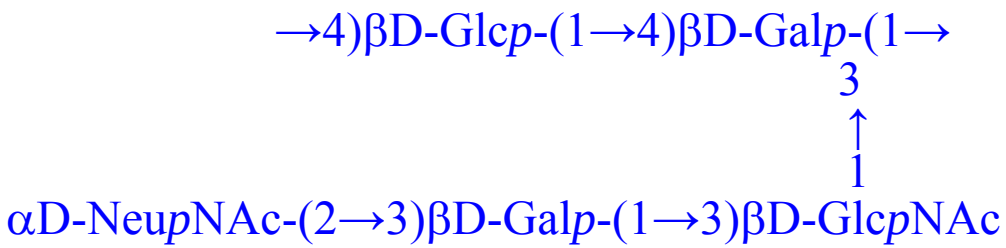
A given species can have different capsular polysaccharides, or different "serotypes"

Group B Streptococcus:

Serotype Ia:



Serotype Ib:



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Group B Streptococcus:

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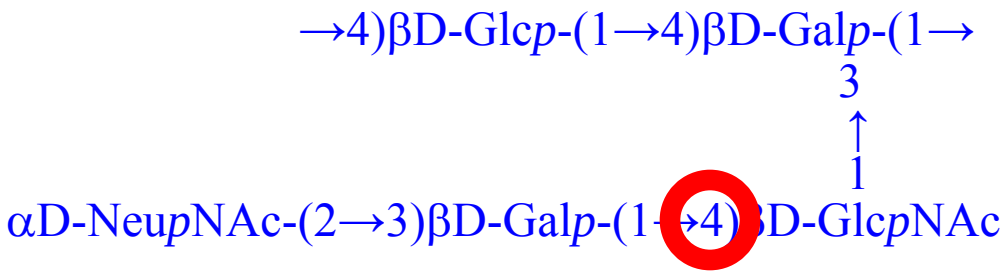
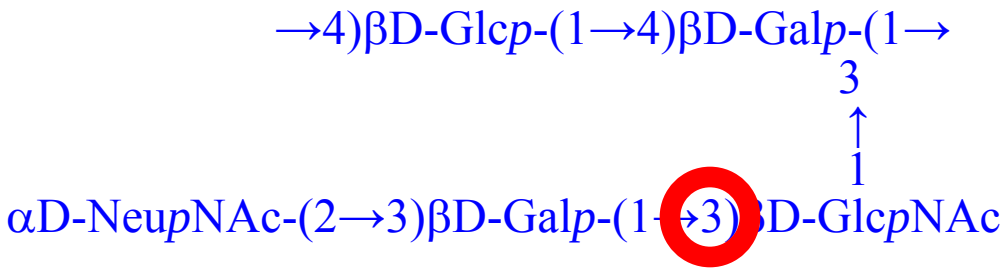
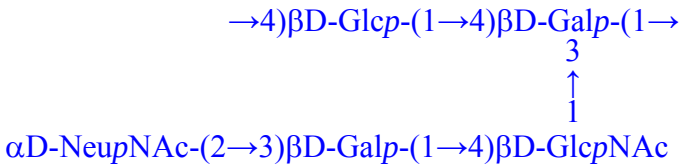


Serotype Ib:

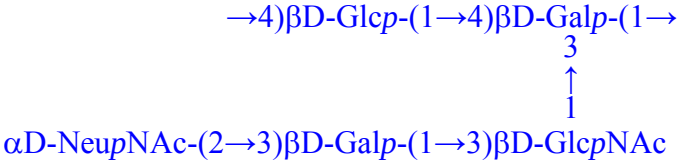


Capsular Polysaccharides of Group B *Streptococcus*

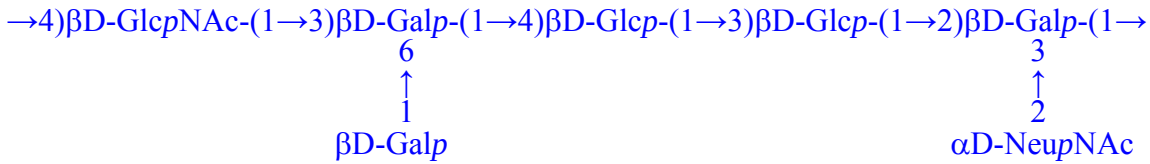
Ia



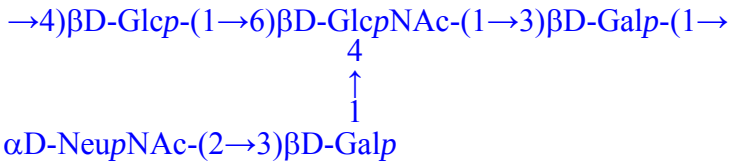
Ib



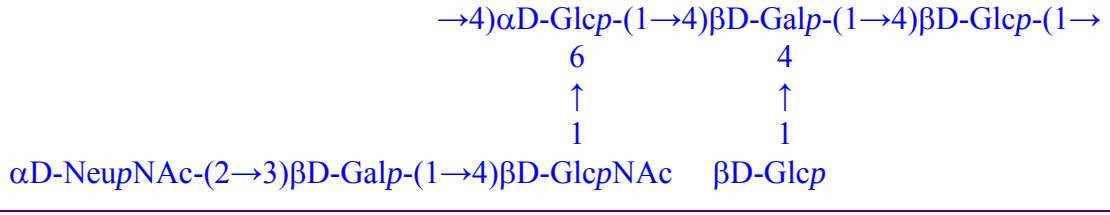
II



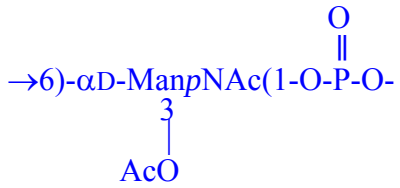
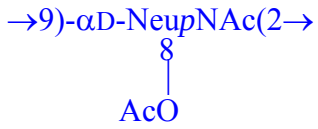
III



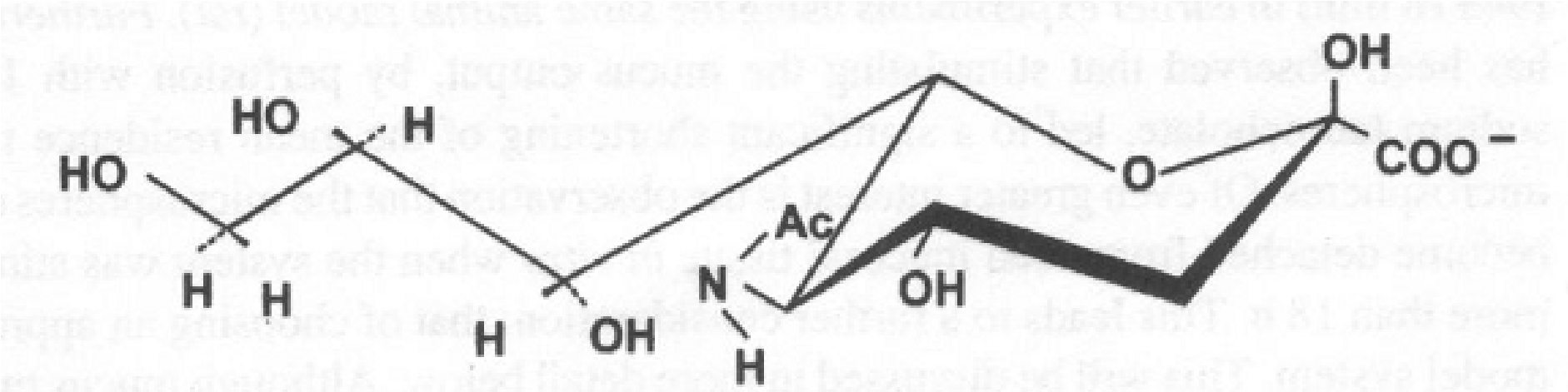
V



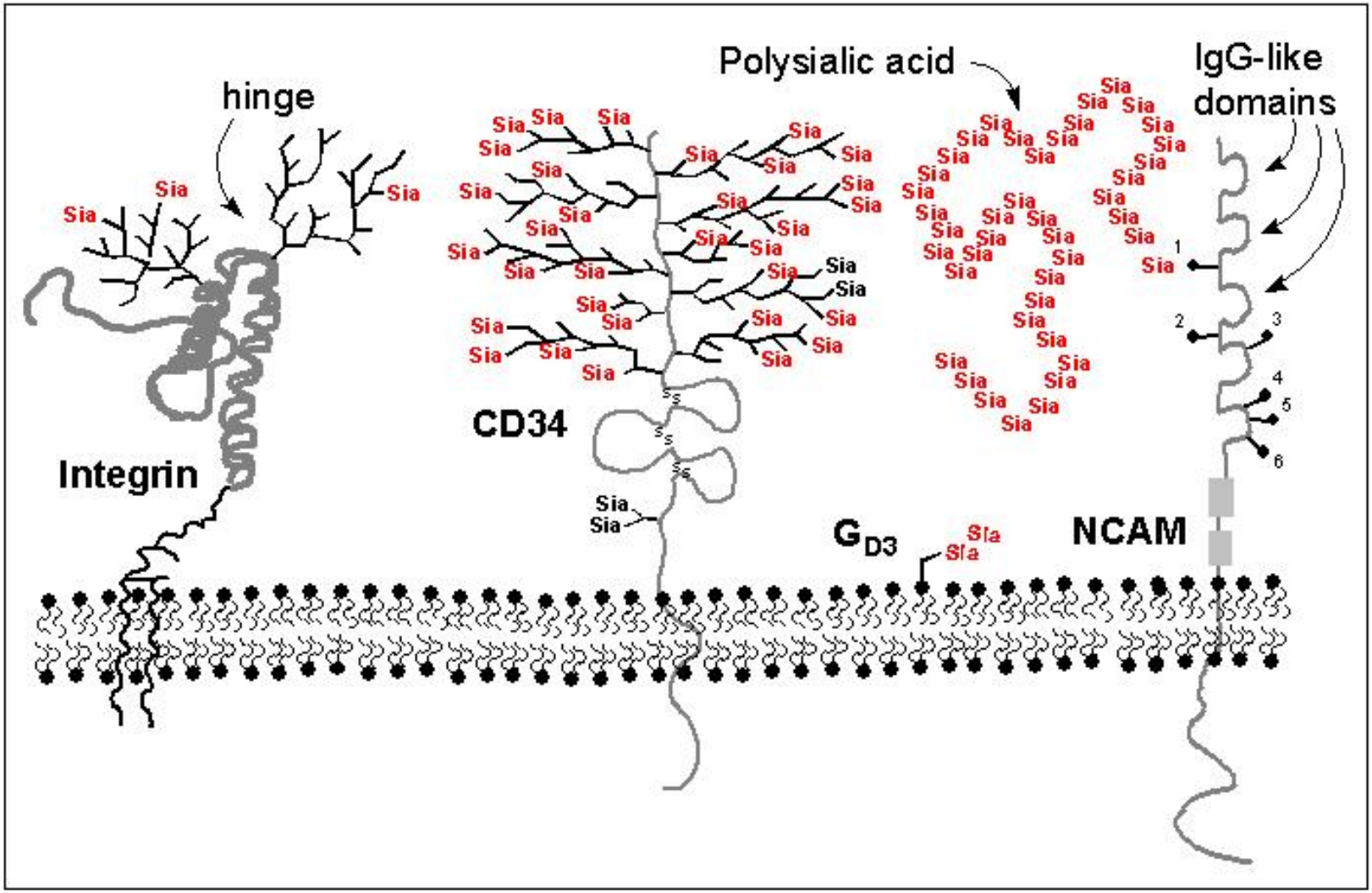
Capsular Polysaccharides of *N. meningitidis*

A	
B	→8)-αD-NeupNAc(2→
C	
C(OAc-)	→9)-αD-NeupNAc(2→
W-135	→6)-αD-Galp(1→4)-αD-NeupNAc(2→
Y	→6)-αD-Glcp(1→4)-αD-NeupNAc(2→(contains OAc groups)

Neu*p*NAc: N-acetyl neuraminic acid – a “Sialic acid”



Sialic acid is often the terminal saccharide in membrane glycoproteins



K. Yarema: *http://www.bme.jhu.edu/~kjyarema/monosaccharides/*

Molecular mimicry in capsular polysaccharides – danger of autoimmune response

- Some bacterial polysaccharides have the same structures as glycans expressed by man, and such polysaccharides have little or no immunogenicity
- Examples include

  - *Neisseria meningitidis* Group B and *E. coli* K1 (same in fetal brain glycoprotein)

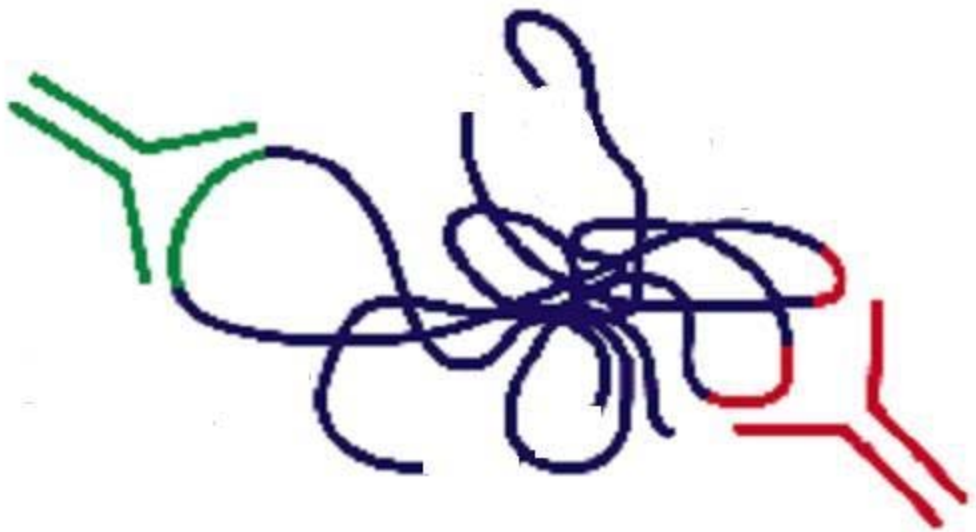
  - *E. coli* K5 (same as precursor of heparin)
- Vaccine manufacture from such polysaccharides is difficult
- Such vaccines carry the risk of a dangerous autoimmune response

Encapsulated bacterial serotypes causing Meningitis in Infants

• *Neisseria meningitidis*	A, B, C, W135 and Y
• Group B *Streptococcus*	Ia, Ib II, III and V
• *Haemophilus influenzae*	b
• *Streptococcus pneumoniae*	4,6,9,14,18,19,23

~100 strains of *Streptococcus pneuomoniae* have been identified and typed, but <20% cause serious disease such as pneumonia and meningitis

Polyvalent antibodies need to be generated against all the (dangerous) serotypes



… and this is a challenge

But this is only one of the challenges!

1. **Effectiveness of polysaccharide vaccine**
2. **Chemical purity of polysaccharide vaccine**
3. **Defined and reproducible molecular weight or molecular weight distribution**
4. **Stability of the preparation**

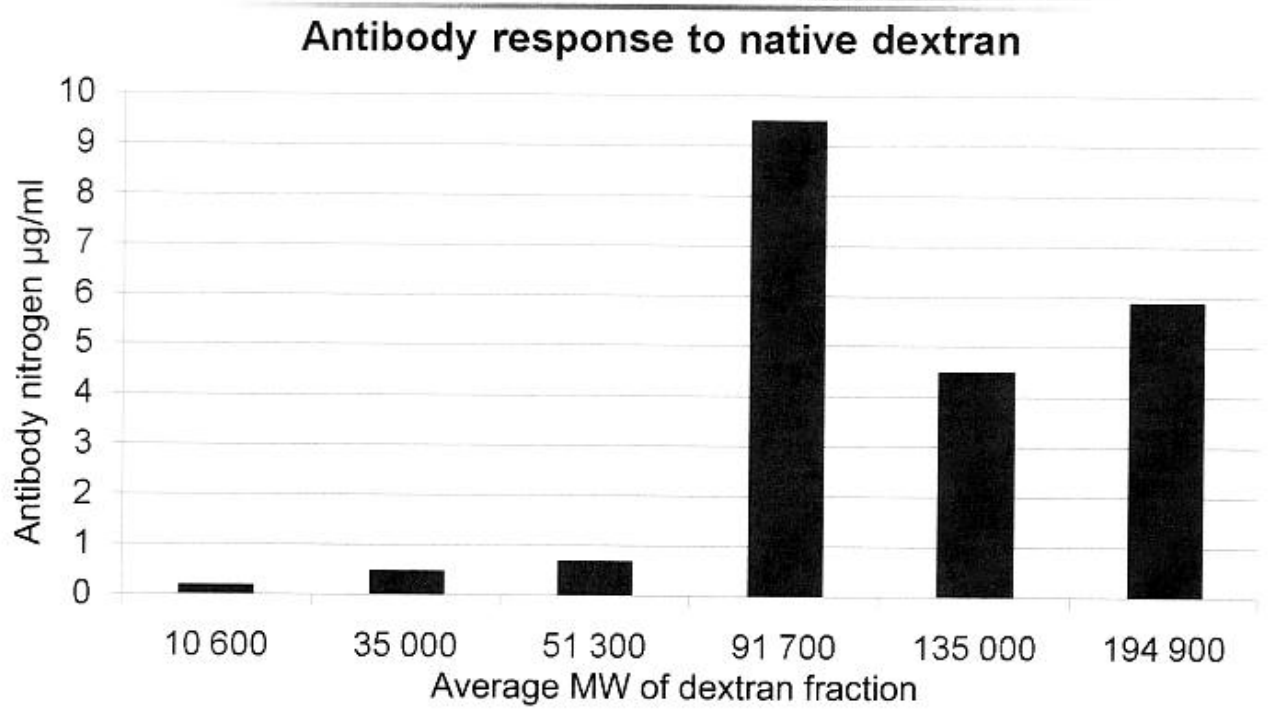
Licensed polysaccharide vaccines

Purified polysaccharide vaccines against three organisms are currently licensed. These are:

- *Salmonella enterica* Serovar Typhi (was *S. typhi*)
- *Neisseria meningitidis*
- Streptococcus pneumoniae (divalent, tri- and tetravalent)

Polysaccharide vaccine against *Haemophilus influenzae* type b (Hib) was briefly available in the USA before the introduction of better glycoconjugate vaccines in the 1980's

Size matters for T cell independent polysaccharides



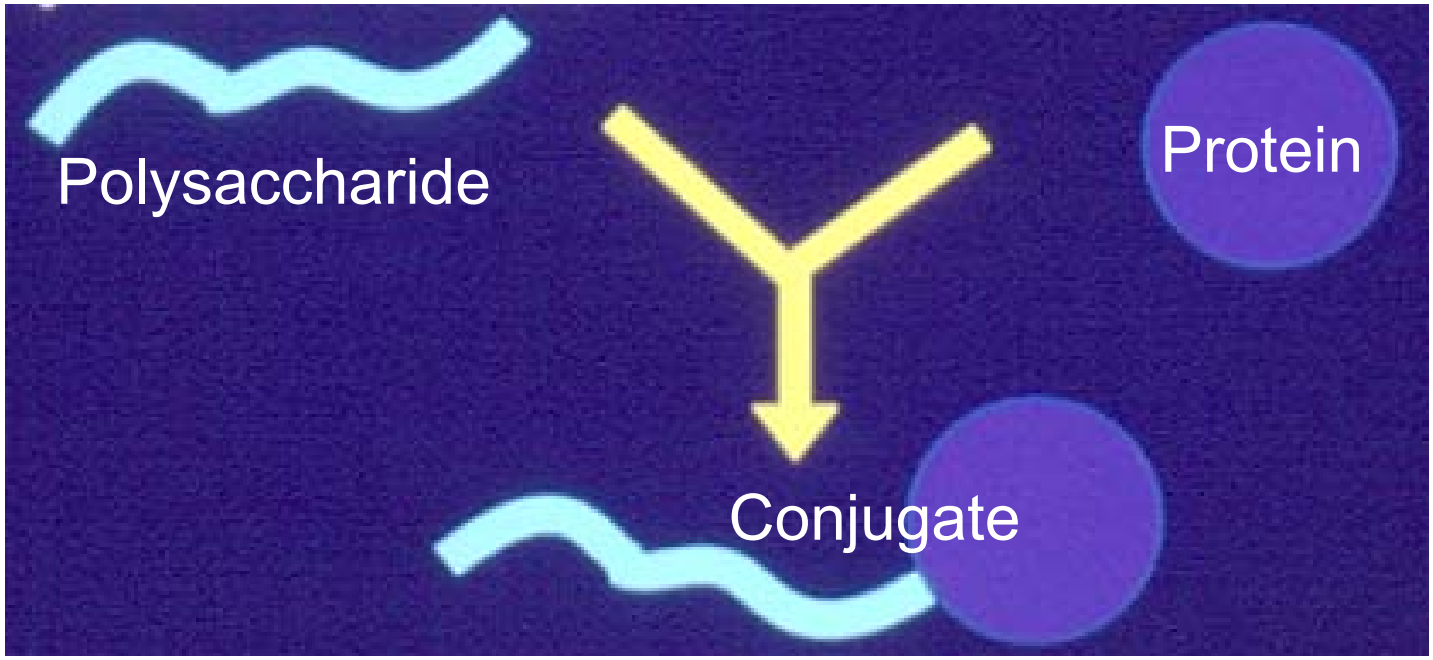
Kabat and Bezer (1958) The effect of variation on molecular weight on the antigenicity of dextran in man. Arch. Biochem. 78: 306-313

courtesy of Dr. Chris Jones, NIBSC London

Flaws in Efficiency of Polysaccharide Vaccines

1. Poor protection in infants (50% of all cases of bacterial meningitis)
2. No long lasting immunity: generate IgM response rather than IgG response in infants (T-cell independent, no memory effect) & continued vaccination can lead to low responsiveness

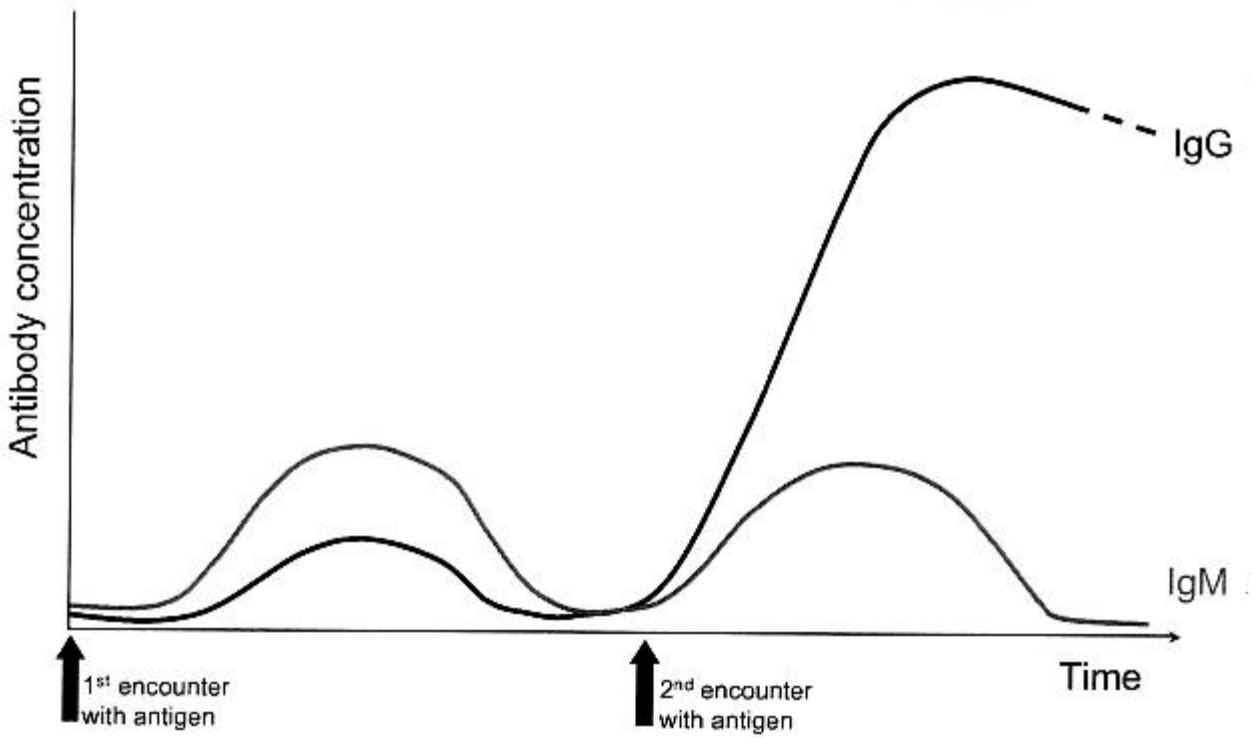
Thus use limited to outbreak control, temporary high risk groups when at risk (military recruits) or every 5 years (typhoid for travellers); for pneumococcal control in elderly, about every 5 years; for endemic typhoid control (every 3 years)



Conjugate polysaccharide vaccines:

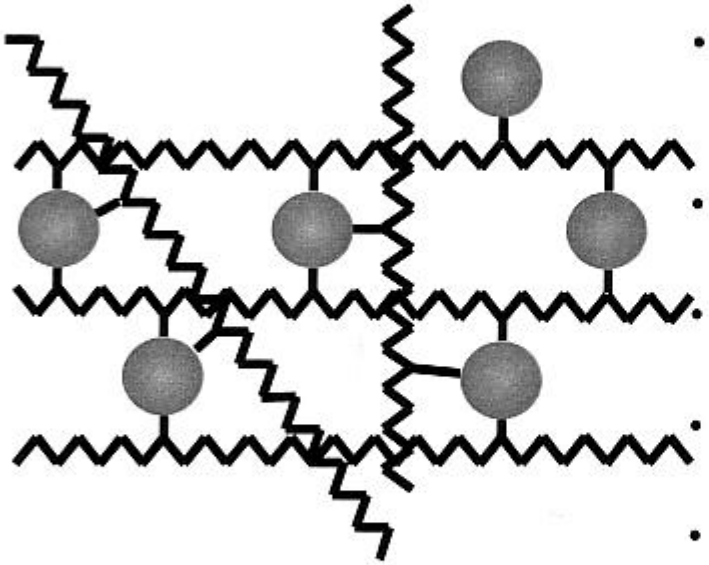
Covalent linking to protein carriers to conjugate vaccines

Dynamics of immune response



courtesy of Dr. Ian Feavers, NIBSC London

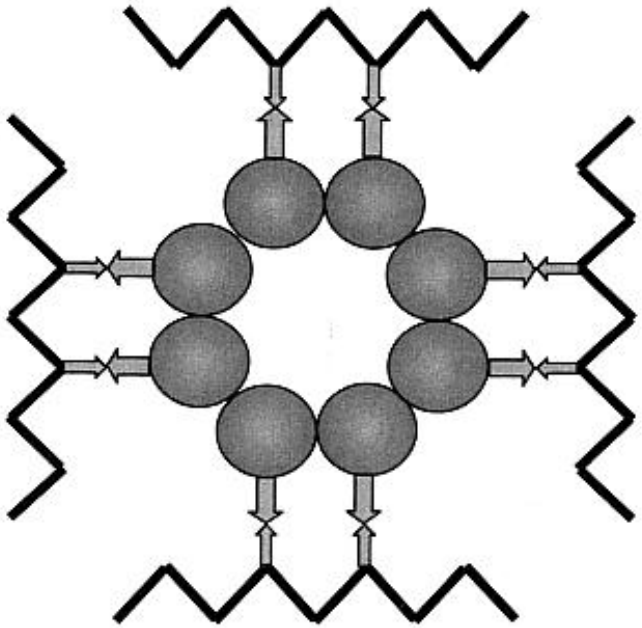
Three structural types of glycoconjugate vaccine – Crosslinked networks



- Produced by random activation of high mass polysaccharides, with multiple activations per chain.
- Coupled to carrier protein through non-specific chemistry
- Each polysaccharide chain attached to multiple carrier proteins
- Each carrier protein coupled to multiple polysaccharide chains
- Often used with tetanus toxoid as carrier protein
- Net work of high mass (typically 5MDa for a Hib conjugate)

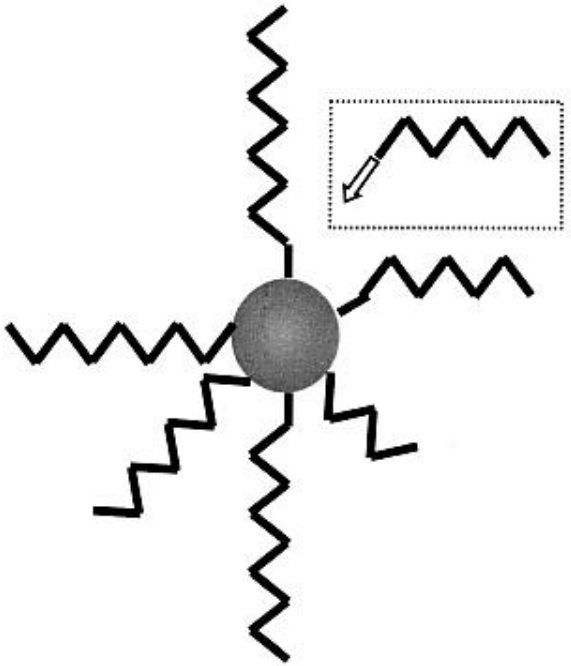
courtesy of Dr. Chris Jones, NIBSC London

Three structural types of glycoconjugate vaccine – Vesicle-based vaccines (PedVaxHib)



- Produced by random activation of reduced mass polysaccharides, with multiple activations per chain.
- "Carrier protein" is an LPS-depleted mixture of outer membrane proteins
- Vesicle nature of OMPs creates a high mass complex.
- OMPs were chosen to provide complementary immunological detection.
- Hard to make materials on a very large scale.

Three structural types of glycoconjugate vaccine – neoglyconjugates



- Produced by coupling monofunctional oligosaccharides
- Produced by coupling bifunctional oligosaccharide at low coupling efficiencies.
- Either direct or indirect attachment to carrier protein (ie. through linker)
- Most often used with CRM197 as carrier, producing a conjugate with MW ca. 90kDa and 30% w/w carbohydrate
- Similar to a typical plasma protein
- Also called fuzzy balls

Polysaccharide Conjugate Vaccines:

1. Stimulate T-dependent immunity
2. Enhanced antibody production, especially in infants
3. Repeat “booster” doses give increased response

An active area for research & development!

Historical timelines for glycoconjugate vaccines

- Discovery that antigens are carbohydrates
- Attempted use of CPS as immunogens
- 1931: Conjugation to protein tried – Avery and Goebel
- 1945: First clinical trial of polysaccharide vaccine - McLeod
- Introduction of pneumococcal CPS vaccine (1970s)
- Introduction of Hib conjugate vaccine (late 1980s)
- Introduction of meningococcal C conjugate vaccine in UK (late 1990s)
- Licensing of heptavalent pneumococcal conjugate vaccine (2000)
- Introduction of quadrivalent Men A,C,Y,W135 conjugate vaccine in USA (2005)
- Now – release of new vaccines by GSK

Meningococcal Conjugate Vaccine

- Menactra™ (Sanofi Pasteur)
- Quadrivalent (serogroups A, C, Y, W-135)
- Approved for persons 11-55 years of age
- Administered by intramuscular injection

Approved by FDA January 2005

Quality control: Characterisation

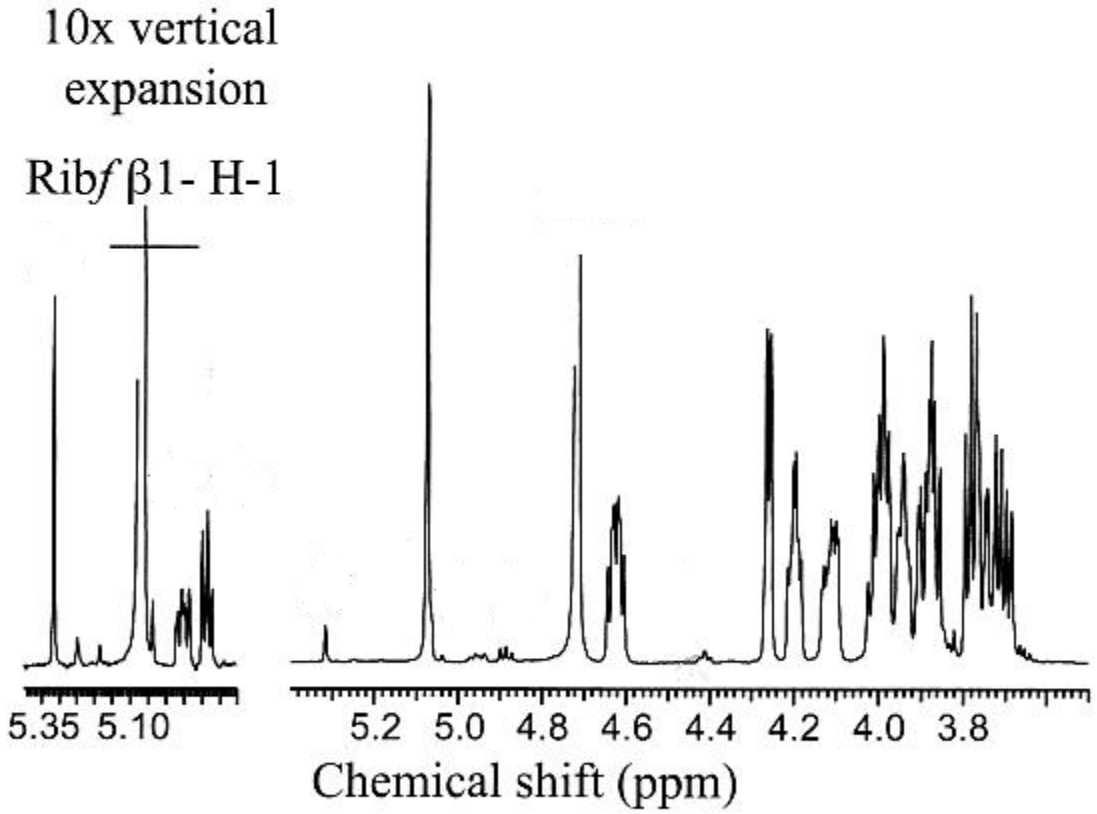
There are increasing demands for detailed characterisation of biopharmaceutical products, particularly vaccines:

- Physicochemical and immunochemical (serology, immunogenicity) characterisation of components – polysaccharide and carrier protein
- Physicochemical and immunochemical characterisation of the conjugate

Physico-chemical Methods:

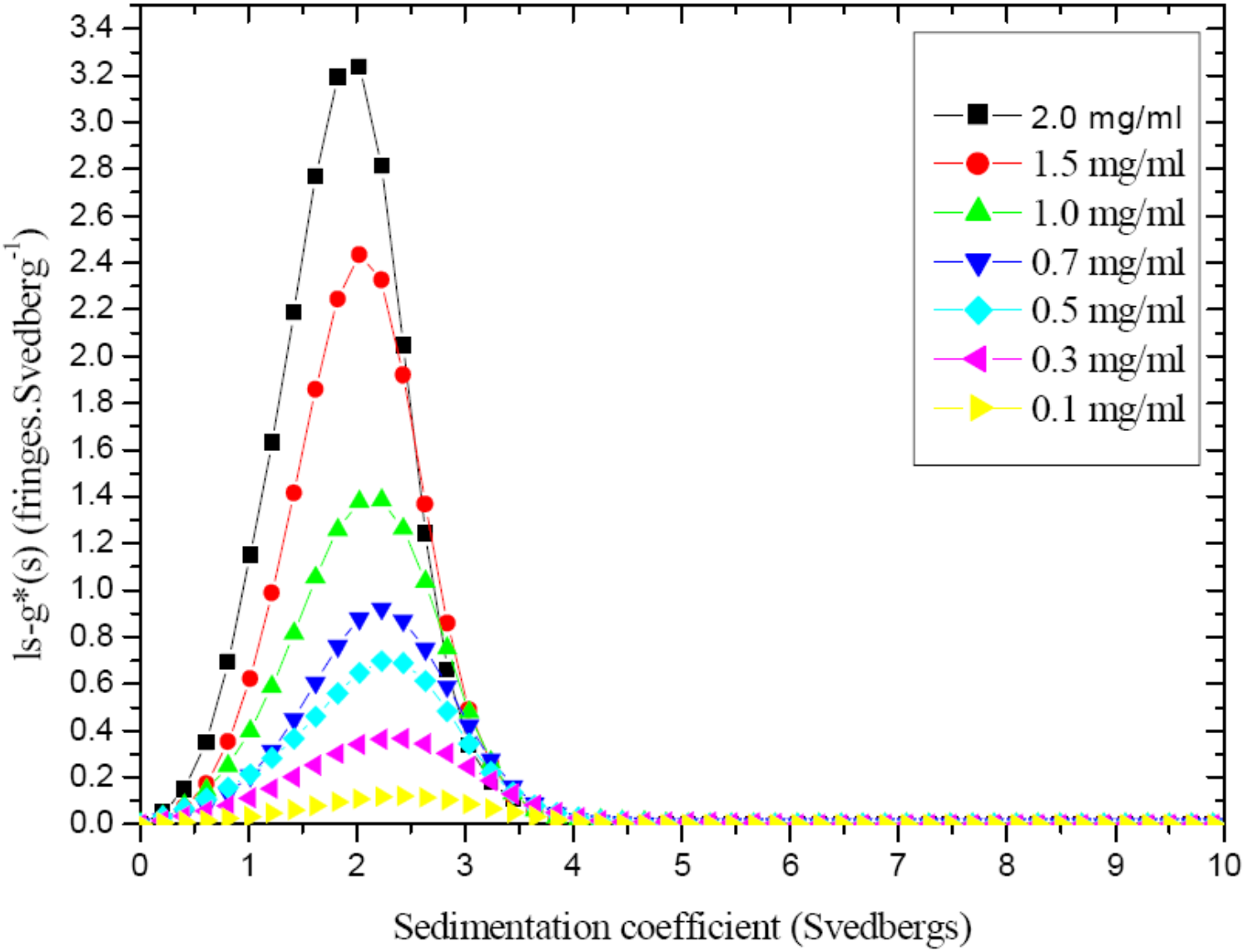
- Identity/Structure: NMR, ESMS (protein component)
- Polysaccharide:protein ration (NMR, HPAE)
- Purity: NMR, AUC
- Size distribution: SEC-MALLS, AUC
- Conformation/Flexibility: Viscometry ([η]), AUC (s, M_w), SEC-MALLs (R_g, M_w)
- Stability: NMR, AUC, GPC, viscometry, CD/fluorescence (protein)
- Location of carbohydrate chains (proteolysis-HPLC, ESMS)
- Amount of unconjugated saccharide (chemical assay)

500MHz Proton NMR Spectrum of Hib PRP



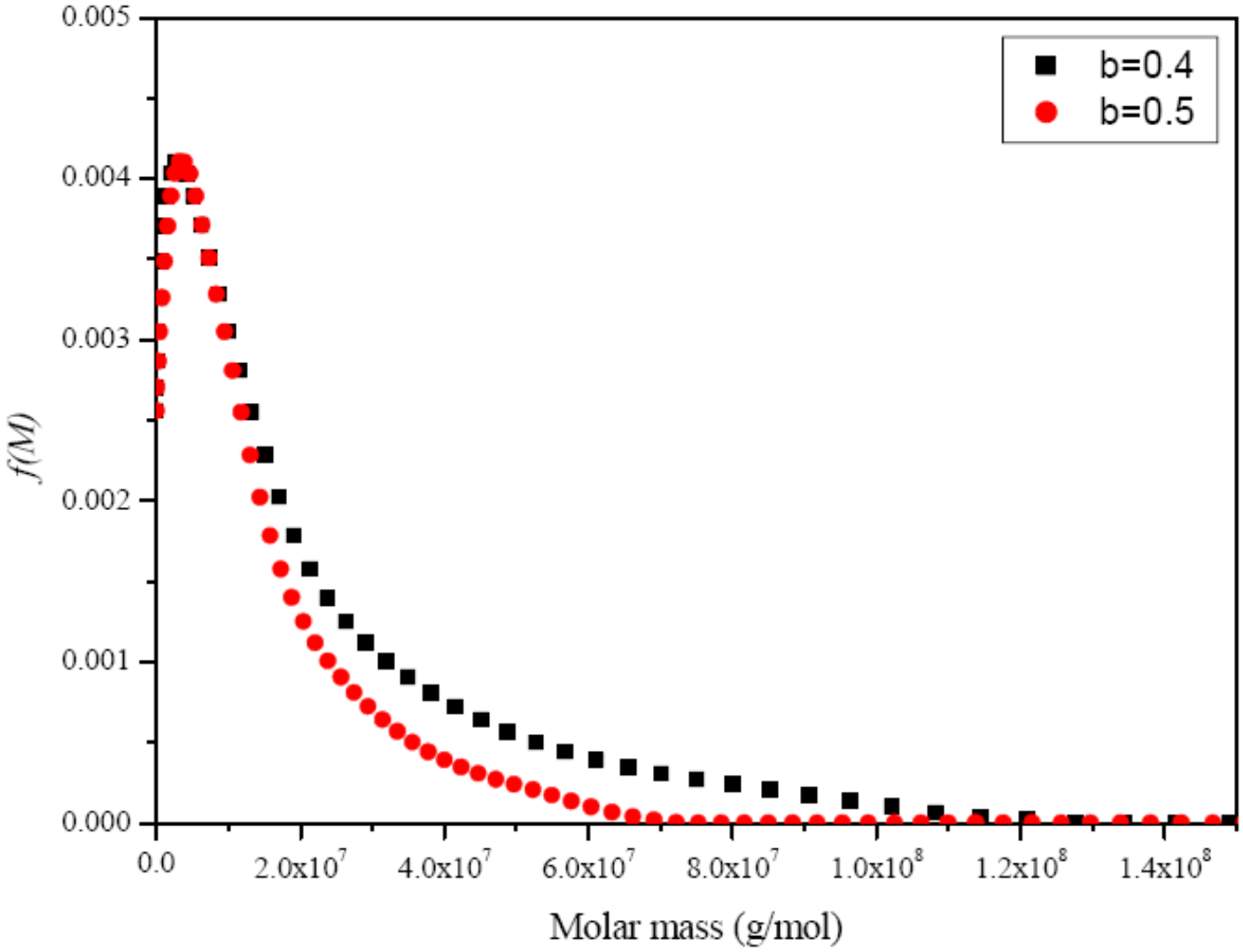
courtesy of Dr. Chris Jones, NIBSC London

Purity: sedimentation coefficient distribution



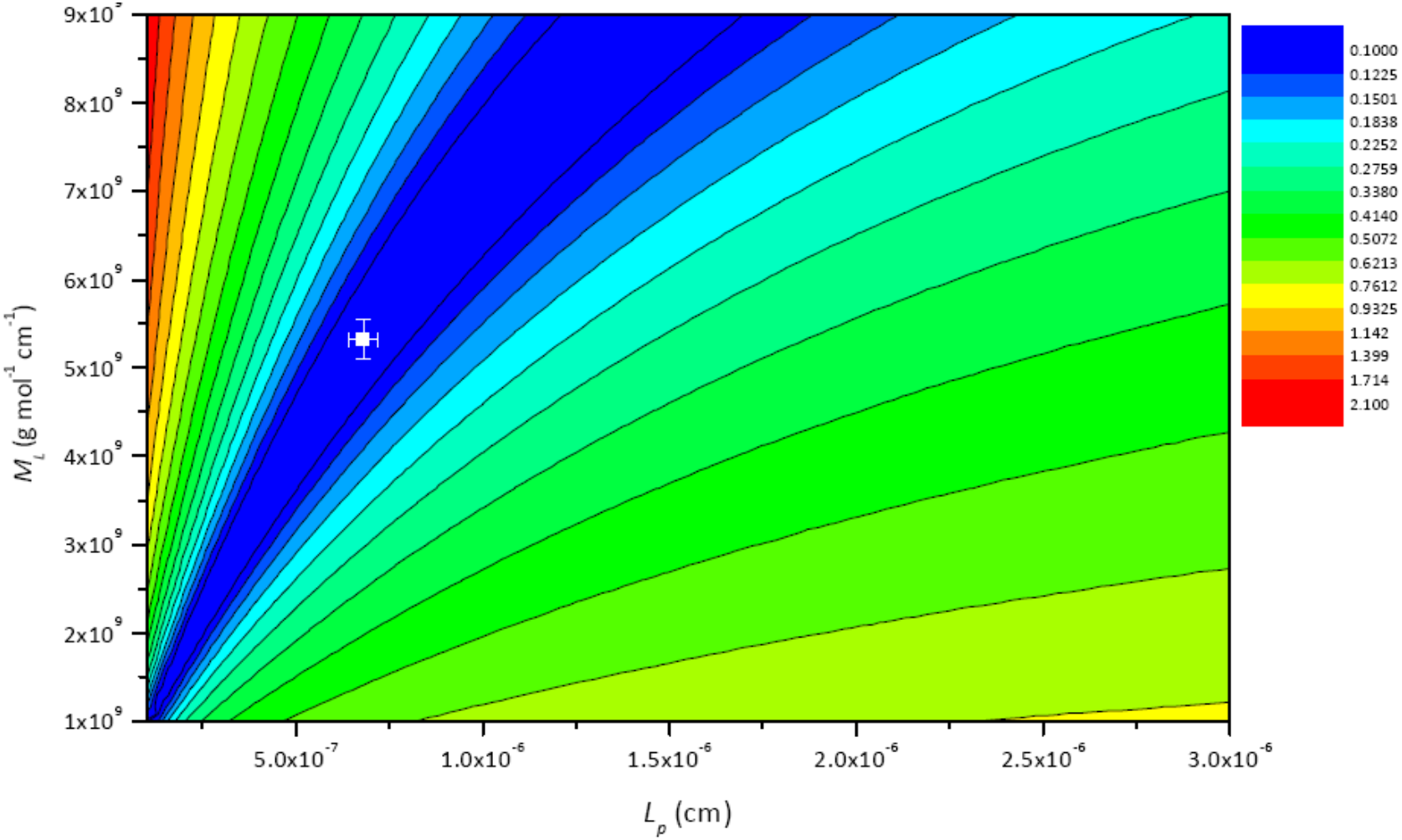
for a glycoconjugate vaccine

Molecular weight Distribution of a very large glycoconjugate vaccine using the f(M) sedimentation velocity method



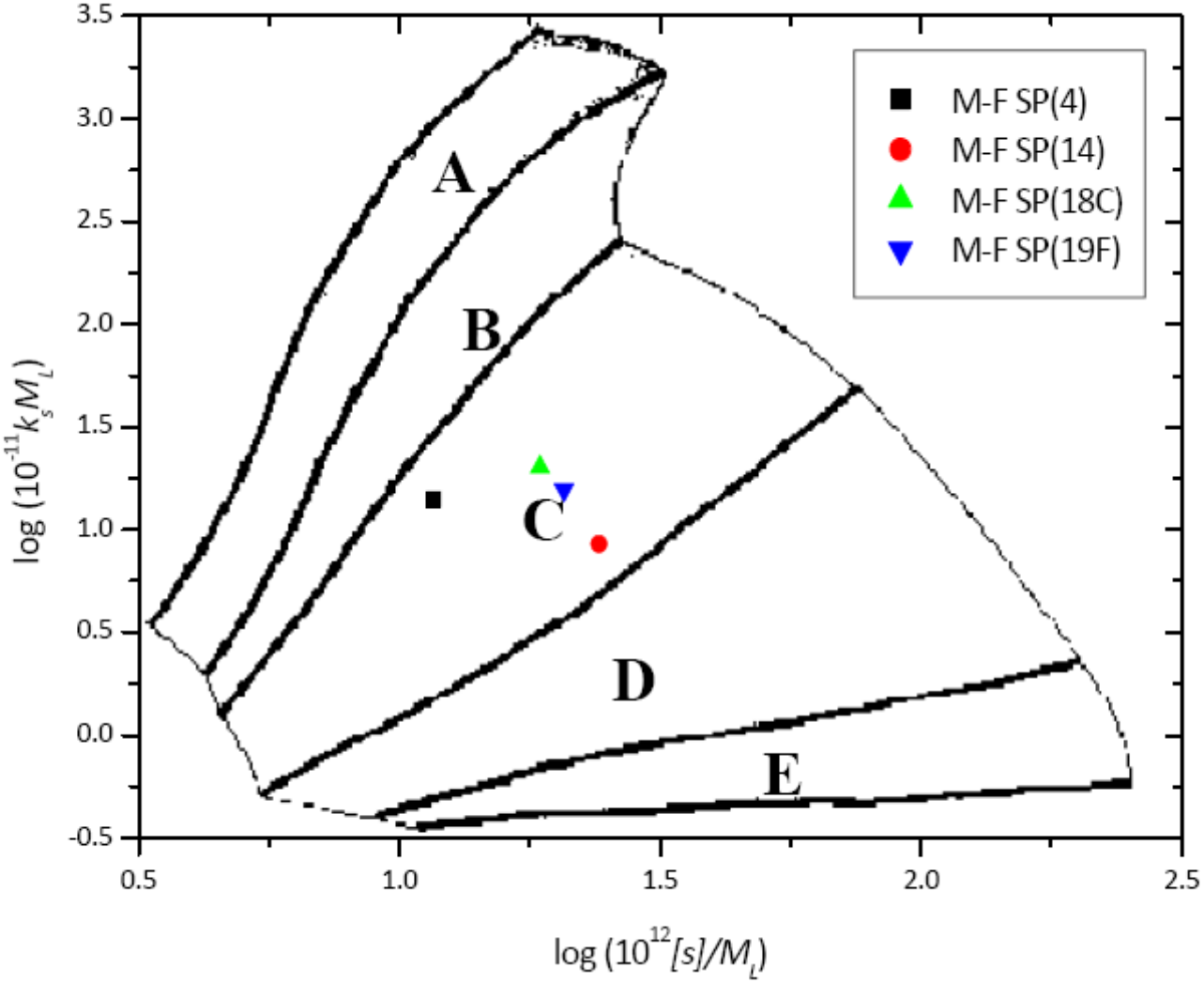
Two plausible values for the conformational parameter b in $s = KM^b$ used. From Harding, Morris and Abdelhameed (2010) *Macromolecular Bioscience* (in press)

Conformational Flexibility: persistence length L_p determination



Global analysis of hydrodynamic data for a *Streptococcal* polysaccharide: L_p ~ 6.8nm, M_L ~ 537 g.mol^{-1}.cm^{-1}: quite flexible!

Conformational zoning plot for 4 different Streptococcal polysaccharides



Based on sedimentation and mass per unit length data.

All are Zone C (Semi-flexible). **A - extra rigid rod; B – rigid rod; C- semi flexible; D- random coil; E: globular/branched**

Reference

J. Suker, M.J. Corbel, C. Jones, I.M. Feavers and B. Bolgiano,"Standardisation and control of meningococcal C conjugate vaccines", *Expert Review of Vaccines*, **2004,** 3, 89-96