

## 4th Year Biochemistry -- Glycobiology option.

### Dr M.R. Wormald -- Structure and conformation of oligosaccharides

The lecture is based loosely on

Glycoproteins: glycan presentation and protein-fold stability.  
M.R. Wormald and R.A. Dwek (1999) *Structure*, 7, R155-R160.

and

Conformational studies of oligosaccharides and glycopeptides: Complementarity of NMR, X-ray crystallography, and molecular modelling.  
M.R. Wormald, A.J. Petrescu, Y.-L. Pao, A. Glithero, T. Elliott and R.A. Dwek (2002) *Chemical Reviews*, published on-line, due out March 2002.

and the references therein. These can be obtained from computers within the Department at <http://www.bioch.ox.ac.uk/glycob/publications.html>, references 324 and 432.

The following are selected other references

- ☒ -- recommended and easy (at least as easy as they come)
- ☐ -- recommended but a bit more advanced

#### **Oligosaccharide conformation/dynamics :-**

##### **General --**

- ☒ Polysaccharide shapes  
Rees (1977)
- ☒ Three dimensional structure of oligosaccharides explored by NMR and computer simulations  
Homans (1995) in "Glycoproteins", pp.67-86, ed. Montreuil, Vliegthart and Schachter.

##### **Computer modeling --**

- Computer modeling of carbohydrates: an introduction  
French and Brady (1990) **Computer modeling of carbohydrate molecules**, 1-19
- ☐ Computational carbohydrate chemistry: what theoretical methods can tell us  
Woods (1998) **Glycoconjugate Journal**, 15, 209-216
- Oligosaccharide structures: Theory versus experiment  
Imberty (1997) **Current Opinion In Structural Biology**, 7, 617-623

##### **X-ray studies --**

- ☐ A statistical analysis of N- and O-glycan linkage conformations from crystallographic data.  
Petrescu, et al. (1999) **Glycobiology**, 9, 343-352.

##### **NMR studies --**

Tertiary structure of N-linked oligosaccharides.  
Homans, et al. (1987) **Biochemistry**, 26, 6553-6560.

Solution conformation of the branch points of N-linked glycans: synthetic model compounds for tri-antennary and tetra-antennary glycans  
Cumming, et al. (1987) **Biochemistry**, 26, 6655-6663

Uncertainties in structural determination of oligosaccharide conformation using measurements of nuclear Overhauser effects.

Wooten, et al. (1990) **Carbohydrate Research**, 203, 13-17

- ⊗ Primary sequence dependence of conformation in oligomannose oligosaccharides.  
Wooten, et al. (1990) **European Biophysics Journal**, 18, 139-148

- ⊗ The solution conformation of the Le<sup>x</sup> group  
Wormald, et al. (1991) **Biochemical and Biophysical Research Communications**, 180, 1214-1221

The systematic use of negative nuclear Overhauser constraints in the determination of oligosaccharide conformations: application to sialyl-Lewis X.

Wormald and Edge (1993) **Carbohydrate Research**, 246, 337-344

- ⊗ The solution NMR structure of glucosylated N-glycans involved in the early stages of glycoprotein biosynthesis and folding  
Petrescu, et al. (1997) **The EMBO Journal**, 16, 4302-4310

The high degree of internal flexibility observed for an oligomannose oligosaccharide is not reproduced in the overall topology of the molecule.

Woods, et al. (1998) **Eur. J. Biochem.**, 258, 372-386.

#### · Others --

Differential flexibility in three branches of an N-linked triantennary glycopeptide

Wu, et al. (1991) **Proceedings of the National Academy of Sciences, USA**, 88, 9355-9359

### **Glycopeptide/Glycoprotein Structure :-**

- ☑ The structural role of sugars in glycoproteins  
Wyss and Wagner (1996) **Current Opinion in Biotechnology**, 7, 409-16
- ☑ Modulation of protein structure and function by asparagine-linked glycosylation  
O'Connor and Imperiali (1996) **Chemistry and Biology**, 3, 803-12

#### · Computer modelling --

- ☑ Protein surface oligosaccharides and protein function  
Woods, et al. (1994) **Nature Structural Biology**, 1, 499-501

#### · X-ray studies --

Crystallographic refinement and atomic models of human Fc fragment and its complex with Fragment B of Protein A from *Staphylococcus aureus* at 2.9 and 2.8 Å resolution

Deisenhofer (1981) **Biochemistry**, 20, 2361-2370

- ⊗ The three-dimensional structure of the carbohydrate within the Fc fragment of immunoglobulin G.  
Sutton and Phillips (1983) **Biochemical Society Transactions**, 11, 130-132

Human leukocyte and porcine pancreatic elastase: X-ray crystal structures, mechanism, substrate specificity and mechanism-based inhibitors.

Bode, et al. (1989) **Biochemistry**, 28, 1951-1963

Structure of a legume lectin with an ordered N-linked carbohydrate in complex with lactose  
Shaanan, et al. (1991) **Science**, 254, 862-866

**NMR studies --**

- ⊗ Monitoring the carbohydrate component of the Fc fragment of human IgG by  $^{13}\text{C}$  nuclear magnetic resonance spectroscopy.  
Rosen, et al. (1979) **Molecular Immunology**, 16, 435-436
- ⊗ Effects of glycosylation on the conformation and dynamics of O-linked glycoproteins: carbon-13 NMR studies of ovine submaxillary mucin  
Gerken, et al. (1989) **Biochemistry**, 28, 5536-5543

The conformational effects of N-glycosylation on the tailpiece from serum IgM.  
Wormald, et al. (1991) **European Journal of Biochemistry**, 198, 131-139

Effects of glycosylation on protein conformation and amide proton exchange rates in RNase B.  
Joao, et al. (1992) **FEBS Letters**, 307, 343-346

- ☑ The conformational effects of N-linked glycosylation  
Edge, et al. (1993) **Biochemical Society Transactions**, 21, 452-455

Effects of glycosylation on protein structure and dynamics in ribonuclease B and some of its individual glycoforms  
Joao and Dwek (1993) **European Journal of Biochemistry**, 218, 239-244

$^1\text{H}$  NMR studies on an Asn-linked glycopeptide  
Davis, et al. (1994) **Journal of Biological Chemistry**, 269, 3331-3338

- ⊗ Variations of oligosaccharide-protein interactions in immunoglobulin G determine the site-specific glycosylation profiles and modulate the dynamic motion of the Fc oligosaccharides  
Wormald, et al. (1997) **Biochemistry**, 36, 1370-1380

Conformation and function of the N-linked glycan in the adhesion domain of human CD2.  
Wyss, et al. (1995) **Science**, 269, 1273-1278.

**Other techniques –**

Internal Movements in Immunoglobulin Molecules.  
Nezlin (1990) **Advances in Immunology**, 48, 1-40

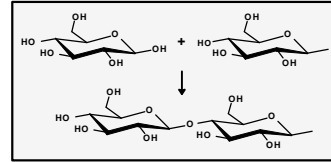
- ⊗ The dynamics of glycan-protein interactions in immunoglobulins. Results of spin label studies.  
Sykulev and Nezlin (1990) **Glycoconjugate Journal**, 7, 163-182

# Structure and Conformation of Oligosaccharides

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## Conformations of oligosaccharides

Formed by linking monosaccharides via a glycosidic linkage



Monosaccharide rings are rigid and have a well-defined conformation independent of environment

The conformational analysis of an oligosaccharide reduces to determining the torsion angles about each glycosidic linkage (2 or 3 torsion angles per residue).

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## Possible roles for protein glycosylation

Alter the biophysical properties of proteins -

- ⇒ Membrane attachment via a GPI anchor
- ⇒ Changes in solubility
- ⇒ presentation at membranes
- ⇒ protein aggregation / association
- ⇒ protease resistance

Alter protein structure / properties -

- ⇒ Changes in tertiary/quaternary structure/flexibility
- ⇒ Restrict or block access to active sites or possible antigenic regions

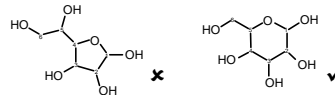
Provide specific recognition targets -

- ⇒ Interactions with calnexin/calreticulin during protein folding
- ⇒ Interactions with lectins
- ⇒ Epitopes recognised by the innate/acquired immune system

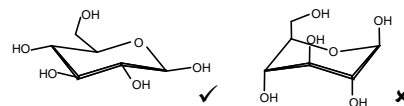
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## Conformations of monosaccharide rings

Ring size – determined by ring strain



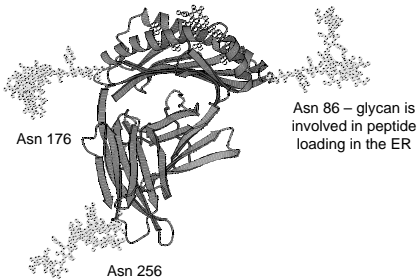
Ring conformation – determined by steric interactions between substituents



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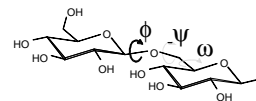
## Mouse MHC Class I

Glycopeptide – presented to T-cell



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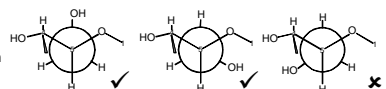
## Conformations of saccharide linkages



$\phi$  – largely determined by the exo-anomeric effect

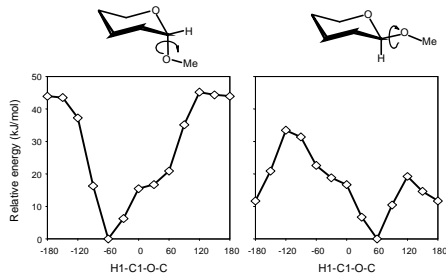
$\psi$  – largely determined by non-bonded interactions

$\omega$  – gauche preference determined by solvation



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## Conformations of saccharide linkages - Exo anomeric effect



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## Conformations of saccharide linkages - information available

### X-ray crystallography -

Most oligosaccharides and glycoproteins either do not crystallise or give no resolvable electron density for the glycan. Glycans that can be seen are incomplete.

⇒ Can give average properties of linkages.

### Nuclear Magnetic Resonance Spectroscopy -

Experimental structural parameters (inter-nuclear distances and torsion angles) averaged on a msec timescale.

⇒ Can be interpreted in terms of a structure if it is assumed that there is a single well-defined conformation.

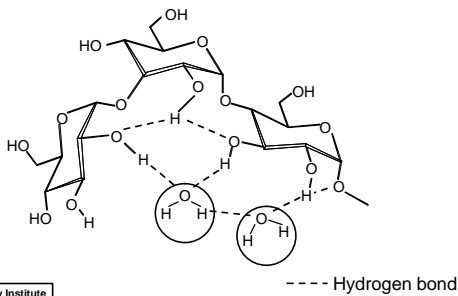
### Molecular Dynamics Simulations -

Theoretical dynamic structures on a nsec timescale.

⇒ Can be interpreted in terms of a structure if it is assumed that the theory is correct.

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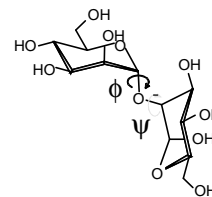
## Conformations of saccharide linkages - Hydrogen bonding and solvent effects



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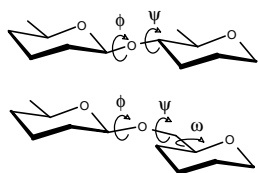
## Man $\alpha$ 1-2 Man linkage

Man $\alpha$ 1-2Man linkages occur in oligomannose type N-glycans, polysaccharides such as mannan and GPI anchors.



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## Conformations of saccharide linkages - information required to define linkage structure



### For each distinct conformer -

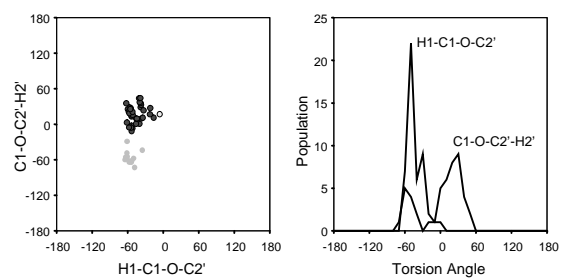
- Average linkage torsion angles
- Fluctuations around the average position

### For the ensemble of conformers -

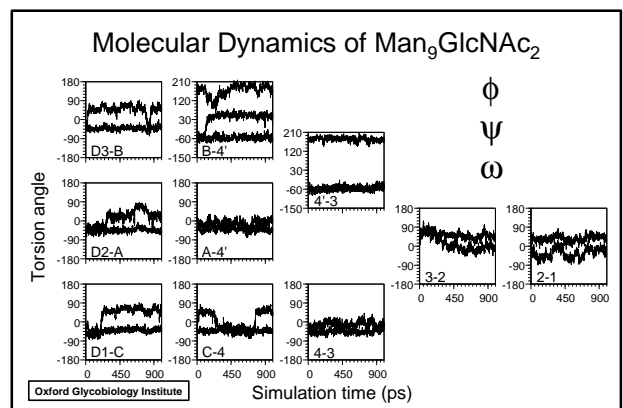
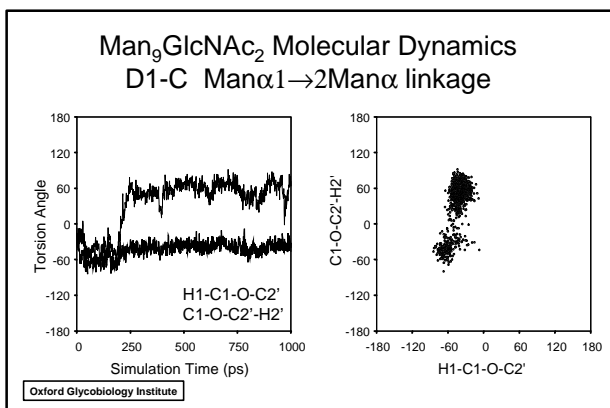
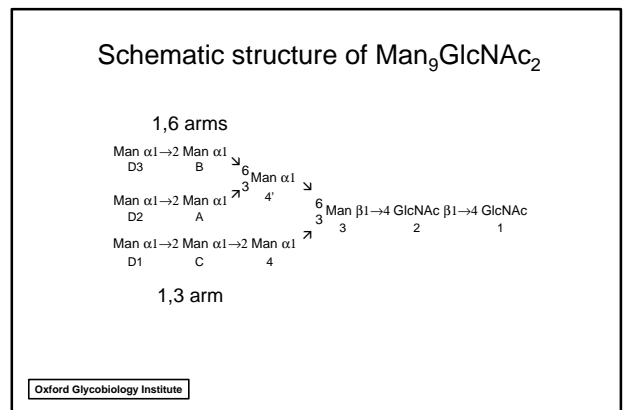
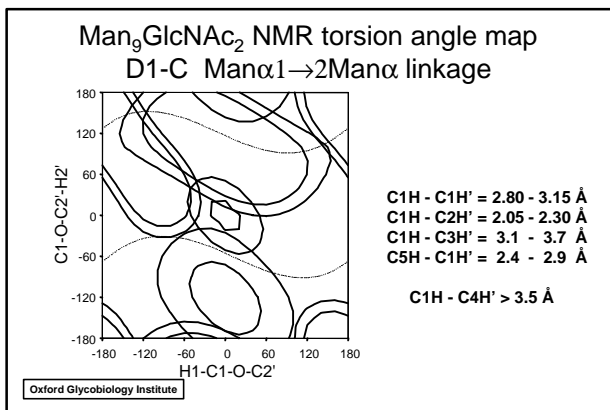
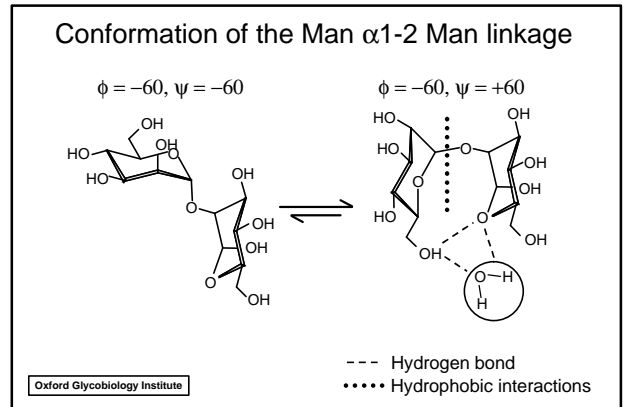
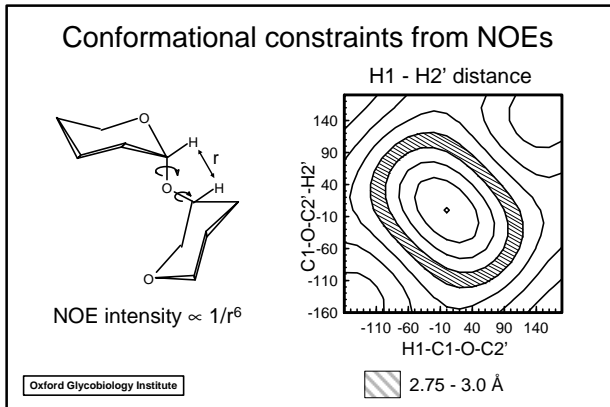
- Relative populations of the different conformers
- Rate of transition between the conformers

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## Crystallographic glycosidic linkage structures- Man $\alpha$ 1 2Man linkage

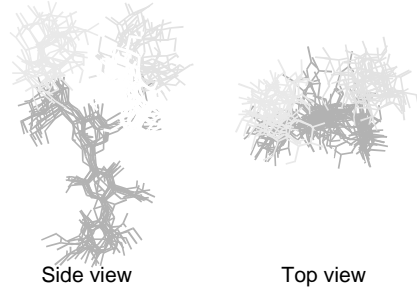


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## Molecular Dynamics of Man<sub>9</sub>GlcNAc<sub>2</sub>

Overlay of structures (all atoms) from 1000 ps



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## Factors determining bio-polymer structure

### Proteins -

Secondary structure – molecular orbital effects and sequential hydrogen bonds.  
Tertiary structure – non-sequential attractive forces and solvent interactions.

### DNA –

Dimerisation – cross-strand hydrogen bonds.  
Tertiary structure – sequential attractive forces and solvent interactions.

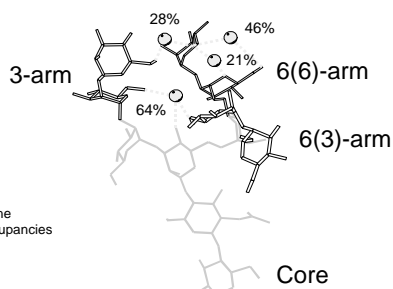
### Oligosaccharides -

Linkage conformation – molecular orbital effects, steric repulsion and solvent interactions.  
Tertiary structure – non-sequential repulsive forces and solvent interactions.

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## Molecular Dynamics of Man<sub>9</sub>GlcNAc<sub>2</sub>

- water mediated hydrogen bonds between the arms



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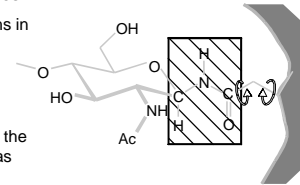
## Conformational properties of the Glycan-Asn linkage

### NMR studies on model peptides -

- H-C-N-H unit is planar and trans in solution

### X-ray crystallography -

- Cα-Cβ and Cβ-Cγ bonds show the same range of conformations as for unsubstituted Asn residues



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## Structure of Man<sub>9</sub>GlcNAc<sub>2</sub>

Individual linkages are flexible on a sub-nsec timescale, many showing more than one conformer.

The properties of a linkage depend on its position in the molecule.

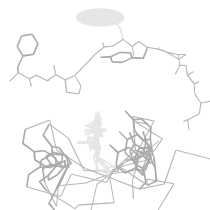
The overall topology of the molecule is more conserved than the structures of the individual linkages.  
⇔ correlated motions of the linkages (due to constraints of the solvent shell ??)

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## Crystal and NMR structures of K2G

X-tal structure of K2G in complex with MHC Class I

Solution structure of K2G from NMR and modelling

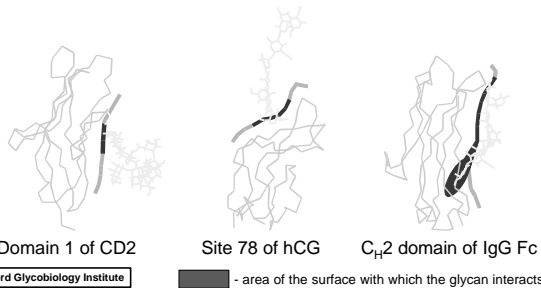


The presence of the GlcNAc causes preferential clustering of aromatic side chains, leading to reduced configurational entropy of the peptide backbone.

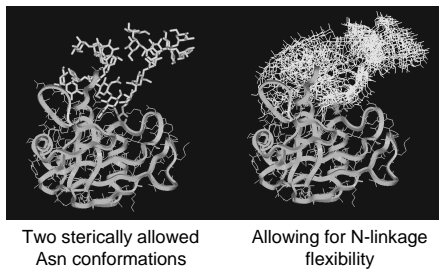
In this case, the clustering places the Phe, Tyr and GlcNAc on the same side of the peptide as seen in the complex with MHC.

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The presentation of the glycan is determined by interactions with the peptide surface



## Ribonucleases B glycan dynamics



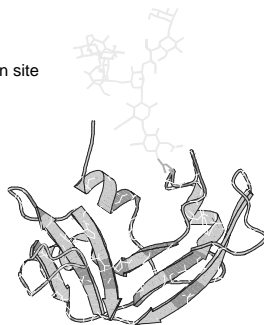
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## Ribonucleases A and B H/D-exchange

Ribonuclease A – aglycosyl  
Ribonuclease B – single glycosylation site

Glycosylation effects the backbone flexibility in all regions of the protein

■ H/D exchange reduced in ribonuclease B compared to A



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