UNIVERSITY OF YORK
BSc Stage 3 Degree Examinations 2017-18

Department:
BIOLOGY

Title of Exam:
Glycobiology

Time allowed: 2 hours
Total marks available for this paper: 100

This paper has two parts:

Section A: Short Answer / Problem / Experimental Design questions (50 marks)
- Answer all questions in the spaces provided on the examination paper

Section B: Essay question (marked out of 100, weighted 50 marks)
- Answer either question A or question B
- Write your answer on the separate paper provided and attach it to the back of the question paper using the treasury tag provided
- The marks available for each question are indicated on the paper

For marker use only:

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Total as %

DO NOT WRITE ON THIS BOOKLET BEFORE THE EXAM BEGINS
DO NOT TURN OVER THIS PAGE UNTIL INSTRUCTED TO DO SO BY AN INVIGILATOR
1. The Glut4 glucose transporter responds to insulin signalling by translocating to the plasma membrane. Once the insulin stimulus stops, Glut4 is endocytosed to be stored in intracellular vesicles awaiting the next insulin stimulus. Glut4 is glycosylated, and the following experiments look at the effects of this glycan on the insulin responsiveness of Glut4.

**Figure 1.** The amount of cell surface Glut4 was determined and normalized to the first bar in each of the two graphs.

(A) Cells were untreated (-KIF) or treated with the mannosidase I inhibitor kifunensine (+KIF). Both sets of cells were serum starved for 3 hours before treatment with insulin (black bars) for 30 min. White bars show controls not treated with insulin.

(B) Cells were serum starved and then insulin treated as in ‘A’. Cells used for the left pair of bars express wild type (WT) Glut4, cells used for the other bars use the N57Q mutant.

a) Explain the significance of the N57Q mutation for this experiment. (4 marks)  
**LO1, 5**

Asparagine (N) can be the site of glycosylation (1 mark). Given the similar results seen with this mutation and an N-glycan processing inhibitor (2 marks), it is very likely that this mutation removes an N-glycan from Glut4 (1 mark).
Answered generally well, but some did not link the two parts of the figure.

b) The level of galectin-3 has been shown to decrease in the serum of diabetes patients. Based on the results of figure 1 formulate a hypothesis to explain what role galectin-3 could be playing in diabetes. (7 marks) 

LO1, 3

The figures show decreased cell surface amounts of Glut4 when its N-glycan is not processed beyond oligomannose state or when it is removed completely (2 marks). Gal-3 binds lactosamine (1 mark), and due to its homo-oligomeric nature clusters lactosamine containing cell-surface proteins (1 mark). Lactosamine is a feature found only in complex or hybrid N-glycans, but not in oligomannose ones (1 mark). Gal-3 could cluster Glut4 and decrease the endocytosis of the transporter following an insulin stimulus (2 marks).

Most highlighted the connection between Gal-3 and lactosamine, and also the fact that kifunensine inhibits mannosidase, but very few took the argument all the way through to comparing the effects seen with kifunensine, how it restricts the glycan from binding Gal-3.

c) Design an experimental strategy to analyse the monosaccharide composition of Glut4’s glycan. (4 marks)

First Glut4 needs to be isolated by immunoprecipitation (1 mark). Then the glycan can be removed from the protein by PNGaseF treatment and collected (1 mark). Finally the glycan can be either treated for total acid hydrolysis and the resulting carbohydrates analysed by GC-MS (2 marks), OR (alternative answer) repeated exoglycosidase treatment followed by MS of the glycan can yield composition (2 marks). Simple MS is not enough for the last part (will only gain 1 instead of 2 marks).

Generally well answered. Partial marks could be gained for mentioning beta-elimination as well as PNGase F (although Glut4 is not O-glycosylated, this was not obvious from the question). Proposing to apply glycosidases to permethylated glycans is not correct, and credit was reduced for these answers.
2. Fibroblasts from a newly discovered *N*-glycosylation CDG patient were analysed by glycan profiling. The profiles were compared to fibroblasts from a healthy volunteer. The table below shows the obtained data for the eight most abundant complex *N*-glycans.

<table>
<thead>
<tr>
<th>N-glycan</th>
<th>Proportion relative to all complex <em>N</em>-glycans</th>
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<tbody>
<tr>
<td></td>
<td>Patient</td>
</tr>
<tr>
<td>Hex₅HexNAc₄NeuAc₂Fuc</td>
<td>4.7 ± 0.6%</td>
</tr>
<tr>
<td>Hex₆HexNAc₄NeuAcFuc</td>
<td>1.3 ± 0.5%</td>
</tr>
<tr>
<td>Hex₅HexNAc₄NeuAc₂</td>
<td>39.0 ± 3.2%</td>
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<tr>
<td>Hex₆HexNAc₄NeuAc</td>
<td>11.2 ± 2.3%</td>
</tr>
<tr>
<td>Hex₅HexNAc₄Fuc</td>
<td>1.1 ± 0.3%</td>
</tr>
<tr>
<td>Hex₆HexNAc₄Fuc</td>
<td>0.6 ± 0.2%</td>
</tr>
<tr>
<td>Hex₆HexNAc₅NeuAcFuc</td>
<td>0.2 ± 0.03%</td>
</tr>
<tr>
<td>Hex₆HexNAc₅NeuAc</td>
<td>12.7 ± 1.1%</td>
</tr>
<tr>
<td><strong>Sum</strong></td>
<td><strong>70.8%</strong></td>
</tr>
</tbody>
</table>

**Table 1.** Relative amounts of the eight most abundant complex *N*-glycans in a healthy control subject’s fibroblasts, and the relative amounts (compared to total complex *N*-glycans) of the same glycans in a CDG patient. The shown *N*-glycans are Hex: hexose, HexNAc: N-acetyl-hexosamine, NeuAc: sialic acid, Fuc: fucose.

a) Which glycosylation enzyme gene is mutated in the CDG? Explain. (4 marks) **LO1, 2, 4, 5**

A fucosyltransferase is mutated (2 marks), because each fucosylated glycan’s relative abundance decreases in the patient (1 mark), and each corresponding non-fucosylated biosynthetic precursor increases (1 mark).

**Answered well**
b) The sum of the glycan amounts in the patient sample is much lower than in the control. Draw one glycan structure that could make up some of this difference. (3 marks) LO1, 2

1 mark for getting the core correct, 1 mark for drawing two antennae, 1 mark for ending in either one or two galactoses.

Answered reasonably well. Some gave structures that are present in the table - this was not given any credit. Oligomannose glycans were also not given credit, as the fate of these cannot be deduced from the presented evidence.

c) This patient could have some problems with their immune function. Explain why. (4 marks) LO3, 4

Fucosylation of the N-glycan core (1 mark) is important for the interactions between IgGs and the Fcgamma receptors as well as complement receptors (2 marks). This means that the decreased fucosylation will impact on the immune responses that the patient will be able to generate (1 mark).

Some credit was also given to discussions of autoimmune reactions, but other answers that described how pathogens are reacting differently with the mutant host were not given any credit, as this was diverging from the question.

3. Sialic acids are important molecules in the interactions between bacterial pathogens and their hosts.

a) Some gut commensal bacteria secrete enzymes that cleave sialic acid from host glycans but cannot use that sugar themselves. Suggest two possible hypotheses as to why this phenomenon might have evolved. (2 marks) LO1, 3, 4

There are two possible ideas for this (1 mark for each, or any other sensible suggestion). The first is that the bacteria are releasing sialic acid for other
commensals to use (there is evidence for this too) and the second is that they are seeking the underlying sugars beneath the sialic acid in the glycan (more limited evidence for this).

b) Outline two methods you could use to determine whether the lipopolysaccharide on the surface of a bacterium has been decorated with sialic acid? (4 marks)  

The approaches you could use are varied, but need to be direct methods that demonstrate biochemically that Neu5Ac is located on the LPS (2 marks for a description of each method). Examples from the lectures are the use of ESI-MS to examine the LPS/LOS. Then need some way to demonstrate species specific to Neu5Ac are present by catalysing their removal (sialidase treatment) or addition (passage through an animal in the case of \textit{H. influenzae}). You could also use a stain of the LOS and show species that are removed upon addition of sialidase. Any other sensible method would suffice, but must involve some direct measurement of outer membrane.

This question was generally answered well with some methods, but often the same methods just repeated. Some students answered a different question about how you would show the sialic acid was important for virulence.

c) Some bacterial sialoglycans mimic host structures. Outline the potential pathological consequences of this phenomenon. (4 marks)  

The best example is that of \textit{Campylobacter jejuni} which has a LOS sialylation pattern that reflects that seen in human gangliosides. This leads to the auto-immune condition Guillaine Barre syndrome (GBS) where antibodies raised against Campy cross react with human gangliosides (2 marks). A second example (which comes from the wider reading alone) is the idea of xeno-autoimmunity whereby Neu5Gc presented by bacteria then results in antibodies being produced that recognise Neu5Gc containing glycans in humans after dietary Neu5Gc is incorporated into glycans, this raising an autoimmune response (2 marks).

This question was generally answered OK for the first part, i.e. Campy and GBS, but not a single student was able to demonstrate that they had read the wider reading and describe the concept of xenoautoimmunity, which was most disappointing.
4. You identify a new pathogenic bacterium that mimics mucin type O-glycans on its surface to evade detection by the host.

a) Based on the following observations draw the structure of the predominant glycan with as much detail as possible, explaining your logic.

i) Glycan profiling shows that the predominant glycan has a size that is consistent with the presence of two hexoses, two N-acetyl-hexosamines, a sialic acid and a fucose.

ii) Monosaccharide analysis confirms the presence of N-acetyl-glucosamine in the glycan.

iii) Treatment with an endo-galactosidase leaves three disaccharides: one consisting of two hexosamines, one with a hexose and a fucose, and one with a hexose and a sialic acid.

iv) Treatment with an endo-glucosaminidase leaves a trisaccharide containing a hexose, a hexosamine and a sialic acid. (6 marks) LO1, 2

The answers to this question were good, much better than for similar questions in the past, well done. Two points to mention:

1. **GalNAc** should be the initiating monosaccharide rather than bacillosamine, since this is an O-glycan.

2. Some came up with a linear glycan that satisfies the points above. While the logic of satisfying the points is correct, this linear glycan cannot be attached to a protein via any of the known mechanisms, therefore it is not correct. Yet, partial credit could be obtained for this.
b) You find that the bacterium binds very strongly to the nasal epithelium, and you suspect this could be dependent on the presence of fucose or sialic acid. How could you test which monosaccharide is involved? (4 marks) LO1, 2, 3

Use a fucosidase and a sialidase to cleave off the respective monosaccharide in two separate experiments (2 marks). Comparing the binding of the bacteria with and without glycosidase treatment to cultured epithelial cells will provide the answer (2 marks).

Alternative experiment would be to use mutant bacteria with the fucosyltransferase and the sialyltransferase knocked out.

This was answered well.

c) Your efforts to bind the isolated glycan to epithelial cells during experiments are unsuccessful. Explain why. (4 marks) LO2, 3

Glycans bind their partners (lectins) generally with low affinity (1 mark). The binding is enhanced by multimerization - having several glycans of the same type on the surface of cell (1 mark) and several receptors (lectins) on the target (1 mark) to lead to high avidity (1 mark).

This was answered well.
SECTION B: Essay question

Answer one question on the separate paper provided

Remember to write your candidate number at the top of the page and indicate whether you have answered question A or B

Mark total for this section: 50

EITHER

A) Explain why it is difficult to control the glycosylation status of biologics, and why do we care?

Answers need to explain the concepts of glycan heterogeneity that stems from the non-templated nature of glycan processing in the Golgi. A brief summary of N-glycan processing from oligomannose to complex glycans is expected. The essay should explain how glycans modulate protein functions, with special focus on the N-glycan found on monoclonal antibodies. This glycan modulates the interactions of the antibody with the Fcγ receptor as well as complement receptor to influence what type of immune response the antibody will elicit and how strong this will be. In addition, a more generic functions of protein linked glycans include the control of the glycoprotein’s half life in serum, and if they generate an adverse immune response against the biologic.

Altering glycosylation on biologics therefore alters their efficacy, and their potential to treat the disease or even cause serious allergic reactions. The essay should then combine the descriptions of heterogeneity and biologic function to discuss the restrictions that current production methods have on controlling drug efficacy due to uncontrolled glycan heterogeneity.

Successful approaches, such as the control of fucosylation and bisecting GlcNAc addition in the case of monoclonal antibodies should be mentioned. Glycan engineering of cell lines, such as in the yeast P. pastoris, or on plant cells can be highlighted. Computational modelling as a tool for informing glycan engineering can also be mentioned.

At least a good description of N-glycan biosynthesis and a discussion of heterogeneity was expected for a 2i essay. Some got most of the points in the specimen answer apart from the examples of altering fucosylation and bisecting GlcNAc addition as engineering strategies.

OR

B) Contrast the functions of cytosolic and cell surface glycans in eukaryotic
physiology and disease.

1. Different types of cytosolic (O-GlcNAc) and cell-surface (O- N-glycans linked to protein, glycolipids). Cytosolic is reversible, cell surface is not. Need generic structures (N-linked beta linked to Asparagine, O-linked alpha-O linked to Ser/Thr, diverse glycolipids). Cellular locations. Roles (n.b N-glycans first in folding and then subsequently in communication).

2. Dysregulation in disease: Cytosolic – O-GlcNAc / phosphate balance in neurodegeneration. Discuss enzymes involved in addition/ removal. Link to tauopathies. Links also to a-synuclein in Parkinson’s stability and amyloid. How small molecule increase in O-GlcNAc combats these three diseases.

3. Dysregulation in Disease: cancer. Changes in cell-surface epitopes on cancer cells linked to invasion, metastasis and diagnosis with examples (e.g., GloboH, Tn, lactosamine repeats etc). Mouse genetic work on GlcNAc TV (MGATV) showing increased proliferation upon increased nutrient availability. ER re-localization of GaINAcT (O-glycan initiation enzyme) causing increased metastasis due to altered Tn levels and consequent altered adhesion.

4. Dysregulation in disease: dystroglycanopathies. Disease of O-linked glycosylation. Genetic basis causes under-glycosylation (O-mannosylation) of alpha-dystroglycan. Notably congenital muscular dystrophy through mutations in the large gene – under glycosylation in its Ser/Thr region disrupts its interactions with laminin (diagram helpful). Also, many other diseases depending on the glycosyltransferase that is dysregulated.

5. Dysregulation in disease: congenital disorders of glycosylation. Many examples Type I involved in synthesis of N-glycan precursors leads to incorrect N-glycans at cell surface. Type II involved in trimming steps (often COG mutants involved in sugar transport, mutants preventing formation of GDP-Man from Maan-1-P etc)

6. [Students may mention, disease of recycling these glycans – the lysosomal storage diseases- although the lysosome itself isn’t cytosolic or surface, but the diseases are about recycling these components, and enzyme replacement therapies rely on the binding of lysosomal enzymes to the cell surface displayed M6PR]

7. Glycans also play key roles in infectious disease – allowing pathogen binding. Examples include, but not limited to, influenza virus, though flu haemagglutinin (H) binding to Sialic Acid-Galactose on cell surfaces (compare human and avian glycans, linkages, species barrier etc). Also demands a neuraminidase (N) to allow viral release – target of anti-virals. Pathogenic E coli and cholera through binding to cell surface GB3 (Gal-Gal-Glc). Leads to ideas of anti-adhesins for therapy.
Similarly, uropathogenic *E. coli* binding to UT via FimH, Helicobacter binding to Sialyllewis X and blood group B.

8. **Summary.** Different types of cell-surface glycans. Play roles in infectious disease, cancer and *many* genetic diseases. Also scope for therapies.

Some very nice answers. While none described all the examples listed above, if the examples used gave a nice range, and were well-argued, this was given first class marks. Including Lysosomal Storage Disorders was a digression from the topic, since these do not involve either of the types of glycans in the question.