

BMB170c Problem Set 3: Glycosylation

Please turn in to **Justin** on **Tuesday, May 11, 2008**

1. Byres *et al.* describe how an ingested glycan can mediate pathogenesis in humans.
 - a. What is the structural basis of substrate specificity in SubAB, and what experiments were conducted as verification?
 - b. Explain why *cmah* cells were not a perfect negative control in Figure 2b. What additional controls could have been readily used?
 - c. Describe the experiment in Figure 3b. Why are the results not very convincing?
 - d. Based upon the results of this paper, would you predict a person suffering from STEC toxicity to recover or become more ill by increasing short-term consumption of Neu5Gc? Propose an experiment to test your hypothesis.

2. Liu *et al.* discovered novel glycosidases that may one day be used to generate universal red blood cells from donated type A, B or AB bloodtypes.
 - a. What properties were screened to identify glycosidases, and how does this screen differ from previous ones? How did the authors justify its superiority?
 - b. Refer to Supplementary Figure 8 and Supplementary Figure 10. Explain why the mechanism for α -N-acetylgalactosaminidase is applicable to the hydrolysis of both GalNAc α -pNP and GalNAc β -pNP. The authors find the α -linked substrate retains its anomeric configuration. Would you expect the β -linked substrate to be retained or inverted?
 - c. Briefly outline the steps used to identify FragA as an α -galactosidase.
 - d. The authors acknowledge a potential shortcoming in removing the A antigen that requires further clinical testing. What is it, and why are the authors optimistic it will not be a problem?

3. Linton *et al.* experimentally determined the function of each gene in the *Campylobacter jejuni* N-linked glycosylation pathway.
 - a. Some of the genes in the *pgl* locus are not required to generate the *C. jejuni* heptasaccharide in *E. coli*. Which genes are they, and why can they be knocked out without a change in phenotype?
 - b. What is actually detected in the gel in Figure 2? Propose an explanation for why no band is observed in lane 11.
 - c. What glycan moiety would you expect if *wecA* and *pglE* were knocked out? Why?
 - d. Name a likely candidate for the identity of the HexNAc transferred in a *pglC* knockout. Why can't the authors make a conclusive identification with the data presented?