Principle Investigator: Alexander R Bennett

Grant Title: Glycycle – Circadian Dynamics of the colonic mucus glycome

Objectives:

(b) Objectives: The objectives of the project are:

1) Develop a biostatistical package for characterizing dual-oscillator waveforms, providing a platform for analysis of the complex diurnal behaviours observed in protein glycan abundances. [Complete + published]

2) Conduct a diurnal sampling regime of the colonic mucus glycome from germ-free C57bl/6 mice, exploring glycome dynamics and contextualising these changes with other molecular modalities. [Sampling + analysis complete]

3) Conduct a diurnal sampling regime of the colonic mucus glycome from microbiome depleted mice (antibiotic-treated, germ-free) and models of altered glycosylation (C1galt1$^{-/-}$, Slc35a1$^{-/-}$, Fut2$^{-/-}$) elucidating the relative contributions of the host and microbiome in modulating temporal dynamics of the mucus glycome. [Sampling + analysis ongoing]

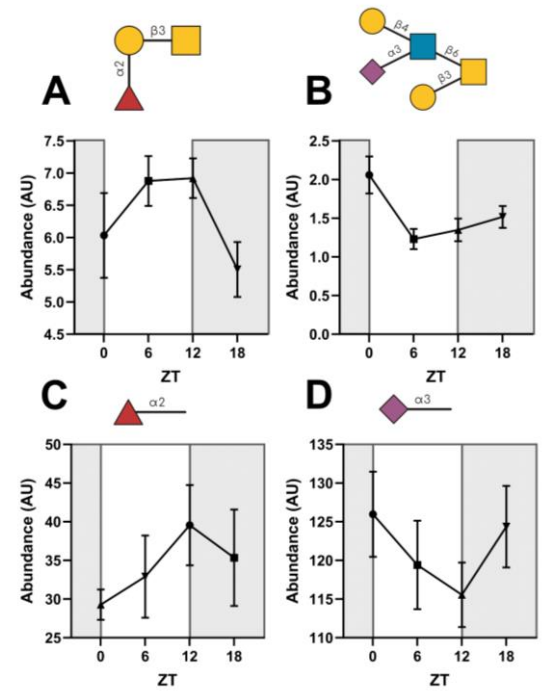

Figure 1: Abundance of glycan sequences (A, B) and motifs (C, D) from colonic mucus of C57BL/6 mice.

Methods:

We have utilized: mass-spectrometry-based glycomics analysis, novel signal processing mathematics in bioinformatics, *ex vivo* mucus measurement and imaging systems, germ-free mice, bacterial immunological challenge models, and genetically altered models of mucin glycosylation in mice during our investigation.

Results:

Parameterisation of dual-oscillator systems:

We have successfully developed bioinformatics algorithms which allow more accurate analysis of temporal molecular biology data (Bennett, *et al*, 2026). This functionality is available in the python package *PyCycleBio*, (github and pypi). Using bounded oscillator equations *PyCycle* efficiently models signals parametrically, and the relationships between oscillator components informs the regulatory mechanisms underlying observed dynamics.

Circadian dynamics in protein glycosylation:

We have also identified circadian rhythms in the abundance of glycan sequences, and motifs within the glycome of C57bl/6 mice **(figure 1: A-D)**. We are now examining what underpins circadian regulation of mucin glycans, with a focus on the combined role of the host goblet cells and microbiome in shaping these dynamics. Analysis of tissues from antibiotic-treated and germ-free mice shows altered dynamics in both glycome and transcriptome, compared to control animals. We have collected tissues from mice lacking the *C1galt1* enzyme in goblet cells, a *Fut2* global knockout and we are breeding a line of mice lacking *Slc35a1* in goblet cells. These will illustrate the role of the host's glycosylation machinery in determining glycan dynamics, and how mucus dynamics shapes the microbiome.