Principal Investigator: Charlie Fehl
Title: Capturing real-time sugar signaling in cells with light-released O-GlcNAc probes

Abstract

Objectives: All cells regulate proteins using glycans to change protein activity and, therefore, cellular behavior. Dynamic glycosylation with O-linked N-acetylglucosamine (O-GlcNAc) occurs on > 7000 proteins and is fundamentally important for mediating cellular responses, for example during signaling (**Figure 1A**). However, our field has very limited knowledge about how O-GlcNAc modifications are regulated in the time following a cellular stimulus (**Figure 1B**). The objectives of this grant are to track the effects of O-GlcNAc on signaling proteins in real-time.

Methods: Our lab has created light-released sugar tools as probes to study O-GlcNAc and other glycosylation processes with rapid (< 30 minute) time control. Briefly, these "PhotoGlcNAc" probes are photocaged GlcNAc metabolites that, when taken up into cells and irradiated with light, cause rapid upregulation of O-GlcNAc modifications (**Figure 1C**). Using these tools, this Mizutani project tracks effects of signaling proteins (Aim 1) and insulin signaling (Aim 2) on O-GlcNAc levels in cell and animal models.

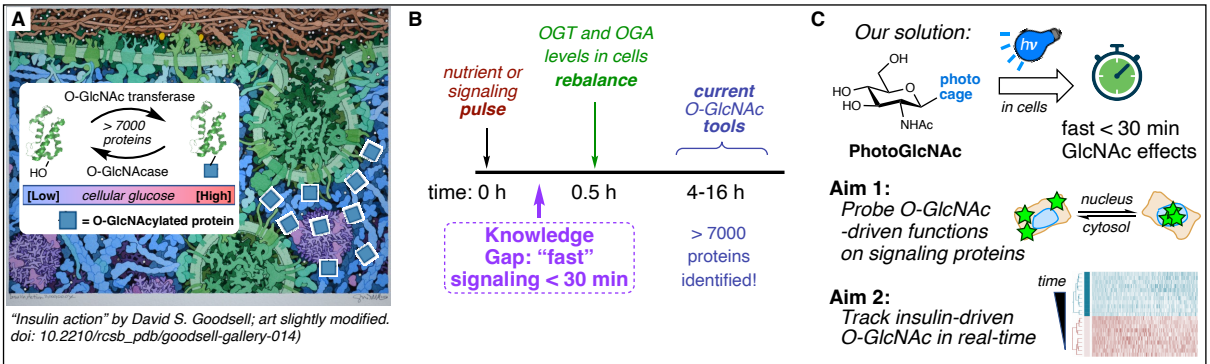

Fig. 1: This proposal creates a **chemical biology** platform to identify real-time O-GlcNAc functions in cells. A) "Global" O-GlcNAc modifications fundamentally remodel the proteome during nutrient sensing and signaling, enabling homeostasis. B) **Knowledge gap** that this proposal seeks to address - no tools exist for probing rapid O-GlcNAc biology in living cells. C) We created PhotoGlcNAc probes to study **real-time** O-GlcNAc effects on key signaling proteins (**Aim 1**) and to track effects of signaling on O-GlcNAc (**Aim 2**).

Results: We synthesized a series of new PhotoGlcNAc probes with varied properties, such as alternative photocaging groups, positions on the sugar ring, and release systems to produce either monosaccharides or phosphosugars following the light exposure as a trigger. The cellular assays we proposed for individual signaling proteins are still in development. However, we were able to use labeled sugars to profile the O-GlcNAcylated proteome of muscle tissue for the first time.

Significance: These novel O-GlcNAc tools are designed to be applied to uncover O-GlcNAc processes that occur over multiple timescales. Time-dependent O-GlcNAcylation of metabolic and signaling proteins may allow cells to dynamically regulate physiological and disease processes.