Chimeric OSTs (oligosaccharyltransferases) to Relax Enzyme Specificity towards Glycosylation Sequons

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Glycosylation is the addition of carbohydrates to a functional group of a protein, and is present in many marketed therapeutic proteins. Historically, glycosylation has been shown to improve the stability, half-life, and effectiveness of these protein drugs. However, glycosylation is typically a process that is only performed exclusively by eukaryotic cells, making many biopharmaceutical companies resort to CHO (Chinese hamster ovarian) cells as a production host. CHO cells, however, require long culturing times, and are easily prone to contamination due to their high doubling times.

Within the past decade, prokaryotic glycosylation was discovered in certain bacterial species such as *C. jejuni*. While the end product post-glycosylation is similar, the functional group being modified on the protein has different requirements, and at times different carbohydrates can be added, depending on the organism. This organization and sequence of the functional groups is referred to as a sequon. In a eukaryotic organism, N-linked or asparagine-line glycosylation occurs along the N-x-S/T sequon, where x can be any amino acid. Prokaryotes, however, have stricter requirements that extend two amino acids prior to the glycosylated asparagine, also referred to as the -2 position. For example, *C. jejuni's* recognition sequon is D/E-x-N-X-T.

Several different prokaryote OSTs have been shown to have a different of more relaxed recognition for the -2 position. The crystal structure of one of these OSTs (*C. lari*) shows that the catalytic domain is actually divided between the transmembrane and periplasmic domains or the OST. Our goal is to investigate how swapping these moieties among other bacterial species will lead to a more relaxed specificity, leading towards glycosylation-capable hosts that can mimic or reach the required level needed in Eukaryotic N-linked glycosylation. This can overcome several bottlenecks and restraints of host selection for therapeutic purposes.