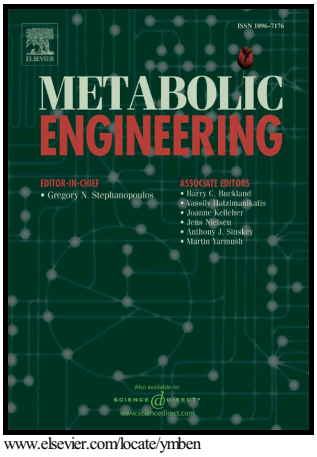

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A flow cytometric approach to engineering *Escherichia coli* for improved eukaryotic protein glycosylation

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Abstract

A synthetic pathway for production of the eukaryotic trimannosyl chitobiose glycan (mannose$_3$-*N*-acetylglucosamine$_2$, Man$_3$GlcNAc$_2$) and its transfer to specific asparagine residues in target proteins was previously engineered in *Escherichia coli*, providing this simple microbe with the ability to perform a complex post-translational protein modification. Here, we leveraged a flow cytometric fluorescence-based assay to improve Man$_3$GlcNAc$_2$ glycan biosynthesis in *E. coli* cells. Specifically, pathway improvements were identified, including reducing pathway enzyme expression levels and overexpressing nucleotide sugar biosynthesis genes, which enhanced production of lipid-linked Man$_3$GlcNAc$_2$ by nearly 50-fold to 13.9 μg/L. In turn, cells producing higher levels of the Man$_3$GlcNAc$_2$ substrate yielded up to 14 times more glycosylated acceptor protein (to ~14 mg/L) than their non-optimized counterparts. These results demonstrate the use of flow cytometry screening as a powerful tool for interrogating the surfaces of glyco-engineered bacteria and identifying meaningful improvements in glycan biosynthesis. We anticipate this approach will enable further optimization of bacterial glycan biosynthesis pathways using new strain engineering tools from metabolic engineering and synthetic biology.