ENGINEERING SULFATE DONOR ACCUMULATION IN *ESCHERICHIA COLI* FOR SYNTHESIS OF SULFATED GLYCOSAMINOGLYCANS

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The model bacterium *Escherichia coli* has been extensively engineered for a variety of applications. However, sulfated biomolecules remain a relatively under-explored domain of biologics that can be synthesized using *E. coli.* An important class in this domain are sulfated glycosaminoglycans (GAGs) which are of great pharmaceutical/nutraceutical interest. On-going studies aim at developing efficient and scalable chemical and chemoenzymatic methods to produce these compounds. However, we propose that engineered *E. coli* capable of entirely *in vivo* synthesis of sulfated GAGs will serve as a great alternative to the current state-of-art.

The biosynthesis of sulfated biomolecules relies on the universal sulfate donor, 3'-phosphoadenosine-5'-phosphosulfate (PAPS). PAPS plays the role of a co-enzyme in enzymatic sulfotransferase reactions and donates the sulfate to the substrate. In the first part of this study, we engineered the metabolism of *E. coli* to improve PAPS accumulation around 10000-fold. In the second part, we applied this engineered strain to *in vitro* biosynthesis of chondroitin sulfate (a sulfated GAG).

Certain strains of *E. coli* possess the ability to biosynthesize unsulfated GAG backbones. These have been well-studied and optimized for GAG production. *In vitro* sulfation requires the addition of purified sulfotransferase and an excess of commercially obtained PAPS to the unsulfated GAG. By utilizing PAPS from our engineered strain in the existing setup, we improve the biotransformation method to one in which all components are synthesized from *E. coli*. We also use this system and its comparison to other *in vitro* systems to identify the bottlenecks for total *in vivo* synthesis of sulfated GAGs in *E. coli*. We show for the first time that with this system, we have achieved the synthesis of chondroitin sulfate A without the dependence on the commercially procured PAPS cofactor.