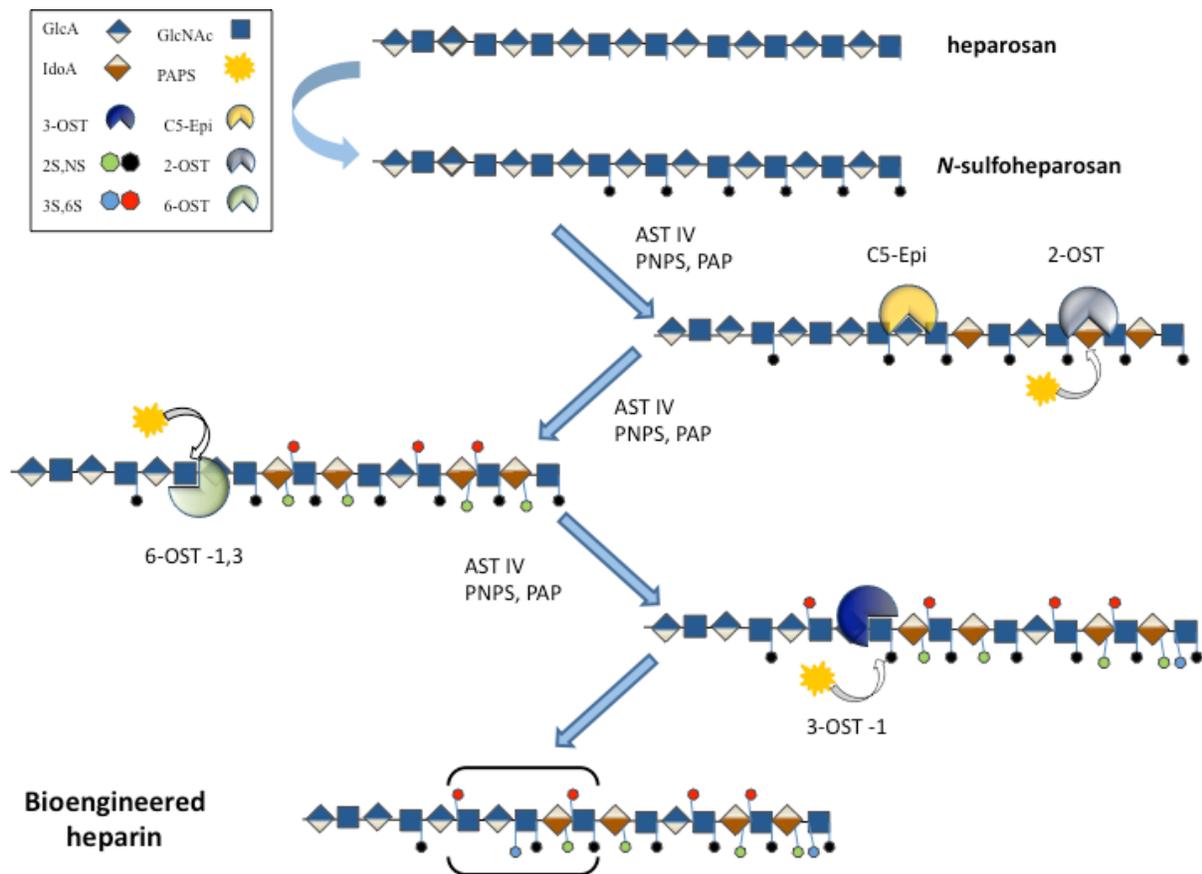


# Glycoengineering

The Glycoengineering subgroup consists of two sections: Bioengineering and Metabolic Engineering. Bioengineering involves the use of microbial, mainly bacterial, processes to generate glycosaminoglycans (GAGs), in fermentation or using enzymology. Metabolic engineering involves the manipulation of the natural metabolic processes of mammalian cells to generate GAGs.

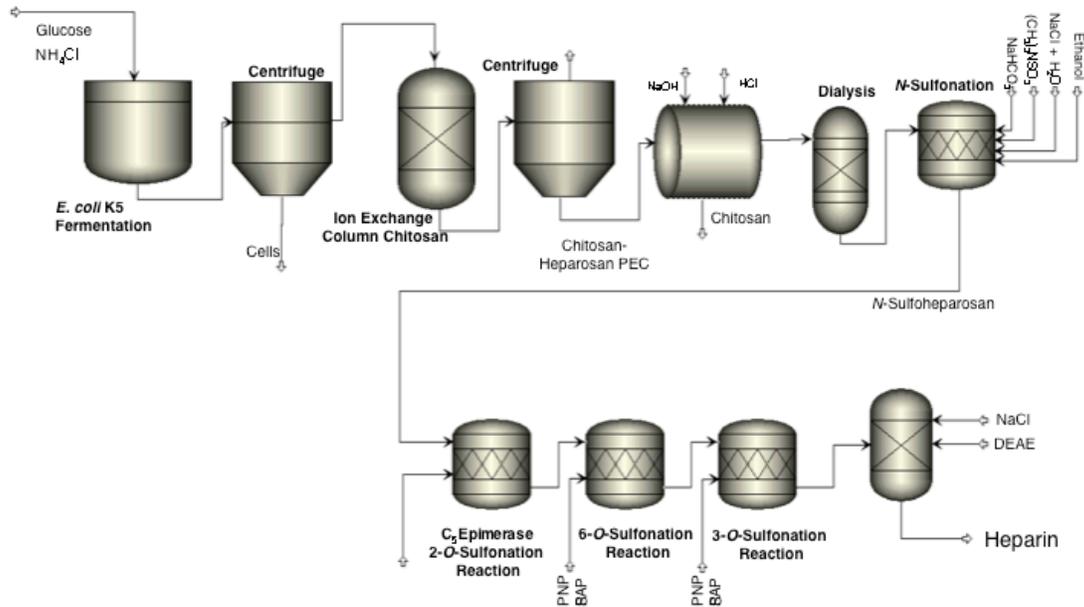
## Bioengineered Heparin Project

Heparin, a highly sulfated glycosaminoglycan (GAG), is used extensively as an anticoagulant. It consists of repeating disaccharide units, containing iduronic acid (or glucuronic acid) and glucosamine, exhibiting variable degrees of sulfation. Heparin, and its analogues, are used during surgery and dialysis, and are often used to coat indwelling catheters and other devices where the vascular system is exposed. Administered parenterally, often continuously due to its short half-life, over 0.5 billion doses are required per year. Currently obtained from mucosal tissue of meat animals, mainly porcine intestine, and to a lesser extent bovine lung, its early stage production is poorly controlled, due to the source of the material. This problem came into sharp focus in 2008 when the presence of contaminating over-sulfated chondroitin sulfate in heparin, sourced from pigs, resulted in almost 100 deaths in the USA. Coupled with the fact that only a few doses are obtained per animal means that the demand for alternative and well-controlled sources of heparin is high.



In an attempt to generate a bioengineered heparin, a biosynthetic pathway was designed that began with bacterial synthesis of the backbone structure. Once extracted from the cell and purified the heparosan polysaccharide undergoes a series of chemoenzymatic modifications to make heparin. Since heparin production would be reliant on microorganisms, instead of animals, scale up could be achieved by increasing fermentation volume.

## Process Flow Chart



## Metabolic Engineering Project

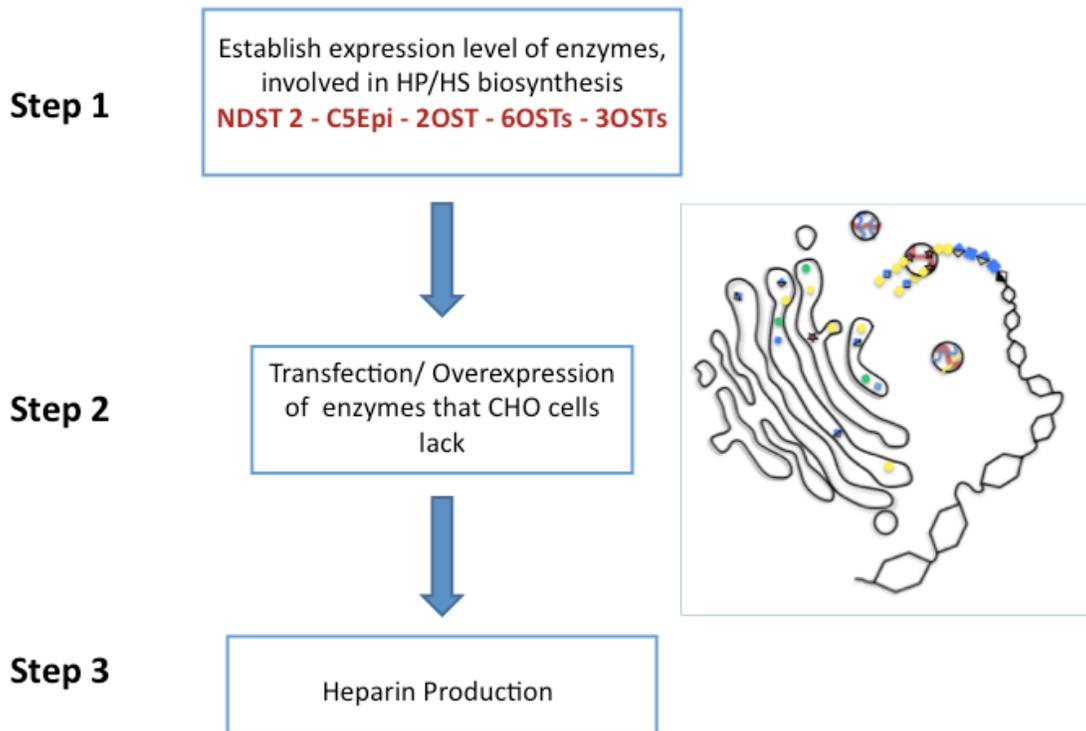
Heparin is a biosynthetic product of mast cells, is stored in mast cell granules and is released with histamine on type-1 allergic response leading to mast cell degranulation. Metabolic engineering offers a potential approach to prepare heparin by fermentation. The metabolic engineering of bacterial cells is relatively straightforward since most of the biosynthetic pathways are relatively simple and well known. While bacteria are both easy and inexpensive to engineer and to grow, they are not compartmentalized, lack a Golgi and thus are incapable of making all but the simplest glycoproteins. Yeast are eukaryotes, and have a Golgi but do not make glycosaminoglycans and are missing the required ability to sulfate polysaccharides. Mast cells cannot be grown in culture and even when converted to mastocytoma cell lines grow very poorly and thus are not good candidates for metabolic engineering.



Associated Press

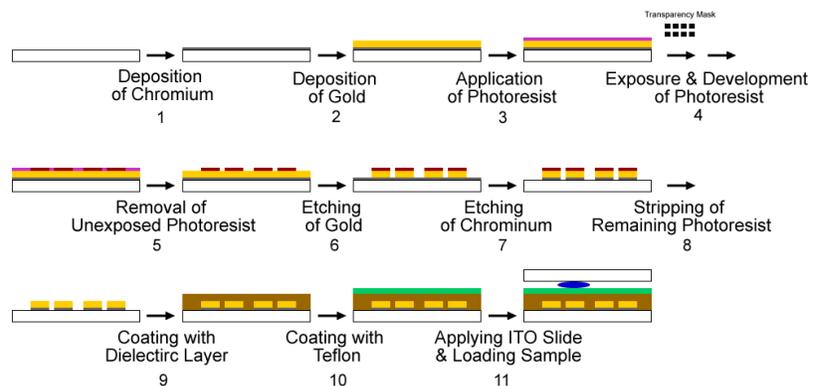
Chinese hamster ovary (CHO) cells have all the appropriate structure and pathways making the related glycosaminoglycan, heparan sulfate, and are used in the commercial production of glycoprotein drugs and thus are the target of our metabolic engineering studies. The project would make use of the enzyme suite already present in the cells and cloning of the enzymes required to complete the heparin synthesis pathway.

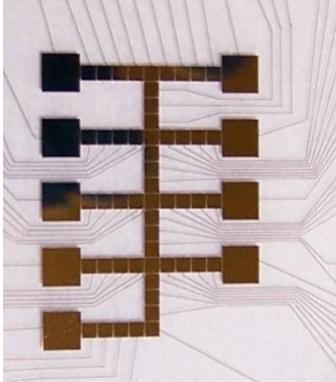
## Bioengineering of Heparin in CHO cells



### Artificial Golgi Project

The Artificial Golgi project utilizes digital microfluidics to drive aqueous droplet suspensions of magnetic nanoparticles to which a core protein has been covalently immobilized. A glycosaminoglycan chain is extended from a serine residue on the immobilized core protein by treating with UDP-sugars in the presence of recombinant polysaccharide synthases. By





applying additional glycosaminoglycan-modifying enzymes including sulfotransferases and C5-epimerase, a fully elaborated glycosaminoglycan is synthesized. This platform is being built and optimized as a test-bed to understand how the natural Golgi organelle uses sequestration of cofactors and enzymes, exposure time and concentrations, to control the structural outcome of these posttranslational modifications. This project includes: the fabrication of digital microfluidics chips using a photoresist process in a Class 100 clean room; the construction of a controller and controller software; droplet visualization and feedback software; robotic reagent dispenser and sampler, applied enzymology, and molecular biology.

