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The Challenges and Potential of Bio-nanomanufacturing of "Humanized" Proteins

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For a protein to be compatible with human biology, it must be posttranslationally modified by the addition of specific sugars, called "glycans" at prescribed positions through a process called protein glycosylation. Protein glycosylation affects myriad biological processes and is critical for the development, growth, function, and survival of the organism. Moreover, changes in glycosylation are often hallmarks of disease. For these reasons, there is strong impetus for development of manufacturing processes to create humanized therapeutic glycoproteins. Glycans profoundly affect protein properties, like folding and function, which ensures it retains its therapeutic potency and dictates its pharmacokinetics, immunogenicity, and biological activity. Thus, therapeutic glycoproteins, ranging from antibodies to vaccines, are important biomanufacturing targets. Currently, in molecular medicine, the vast majority of the 64 protein products approved by U.S. and European regulatory agencies and the ~500 candidates in clinical trials are glycosylated, with a market valued near $140 billion annually. However, even more exciting are the possibilities of intentionally engineering of a protein's glycosylation pattern, which may lead to drugs with improved efficacy or new targets.

There are two main approaches for manufacturing therapeutic glycoproteins at present: one relies on producing proteins in bacteria cell culture, which suffers from limited ability to engender those proteins with sugar groups important to their "humanization". The second is a cell-free manufacturing approach. Cell-free protein synthesis holds great potential for producing high-value, biotherapeutic nanomaterials without the complications of cell culture and benefitting from chemical manufacturing know-how. Here, raw materials and biological enzymes are mixed in 'one-pot' to produce biological products. But current shortcomings of this approach are competing reactions, side products, and low yields, which require extensive and expensive separation schemes to harvest products. Mammalian cells avoid these shortcomings by localizing reactions within subcellular compartments and orchestrating the reaction sequences. In addition, the biocatalysts that give the final molecule its essential posttranslational features are compartmentalized in the cell's organelle membranes of the Golgi apparatus and endoplasmic reticulum. Handling enzymes outside of their native membrane environment in the one-pot approach drastically reduces their activity. Thus, in vitro, sequential, bio-enzymatic reactions have never been achieved in a cell-free manufacturing approach to date.

The cutting edge of bio-manufacturing is to mimic the elegant compartmentalization strategies used by cells. I will highly our approach as an example of such a strategy where we create a microfluidic biomembrane device that organizes biological reactions in proper spatial and temporal sequence, giving supreme flexibility in optimizing individual reactions and constructing authentically glycosylated proteins with high specificity. Through continued assessment of nanostructure product architectures, this work will advance understanding of nanoscale phenomena and processes for nanomaterials manufacture and discovery. This cell-free device concept will enable facile optimization of glycosylated protein production, and provide a framework for understanding how experimental conditions affect product yield and quality that is broadly applicable to the bio-nanomanufacturing of virtually any posttranslationally-modified protein (not just human therapeutic targets). The benefits of this manufacturing paradigm to society are reducing the cost of these drugs and providing scientists an avenue to design and develop synthetic drug compounds that may or may not exist in nature to treat disease.