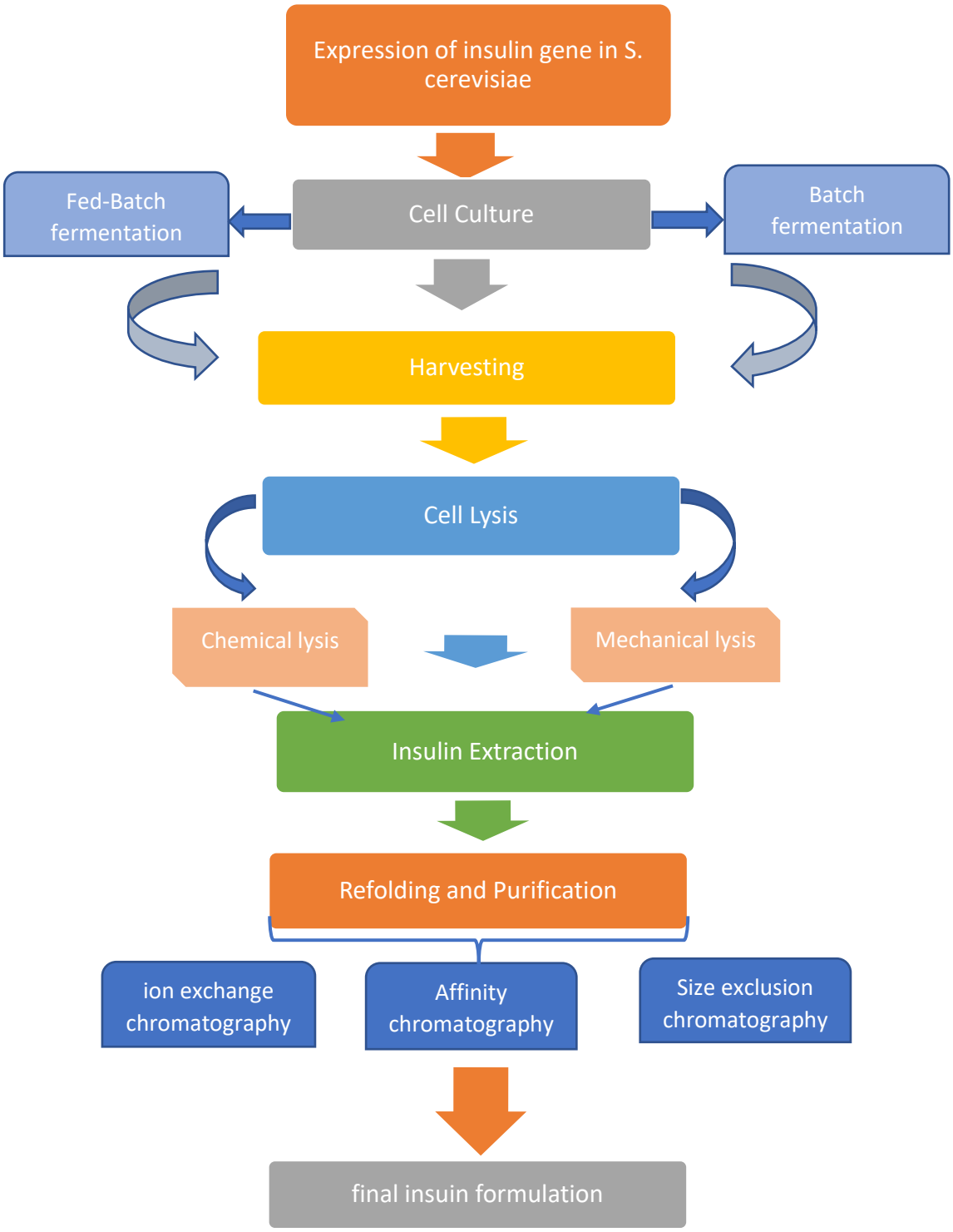
**Produce a single flowsheet detailing possible routes for the production and downstream purification of recombinant insulin, from either S. cerevisiae**



Insulin is a protein hormone produced by the beta cells of the pancreas that plays a critical role in regulating glucose homeostasis in the body. The insulin protein is made up of two polypeptide chains, an alpha chain of 21 amino acids and a beta chain of 30 amino acids, connected by disulfide bonds (Wilcox, 2005). Insulin is manufactured using recombinant DNA technology, which involves inserting the human insulin gene into a bacterial or yeast host cell. The host cell then produces the insulin protein, which is harvested and purified for use as a medication (Steiner *et al.*, 2009).

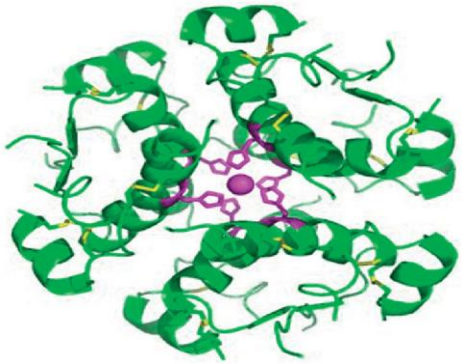


Figure 1: the protein structure of insulin (Huiying, Guangying and Shiyang, 2019)

Both prokaryotic and eukaryotic systems are capable of expressing insulin. The quantity and quality of recombinant insulin produced are greatly influenced by the selection of a suitable expression system. The yeast Pichia pastoris is one of the most often utilized expression systems for producing insulin. The ability of P. pastoris to carry out post-translational alterations is one of the main justifications for adopting it as an expression system for insulin (PTMs). PTMs are essential for insulin's correct folding, stability, and performance. Disulfide bond formation, a PTM necessary for the correct folding and functioning of insulin, can be carried out by P. pastoris. As a result, appropriately folded and bioactive insulin is produced. on the other hand, Prokaryotic organism like E.coli, and lacks the machinery to perform post-translational modifications (PTMs) like disulfide bond formation. This means that insulin produced in E. coli may not be properly folded or biologically active. E. coli is prone to proteolytic degradation, and the host cells may break down the insulin molecule during expression and purification. This can result in a low yield of properly folded and active insulin (Porro *et al.*, 2011). Furthermore, yeast offers several advantages over other expression systems, including ease of genetic manipulation and high productivity. E. coli is known to produce endotoxins, which are released when the cells are lysed.

Endotoxins can trigger a strong immune response in humans which means that the purification of insulin produced in E. coli must be carefully optimized to remove endotoxins, which can be time-consuming and costly. Yeast is a well-established model organism with a fully sequenced genome, making it easy to manipulate its genetic makeup to optimize insulin production (Baeshen *et al.*, 2014). Additionally, yeast can be grown to high cell densities in bioreactors, enabling high levels of insulin production per unit of cell culture. Moreover, yeast such as P. pastoris has a robust secretory system, which enables efficient secretion of insulin into the extracellular space. The secretion system of P. pastoris is highly active and efficient, resulting in high yields of properly folded and active insulin. This secretory system is based on the secretion of proteins into the endoplasmic reticulum (ER), where they undergo PTMs before being transported to the Golgi apparatus and then secreted into the extracellular space. In conclusion, P. pastoris is a suitable expression system for insulin production due to its ability to perform PTMs, high yield of protein production, robust secretory system, eukaryotic nature, and potential for genetic engineering. P. pastoris holds great promise for the development of new and improved insulin therapies (Huang, Lin and Yang, 2012).

In this process, the insulin gene is created and optimized for Pichia pastoris. The insulin gene is cloned into a suitable Pichia pastoris expression vector. Pichia pastoris cells are transformed with the insulin expression vector. Shake flasks or small-scale bioreactors are used but they are not feasible for large-scale production. S. cerevisiae cells are then cultured in a 1000L bioreactor in a suitable media, such as YPD or M9 media, and incubated at a temperature of 25-30°C, with agitation and aeration to promote cell growth and protein expression.

Once the cells have reached a certain density, they are harvested by centrifugation or filtration. Centrifugation is preferred over filtration because it is faster and more efficient (Polez *et al.*, 2016; Ramapriya *et al.*, 2018). The harvested cells are lysed using a suitable method. Both chemical and mechanical cell lysis methods can be used. Chemical cell lysis involves the use of chemicals such as detergents or chaotropic agents to disrupt the cell membrane and release the cellular contents. This method is relatively easy to perform, does not require specialized equipment, and can be effective at releasing both soluble and insoluble proteins. However, the use of chemicals can affect the activity and stability of the target protein and may require additional purification steps to remove any remaining contaminants. Mechanical cell lysis on the other hand involves physically

breaking open the cells using methods such as sonication or homogenization. This method can be highly effective at releasing insulin proteins and does not require the use of chemicals that can affect the target protein's activity and stability. However, mechanical cell lysis requires specialized equipment, can be time-consuming, and may generate excessive heat that can denature the protein. In general, mechanical cell lysis is preferred over chemical cell lysis for the production of recombinant proteins such as insulin, as it provides a higher yield and is less likely to affect protein activity and stability (Huang, Lin, and Yang, 2012).

The lysate is subjected to a series of purification steps to isolate the insulin. The first step is typically a chromatography step such as ion exchange, size exclusion, or affinity chromatography. This step separates the insulin from other cellular components such as proteins, DNA, and RNA. The choice of chromatography step depends on the specificity and yield of insulin. Ion exchange chromatography (IEX) is a commonly used purification step for insulin, which separates insulin from charged impurities based on their charge differences. Size exclusion chromatography (SEC) can separate insulin from high-molecular-weight impurities based on their size differences. Affinity chromatography can capture insulin with high specificity using an immobilized ligand that recognizes insulin. In general, a combination of these chromatographic steps is used to achieve high-purity insulin (Lowe, Lowe, and Gupta, 2001). Insulin purity, potency, and identity are analyzed using techniques such as HPLC and mass spectrometry (Mandrup, 1992; Sergeev *et al.*, 2001). The purified insulin protein is then formulated into its final dosage forms, such as a liquid injection or a lyophilized powder.

In conclusion, the production and downstream purification of recombinant insulin from S. cerevisiae involves a series of unit operations, including cell culture, cell harvesting, cell lysis, purification, and formulation. The choice of expression host is critical in determining the success of the production process, and S. cerevisiae is a suitable choice due to its high expression levels, scalability, and post-translational modification capabilities. While there are several challenges and considerations in recombinant insulin production, including product yield, quality, and purity, these can be addressed through optimization of the various unit operations involved. Overall, the production of recombinant insulin from S. cerevisiae offers a promising approach to address the increasing demand for this important therapeutic protein.

## References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