ALTERNATIVE STRATEGY ENABLES AUTOMATION OF UP- AND DONWSTREAM PROCESSES FOR RECOMBINANT PRODUCTION OF AN ANTIMICROBIAL PEPTIDE IN ESCHERICHIA COLI

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Standard production processes for antimicrobial peptides (amps) comprise of batch cultivations in upstream and chromatographic purification in down-stream. Although chromatography in most variations is limited in scale-up and one of the most expensive steps in the production chain it still remains the industrial gold standard in downstream processing. To overcome this bottleneck in the production chain, alternative column-free purification strategies with aggregating tags are a promising approach. Such a process can be automated and scaled-up in consent with reasonable expenses. However, new processes involving alternative downstream strategies have to be developed starting from scratch. In regards to vector design it must be considered whether the product is intended to be expressed in a soluble or insoluble form, in which cell compartment it shall be synthesized or what kind of plasmid features are needed for the realization of an automated downstream process. In this study the soluble production of an amp with several structural disulfide bonds was conducted in an *E. coli* strain with an oxidizing cytoplasm. To generate the expression vector a unique combination of plasmid features was assembled by Golden Gate cloning to enable an automated downstream procedure with aggregating tags for column-free purification. The junction of a thioredoxin-tag (trxA) with elastin-like-polypeptides (ELPs) and self-splicing inteins allows the release of the protein from the cytoplasm into the extracellular space and the separation from host cell proteins (HCP) in the bioreactor vessel. The trxA-tag acts as a solubility enhancer to the product and facilitates the release into the extracellular space via simple osmotic shock procedure. Reversible, temperature dependent phase transition of the ELPs enables purification of the desired product from impurities such as HCP and, after cleavage by self-splicing inteins, to separate the amp from the tags itself.

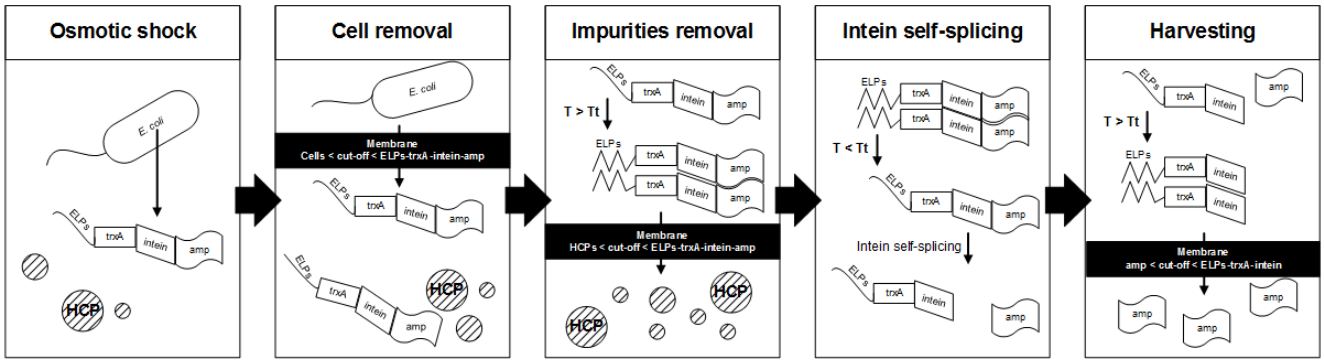

Figure 1 - Workflow of downstream processing using an ELP-thioredoxin-intein-amp construct for purification

This interaction of thioredoxin-, ELP- and intein-tag facilitates scalability and economic feasibility with rendering cell disruption apparatuses, chromatographic columns and enzymatic digestion obsolete. In addition to the alternative downstream strategy, the integration of process analytical technology (PAT) in the upstream process and the implementation of a defined media generates a production process with high potential for application in pharmaceutical industry in regard of GMP-compliance. The presented bioprocess for amp production addresses crucial requirements for process automation in the up- and downstream sector and promotes alternative strategies in process design.