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Current research approaches in downstream processing of pharmaceutically relevant proteins

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Biopharmaceuticals and their production are on the rise. They are needed to treat and to prevent multiple diseases. Therefore, an urgent need for process intensification in downstream processing (DSP) has been identified to produce biopharmaceuticals more efficiently. The DSP currently accounts for the majority of production costs of pharmaceutically relevant proteins. This short review gathers essential research over the past 3 years that addresses novel solutions to overcome this bottleneck. The overview includes promising studies in the fields of chromatography, aqueous two-phase systems, precipitation, crystallization, magnetic separation, and filtration for the purification of pharmaceutically relevant proteins.

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Introduction

Owing to a rapidly increasing market demand for therapeutic proteins, for example, monoclonal antibodies (mAbs), the industry is facing the challenge of efficiently manufacturing large-product amounts [1]. After recent process-intensification improvements in the upstream processing (USP), the current bottleneck of production processes shifts to in the purification of protein products in the subsequent downstream processing (DSP) [2]. In general, DSP of therapeutic proteins begins with clarification by filtration and/or centrifugation, followed by capture, purification, and polishing steps mainly done by chromatography and filtration techniques as shown in Figure 1 [3]. The goal of DSP is to improve the purity and increase the concentration of target molecules.

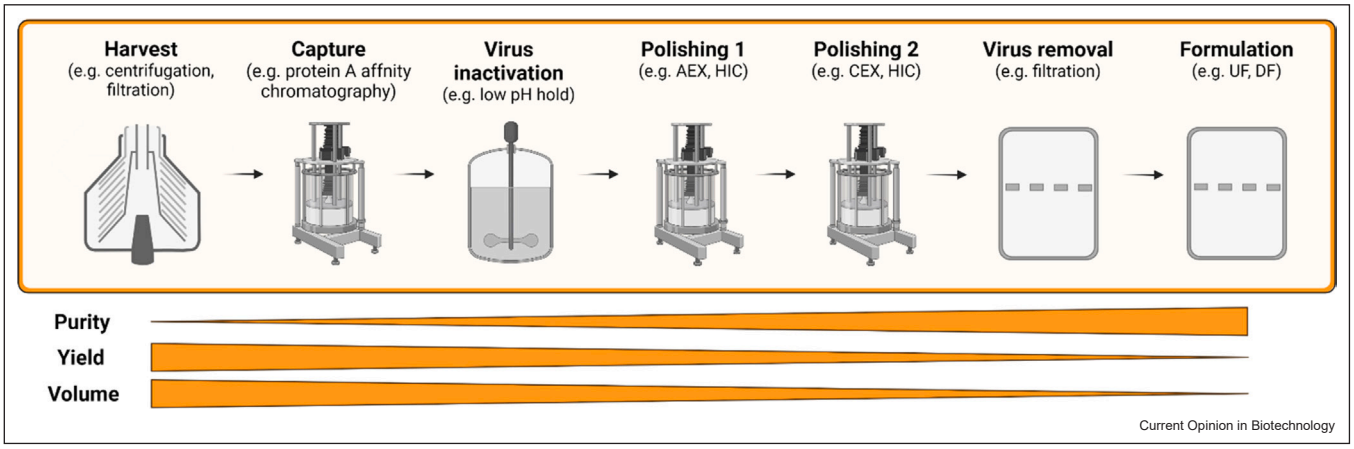
The DSP accounts for a significant part of the total production cost of biopharmaceuticals, mainly driven by the expensive chromatography steps [2,4,5]. Packed-bed chromatography has been the industrial workhorse for decades as it achieves excellent yields and purities [2]. However, conventional chromatography might reach its limits in processing the increasing titers and volumes of USP. Therefore, new and alternative technologies that allow for process intensification are increasingly being investigated [5,6]. Current process-intensification strategies include the transition from batch to continuous integrated processes, the use of single-used equipment, the improvement of process control, and the use of scale-down models for more efficient process development [7,8]. A summary of innovative DSP strategies is highlighted in Figure 2.

Most downstream processes will always need multiple steps and purification cascades, however, we want to highlight the current trends for different separation procedures as well as novel processing concepts.

Precipitation

The main advantages of precipitation include fast and robust processing of high titers and large volumes, scalability, high yields, and low costs, making it a promising alternative technique [9]. Several studies recently demonstrated the applicability of precipitation for mAb capturing as a valuable alternative to the currently used and limited protein-A chromatography (see Figure 1) [10–12]. Continuous processes using tubular reactor designs can be implemented to address the need for process intensification. Furthermore, the integration of precipitation with subsequent washing (and resolubilization) steps into the current mAb-purification platform process is possible [13]. However, precipitation can influence other purification steps significantly [14•]. An efficient precipitation process of mAbs using $ZnCl_2$ and polyethylene glycol (PEG) has been demonstrated recently [10,11]. Dutra et al. developed a precipitation process based on $ZnCl_2$ without PEG, which reduces the

Figure 1



Schematic sequence of unit operations constituting the platform approach employed in the DSP of pharmaceutically relevant proteins (e.g. mAbs). AEX: anion-exchange chromatography; CEX: cation-exchange chromatography; HIC: hydrophobic-interaction chromatography.

viscosity of the processed fluid for improved harvesting and washing of precipitates [12].

Besides precipitation with PEG and salts, affinity precipitation using, for example, stimulus-responsive elastin-like polymers [15] or Ca^{2+}-dependent calsequestrin fused to affinity peptides, was recently successfully employed for selective capture and purification of mAbs and other therapeutic proteins [16•]. Affinity precipitation is promising as optimized peptides allow the construction of a robust platform-compatible process, for example, similar to protein-A affinity chromatography used in current mAb-purification platform processes.

Current research in the field of process analytical technology (PAT) to approximate the "Quality by Design" (QbD) concept introduced by the FDA could help speed up precipitation-process development and ensure product quality in the future [17•].

Crystallization

Protein crystals possess ordered protein configurations that generally have higher physicochemical stability and purity than amorphous precipitates. Therefore, crystallization can be used in intermediate and polishing steps in DSP [8]. Another advantage is the possible timely controlled release of therapeutic proteins from the crystal lattice when used as drug formulation.

Protein crystallization, such as antibody crystallization, is a thermal process that depends on the supersaturation of the protein. The crystallization of proteins is similar to the crystallization of small molecules, but needs mild-condition changes such as salts or polymers, as well as slow pH, ionic strength, and/or temperature changes (cooling crystallization). However, a major obstacle of the crystallization process is the difficulty of its implementation. The large size and complex configuration of proteins, especially of mAbs with their flexible hinge region, hinder a simple crystallization process [18••]. Therefore, current studies focus on a better understanding of nucleation and crystal growth through mathematical approaches [19,20] and empirical high-throughput screening [21,22].

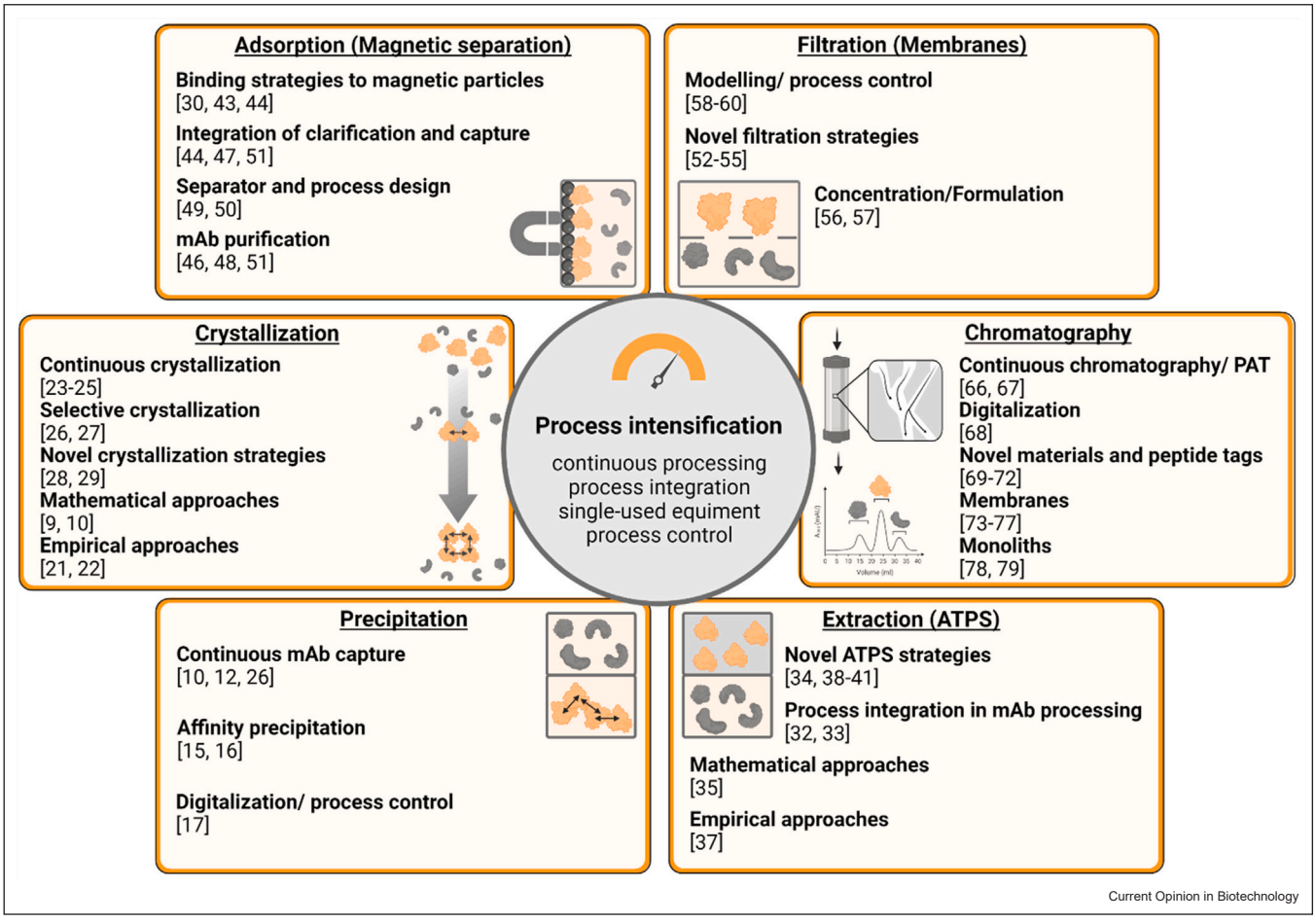
In the last decades, much research effort has been devoted to lysozyme as a model protein in mainly batch operations from solutions with already high purity. However, for the intended use in biopharmaceutical DSP, research focuses on continuous [23–25] and selective crystallization [22,26,27] of proteins such as mAbs. Selective crystallization from impurities is a possible alternative for early applications in DSP [22•].

Grob et al. recently demonstrated the enhancement of crystallizability of an alcohol dehydrogenase by rational crystal-contact engineering [28], which could open up a broader use of crystallization as a purification technique. Moreover, nanoparticle-induced precipitation and crystallization are an upcoming trend for pharmaceutical protein purification. Especially, iron oxide nanoparticles play a great role in the precipitation and crystallization of proteins such as lysozyme or trypsine [29] and nanobodies [30].

Extraction (aqueous two-phase system)

Aqueous two-phase systems (ATPS) are due to their beneficial aqueous nature by far the most researched extraction technique for proteins. A spontaneous formation of an ATPS can be observed, when two

Figure 2



Schematic overview of current research topics and studies in biopharmaceutical DSP. Process intensification is the overarching goal of all current innovations in DSP.

hydrophilic phase-forming components remixed above a critical concentration [31]. The advantages concerning biopharmaceutical DSP applications include high biocompatibility, high recovery yields and selectivity, low cost, fast equilibrium adjustment, and scalability.

Researchers recently demonstrated the integration of mAb clarification and capture steps [32] and proposed the direct integration of ATPS operations into current industrial mAb platform processes [32–34].

The main reasons why ATPS is not yet established in DSP are discussed in the literature. The underlying mechanisms of ATPS on biomolecule partition are not yet wholly understood and there are no large-scale studies and promising continuous application process designs for ATPS [31]. However, researchers aim to fill these knowledge gaps. The prediction of partition coefficients is currently investigated [35]. Different reactor systems, such as the use of coiled flow inverters for extraction processes, are tested [36]. Understanding and mathematical modeling are supported by the development of (continuous) microfluidic screening [37]. New phase-forming components, such as ionic liquids [38••] and the improvement of the process strategy (e.g. via multistage extraction or phase/component recycling), are currently being studied [39•]. Moreover, with reactive ATPS [40] and magnetic-assisted ATPS [41], there are further approaches to process intensification. In addition to ATPS, also three-phase partitioning processes show advantages for protein purification processes [42]. A systematic understanding of ATPS based on small-scale screening is considered key for future process development and scale-up.

Adsorption (magnetic separation)

The binding of a pharmaceutically relevant protein such as a mAb to a solid phase is a classical separation mechanism

that is very often used in chromatographic processes as well. Moreover, there are multiple batch-adsorbent materials and adsorption processes that can be used for DSP and antibody purification. However, in this section, the focus is mainly on batch-adsorption systems based on magnetic separation in contradiction to packed-bed systems. A current trend for these adsorption processes is the use of ferromagnetic, ferrimagnetic, or superparamagnetic carriers, which carry a specific binding site, for example, protein-A or protein-G domains. The advantage of magnetic-based processes is the simple separation of the bound and the nonbound phase. Therefore, this mechanism is well-suited for protein-capture steps. The recent trends on larger scales for protein purification have been reviewed by Schwaminger et al. [43]. New adsorbents and binding strategies to magnetic particles were developed in recent years [30,44,45]. Zanker et al. showed that a direct capture of nanobodies containing an affinity tag is possible with magnetic nanoparticles [30•]. Especially protein-A-based magnetic beads can be used for purification processes and capture of mAbs in analogy to protein-A chromatography [46–48]. Moreover, magnetic separation processes and especially high-gradient magnetic separation processes are continuously improved toward separator and process design [49,50], as well as toward process integration [43,47,51]. Brechmann et al. showed that magnetic beads can be used efficiently at very high cell densities for antibody capture [51•]. Magnetic separation processes might provide a sustainable alternative to protein-A chromatography for future DSP due to the energy-efficient separation that can be used in early stages of the DSP without previous harvest filtration and centrifugation steps that are necessary for packed-bed systems.

Filtration

Filtration is a separation step that allows for separating proteins according to their size. Moreover, filtration is used for concentration of proteins and buffer exchanges. Thus, depending on the protein size and the processing step, microfiltration, ultrafiltration (UF), nanofiltration, and reverse osmosis play a role in the purification of pharmaceutically relevant proteins.

Recent trends in filtration are often related to process intensification [52••] and to the processing strategy, depending on the order of unit operations [53]. A current study showed how UF affects the purification process of the protein C-phycocyanin and highlights the importance of the filtration step and its position in the DSP [53]. High-performance countercurrent membrane purification has been introduced for protein purification using bovine serum albumin as a model protein [54•]. Also, the coupling of separation driven by electric charge and filtration is an ongoing trend for protein purification [55].

Moreover, filtration is of great importance for protein formulation, since it is very often used as a final step in a purification cascade (see Figure 1) [56]. Thus, the understanding and design of this last process step are of great importance. A recent study investigated the modeling and optimization of single-pass tangential flow UF for mAb purification [57•].

New modeling approaches are developed and used for filtration processes recently. A new model for electrostatic effects has been developed by Briskot et al., which allows for better pH control for UF and diafiltration (DF) processes [58]. Ambrožič et al. used a new mathematical approach to model UF and DF with the aim to approximate QbD for filtration technologies [59]. Along with improved mechanistic model concepts, also digital twins and hybrid models are developed for filtration processes for protein purification [60•]. These ongoing trends in filtration will also lead to more efficient purification processes.

Chromatography

Chromatography is conventionally used in multiple operational units for protein separations. Chromatography describes the process of dynamic separation of mixtures. The versatility of this technology is dependent on different separation mechanisms such as specific interactions with a solid phase or different diffusivities. Chromatography is not only a process step, but depicts a lively research field with novel studies, for example, on new materials [58,61–65], new stationary phases such as membranes and monoliths, continuous processing and PAT [66,67], process modeling [68•], and new approaches such as the development of novel affinity materials [69,70] or novel peptide tags for stationary phases [69–72]. In this short review, we focus on unconventional promising alternatives to packed-bed chromatography such as membrane and monolith chromatography.

The main advantage of membrane chromatography is higher flow rates compared with conventional chromatography, which benefits productivity [73]. Cost-effective manufacture of membrane adsorbents offers the possibility of single-use, reducing time-consuming and costly cleaning and validation procedures of packed-bed columns in biopharmaceutical applications [74•]. Much research focuses on increasing binding capacities, which has long been a major drawback of membranes. The use of nonwovens [74,75] and electrospun nanofibers [76] showed success in purifying therapeutic antibodies and other proteins. Roshankhah et al. developed a cation-exchange z^2 laterally fed membrane chromatography (z2LFMC) process with three times higher productivity compared with conventional protein-A chromatography for antibody purification [77•].

Monolith chromatography is also characterized by high mass transfer efficiency. Simon et al. recently

demonstrated the cheap, robust, and customized fabrication of monolith columns by 3D printing [78•]. Their anion-exchange monoliths exhibited static binding capacity comparable to that of commercial material, indicating the great potential of monoliths and additive manufacturing for biopharmaceutical DSP. The great potential of monoliths was confirmed, for example, by Wilke et al. who demonstrated purification of IgG from human plasma with high productivity using sintered glass monoliths with immobilized protein A [79].

In addition to the development of novel chromatography processes, multiple recent studies also focus on the understanding of the binding and elution mechanisms of mAbs [80–84].

Even though there are multiple novel approaches in DSP, chromatography remains the working horse for the purification of pharmaceutically relevant proteins.

Conclusion

The reviewed studies suggest exciting developments in biopharmaceutical DSP in the coming years. We are confident that some of the mentioned approaches will find their application and lead to improved and more sustainable production of pharmaceutically relevant proteins. Nevertheless, convincing regulatory authorities of new purification methods as an alternative to established chromatography methods will be a challenging future task.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

All figures have been created with BioRender.com.

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