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High Throughput Downstream Screening System for Protein Purification

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Abstract

The aim of the project was the development of a modular construction system concept based on membrane adsorber technology for protein purification. The approach enables a high throughput screening for the determination of the optimal downstream conditions of several bioproducts simultaneously. An easy scale up of the purification results from microtiter to industrial scale can be performed.

HTDSS

In order to screen different target proteins and to devise the most efficient strategy for their fast and direct separation and purification, we developed a modular construction system concept based on membrane adsorber technology. Figure 1 exemplifies this concept of the so called high throughput downstream screening system (HTDSS).

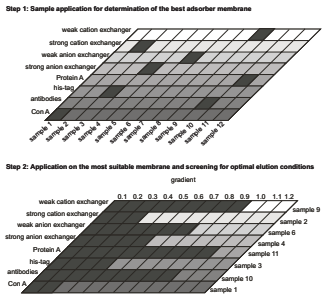


Figure 1. Modular concept for a screening experiment

Easy Scale-up

For the implementation of the HTDSS 8-strip devices were designed. Each 8-strip represents a certain membrane type (e.g. cation exchanger, metal chelate) and can be arranged in the 96-well format and processed by centrifugation.
After the determination of the optimal purification strategies for the target proteins scale-up experiments can be performed using membrane modules in various formats (Figure 2).

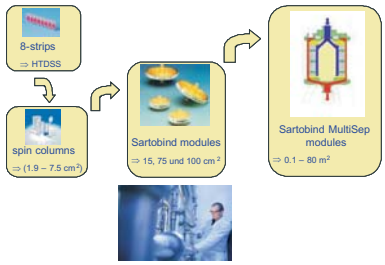


Figure 2. Membrane adsorber in various formats

Separation of model proteins

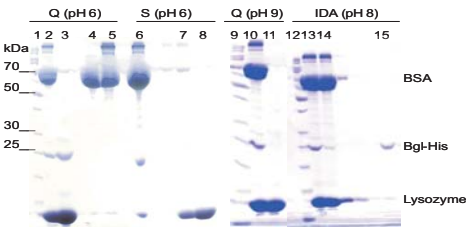
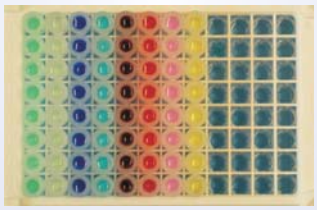


Figure 3. Separation of BSA, lysozyme and Bgl-His on Q, S and IDA membrane adsorber.

The efficiency of the used adsorber membranes were tested by the separation of a BSA/lysozyme/Bgl-His. The different pI values and the distinct affinities for the metal chelate allow the separation of BSA, lysozyme and Bgl-His on ion exchange and IDA membrane adsorbers. The isolation of BSA was carried out at pH 6 on Q membrane (Lane 4). S membrane retained lysozyme at this pH value (Lane 7). Lysozyme was also fractionated at pH 9 on Q membrane, where BSA and Bgl were completely adsorbed on the membrane and pure lysozme passed through (Lane 11). The isolation of Bgl-His from the mixture succeeded only with IDA membrane. Bgl-His was purified on IDA membrane loaded with Cu^{2+}-ions (Lane 15).



Purification of IgG_1

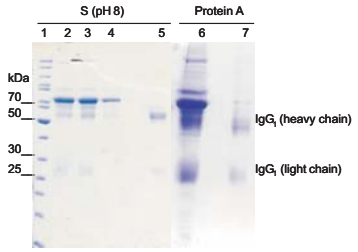


Figure 4. Purification of IgG_1 from cell culture supernatant using S and Protein A membrane.

As an other example for the successfull application of HTDSS the purification of IgG_1 from cell culture supernatant is presented. The antibody could be purified on S membrane at pH 8 (Lane 5) and by appying on Protein A membrane (Lane 7).

Purification of His-tagged proteins

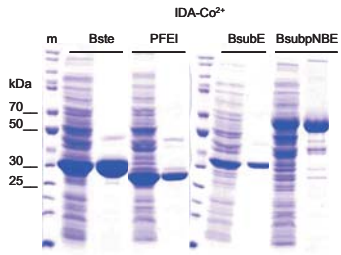


Figure 5. Purification of proteins with polyhistidine tags using metal chelate membrane.

The purification of recombinant proteins engineered with polyhistidine, $(His)_6$ tags from crude fermentation broths and cell lysates using Immobilized Metal-Affinity Chromatography (IMAC) provides a one-step isolation of proteins at over 90 % purity. 8-strips allow a screening of different metal ions and matrices simultaneously. To obtain pure His-tagged proteins, the 8-strips are loaded with Ni^{2+}, Co^{2+}, Cu^{2+} and Zn^{2+} solutions and the proteins are separated from cell lysate by applying on the charged membranes. According to the analysis by SDS-PAGE of four enzymes with histidine tags, Co^{2+}-ions are most suitable for the isolation.

Conclusion

The applicability of the developed membrane adsorber based devices in HTDSS, the selectivity of the adsorber membranes and the effect of loading-elution conditions were investigated and successfully applied for the purification of different proteins from model protein mixture, fermentation broth and cell culture supernatant.
In summary the HTDSS facilitates a fast and parallel determination of the optimal purification conditions of the target proteins.

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