

Preparative Parallel Protein Purification (P4)

The structural analysis of pharmacologically relevant target proteins is one of the key elements in the drug discovery process [1]. Therefore the demand to supply large numbers of different proteins at the right quantity and quality has dramatically increased. This can only be met by high throughput (HT) approaches. One prerequisite is the availability of complete genomic sequences of human and many other species, which set the molecular basis for recombinant expression of proteins [2]. Another important attribute is the possibility to fuse the recombinant protein to tags thus enabling simplified and uniform affinity purification [3, 4].

The process of protein expression and purification has been automated either in modules focusing on liquid handling robots [5–7] or as fully integrated platforms [8]. Currently, most HT platforms use the micro-titre plate (MTP) format and are therefore limited to small-scale not being able to deliver the amounts required for crystallography. Those approaches based on standard robots also suffer from drawbacks such as the absence of online UV monitoring and gradient forming capabilities. Often negative pressure in form of vacuum as driving force is applied thus deteriorating the flow characteristics and leading to poor resolution. The currently most integrated system, which is based on a programmable single channel LC system containing features like gradient forming and UV monitoring, is capable to perform multi-step purifications from up to

six samples without user interference [9]. Further development of that device led to the integration of up to twelve modules to obtain a true parallel purification system. So far real multi-channel systems only existed for analytical purposes [10] or for purifying small organic compounds. Here we describe the modification of a ten-channel parallel flash chromatography system (CombiFlash OptiX 10, Isco Inc.) for automated parallel protein purification applications. This system enables us to perform ten different purifications in parallel with individual gradients and UV monitoring. We present data from a typical application including a capture step using affinity chromatography, polishing step with ion exchange chromatography, and desalting/buffer exchange by gel filtration.

All parallel purifications were performed with the CombiFlash OptiX 10 system (Isco Inc., Lincoln, USA) equipped with the X-Y fraction collector Foxy 200 and controlled by the PeakTrak software. This instrument features ten separate channels for parallel flash chromatography with individual gradient formation and UV monitoring. The OptiX 10 instrument was modified in several ways to facilitate typical protein purification applications. The minimal flow rate was lowered from 5 mL min⁻¹ to 1 mL min⁻¹ and the UV flow cell was exchanged to one with a path length of 4 mm (standard is 0.2 mm). The metal plate covering the back unit of the OptiX 10 was replaced with Plexiglas to monitor the absence of air in the pump cylinders. The system was further stripped from sample loading parts, except for the manual 4-way injection valves to which a MCP Standard

multi-channel peristaltic pump (Ismatec, Glattbrugg, Switzerland) was added. The peristaltic pump was set up with a 12-cassette multi-channel pump head (CA 12) with 1.02 mm I.D. Tygon tubing capable of delivering flow rates between 0.13–31 mL min⁻¹. All external tubing fittings were replaced with Luer or M6 connectors and union adaptors.

We expressed proteins from ten different plasmids in one-litre cultures of *E. coli*. The protein products were His-tagged variants of seven different nuclear receptor ligand binding domains with molecular weights from approximately 25–40 kDa and pI values ranging from 5.5–9. The lysates of the harvested bacterial cells were applied onto ten 7 mL Ni-NTA columns equilibrated with 20 mM Tris pH 8.0, 200 mM NaCl via the peristaltic pump. Elution was conducted using a three-step gradient (two wash steps and a final elution step with 200 mM imidazole) and peak fractions were analysed by SDS-PAGE (data not shown) (Fig. 1). After subsequent desalting the Ni-NTA pools were loaded onto 5 mL Q sepharose FF columns equilibrated with 20 mM Tris pH 8.0 and elution was achieved with a linear gradient of increasing concentration of NaCl (Fig. 2). The flow rate during these procedures was 5 mL min⁻¹. Fractions of 5 mL each were collected and A280 was monitored during the entire chromatographic procedure. Using this protocol, nine of the cultures yielded purified His-tagged protein. One protein seemed not to be expressed at all since only contaminant proteins eluted from the Ni-NTA step. The protein content in the final ion exchange pools was estimated with the Bio-

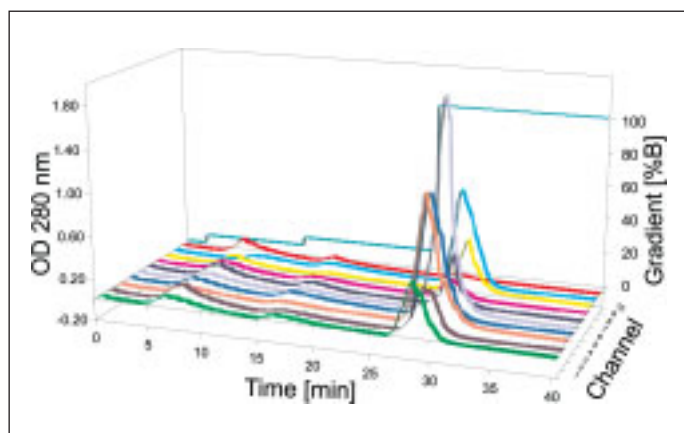


Fig. 1.: Chromatogramm of the Affinity chromatography

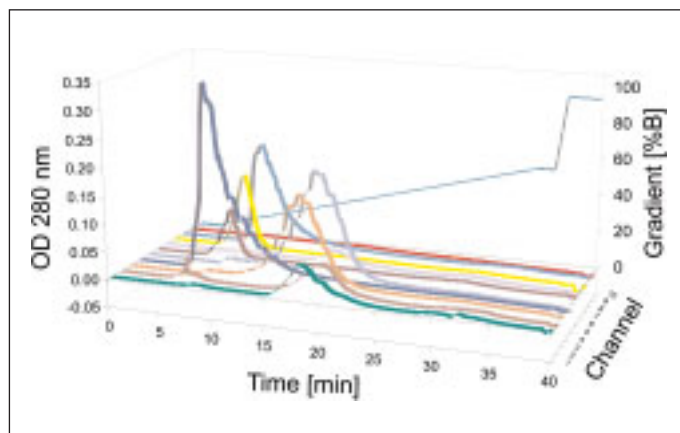


Fig. 2.: Chromatogramm of the Ion exchange chromatography

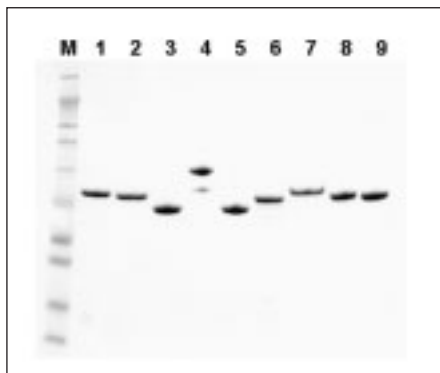


Fig. 3: SDS-PAGE analysis of purified His-tagged sample proteins

Rad protein assay and yields from the different cultures varied from 6–170 mg L⁻¹. Purity was assessed with SDS-PAGE (Fig. 3).

The experiments described above demonstrate that the modified OptiX 10 is a suitable instrument for a preparative parallel protein purification platform. In a sample experiment, proteins of high purity were obtained from nine out of ten cultures expressing different His-tagged proteins, using a three-step parallel purification procedure. However, there are still some flaws that have to be improved. Solvent exposed material should be replaced with inert metal-free parts to tolerate long-term usage of aqueous solutions and salts; optimization of the gradient mixing to improve gradient formation, collecting fractions in MTP deep-well blocks instead of tubes. The software should be adapted for the present applications and the external peristaltic pump should be amenable to software control or separate loading tubes operated via the inbuilt pump should be installed. Based on our specifications most of these features have now been included in the recently released BioOptix10.

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