Practical aspects of sample concentration and buffer exchange utilizing a miniaturized tangential flow filtration (TFF) system

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The most time-consuming steps in any preparative protein production are concentration and buffer exchange, whether for structural biology or general biochemistry. These steps must be done at least once at the end of a purification protocol but often must also be done between chromatrography steps.

Ultrafiltration using disposable centrifuge-driven devices is the standard for concentrating proteins on a lab scale. These devices use dead-end filtration where flow is perpendicular to the molecular weight cutoff membrane. This creates a concentration gradient, causing the flow to slow down and the concentration at the membrane to exceed the target, leading to aggregation or precipitation. The process can only be monitored by interrupting the centrifuge to examine the progress and/or mix the sample. This is tedious and inefficient.

The most popular buffer exchange method is membrane dialysis, which also uses a molecular weight cutoff membrane, but is driven by osmotic pressure. This method requires little monitoring but takes up to 18 hours, and requires large volumes of dialysis buffer and a final concentration step.

Industrial scale systems use tangential flow filtration (TFF) to perform both buffer exchange and concentration. They circulate the sample (retentate) as it flows parallel (tangential) to the membrane. This mixes the retentate as it is concentrated, which reduces the concentration gradient and thus the likelihood of aggregation or precipitation. Filter dialysis (diafiltration) is performed by adding a buffer during this process. However, the downside to these systems is that they usually work with liters of sample, and have dead volumes of tens to hundreds of milliliters, making them unsuitable for lab scale.

We present experimental data obtained with our previously described TFF system. In brief, it is one of the smallest devices of its kind, intended to make lab-scale purification faster and easier. It uses a consumable cartridge with an integrated microfluidic diaphragm pump and a filter membrane. The system is capable of completely automated buffer exchange and concentration using non-contact liquid sensing to track volume. In a single run, it can perform up to 50mL of concentration with diafiltration, or 100mL without, and has virtually no dead volume. We discuss concentration speed, sample recovery, as well as the impact of concentration, viscosity and temperature on the process using commercially available proteins. In all benchmarks, the miniature TFF device performs as well as, if not better than the largest commercially available dead-end centrifuge-driven devices.