The UNIVERSITY of OKLAHOMA | **The Wu Lab | Top-down MS and Functional Proteomics**

Construction of a Reusable Gel-Eluted Liquid Fraction Entrapment Electrophoresis (GELFrEE) Using 3D Printing

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1 Introduction

Gel-Eluted Liquid Fraction Entrapment (GELFrEE) is commonly utilized in proteomics research for size-based separation and fractionation of proteins. According to **Figure 1**, a complex sample is loaded onto the gel and a current is applied across. The negatively SDS coated proteins will be drawn toward the positive anode. The proteins are separated based on size as the proteins migrate through the gel. Smaller proteins move through the pores of the gel more easily and will elute first.

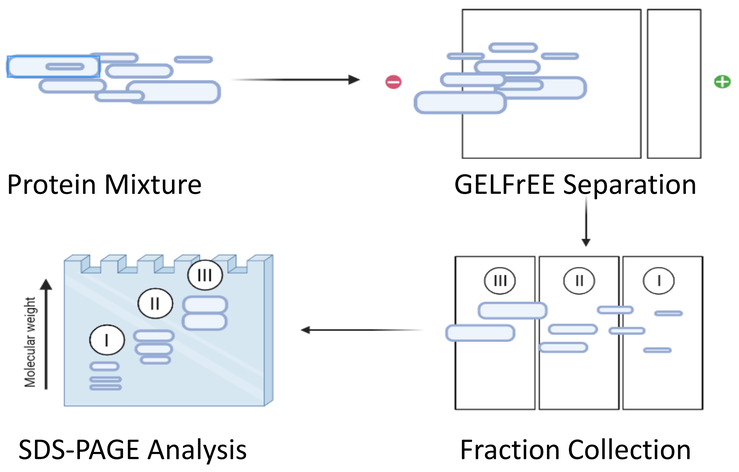


Figure 1: Schematic of GELFrEE separation and fraction collection. GELFrEE is a sized based separation technique that utilizes polyacrylamide gel to separate proteins on molecular weight with smaller proteins eluding first when collecting fractions. A current pulls the proteins across the gel.

Commercial GELFrEE cartridges are wasteful due to their single-use application and, furthermore, are being discontinued for leaving researchers that utilize the technique without needed resources. We have designed a 3D-printed, reusable GELFrEE cartridge that can be utilized with existing GELFrEE instruments. This cartridge allows the user to clean and repeatedly refill the chambers with new gels eliminating unnecessary plastic waste. The 3D-printed cartridge will further allow us to optimize the composition of the separating gel according to the experimental purposes.

2 Methods

2.1 3-D Printing the reusable GELFrEE Cartridge

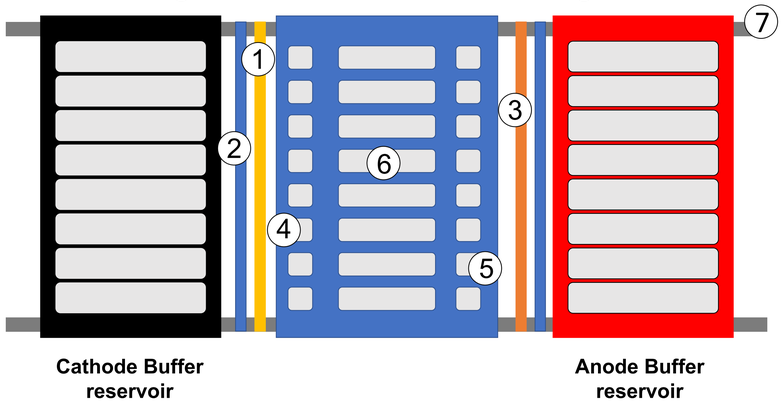


Figure 2: Top-down schematic of 3-D printed GELFrEE cartridge. (1) Whatman filter paper; (2) Nano sticky tape (sealant); (3) Snakeskin (10 kDa); (4) Sample loading chamber; (5) Sample collecting chamber; (6) Polyacrylamide gel; (7) steel rods.

2.2 Optimizing polyacrylamide filling and separation

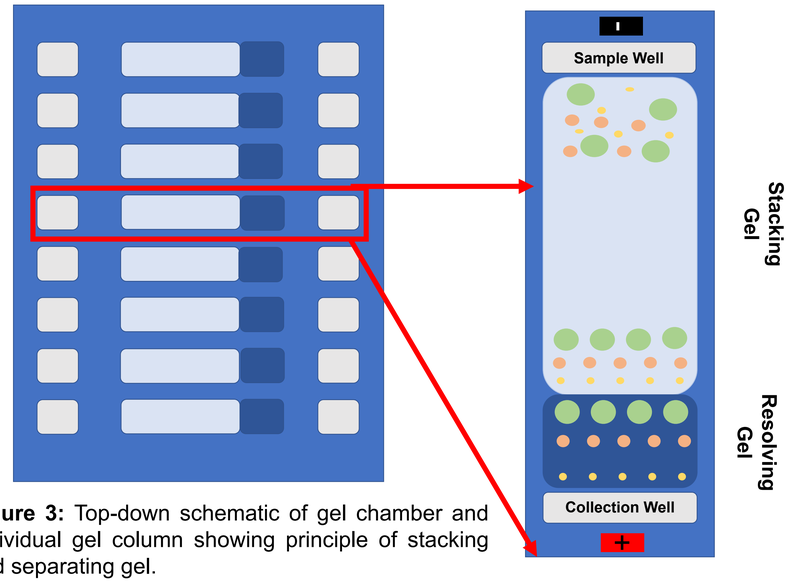


Figure 3: Top-down schematic of gel chamber and Individual gel column showing principle of stacking and separating gel.

Figure 4: Picture of 3D printed cartridge utilized in collecting data.

3 Results

3.1 Proof-of-concept. *E. coli* cell lysate separation using 4% stacking gel

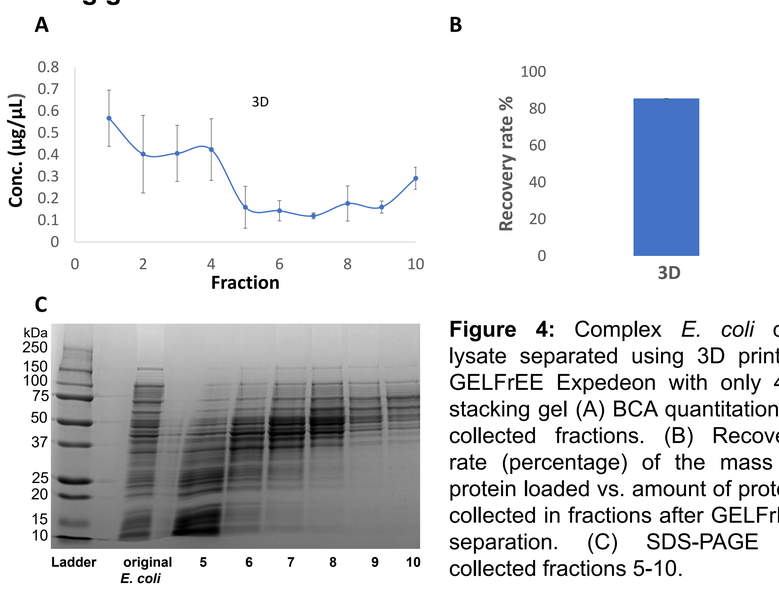
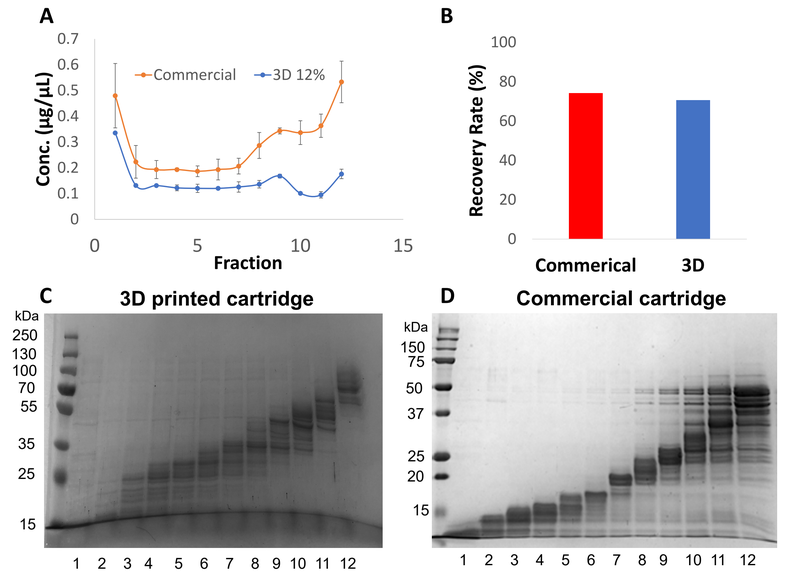


Figure 4: Complex *E. coli* cell lysate separated using 3D printed GELFrEE Expedeon with only 4% stacking gel (A) BCA quantitation of collected fractions. (B) Recovery rate (percentage) of the mass of protein loaded vs. amount of protein collected in fractions after GELFrEE separation. (C) SDS-PAGE of collected fractions 5-10.

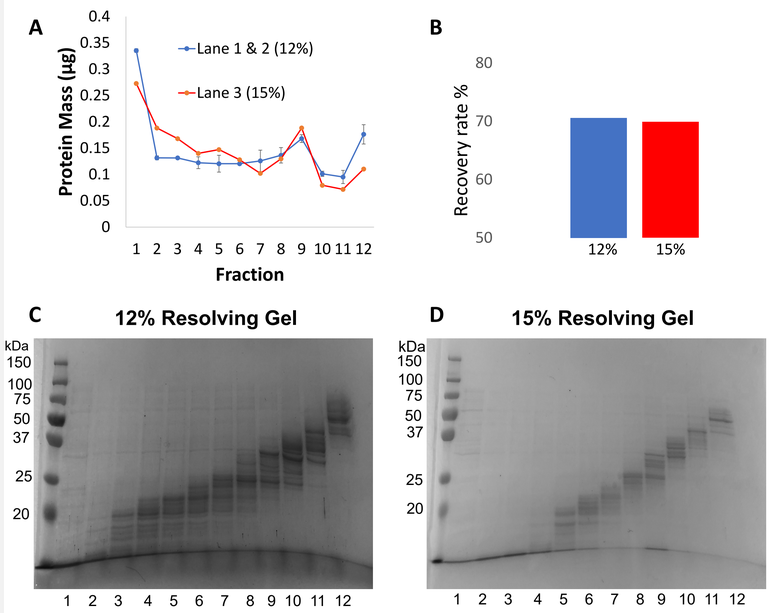
3.2 12% commercial cartridge vs. 12% 3-D printed cartridge

Commercial cartridge and 3D printed cartridge using 12% resolving and 4% stacking gel separating complex *E. coli* lysate. (A) BCA quantitation of fractions collected. SDS-PAGE of fraction collected using the (C) 3D printed cartridge and (D) commercial cartridge.



3.3 3-D printed cartridge with 12% and 15% resolving gel

Comparison of 3D printed cartridge using 12% and 15% resolving gel (4% stacking for both). 500 ug of *E. coli* lysate was separated. (A) BCA quantification to determine mass eluted. (B) Recovery rate (%Recovery = Mass Recovered/Initial Mass). SDS-PAGE for collected fractions, (C) 12% resolving; (D) 15% resolving.



4 Summary

- The 3D printed GELFrEE cartridge was capable of sized-based separation of complex protein mixtures.
- The 3D printed cartridge also facilitates customized protein separation via the optimization of polyacrylamide concentration.

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Reference
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