# SIZE-BASED PROTEIN FRACTIONATION IN NANOFLUIDIC CHANNEL ARRAYS

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## ABSTRACT

Separating and quantifying <100 nm bioparticles is an important research area; unfortunately, current methods for this have disadvantages. We have used standard microfabrication techniques to create nanofluidic sieving devices that address limitations of present approaches. Our system has an array of parallel nanochannels, with each channel having a height step. These height steps trap proteins based on size when capillary action draws solution through the nanochannels. We tested the setup with different sizes of proteins to assess trapping. The devices clearly provide size-dependent protein capture within nanochannels. This work demonstrates important progress in nanofluidic molecular fractionation.

**KEYWORDS:** Nanofabrication, Nanofluidics, Nanoparticles, Protein Separations

## INTRODUCTION

The separation of biomolecules, particles, etc. in the size scale of 10-100 nm is an important analysis area, with applications in determining particle size distributions, classifying lipoprotein fractions, and sizing DNA fragments. Various methods are used to characterize such particles, including electrophoresis, ultracentrifugation, size-exclusion chromatography, field-flow fractionation, and electron microscopy. However, existing approaches have limitations in applicability to many particle types (electrophoresis), resolution (size-exclusion chromatography and field-flow fractionation), speed (ultracentrifugation) and throughput (electron microscopy). Thus, we have focused on developing a capillary flow nanofluidic system that provides rapid and straightforward size-based separation of viruses, nanoparticles and proteins.

We previously demonstrated a process for making nanofluidic channel arrays through thin-film microfabrication [1]. These nanofluidic systems had height steps that took each channel from a critical dimension (height) of ~200 nm down to ~30 nm. As illustrated schematically in Figure 1, when solution flows through these nanochannels by capillary action, components that are smaller than the height step pass through and accumulate at the exit, while structures larger than the height step are trapped. We previously showed that these nanofluidic arrays size-selectively collect nanoparticles and viruses at height steps [1,2]. Herein, we demonstrate two key advances in nanofluidic separation: (1) we have improved device fabrication, enabling structures with shorter (15 nm) height steps that significantly extend the size range of particles/molecules that can be fractionated; and (2) we have characterized protein separations in these nanofluidic channels.

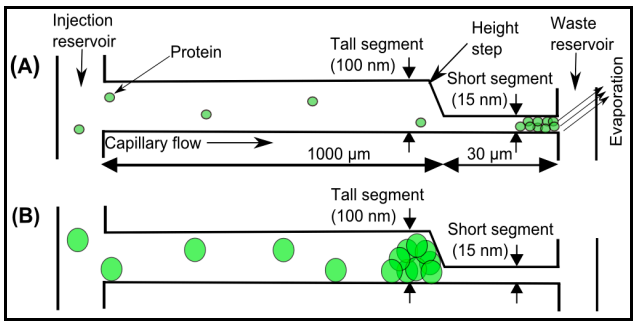


*Figure 1: Side-view schematic of size-based trapping of proteins in a dual-height nanochannel. (A) 9 nm diameter proteins pass through a 100-15 nm height step and accumulate at the exit. (B) 17 nm diameter proteins trap at the same height step (channel lengths and heights are not drawn to scale).*

## EXPERIMENTAL

Devices are constructed on a 4"-diameter Si wafer using standard microfabrication techniques, including photolithography, thin-film deposition, chemical vapor deposition, and wet/dry etching. We generated 49 chips/wafer, with each chip having a ~1 $cm^2$ footprint and consisting of 200 parallel nanochannels. Channels start from the injection reservoir with a 100 nm height and step to a shorter, 15-30 nm height ~30 µm before the waste reservoir, as shown schematically in Figure 1. The details of our fabrication approach are illustrated in Figure 2; devices were made similarly to our previous method [1], but with two key improvements. First, we protected the Al with a thin Cr layer to prevent undesired etching in the photoresist developer. Second, we reduced the length of the shorter channel segments to ~30 µm, which allowed bubble-free capillary flow, even with 15 nm height channels.

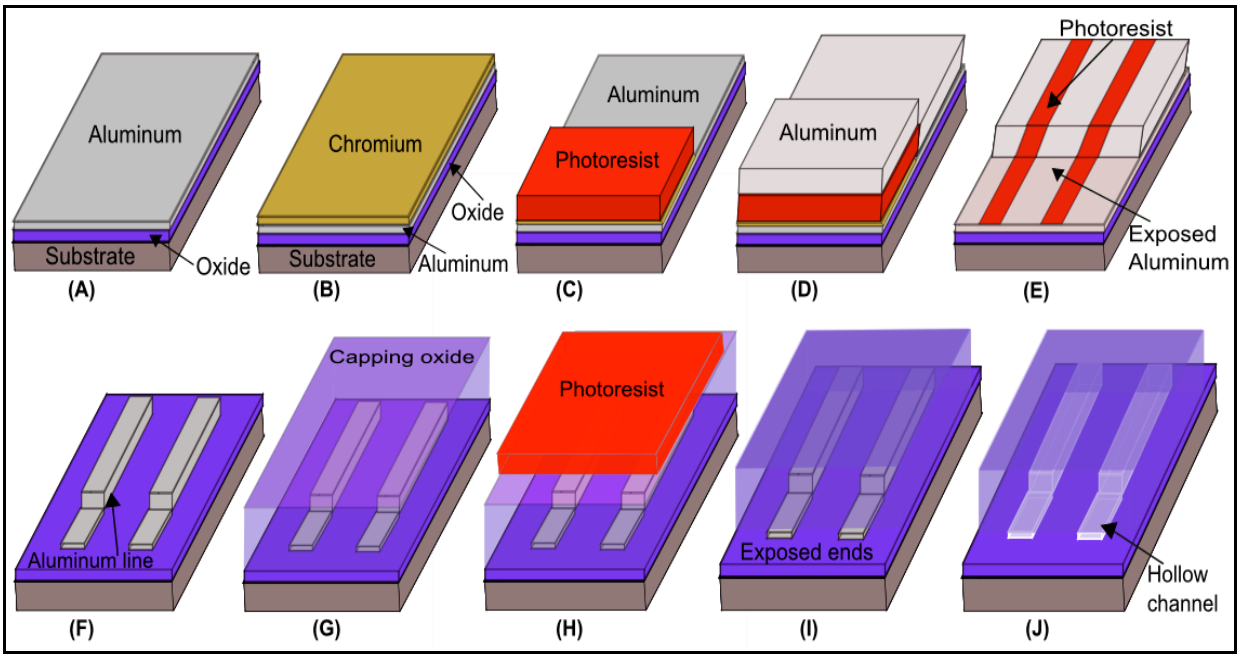


*Figure 2: Fabrication of dual-height nanochannels. (A) A ~100 nm thick oxide layer is deposited on a 4" Si wafer followed by a thin (15-30 nm) Al layer. (B) A ~15 nm Cr layer is deposited. (C) Photoresist is coated and patterned, and the exposed Cr is removed. (D) A second Al layer is deposited to make the taller segment. (E) Al is lifted off and Cr is removed; then photoresist is coated and patterned. (F) Exposed Al is removed, followed by photoresist stripping. (G) A capping layer of ~4 µm thick oxide is deposited to cover core lines. (H) Photoresist is coated and patterned. (I) Exposed oxide is etched to open the ends. (J) Al lines are etched using aqua regia, and the channels are then filled with water.*

*Table 1. Physical properties of proteins analyzed.*

| Protein | Abbreviation | Molecular Weight (kDa) | Diameter (nm) |
|---|---|---|---|
| Myoglobin | Mb | 18 | 3.5 |
| Hemoglobin | Hb | 68 | 5.5 |
| Catalase | Ct | 250 | 10.5 |
| Thyroglobulin | Tg | 660 | 17 |

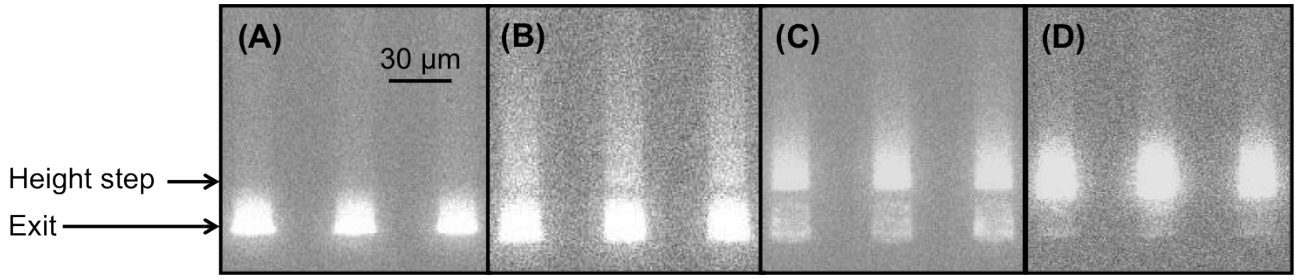


*Figure 3: Fluorescence images showing an increase in trapping at a 100-15 nm height step as protein size increases from (A-D). (A) Mb, (B) Hb, (C) Ct, and (D) Tg. All protein concentrations are 0.1 mg/mL.*

## RESULTS AND DISCUSSION

We have carried out size separation experiments on the proteins whose sizes and physical properties are listed in Table 1. Fluorescence images after flow of different protein solutions in nanofluidic channels that step from 100 to 15 nm are shown in Figure 3. Smaller proteins (Mb and Hb) mostly pass through the height step, while larger proteins (Ct and Tg) are largely trapped at the step. We have also studied the trapping of Tg as a function of step height from 15-29 nm (Fig. 4). With shorter height steps Tg is trapped at the interface, while for taller height steps Tg passes through and accumulates at the channel exit. Figure 5 summarizes the results of hundreds of flow experiments done with four different proteins and five different channel step heights. The smallest protein (Mb) has a low trapped/total ratio for all channel step heights. An intermediate size protein (Hb) is partially trapped at shorter steps and less trapped at taller steps. Larger proteins (Ct and Tg) are mostly trapped for shorter steps, partially trapped for intermediate height steps, and minimally trapped for the tallest steps.

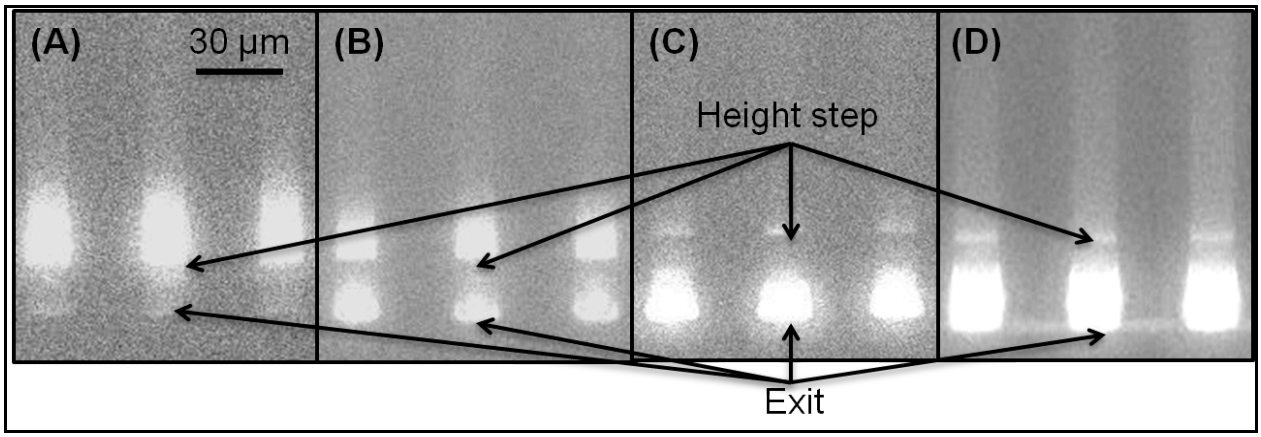


*Figure 4: Fluorescence images showing change in trapping pattern of Tg (0.1 mg/mL) with height step size. (A) 15 nm, (B) 18 nm, (C) 22 nm, and (D) 29 nm.*

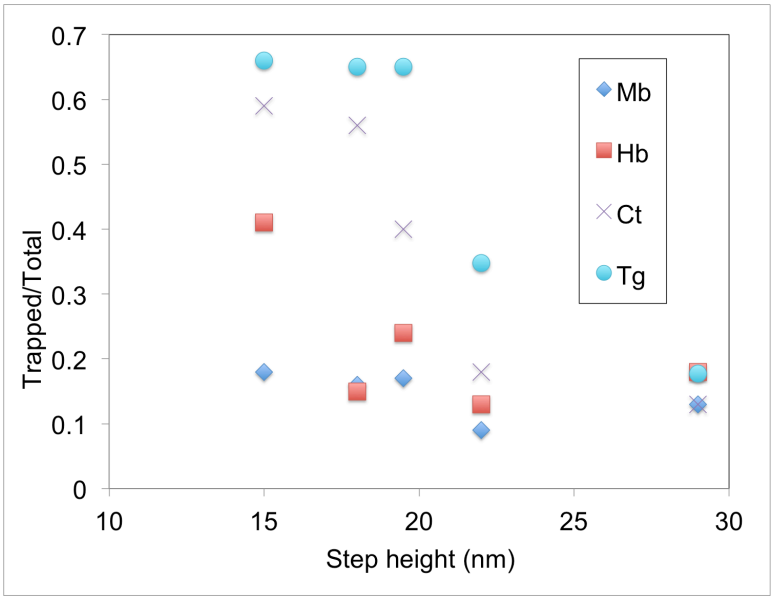


*Figure 5: Plot of the ratio of fluorescence signal at a height step (trapped) divided by the total signal in the channel for 4 proteins and 5 different step heights. Each data point shows the average from 9-12 separate flow experiments.*

## CONCLUSION

We have created and evaluated nanosieving devices for separating proteins based on size. We improved over our previous processes to enhance fabrication reproducibility and create channels with heights as short as 15 nm. These advances permitted studies on the effects of protein diameter on trapping. The ratio of trapped to total protein at these nanoscale steps depended on protein size and step height. The ratio of trapped to total protein increased with protein size for devices that had the same step height. These results provide the framework for fractionating multiple proteins in nanosieving systems, with potential for application in lipoprotein analysis, sample preconcentration, and isolating protein aggregates that can occur in protein drug formulations.

## ACKNOWLEDGEMENTS

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## REFERENCES

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