Nanofluidic Device for Continuous-Flow Size- or Charge-Based Separation of Native Proteins

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A typical proteome consists of ~10,000 different proteins, and such a complex protein mixture must be separated into simpler mixtures before bioanalysis and detection. Protein separation is routinely accomplished with two-dimensional gel electrophoresis, a labor-intensive procedure which can take many hours.

We have fabricated an anisotropic two-dimensional periodic nanofluidic filter array (Anisotropic Nanofilter Array: ANA) which is operated with two orthogonal electric fields. The ANA quickly separates in continuous-flow different-sized or -charged biomolecules, dependant on the buffer ionic strength. If the thickness of the electrical double layer is modulated to be comparable to the 55-nm-high nanofilters (e.g. at low ionic strength), electrostatic interactions of the molecules with the charged nanofilter walls are dominant, resulting in charge-based separation of the biomolecules, dependant on their isoelectric point (pI) and the pH value of the buffer. At high ionic strength the electrical double layer thickness is negligible, leading to pronounced steric interactions of the proteins with the ANA, and therefore separation based on their molecular size.

Size-based separation of fibrinogen (MW 340 kDa), B-phycoerythrin (MW 240 kDa), and lectin (MW 49 kDa) was obtained at TBE 5× buffer, pH=9.6. Three distinct streams of native proteins can be distinguished on the ANA, corresponding to the molecular size of the biomolecules. This separation mechanism is described by Ogston sieving.

Charge-based separation of native proteins has been achieved at TBE 0.05× buffer, pH=9.6. To exclude size-based separation effects, two biomolecules with approximately the same MW and different pI values were investigated: streptavidin with MW 52.8 kDa and pI=5–6, and lectin with MW 49 kDa and pI=8.0–8.8. At TBE 0.05×, streptavidin bears a higher negative net charge than lectin, and therefore passes through a lower number of cation-selective nanofilters, resulting in separated protein streams on the ANA. At TBE 5× no separation was observed which shows that the size difference between these two proteins can not be resolved on this particular ANA device, confirming that the separation at low ionic strength is indeed charge-based. B-phycoerythrin (pI=4.2–4.4), fibrinogen (pI=5.5), and lectin (pI=8.0–8.8) were also separated based on their net charge, and the separation resolution is increasing with an increasing electric field through the nanofilters.

Typical separation times with TBE 0.05× were 13s, and 400s with TBE 5×. This shows that rapid separations are possible, which hold great promise for proteomics.

References
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