366f Ultra-Fast DNA Sequencing by Microchannel Electrophoresis with a Dynamic Coating: 540 Bases in 4.5 Minutes

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Microfluidic chip-based electrophoresis for DNA sequencing represents the future for high-throughput sequencing projects due to its reductions in cost, time and reagent consumption and the possibility of integrating sequencing with other steps of genetic analysis into a total micro-analytical system. To this end, the development of optimal polymeric separation matrices and wall coatings for DNA sequencing on microfluidic chips is crucial. Moreover, for the practical implementation of chip-based sequencing in a high-throughput environment, a dynamically adsorbed polymeric wall coating is preferred, although to date only covalently applied wall coatings have been demonstrated in these sequencing devices. While linear polyacrylamide has been the common choice for the separation matrix, other novel acrylamide-based polymers including matrices developed in our lab have shown potential as high performance DNA sequencing matrices. While most published data on microchip-based DNA sequencing have reported read lengths of greater than 400 bases, sequencing times on chips generally have ranged from 18-30 minutes. (Note that capillary electrophoresis requires about 60-90 minutes to give comparable results.) In this presentation, modified acrylamide-based separation matrices are combined with more hydrophilic channel-coating acrylamide polymers to achieve DNA sequencing read lengths greater than 540 bases, at 98.5% accuracy, with a total separation time of approximately 8 minutes, and detection of the 540-base DNA fragment at 4.5 minutes. Further optimization of these separation media with respect to polymer properties such as molecular weight, composition, and solution concentration, as well as optimization of the wall-coating polymers, may allow for even longer reads at this reduced time. This results we have obtained represent the fastest sequencing time for such a long read reported to date, and hence provide a step forward in the development of microchannel-based sequencing technologies.