249a Isolating Unlabelled Biomolecules in a Microfluidic System – Switching Samples for Multiple Downstream Procedures

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Label Free Intrinsic Imaging (LFII) combines UV absorption, photodiode array detection, automated fluidic handling and digital signal processing to map the space-time trajectories of bioanalytes and chemicals in capillaries. This direct absorption technique eliminates labels and dyes by using signal-to-noise enhancing image processing algorithms, and has been successfully applied to protein sizing, DNA fragment analysis, chemical detection and DNA sequencing. Imaging the molecule itself, rather than the label chemically attached to it, significantly improves resolution while controlling migration time. There are also advantages in the speed of analysis, health and safety issues, reagent costs and reduced sample size. By working with unlabeled molecules, LFII also allows real-time fraction collection in the system. This gives us the ability to actively manipulate analytes, for example by switching an identified protein for subsequent analysis and the potential to identify using advanced data-mining tools. This paper will describe how LFII can be applied to the isolation of protein samples. A system of bifurcated channels in a plastic microfluidic chip has been designed. The branches in the chip have embedded exclusion electrodes to facilitate the switching, into an isolation channel, of single or multiple bands of a specific biomolecule from a complex mixture migrating by capillary electrophoresis. Initial migration in the separation zone will be controlled by standard CE electrodes in the reservoir wells at either end of the chip channels. The label-free proteins are imaged in real-time and their entry into the bifurcation zone accurately monitored. A switch will control the CE electrodes switching the anode from the separation phase to the collection channel moving the target protein into the side channel collection zone. The internal electrodes will be positioned so that closely migrating bands are excluded from contaminating the selected sample, and so that it can't to move back into the separation phase. This will be achieved by causing a voltage drop across the entrance to the collection zone after target selection. In-house software linked to deltaDOT's Label Free Intrinsic Imaging technology will control the switch. The anode will then switch back to the separation channel. The selected, unlabelled, biomolecules can then be recovered for further analysis, such as Mass Spectrometry or other technologies such as deltaDOT's Protein Folding Chip system.