### Three minutes-long electrophoretically assisted zeptomolar microfluidic immunoassay with magnetic-beads detection. Journal of the American Chemical Society 129:12628-12629

An ultrasensitive two-step immunoassay was developed for rapid assay of proteins and viruses. In the first step, electrophoresis was used to quickly bring soluble analytes from a flowing solution to a microarray of probe molecules immobilized on a semi-permeable membrane. In the second step, the captured analyte was detected by scanning the microarray with functionalized magnetic beads passed over the array surface by shear flow and pressed to the array surface by a magnetic field.
Three Minutes-Long Electrophoretically Assisted Zeptomolar Microfluidic Immunoassay with Magnetic-Beads Detection

Victor N. Morozov,* † ‡, Stephanie Groves, † Michael J. Turell,§ and Charles Bailey†

The National Center for Biodefense and Infectious Diseases, George Mason University, Manassas, Virginia 20110, Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino, Moscow region, Russia 142290, and U.S. Army Medical Research Institute of Infectious Diseases, 1425 Porter Street, Frederick, Maryland 21702

Received July 9, 2007; E-mail: vmorozov@gmu.edu

Development of protein-detection techniques as sensitive as PCR-based assays presents a tremendous challenge in diagnostics. With benefits of amplification being unfeasible for proteins, limit of detection (LOD) may only be decreased by increasing detection sensitivity. However, even an ultimate nanoscale biosensor capable of detecting a single analyte cannot have LOD much lower than femtornolar2 because of the impractically long time needed for an analyte to encounter the biosensor at lower concentrations. To overcome this problem and further decrease LOD while accelerating the assay, a new approach was suggested recently in which passive diffusion of analytes is replaced by their active delivery to the sensor with an external force3 (electric, magnetic or mechanical). LOD in such active assays may be extremely low because of analyte preconcentration and because of background reduction resulting from removal of weakly bound analytes with the force direction reversed.

Though different elements of the active assay were reported previously,1 this Communication describes for the first time an immunoassay method in which all the assay steps were performed actively in a single microfluidic device (schematically illustrated in Figure 1, panels A and B). The solution to be analyzed moves through a microfluidic channel (1.2 × 8 × 0.1 mm3) whose upper and lower walls are made of dialysis membranes. The bottom membrane has an antigen or antibody microarray on its upper surface. The membranes, penetrable to small ions, allow application of a normal electric field which draws charged analytes to the array surface, as illustrated in Figure 1A. While other similarly charged macro-ions are also brought to the microarray surface, they cannot establish strong bonds and are eventually removed by flow. The collected analytes are then detected with magnetic beads, as illustrated in Figure 1B. A suspension of magnetic beads, functionalized with probe molecules that specifically bind the analytes, flows through the cell while a magnet attracts the beads to the surface, causing them to slide over the array surface. When a bead meets a captured analyte, it becomes tethered temporarily or permanently. By choosing the correct shear rate, beads can be removed from the areas between spots to reduce background as well as from spots of weakly binding cross-reactive antibodies, thus improving assay specificity.

A detailed description of the cell design, peripheral devices, preparation of beads and arrays is presented in Supporting Information. The method was tested using both antigen and antibody arrays. For the first test, an anti-streptavidin (SA)-IgG array was manually prepared by electrospray deposition5 on a dialysis membrane coated with a layer of oxidized dextran6 and SA was electrophoretically

Figure 1. (A) Schematic of the electrophoretic capturing of charged analytes on an antibody array from flow. Green arrows denote flow of analyte, blue arrows denote flow of electrode buffer, red lines are platinum electrodes forming an electric field marked by red arrows. (B) Detection of captured analytes by scanning with functionalized magnetic beads. Suspension flow is marked by green arrows. (C) Dark-field images of an anti-SA-IgG array with SA molecules captured from 2 × 10−17 M solution of SA and detected by scanning with biotinylated magnetic beads. Distance between neighboring spots is 150 µm. Number in the upper right corner of each image in panel C indicates total time (in min) of capturing SA at a flow rate of 20 µL/min. Control image was obtained after capturing for 8 min under identical conditions from the same buffer solution without SA.

As illustrated in Figures 1C and 2A, a recognizable signal was obtained after 2−4 min of collection when 400−800 SA molecules had passed through the cell. Adding 1% serum (≈5 × 1010 molar excess of proteins) reduced the signal by only 10−15%, while adding 10% serum resulted in 50% inhibition of the signal. We
Figure 2. Dependence of spot brightness on the number of analyte molecules passed through the flow cell and on shear flow. (A) Streptavidin molecules captured on anti-SA-IgG array from buffer solution (empty circles), from the same buffer containing 1% dialyzed chicken serum (filled circles) and 10% chicken serum (filled squares) at a flow rate of 20 µL/min. (B) Dependence of the signal on the shear flow upon electrophoretic capturing of SA for 4 min. Numbers over points denote flow rate in µL/min. (C) Anti-Ova-IgG (commercial rabbit anti-Ova-IgG from Chemicon Intl., Temecula, CA, diluted 1012-fold) captured on an ovalbumin array at a flow rate of 60 µL/min. (D) Assay of viruses in serum from West Nile virus-infected chickens. The serum was dialyzed against water, diluted 1:200 with buffer, and WNV antigens were captured on an array of anti-WNV-IgG (solid circles). The same procedure was followed for a sample purified by exclusion chromatography (empty circles). The flow rate of the sample solution was 20 µL/min. The numbers above the experimental points in panels A, C, and D denote the time of capture in min. Other capturing parameters were a voltage of 110 V/10 cm, a flow cell design, peripheral devices, and general performance. This technology can be used in numerous applications where speed and/or extreme sensitivity are required such as in “warning”-type pathogen detectors, rapid immunoassay devices in emergency or surgery cabinets, or in an assay of biomarkers present at very low concentrations in biological fluids.

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Supporting Information Available: Detailed description of the flow cell design, peripheral devices, and general performance. This material is available free of charge via the Internet at http://pubs.acs.org.

References
4. (a) Flow rate of suspension of magnetic beads $Q = 5–10$ µL/min corresponded to a shear rate, $\gamma = 40–80$ sec$^{-1}$. At this shear-rate, a force applied to a single 20 nm long tether holding a magnetic bead, 1 µm in diameter, on the array surface is estimated as $T = 1.6–3.2$ pN.

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