An Integrated Microelectronic Device for Label-free Nucleic Acid Amplification and Detection

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While there have been extensive advances in miniaturized polymerase chain reaction (PCR) systems, progress on integrated microfabricated readout mechanisms has been rather limited, and most systems rely on off-chip optical detection modules to measure the final product. Existing optical detection platforms typically include CCD cameras, photodiodes, and photomultiplier tubes. While such hardware has adequate sensitivity for detecting PCR products in sample volumes significantly lower than that of bench-top systems, most are difficult to miniaturize and integrate into a compact analytical system. For example, some portable systems incorporating external LEDs and photodetectors can weigh between 1 kg and 4 kg each. To address these limitations, several groups have successfully embedded photodetectors within integrated PCR platforms. However, these devices still rely on external excitation sources.

To address this limitation, we have developed an integrated microelectronic device for amplification and label-free detection of nucleic acids (Figure 1) [1]. Amplification by PCR is achieved with on-chip metal resistive heaters, temperature sensors, and microfluidic valves. We demonstrate a rapid thermocycling with rates of up to 50°C/s and a PCR product yield equivalent to that of a bench-top system. Amplicons within the PCR product are detected by their intrinsic charge with a silicon field-effect sensor. Similar to existing optical approaches with intercalators such as SYBR Green, our sensing approach can directly detect standard double-stranded PCR products while in contrast our sensor occupies a micron-scale footprint, dissipates only nano-watt power during operation, and does not require labeling reagents. By combining amplification and detection on the same device, we show that the presence or absence of a particular DNA sequence can be determined by converting the analog surface potential output of the field-effect sensor to a simple digital true/false readout.

REFERENCES

Monitoring of Heparin and its Low Molecular Weight Analogs by Silicon Field Effect

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Heparin is a highly sulfated glycosaminoglycan that is used as an important clinical anticoagulant. Monitoring and control of the heparin level in a patient’s blood during and after surgery is essential, but current clinical methods are limited to indirect and offline assays. We have developed a silicon field-effect sensor for direct detection of heparin by its intrinsic negative charge [1]. The sensor consists of a simple microfabricated electrolyte-insulator-silicon (EIS) structure encapsulated within microfluidic channels (Figure 1). As heparin-specific surface probes, we used the clinical heparin antagonist protamine or the physiological partner antithrombin III. The dose-response curves in 10% PBS revealed a detection limit of 0.001 U/ml, which is orders of magnitude lower than clinically relevant concentrations. We also detected heparin-based drugs, such as the low-molecular-weight heparin enoxaparin (Lovenox®) and the synthetic pentasaccharide heparin analog fondaparinux (Arixtra®) (Figure 2), which cannot be monitored by the existing near-patient clinical methods. We demonstrated the specificity of the antithrombin III functionalized sensor for the physiologically active pentasaccharide sequence. As a validation, we showed correlation of our measurements to those from a colorimetric assay for heparin-mediated anti-Xa activity. These results demonstrate that silicon field-effect sensors could be used in the clinic for routine monitoring and maintenance of therapeutic levels of heparin and heparin-based drugs and in the laboratory for quantitation of total amount and specific epitopes of heparin and other glycosaminoglycans.

REFERENCES
Weighing of Biomolecules, Single Cells and Single Nanoparticles in Fluid


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Nanomechanical resonators enable the measurement of mass with extraordinary sensitivity. Previously, samples as light as 7 zeptograms (1 zg = 10^-21 g) have been weighed in vacuum, and proton-level resolution seems to be within reach. Resolving small mass changes requires the resonator to be light and to ring at a very pure tone—that is, with a high quality factor. In solution, viscosity severely degrades both of these characteristics, thus preventing many applications in nanotechnology and the life sciences where fluid is required. Although the resonant structure can be designed to minimize viscous loss, resolution is still substantially degraded when compared to measurements made in air or vacuum. An entirely different approach eliminates viscous damping by placing the solution inside a hollow resonator that is surrounded by vacuum (Figure 1). We have recently demonstrated that suspended microchannel resonators can weigh single nanoparticles (Figures 2), single bacterial cells, and sub-monolayers of adsorbed proteins in water with sub-femtogram resolution (1 Hz bandwidth). Central to these results is our observation that viscous loss due to the fluid is negligible compared to the intrinsic damping of our silicon crystal resonator. The combination of the low resonator mass (100 ng) and high quality factor (15,000) enables an improvement in mass resolution of six orders of magnitude over a high-end commercial quartz crystal microbalance [1]. This gives access to intriguing applications, such as mass-based flow cytometry, the direct detection of pathogens, or the non-optical sizing and mass density measurement of colloidal particles.

**Figure 1**: A suspended microchannel translates mass changes into changes in resonance frequency. Fluid continuously flows through the channel and delivers biomolecules, cells, or synthetic particles. Sub-femtogram mass resolution is attained by shrinking the wall and fluid layer thickness to the micrometer scale and by packaging the cantilever under high vacuum. In one measurement mode, particles flow through the cantilever without binding to the surface, and the observed signal depends on the position of particles along the channel (inset 1–3). The exact mass excess of a particle can be quantified by the peak frequency shift induced at the apex.

**Figure 2**: Synthetic particles of known size and density were measured to calibrate the mass sensitivity of the device. Gold nanoparticles (100 ± 8 nm) weighing 10 fg more than the water they displace produced a mean frequency shift of 36 mHz with a standard deviation of 6 mHz. On a different device, we measured a frequency shift of 310 ± 30 mHz for polystyrene microspheres (1.51 ± 0.01 um) with 90.1 fg mass excess.

**REFERENCES**

Integrated System for Cancer Biomarker Detection

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There is evidence to suggest that the next generation of cancer-screening tests may employ not just one, but a small panel of less than ten biomarkers that together add statistical power to the detection of specific cancers. While immunoassays such as ELISA are well established for detection of antigen-based biomarkers, the fidelity of the assay is governed by the disassociation constant, $K_d$, of the antibody-antigen complex. If the antigen concentration is significantly below $K_d$, then the binding kinetics are slow and readout precision of the antigen-antibody complex can be degraded by noise.

We propose a general approach for improving the performance of ligand-receptor assays. The approach is based on a nano-fluidic device that controllably concentrates a dilute sample and an ultra-sensitive suspended microchannel resonant mass sensor that detects specific biomarkers within the concentrate. Since the amplification (or gain) of the concentrator is adjustable, the dynamic range and detection limit of the immunoassay can be governed by the properties of the concentrator and not $K_d$. Since the integrated concentration/detection system is batch-fabricated by conventional foundry-level processing techniques, the cost per device could potentially be less than ten dollars.

Over the past year, we have fabricated the first generation of integrated systems (Figure 1). The devices appear to be functional based on initial visual inspections. We are currently validating the performance of the system by using quantum dots for a calibration assay. We are also in the process of validating the performance of the concentrator and mass sensor (as individual components) with prostate-specific antigen so that we can make comparisons to existing methods in terms of sensitivity and selectivity.

Figure 1: Integrated system for concentration and detecting biomolecules.