DIAGNOSTICS

Fast and sensitive electromechanical sensing

An electromechanical chip consisting of self-assembled DNA-based cantilevers immobilized on a liquid-gated graphene field-effect transistor can be configured to rapidly detect ultra-low concentrations of biomolecules, including viral nucleic acids, in biological fluids.

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The coronavirus disease 2019 (COVID-19) pandemic has highlighted the need for innovation in the disease-diagnostics toolbox. The gold-standard method for the detection of ultra-low amounts of viral infection is the quantitative polymerase chain reaction with reverse transcription (qRT–PCR), which requires heavy equipment and specialized operators and has turnaround times of tens of hours (from sample collection to the reporting of the result). Rapid antigen tests using the lateral-flow format can be self-administered with minimal training; however, they are not sufficiently sensitive to detect the infection in its early stages. Diagnostic tests that combine the speed of lateral-flow assays and the sensitivity of qRT–PCR would therefore be highly beneficial. Reporting in *Nature Biomedical Engineering*, Dacheng Wei, Zhaoqin Zhu and colleagues now show that self-assembled DNA structures functioning as molecular cantilevers and immobilized on liquid-gated graphene field-effect transistors can be used to detect, within a few minutes, ultra-low concentrations (near the single-molecule limit) of ions and biomolecules in diluted complex fluids, and RNA molecules of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in heated nasopharyngeal swab samples[1].

Building a sensor for the detection of single-molecule-binding events in unprocessed biofluids is challenging[2]. Wei and co-authors' strategy is based on the immobilization of a dense layer of precisely designed molecular biorecognition units on an electronic surface. They designed a DNA-based biorecognition unit consisting of a stiff pyramidal base of double-stranded DNA connected to a flexible cantilever made of single-stranded DNA and ending with an aptamer — a short single-stranded oligonucleotide — of complementary oligonucleotides designed to specifically bind to an analyte (the authors used thrombin, ATP, Hg^{2+}, single-stranded DNA, and RNA sequences from SARS-CoV-2). When a molecule of analyte binds to the aptamer, the cantilever bends (Fig. 1a), and such mechanical perturbation triggers an electrical signal at the underlying graphene surface of the transistor (Fig. 1b). The net effect of the binding event at the cantilever tip is a reduction in the current at the transistor's channel.

Transistors can be powerful amplifiers of weak biological signals[3]. Yet they are only sensitive to electrostatic changes near their electronic surface — that is, within the Debye length, which is typically less than 1 nm in biological media[4]. Hence, a binding event that occurs beyond this distance from the transistor's surface will not be detected. Wei and co-authors minimized the effect of this problem by using electrical fields to control the position of the cantilevers: by applying a negative voltage at the transistor's gate through the electrolyte covering the transistor's channel (the graphene layer), the negatively charged cantilevers are 'pushed' closer to the graphene surface (Fig. 1c). However, for the aptamers to effectively bind to molecules of the target analyte, they need to be oriented upright (away from the graphene surface). The authors also used electrostatic actuation to control the orientation of the aptamers by applying a positive voltage at the gate electrode (thus lifting the cantilevers, whose positions they monitored by tagging a fluorescent dye at their tip). A recently developed sensor has also used 'upright' biorecognition units consisting of surface-immobilized self-assembled oriented layers of nanobodies

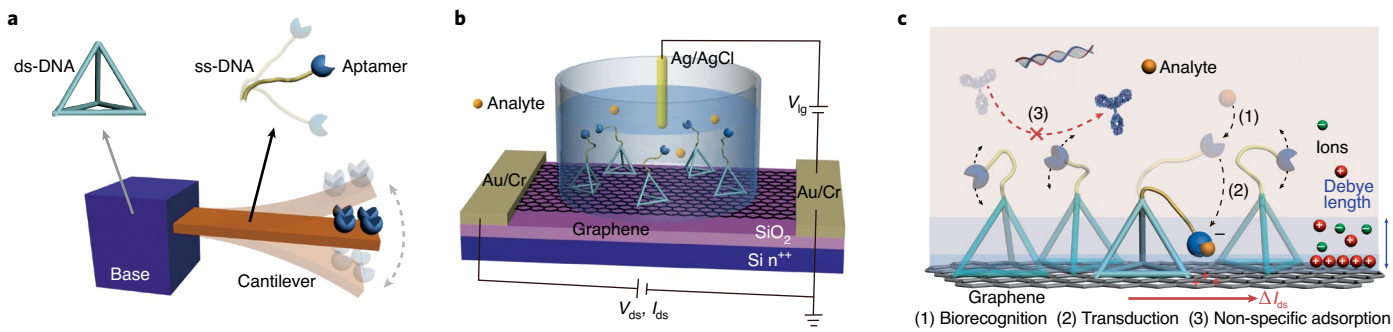

Fig. 1 | An electromechanical sensor for the rapid and sensitive detection of biomolecules. a, Sensor components: a rigid, double-stranded-DNA (ds-DNA) pyramidal base, a flexible single-stranded-DNA (ss-DNA) mechanical cantilever, and an aptamer as the recognition element. **b**, The base is immobilized on the graphene surface of the channel of a field-effect transistor. The channel is gated with an Ag/AgCl electrode through an aqueous electrolyte. **c**, When the analyte binds to the aptamer (1), a negative gate voltage (V_{lg}) brings the analyte-bound cantilever towards the channel surface and within the Debye length (2). The charged complex changes the source–drain current (I_{ds}) recorded at a voltage applied at the contacts (V_{ds}). The presence and height of the pyramidal base minimizes non-specific adsorption (3). Figure reproduced with permission from ref. [1], Springer Nature Ltd.

for the detection of the spike protein of SARS-CoV-2 (ref. [5]).

Complex biofluids can passivate biorecognition surfaces, compromising the sensitivity and robustness of sensors and leading to false-positive readings. Therefore, most sensors integrate antifouling films (typically made of bovine serum albumin or zwitterionic polymers) on the sensor's surface[6]. However, the films can decrease the sensor's sensitivity. Instead, Wei and co-authors produced a dense layer of biorecognition units, with the pyramid bases (spanning 5.3 nm above the graphene surface) 'repelling' biomolecules of diameters larger than 1.5 nm from the surface (Fig. 1c). Hence, the DNA bases acted as an in-built antifouling layer, preserving the sensitivity and operational robustness of the sensor when used with complex media, such as serum, viral transport medium and artificial saliva.

Wei and colleagues' sensor detected SARS-CoV-2 RNA in 60 seconds on average (at most, in about 4 min), and the lowest concentration of SARS-CoV-2 RNA that led to a change in current was about 2 molecules in 100 µl of a nasopharyngeal swab sample placed in viral transport medium. Moreover, the sensor correctly classified all nasopharyngeal samples from 33 individuals with COVID-19 and from 54 COVID-19-negative controls (the COVID-19-positive samples had qRT–PCR cycle-threshold values of 24.9–41.3). However, before taking the measurements the devices had to be immersed in testing solution to aid the stabilization of the source–drain current, and the clinical samples had to be heated for 30 min to release the nucleic acids. These preparation steps may hinder point-of-care uses.

For real-world use, the sensor would need further optimization, in particular with regard to the design of the signal amplifier and the operating conditions. First, because the operating voltage is different from the voltage required to maximize the transconductance (the gain) of the transistor, it is not operating at ideal conditions when a negative gate voltage is applied to bring the cantilevers close to the graphene surface. Electrochemical transistors[7] can detect binding events far from the electrode or semiconductor surface, and hence they may obviate the need to operate the transistor at a non-ideal voltage bias. Another alternative would be to change the transistor's geometry or the materials used to generate the maximum transconductance at the voltage that bends the cantilevers towards the surface. Second, for certain analytes, large analyte-concentration differences led to only small changes in the measured current (this was the case for samples with human complementary DNA, for which a three-orders-of-magnitude increase in concentration caused an approximately 0.7% change in the measured current). Third, device-to-device variations for devices fabricated at the laboratory scale may result in differences in each transistor's output current that would impair the technology's ability to be reproduced by other researchers. Titanium carbides and other materials may provide larger changes in the output signal[8] and could thus increase the reliability of the devices. Automation in operation and standardization in device fabrication will also enhance the robustness of their performance.

The nearly single-molecule level of detection (two SARS-CoV-2 RNA molecules in 100 µl of viral transport medium) of the sensor and its miniaturized dimensions make Wei and co-authors' technology particularly promising for wide applicability in biochemical sensing. And because the technology's speed does not compromise the sensitivity of detection and can be used with saliva samples, it could become a strong candidate for replacing rapid antigen kits for COVID-19 testing. ❐

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Competing interests

The author declares no competing interests.