Electronic Supplementary Material (ESI) for Nanoscale.

Supplementary Information

Towards personalised rapid label free miRNA detection for cancer and liver injury diagnostics in cell lysates and blood based samples

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Methods

Cantilever prototype device was designed and built according to Walther et al.[35] and Noy et al. [30] Presented below are further details regarding the technical aspects of the prototype device and experimental procedures. Preparation techniques for SPR chips and cantilever array sensor are detailed. The cell lysates preparation is also described.

Cantilever measurement device

Enhancements made to the device referenced in Walther et al. [35] are: (1) Larger surface area position sensitive detector (PSD) (1L20-20-A_SU9 SiTek Electro Optics), (2) Data acquisition board with higher sampling rate (PCIe-6361, National Instruments), (3) Improved thermoregulation system (miniStat 125, Huber Kaltemaschinenbau GmbH), (4) dual syringe pump with solutions and samples contained inside the thermoregulation system (neMESYS system, Cetoni GmbH). The entire experimental set up and data acquisition is controlled by custom made LabVIEW (Version 14.0.1f3 64-bit) software interface.

Cantilever Array Preparation

Vacuum deposition rates of 0.2 Å /s and 0.5 Å /s were used for titanium (3 nm) and gold (23 nm) respectively before being stored under argon. Thiolated ssDNA probes arrived in a protective 0.1mM dithiothreitol (DTT) solution. This DTT needed to be removed prior to functionalisation via liquid-liquid phase separation (4 times) using ethyl acetate (Sigma Aldrich). After DTT extraction probe solutions of 20 µM in 50mM trithylammonium acetate are stored under argon at -20 °C. All solutions were made with twice filtered (0.2µm), twice autoclaved nanopure H_2O.

Cantilever measurement protocol

After priming the device with DPBS+ buffer and sample, the chip was mounted into the measurement chamber. Laser focusing onto the tips of the cantilevers was performed automatically. Once the internal temperature has stabilised, the experiment begins by testing the cantilever mechanical response to a heat pulse of +2 °C above the stable set point and a flow pulse of 38 µl at 150 µl/min. The downward deflection observed during the heat pulse or peltier test is used to normalise the mechanical response of the cantilevers during analysis. Sample injection of 38 µl at 150 µl/min takes place when the cantilevers are stable post peltier test. Incubation time is 20 minutes, allowing reactions to occur on the cantilevers surface. Sample is then removed by a 300 µl buffer wash at 150 µl/min. All events are controlled automatically, to ensure reproducibility and reduce user error.

SPR chip preparation

As sensor chips, we used either recycled CM5 or SM Biacore sensor chips or commercially bought Biacore Au sensor chips (BR-1005- 42, Biacore AB, Sweden). To enable recycling the CM5 and SA chips were rinsed in and stored in PBS (Sigma Aldrich). Then the chip holder containing the chips was placed in Deconex 12PA (Borer Chemie AG, Switzerland) in an ultrasonic bath for 15 minutes at 50 °C. Following this, they were rinsed with and stored in nanopure water for 10 minutes. The chip holder was then filled with Deconex 20NS (Borer Chemie AG, Switzerland) and placed in an ultrasonic bath for 15 minutes at 50 °C. Finally, the chips and chip holder were rinsed with and stored in nanopure water for 30 minutes before drying with nitrogen gas flow. Prior to the functionalisation the chips were treated with UV/O_3 for 10 min. The SPR chip was functionalised outside the instrument. The ssDNA probes were

immobilized onto the surface by pipetting 50 µl of 10 µM thiolated ssDNA in 50 mM TEAA buffer. The solution was incubated on the chip overnight (**>**10 h). The chip was rinsed with TEAA buffer for 30 seconds, respectively 3 times with buffer. Afterwards the chip was incubated for 1 hour with 50 µl of 10 µM 11-Mercapto-1-undecanol (Sigma) solution. The chip was rinsed with H_2O 3 times 30 seconds and dried in an air-stream of N_2 then stored under Argon at 4 °C.

Cell sample preparation

1 ml cell stock of Huh7 or Me15 cells was seeded into cell culture flasks (BD Biosciences) with 10 ml Dulbecco's Modified Eagle Medium + 10% Fetal Bovine Serum medium (Gibco/Thermofisher). When the cells were fully confluent the flask was drained and washed with 5 ml DPBS+. The DPBS+ was then removed and 500 µl trypsin was added to the flask for 5 minutes incubation. Following this, 5 ml medium was added and the cells were transferred to a medium flask with 20 ml of medium. For transfer to larger flask 1 ml trypsin was used to dissociate the cells. In the large flask 4 ml of cell solution was added to 26 ml medium. Dissociation from the large flasks was performed using 2 ml trypsin after rinsing with DPBS+. Cells were aspirated into a falcon tube using 10 ml medium. An additional 25 ml of medium was then added to ensure the trypsin was inhibited. A cell count using 10 µl was performed. Two fractions of cell solution containing each 10 x 10^6 cells were transferred to two 15 ml falcon tubes. The tubes were centrifuged for 5 min at 400 rpm. Buffer was removed and the pellet was resuspended in 10 ml lysis-buffer (8 ml H_2O and 2 ml Lysis-buffer (Passive Lysis Buffer 5x, Promega)) to a final concentration of 10^6 cells/ml. Similar to serum samples the cell lysate was expanded from 10 µl to 75 µl (protein concentration upon injection was 28.3 µg/ml).

Direct cancer related miRNA detection in cell lysates

In the set of experiments presented here we performed control measurements on two cell lines investigated. Me15 cell lysate are miRNA-122 (target) negative while Huh7 cell lysate are miRNA-122 positive. The control cell lysate (Me15) produced a nanomechanical differential signal of 100 nm due to interference from background molecules. This offset serves as a baseline for Me15 miR-122 spike in experiment as shown in Fig. 2 of the main text. The specific annealing of the miRNA target in Huh7 cell lysate generates extra surface stress resulting in approximately 200 nm differential deflection indicating a target concentration of roughly 500 pM.

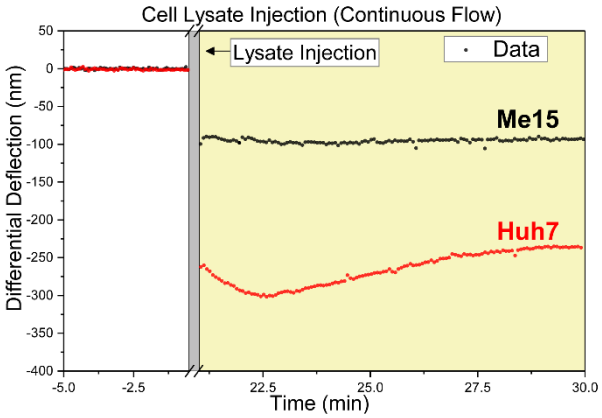

Figure S1. Overlay of two consecutive continuous flow experiments to investigate the detection of miR-122 in Me15 and Huh7 cell lysate: The graph shows the significant difference between the injection of miRNA-122 positive Huh7 cell lysate (red curve) and the injection of Me15 cell lysate (black curve) which is miR-122 negative. Two injections were performed in series on the same cantilever array chip. The line break (grey area) represents a series of fluidic events including sample introduction involving flow of 600 µl at 150 µl/min, 10 minutes of incubation and a buffer wash of 450 µl at 150 µl/min. Yellow area depicts sensor response post wash. Sample was not diluted resulting in particles scattering the optical signal

during incubation, creating fluctuations in the deflection reading. The reference sequence used was a firefly luciferase gene segment. miR-122 target sensitive cantilevers response are averaged form multiple cantilevers as is the reference response. Post fluidic events the Huh7 sample induces ~200 nm differential deflection whereas the injection of the negative control (Me15) leads to ~100 nm differential deflection signal. The response induced by the Me15 cell lysate is due to nonspecific interactions and is stable post wash while the Huh7 cell lysate response is greater post wash as miR-122 are present to occupy surface bound probes.

Surface plasmon resonance dose response

These experiments were performed on Biacore's surface plasmon resonance (SPR) system to establish a dose response curve for miRNA hybridisation. We utilized SPR chips with blank gold surfaces that were functionalised with oligonucleotides. Various concentrations of pure miRNA target were injected as background interference would mask the miRNA hybridisation signal. While the corresponding cantilever measurements were conducted in cell lysates. This data is summarized in Figure 2 of the main text.

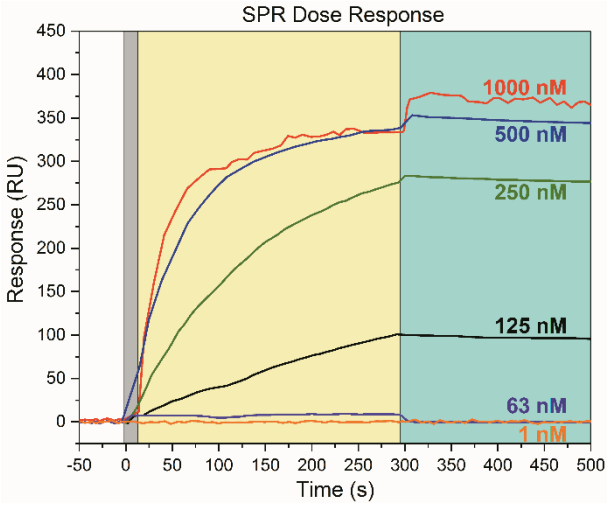

Figure S2. Overlay of multiple SPR hybridisation dose response experiments using Biacore sensor chip functionalised with 1 µM thiolated probe for 1 hour. Grey area represents 25 µl injection of pure miRNA target (various concentrations). Incubation is indicated by the yellow area and rinsing with PBS buffer at 25 µl/min is depicted in the blue area. The result value for each concentration was taken just after the rinse began as excess molecules which may interfere with the signal are removed. 1000 nM and 500 nM seem to reach saturation and therefore have similar amplitudes. Signals from lower concentrations are ambiguous.

Cantilever array dose response

These experiments were all performed on separate cantilever arrays with four target sensitive and four control cantilevers. Me15 cell lysates were spiked with various centration's of target miRNA. The sensor was then exposed to undiluted sample in flow through conditions. Following this a buffer rinse was performed to reveal the result.

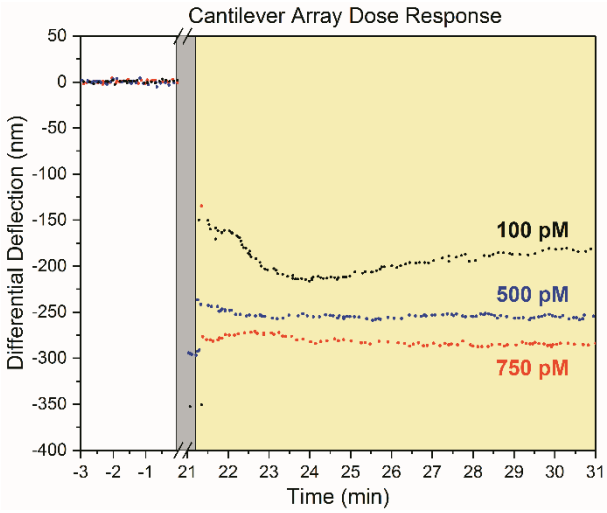

Figure S3. Overlay of multiple cantilever array dose response experiments. Cantilever array annealing data obtained from three miRNA target spike-in concentration experiments in Me15 cell lysate background in flow-through conditions. The line break (grey area) represents a series of fluidic events including sample introduction involving flow of 600 µl at 150 µl/min, 10 minutes of incubation and a buffer wash of 450 µl at 150 µl/min. Yellow area depicts sensor response post wash. Sample was not diluted resulting in particles scattering the optical signal during incubation, creating fluctuations in the deflection reading. The differential signal was created by subtracting the average response of four target (miR-122) sensitive cantilevers from the averaged response of four control sensors. A Differential signal from 100 pM non amplified target in undiluted cell lysates was achieved using the label free cantilever array diagnostic platform.