Supporting Information

Fast diagnostics of BRAF mutations in biopsies from malignant melanoma

François Huber[*†], Hans Peter Lang[†], Katharina Glatz[‡], Donata Rimoldi[§], Ernst Meyer[†], Christoph Gerber*[*†]

† Swiss Nanoscience Institute, Department of Physics, University of Basel, CH-4056 Basel, Switzerland

‡ Institute of Pathology, University Hospital Basel, CH-4031 Basel, Switzerland

§ Ludwig Center for Cancer Research, University of Lausanne, CH-1066 Epalinges, Switzerland

*Corresponding authors: francois.huber@unibas.ch, christoph.gerber@unibas.ch

Methods

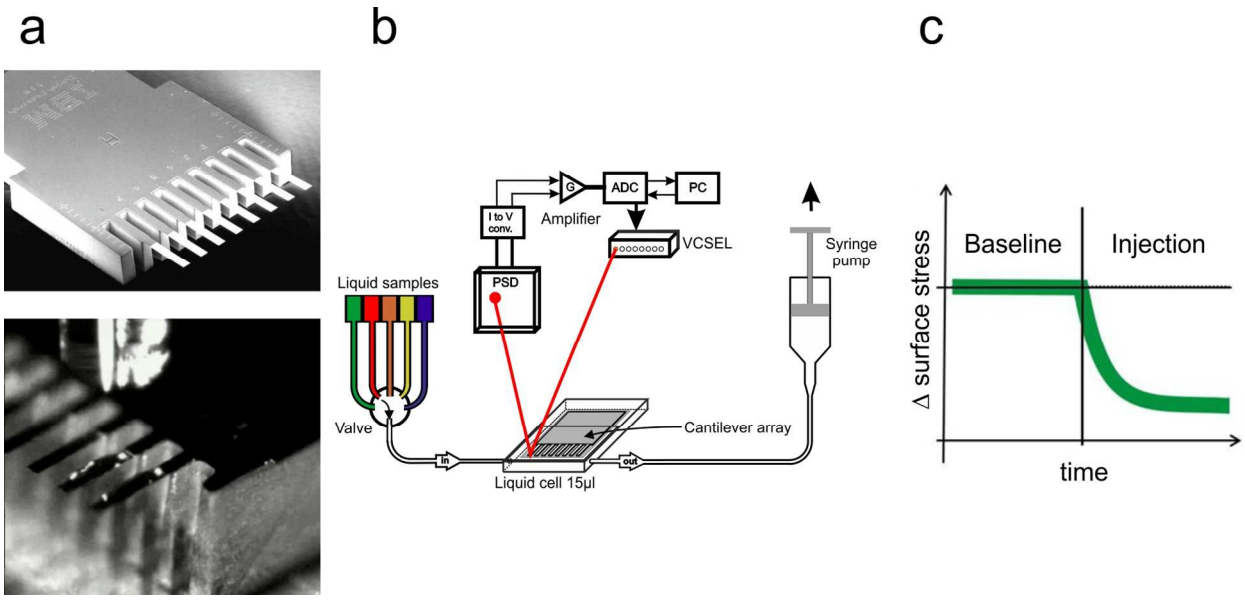

Figure S1. Experimental setup. (a) A microcantilever array (scanning electron microscope image shown above) is functionalized using an inkjet spotter and mounted into the liquid measurement chamber (volume of 15 µl). (b) Schematic drawing of the sensor instrument: Liquid cell with a mounted microcantilever array. Optical read-out system comprising of vertical cavity surface emitting lasers (VCSELs) and a position sensitive detector (PSD). Data acquisition and operation of the liquid handling system consisting of a multi valve selector, a syringe pump and temperature control are operated by a PC. The multi valve selector allows for different samples to be investigated and the syringe pump delivers liquids at 10 µl/min to the liquid measurement cell. A minimum of 500 ng sample can be used. (C) Progression of experiment; after equilibrating the system (baseline) with fresh 0.13 × SSC at 36 °C a sample is injected upon which a signal can be observed.

A microfabricated array of eight identical silicon cantilevers with a pitch of 250 µm, a length of 500 µm, a width of 100 µm, a thickness of 1 µm and a spring constant of 0.02 N/m were provided by the Micro-and Nanomechanics group at the IBM Research GmbH. The cantilever arrays were cleaned using an UV/Ozone cleaner (Jelight Company, Inc. Laguna Hills, California). Thiolated oligonucleotides (Microsynth AG, Balgach, Switzerland) were treated with 1 mM TCEP (Tris(2-carboxyethyl)phosphine, Sigma-Aldrich Chemie, Buchs, Switzerland) thereby reducing disulfide bonds to ensure efficient self-assembled monolayer formation. Functionalization was carried out at a concentration of 40 µM thiolated oligonucleotide in 50 mM TEAA buffer (triethyl ammonium acetate, Fluka, Buchs, Switzerland) using an MD-P-705-L inkjet dispensing system (Microdrop, Norderstedt, Germany). Afterwards the array was washed once with water and in buffer and mounted in the 15 µl volume measurement chamber. Samples were injected at 10 µl/min. Total RNA was extracted from tissue culture samples and biopsies using standard methods. The deflection signal in nm can be converted into a surface stress in mN/m using Stoney's equation and Sader's correction[1] with a Young's modulus of 1.2×10^{11} Pa for silicon, a cantilever thickness of 0.5×10^{-6} m and a Poisson ratio of 0.25.

RNA purification from cell cultures: Total RNA was extracted using Trizol reagent (In vitrogen) following manufacturer's instructions. RNA was further purified by ammonium acetate/ethanol precipitation and dissolved in DEPC-treated H_2O.

RNA purification from FFPE tissue: A 20 µm section was cut from each FFPE (formalin-fixed paraffin-embedded) block and collected in a tube. The sections were deparaffinized by two rinses in xylene. After paraffin solubilization, the tissue was rinsed twice in 100% ethanol and collected by centrifugation at 10,000 × g for 10 min. Alcohol was aspirated and the tissue pellets were resuspended in 480 µl of digestion buffer (Lysis buffer: 45 ml H_2O distilled + 5 ml 10X PCR

Buffer_AmpliGoldTaq + 50 µl ATL_Qiagen) and 20 µl of proteinase K (Promega 20 mg/ml) were added. Sections were incubated at 56°C overnight. Prior to RNA purification we inactivated proteinase K at 95°C for 10 min. Subsequently 1 ml of TRIzol® Reagent (Ambion Catalog number: 15596-026) was added per tube. The solution was vortexed and incubated at room temperature for 5 min. 1 µl of Glycogen (ThermoFisher Catalog number: R0551) was added to improve the RNA recovery, followed by 200 µL of BCP (Phase Separation Reagent MRC Catalog number: BP151). Each tube was vortexed vigorously for 30 sec and allowed to sit at room temperature for 3 min. Phase separation was achieved by centrifuging the sample at 12,000 × g for 15 min at 4°C. After centrifugation, the aqueous phase (containing the RNA) was carefully transferred to a new 1.5 ml Eppendorf Tube. The RNA from the aqueous phase was precipitated by mixing with 0.6 ml of isopropanol (99,9% pure) and stored at -20°C for 30 min. After that the sample was centrifuged at 12,000 × g for 30 min at 4°C. At this point the supernatant was removed and the RNA pellet was washed in 75% ethanol by vortexing. A final centrifugation step at 12,000 × g for 10 min at 4°C was performed. The ethanol was removed and the RNA pellet was briefly air-dried for 5 min. The RNA was re-suspended in 50 µl of RNase-free water (DEPC treatment) and incubated for 10 min at 60°C. RNA purity and concentration were determined by the absorbance at 260 nm (A260) and 280 nm (A280) using NanoDrop ND-2000 (Thermo Fisher Scientific, MA).

For experiments using total RNA from cell culture and melanoma tissue samples were diluted to 20 ng/µl in a 0.13 × SSC buffer equalling 20mM NaCl.

Table S1. List of oligonucleotides used for this work

Probe	Sequence	Experiment
BRAFV600E	5'-GAGATTTC**T**CTGTAGCTA-3'	Detection of BRAFV600E in total RNA
BRAFV600K	5'-GAGATTTC**TT**TGTAGCTA-3'	Distinguishing BRAFV600E from BRAFV600K
BRAFwildtype	5'-GAGATTTC**A**CTGTAGCTA-3'	Reference in BRAFV600E detection
polyAC	5'-ACACACACACACACACAC-3'	Reference for BRAF RNA detection
BRAFV600E complement	5'-TAGCTACAG**A**GAAATCTC-3'	Optimization of binding conditions
BRAFV600K complement	5'-TAGCTACA**AA**GAAATCTC-3'	BRAFV600K control experiment

Probe oligonucleotides and the corresponding experiments where they were used. The important bases for the detection are labelled in red.

Cantilevers were functionalized with corresponding 18mer probe thiol oligonucleotides (Table S1) and a 18mer reference oligonucleotide (polyAC). Initial experiments investigated optimal hybridization conditions and minimum concentrations for the detection of the mutation in total RNA extracted from biopsies. The most efficient hybridization temperature where only binding of $BRAF^{V600E}$ mutant to the mutant BRAFV600E oligonucleotide occurs was evaluated (Figure S1). The experiments were based on theoretical estimates of the melting temperature and salt concentration for the BRAFV600E oligonucleotide (36.8 ±1.2 °C in 20mM NaCl). 36 °C and a saline sodium citrate (SSC) buffer containing 20mM NaCl turned out to be the most promising hybridization condition with the highest specificity. Whereas at 34 °C the $BRAF^{V600E}$ signal is higher, we observe also a higher nonspecific wild type *BRAF* binding signal corresponding to

77% of the $BRAF^{V600E}$ signal. No binding to the wild type reference by the mutated oligonucleotide is observed at 36 °C. At 38 °C the $BRAF^{V600E}$ signal is diminished by 44% and at 40 °C is less than 10% of the signal at 36 °C. These studies suggest choosing 36 °C as the optimized temperature for the binding experiments.

Data analysis was done using OriginPro 2015 (OriginLab Corporation, Northampton, MA 01060, USA). Data were flattened using the locally weighted scatterplot smoothing (LOWESS) function and then a Langmuir function was fitted to the smoothed data. The dendrogram was computed using the hierarchical cluster analysis tool applying the Euclidian sum of distances method. The endpoints of the Langmuir fits right before the washing step with buffer were used for the creation of the dendrogram.

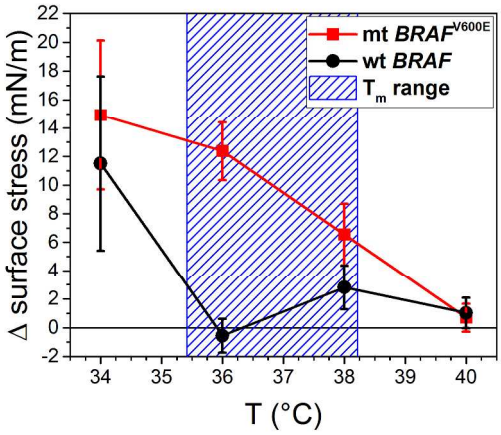

Figure S2. Temperature dependence of oligonucleotide binding: The red curve shows binding of the mutant oligonucleotide to the complement functionalized cantilever in dependence of the hybridization temperature whereas the black curve depicts binding of the wild type oligonucleotide to the cantilever functionalized with the mutated oligonucleotide. The blue

hatched area indicates the melting temperature (T_m) range. The results imply 36 °C as the most promising temperature for binding experiments.

REFERENCES

1. Sader, J. E. *J. Appl. Phys.* **2001**, *89*, 2911–2921.