Supporting Information: Label Free DNA Based Detection of *Mycobacterium tuberculosis* and Rifampicin Resistance through Hydration Induced Stress in Microcantilevers

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We provide here an expanded description of the experimental procedures described in the main text. Also, we include a brief explanation of the interpretation of the surface stress curves.

S1. Bacterial cultures

Two clinical isolates were obtained from the collection of the Microbiology Department of Hospital La Paz (Madrid, Spain) from positive mycobacteria growth indicator tubes (MGIT). One of them (wt) was susceptible to all antibiotics, and the other (mut531) had the mutation S531L (TCGŸ TTG) in the *rpoB* gene and was resistant to rifampicin.

S2. Probe DNA sequences

All thiol modified single stranded DNA (Table 1) were purchased from Stab Vida (Caparica, Portugal). In order to increase the hybridization efficiency, the thiolated sequences have a tail of 5 thymines between the C_3H_6 spacer and the sequence of interest in order to keep the hybridization bases away from the gold surface, due to the low affinity for gold of thymine.

S3. Reagents

Toluene, methanol, tris-ethylenediaminetetraacetic acid (Tris-EDTA), sodium chloride (NaCl), 20x saline sodium citrate (SSC), hydrochloric acid (HCl), 10% sodium dodecyl sulfate (SDS), commercial 2x prehybridization solution, commercial hybridization buffer PerfectHybTM Plus Hybridization Buffer, ethanol and the kit for creating hydrophilic polydimethylsiloxane (PDMS) surface were purchased from Sigma Aldrich (St. Louis, USA). The FastDigest AluI kit was acquired from Thermo Scientific (USA). UltraClean® Microbial DNA Isolation Kit was purchased from MoBio (Carlsbad, CA, USA).

S4. Buffers and solutions

All buffers were prepared using molecular biology grade water. The immobilization buffer consisted on 1x Tris-EDTA (which consists in 10 mM Tris-HCl and 1 mM disodium EDTA) with NaCl 1 M and pH 7.5. In order to remove the dissolved oxygen and minimize thiol oxidation the buffer was degassed by simultaneous sonication and bubble extraction with a vacuum pump for 20 min. Thiolated DNA aliquots were prepared with the degassed 1x TE-NaCl 1 M buffer and stored at -20ºC until its use. Low and high stringency wash buffers contained 0.1% SDS and 2X SSC and 0.5X SSC, respectively. 1x SSC buffer was prepared by diluting 20x SSC in water.

S5. Surface functionalization

Arrays of eight silicon microcantilevers with 20 nm gold coating were purchased from Concentris (Basel, Switzerland). The cantilevers are 500 µm long, 100 µm wide and 1 µm thick. Since the cleanliness of the gold surface affects the surface stress response of the microcantilevers,[S1] prior to its use, cantilever arrays were deeply cleaned by two consecutive procedures that remove organic contaminants. They were immersed in toluene,

methanol and Milli-Q water; dried under a stream of dry nitrogen and irradiated in a UV-Ozone cleaner for 1 hour. Then, the corresponding thiolated DNA was diluted in the degassed immobilization buffer to a final concentration of 5 µM. The cantilever arrays were incubated in each DNA solution overnight at 25 ºC under agitation. Afterwards, the arrays were cleaned with low and high stringency hybridization wash buffers for 5 and 20 minutes, respectively, to wash out the physisorbed DNA away from the microcantilever surface and finally rinsed with plenty of Milli-Q water. Cleansing steps were carried out at 25 ºC as well.

S6. Surface blocking and chip preparation

Prior to hybridization, the microcantilever backside and the voids on the gold surface of the functionalized microcantilever chips were blocked by incubation in a 1x prehybridization solution (Sigma-Aldrich) for 30 minutes at the hybridization temperature (30 ºC). The excess of blocking agent was removed by incubating the samples in SSC 1x buffer for 15 minutes at 25 ºC. Then, the samples were rinsed with Milli-Q water. The microcantilevers were then dried under a stream of nitrogen and mounted on a 96-well array format plate covered with polydimethylsiloxane (PDMS).

S7. Preparation of target DNA samples

For genomic DNA purification several MTB colonies were taken from Lowenstein-Jensen slant cultures, resuspended in phosphate buffered saline (PBS) and boiled for 15 minutes. DNA was purified using UltraClean® Microbial DNA Isolation Kit (MoBio). IS6110 was amplified by PCR using the oligonucleotides and conditions described in Eisenach et al.[S2] The concentrations of the gDNA and the PCR product were measured with a spectrophotometer, NanoDrop 2000 (Thermo Scientific, USA), by means of its absorbance at 260 nm (1 Abs_{260nm} unit is equivalent to 50 µg/mL for dsDNA). The gDNA was partially digested with the restriction enzyme FastDigest AluI (Thermo Scientific, USA) following the recommendations given by the manufacturer. The FastDigest AluI restriction enzyme recognizes AG^CT sites, leaving a 554b fragment containing the complementary region to the sequence called here ISseq and a 586b fragment containing the complementary fragment to the rpoB_mut531 sequence. Prior to hybridization, the DNA was denatured at 95ºC for 5 min and placed in ice for 2 min. Then, it was diluted directly to the corresponding concentration in PerfectHybTM Plus Hybridization Buffer (Sigma-Aldrich) and mixed vigorously to homogenize the solution.

S8. Measurement setup

The experiments were performed in a SCALA-Bio platform (MecWins, Spain) where read-out was made directly from a 96-well plate coated with polydimethylsiloxane (PDMS) to attach the microcantilever chips to its surface. The read-out of the microcantilever

deflections is based in the automated two-dimensional scanning of a laser beam across the surface of each microcantilever, and the collection of the reflected beam on the surface of a two-dimensional position sensing linear detector (PSD) orthogonally oriented to the reflected beam. The system was equipped with an environmental chamber with capability to keep temperature at 25.00 ± 0.02 ºC and to change relative humidity at a rate of 10.00 ± 0.08% min-1. Prior to the measurement of the surface stress, the cantilevers were equilibrated at 0% relative humidity under a flow of dry nitrogen for one minute. The system was calibrated as described in reference S3 in order to attain accurate values of the surface stress.[S3]

S9. Hybridization experiments

In the set of experiments intended to detect the presence of the bacillus, we functionalized the microcantilever chips with the sequences ISseq and ControlOligo. This last sequence was used as a negative control. Then, we incubated both the test and control chips attached to the PDMS surface of the 96 microwell plate with the same sample solution containing the IS6110 amplicon or the gDNA. In the experiments designed to detect the antibiotic resistance, test and negative control chips were sensitized with rpoB_531 (probe sequence) and rpoB_wt (control sequence), respectively, and incubated with the same sample solution containing the gDNA from the mutant 531. Note that the sequence rpoB_wt, complementary to the gDNA of the wild type bacteria, differs from rpoB_531 in just one single base. Hybridization was performed at 30 ºC for 1 hour under agitation at final concentrations ranging from 10 ng/mL to 1 fg/mL for the experiments with the IS6110 PCR product and from 20 ng/mL to 200 fg/mL for the experiments with the gDNA. After hybridization, the cantilever arrays were cleaned with low and high stringency hybridization wash buffers for 5 and 30 minutes, respectively, for the experiments with the PCR product while 5 and 15 minutes washing suffice for the experiments with the gDNA, always under agitation. Then, the chips were extensively rinsed with plenty of Milli-Q water. The higher stringency wash was carried out at the hybridization temperature (30ºC), while the lower stringency wash was performed at 25ºC. The response of each microcantilever before and after incubation with the problem solution was measured, as shown in Figure 1a in the main text, and recorded. This response comparison serves as an excellent reference to filter out any spurious mechanical signals and deviations in the response between cantilevers with different mechanical properties.

S10. Hydration cycle and surface stress.

Figure S1a shows a schematic depiction of the bending originated at different times during a hydration cycle. Each stage is numbered and relates with the corresponding surface stress curves shown in Figure 1b, being #1 the starting point and #3 the end. Stages #2a and #2b correspond to the hydration cycle of ssDNA and dsDNA monolayers, respectively.

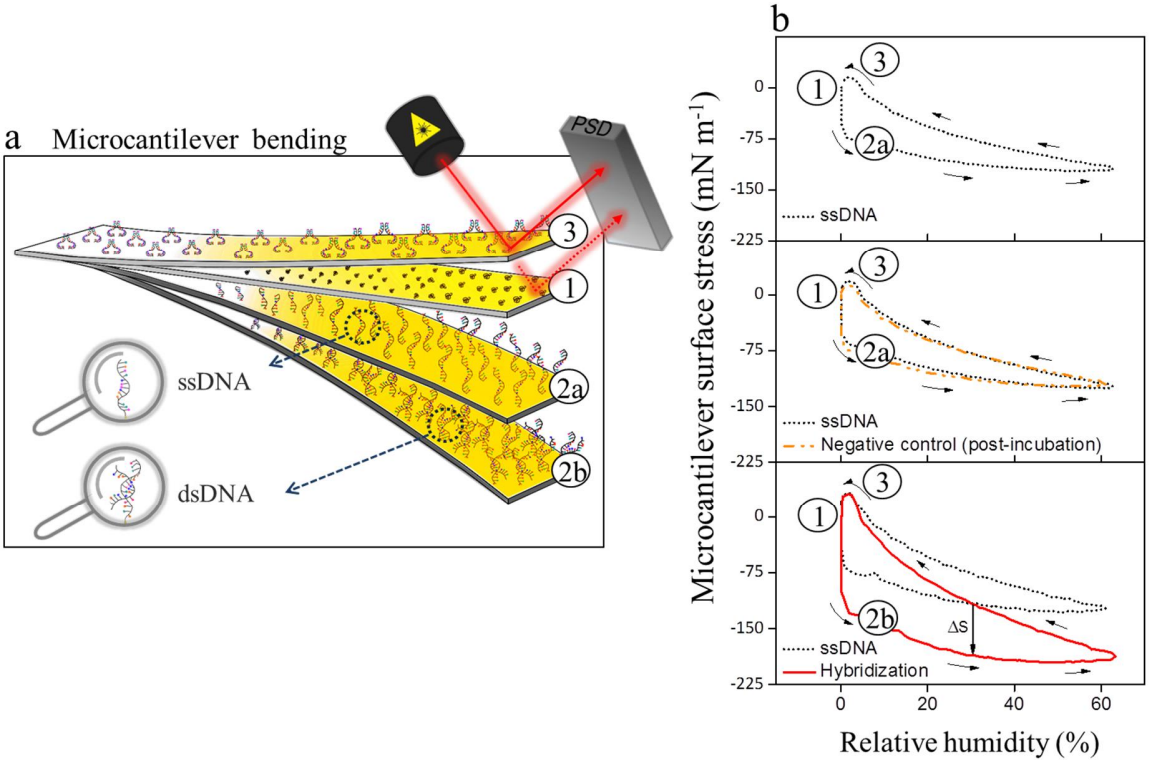

Figure S1. a) Schematic depiction of the bending suffered by the microcantilevers sensitized with DNA in response to a humidity cycle. Stage 1 represents the starting point for both non-hybridized and hybridized microcantilevers. Stage 2a and 2b represent the bending originated from the hydration of the ssDNA and dsDNA monolayers, respectively. Stage 3 depicts the tensile peak generated at the end of dehydration for every DNA monolayer. b) Representative curves of the surface stress as a function of RH for a ssDNA monolayer (black dotted line), a ssDNA monolayer after incubation with a non-complementary negative control sequence (orange dashed line) and a dsDNA monolayer after hybridization with complementary strands (red solid line).

The ssDNA monolayers show highly reproducible surface stress hydration/dehydration patterns. Further details can be found in Domínguez et al.[S3]

Supporting Information References

S1. Tabard-Cossa, V.; Godin, M.; Burgess, I. J.; Monga, T.; Lennox, R. B.; Grütter, P. Microcantilever-based sensors: effect of morphology, adhesion, and cleanliness of the sensing surface on surface stress. *Analytical chemistry* **2007,** *79* (21), 8136-8143.

S2. Eisenach, K. D.; Cave, M. D.; Bates, J. H.; Crawford, J. T. Polymerase chain reaction amplification of a repetitive DNA sequence specific for Mycobacterium tuberculosis. *The Journal of infectious diseases* **1990,** *161* (5), 977-81.

S3. Domínguez, C. M.; Kosaka, P. M.; Mokry, G.; Pini, V.; Malvar, O.; del Rey, M.; Ramos, D.; San Paulo, Á.; Tamayo, J.; Calleja, M. Hydration induced stress on DNA monolayers grafted on microcantilevers. *Langmuir* **2014**, *30* (36), 10962ï 10969.