SUPPORTING INFORMATION

Detection of multiple DNA-targets using a single probe during the acoustic sensing of the conformation of the hybridized products

Achilleas Tsortos[a], Aristea Grammoustianou[a,b], Rena Lympouridou[a], George Papadakis[a] and Electra Gizeli[a,b]

[a]IMBB, FORTH, 100 N. Plastira str., Heraklion, Crete, 70013, Greece
[b]Dept. of Biology, University of Crete, Vassilika Vouton, Heraklion Crete, 71110, Greece

Materials and Methods

1. Chemicals

PBS (0.01 M phosphate buffer, 0.0027 M KCl and 0.137 M NaCl, pH 7.4) was purchased from Sigma-Aldrich (Germany).

2. Oligonucleotide probes and targets

A ssDNA probe of 86nts (Probe-86) with a polyC tail in between its target hybridization regions and a ssDNA probe of 86nts with a random sequence in between its target hybridization regions (Probe-86(1)) were purchased from Integrated DNA Technologies (IDT, Belgium). Both oligonucleotide probes were synthesized at the 200nmol scale and purified by high-performance liquid chromatography (HPLC).

Target oligonucleotides consisted of 14nts of a miRNA biomarker (i.e., T1: miRNA-21; T2: miRNA-155; T3: miRNA-150; and, T4: miRNA-107) and a tail (underlined below) comprising primarily a variable number of thymines and 5 cytosines (polyT tail). Each target was synthesized combined with a polyT tail comprising 20, 30, 40 and 50nts. Oligonucleotide targets used in the experiments were ordered from Microchemistry Laboratory (FORTH, Greece), synthesized at the 200nmol scale and purified by high-performance liquid chromatography (HPLC).

The sequences of oligonucleotides used in this work are listed in the table below; note that each one of the targets is depicted combined with one of the available polyT tails.

Probes

Probe-86: 5'-biotin-AGC TTA TCA GAC TGC CCC CCC CCC CTC CTA CAT ATT AGC CCC CCC CCC CTC CCA ACC CTT GTC CCC CCC CCC TAT CAA AGC ACA GA -3'

Probe-86(1): 5'-biotin- AGC TTA TCA GAC TGG CAT CAT CAA CTC CTA CAT ATT AGT CAT CAT CAA CTC CCA ACC CTT GTT CAT CAT CAA TAT CAA AGC ACA GA -3'

Targets

miRNA-21+(T1)+polyT20: 5'- TTT TTT TTT TTT TTT CCC CCC AGT CTG ATA AGC T -3'

miRNA-155(T2)+polyT30: 5'- TTT TTT TTT TTT TTT TTT TTT TTT TCC CCC CTA ATA TGT AGG AG -3'

miRNA-150(T3)+polyT40: 5'- TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTC CCC CAC AAG GGT TGG GAG -3'

miRNA-107(T4)+polyT50: 5'- TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT CCC CCT CTG TGC TTT GAT A -3'

3. Acoustic device

A Q-Sence E4 (Biolin Scientific, Sweden) acoustic device was employed for real-time simultaneous measurement of frequency and dissipation changes. Au-coated 5 MHz AT-cut quartz crystals were used. The frequency and dissipation responses were recorded at 35 MHz overtone. Prior to the experiments, the QCM crystal chips were first subjected to plasma cleaning for 3 min using a Harrick plasma cleaner PDC-002 ("Hi" setting) in order to obtain a clean surface. All measurements were taken at 25 ^{o}C.

4. Surface hybridization of target oligonucleotides

A continuous flow of PBS (0.01 M phosphate buffer, 0.0027 M KCl and 0.137 M NaCl, pH 7.4, Sigma) was flushed through the system at a flow rate of 50 μL/ min and allowed to equilibrate prior to the first addition. Neutravidin (Life Technologies), dissolved in PBS in a total volume of 200μL, was physically adsorbed on the Au surface at a concentration of 3.3 μM in order to produce a fully covered protein layer. After buffer rinse, the QCM sensor was exposed to the oligonucleotide probe (Probe-86 or Probe-86(1)) which was introduced at a concentration of 0.2 μM in PBS and in a total sample volume of 200 μL. Probe-immobilization, occurred through avidin-neutravidin interaction, was followed by buffer rinse and the addition of the target oligonucleotides. Each one of the miRNA-21+polyT, miRNA-155+polyT, miRNA-150+polyT and miRNA-107+polyT targets was diluted in PBS at a final concentration of 4 μM and was added in a volume of 200 μL; hybridization took place at room temperature. Before applying the probe and target oligonucleotides on the device surface, both were heat-denatured at 95 ^{o}C and then placed immediately on ice for a minimum of 5 min before introducing them to the sensor surface.

To detect the simultaneous binding of two oligonucleotide targets on the same probe, the same procedure was followed. After neutravidin binding and probe attachment, targets T1-polyT20 (4 μM) and T4+polyT50 (4 μM) were mixed in PBS buffer in two ratios (1:1 and 1:10) and applied in a final volume of 200 μL. The sensor surface was exposed to the sample solution and the targets were let to hybridize at room temperature. Prior to their addition to the QCM sensor, probe and target oligonucleotides were heat-denatured as described above.

SUPPLEMENTARY FIGURE

To investigate the effect of the probe sequence, Probe-86(1) was constructed and immobilized on the sensor surface as described before (see materials and methods, section 4). Probe-86(1) was designed to have a random sequence between the target recognition regions.

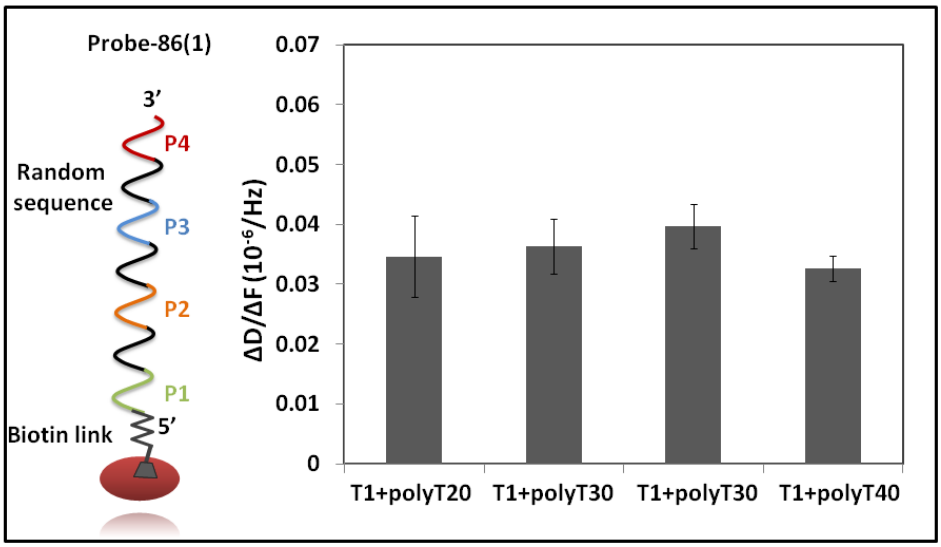

Fig. S1 (Left) Schematic representation of probe-86(1) consisting of a random DNA sequence between the target binding positions. **(Right)** Acoustic ratios obtained for the hybridization of target oligonucleotides T1+polyT$_{20}$, T1+polyT$_{30}$, T1+polyT$_{40}$ and T1+polyT$_{50}$ at position P1 of Probe-86(1).

Results show that the acoustic ratios observed during the hybridization of target T1 combined with a different size of polyT tail to position 1 of Probe-86(1) cannot be distinguished. This observation agrees with the one obtained for Probe-86 (Fig. 2), implying that the sequence composition of the probe does not affect the measurements.