Electronic Supplementary Information

A self-assembled DNA nanostructure-amplified quartz crystal microbalance with dissipation biosensing platform for nucleic acids

Wei Tang, Dingzhong Wang, Yi Xu, Na Li and Feng Liu*

Beijing National Laboratory for Molecular Science, Key Laboratory of Bioorganic Chemistry and Molecular Engineering of Ministry of Education, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, China.

*To whom correspondence should be addressed. Fax: 86-10-62751708;

Tel: 86-10-62761187; E-mail: liufeng@pku.edu.cn

Materials

Tris(2-carboxyethyl)phosphine (TCEP), 6-mercapto-1-hexanol (MCH), tris(hydroxymethyl)aminomethane (Tris), and 3-aminopropyltriethoxysilane (APTES) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

RIPA cell lysis solution, Gel-Safe dye, and 50×TBE buffer were purchased from YuanPingHao Bio. (Beijing, China). DNA Ladder and all HPLC-purified oligonucleotides were purchased from Sangon Inc. (Shanghai, China). The sequences of oligonucleotides are listed in Table S1.

The oligonucleotides were dissolved in Tris buffer (20 mM Tris, 300 mM NaCl, 5 mM $MgCl_2$; pH 8.3) and stored in the dark at –20 ^{o}C. All oligonucleotides were heated at 95 ^{o}C for 5 min and cooled to 25 ^{o}C in 1 hour before use. All other chemicals employed were of analytical grade, and double distilled water was used in all experiments.

Table S1. DNA oligonucleotide sequences.

Name	Sequence (from 5' to 3')
Thiolated capture probe (CP)	HS-$(CH_2)_6$TT AAACAC GCACCT CAAAGC TGTTCC *CACAGT* GGAACA GCTTTG AGGTGC
target p53 (G)	GGAACA GCTTTG AGGTGC GTGTTT
T mutation (T)	GGAACA GCTTTG AGGTGC TTGTTT
A mutation (A)	GGAACA GCTTTG AGGTGC ATGTTT
C mutation (C)	GGAACA GCTTTG AGGTGC CTGTTT
Random sequence (R)	GGAGAG TAGGTG GTAAGT AGCCAT
H1	GGAACA GCTTTG AGGTGC *CATCTCG* CACCTC AAAGCT GTTCCA CTGTG
H2	GAGATG GCACCT CAAAGC TGTTCC *CACAGT* GGAACA GCTTTG AGGTGC

In the hairpin sequences, loops are italicized and sticky ends are underlined.

QCM-D experiments

All binding processes were monitored on-line by using a Q-Sense E4 QCM-D instrument (Q-Sense AB, Västra Frölunda, Sweden). Prior to modification, crystal chips (5 MHz, AT-cut) (Hrbio Co. Ltd, Beijing, China) used were immersed in a boiling solution (30% H_2O_2, 28% ammonia, and double distilled water in a volume ratio of 1:1:5) for 10 min. Then the cleaned chips were rinsed thoroughly with double distilled water, and dried by nitrogen gas prior to use.

The QCM-D measurements were performed at 20 ^{o}C with a flow rate of 10 μL min^{-1}. All experiments were carried out in Tris buffer. A 0.1 μM CP solution was pretreated by 1 mM TCEP for 30 min, then injected into the QCM-D chamber for on-line self-assembly on the gold electrode of a crystal chip for 50 min. Subsequently 1 mM MCH was injected for 20 min to remove the nonspecific CP adsorption and block the bare gold surface. Then, various concentration of target p53 was injected and

hybridized with the capture probe for 150 min. At last, a premixed solution contained 0.5 μM H1 and 0.5 μM H2 hairpins was injected for 50 min. The QCM-D data simultaneously measured in frequency and energy dissipation changes were obtained at 5 overtones.

Gel electrophoresis

50 μL sample with a mixture of 1 μM H1, 1 μM H2, 0.2 μM CP, and 0.1 μM target p53 was prepared and incubated overnight at room temperature. The 1.5% agarose gels contained 0.1 μL Gel-Safe dye per milliliter of gel volume were prepared by using 1×TBE buffer. The gel was run at a constant voltage of 110 V for 1.5 h and scanned by a Tanon 1600 imager.

AFM experiments

Sample was prepared the same as gel electrophoresis experiments. 25 μL of sample were deposited on the freshly cleaved micas for 20 min, the surface was gently rinsed and dried. AFM images were taken out using a SPI3800/SPA400 atomic force microscope (Seiko Inc., Tokyo, Japan) in taping mode to simultaneously collect height and phase data. A Si cantilever with an oscillation frequency of 125 kHz and a spring constant of 14 N/m (SI-DF20, Seiko Inc., Tokyo, Japan) was used for AFM images.

Preparation of HeLa cells lysates

Incubated HeLa cells were collected by trypsinization and centrifugation, and pelleted at 3500 rpm for 5 min at 4 ^{o}C. The cells were resuspended in RIPA cell lysis at a concentration of 5×10^{6} cells/mL, incubated for 10 min in ice, and then centrifuged at 12 000 rpm for 30 min at 4 ^{o}C. The supernatant was collected and filtered by 0.45 μm filter membrane prior to store at –20 ^{o}C.

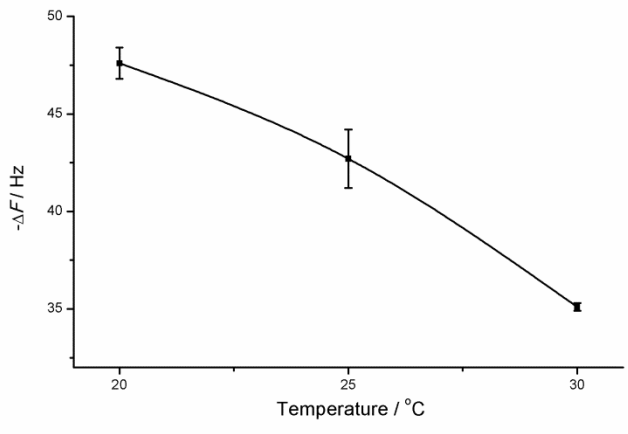

Fig. S1 The amplified frequency response of 30 nM target p53 at different experimental temperature. The most used temperature of 20, 25, or 30 ^{o}C in biosensing was investigated. The frequency change fell down with the temperature raised due to the lower stability of DNA polymer at a higher temperature. 20 ^{o}C was chosen as the optimum temperature condition according to the result.

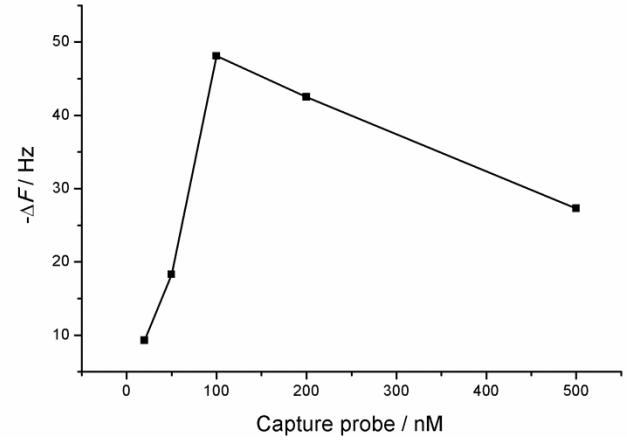

Fig. S2 The amplified frequency response of 30 nM target p53 with different concentration of capture probe. The capture surface density was controlled by changing the concentration of capture probe from 20 nM to 500 nM. With the capture surface density increase, the binding response increases at first and then decreases after the concentration of capture probe increase to 200 nM. This phenomenon indicates that the crowed packed capture hairpins prevent the target sequence from approaching their toehold binding sites on surface efficiently. 100 nM capture probe

was chosen as the optimum condition for biosensing layer construction according to the result.

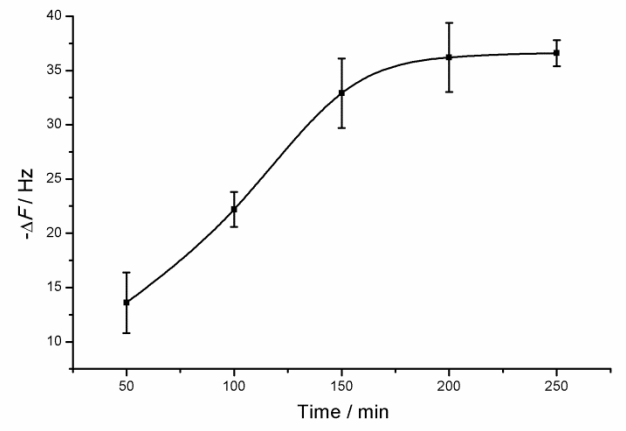

Fig. S3 The amplified frequency response of 30 nM target p53 with different reaction time of target p53 hybridization. The reaction time was changed to 50 min, 100 min, 150 min, 200 min, and 250 min, respectively. After hybridization for 150 min, the frequency change reached equilibrium. Further reaction was not capable of leading to the obvious signal change.

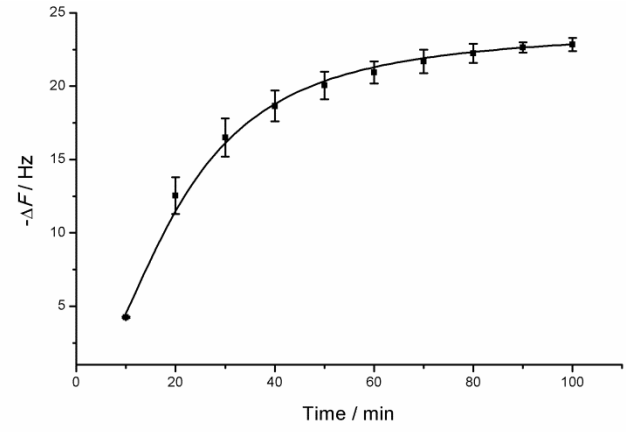

Fig. S4 The amplified frequency response of 30 nM target p53 with different reaction time of HCR operation. The reaction equilibrium was reached about 50 min. Further reaction was not capable of leading to the obvious signal change.