Electronic Supplementary Information

In situ assembly of DNA-streptavidin dendrimer nanostructure: a new amplified quartz crystal microbalance platform for nucleic acid sensing

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Materials and Reagents

Tris(2-carboxyethyl)phosphine (TCEP), 3-aminopropyltriethoxysilane (APTES), 6-mercapto-1-hexanol (MCH), tris(hydroxymethyl)aminomethane (Tris), and streptavidin (SA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). RIPA cell lysis solution was obtained from YuanPingHao Bio. (Beijing, China). Concentrated sulfuric acid, H_2O_2 (30%), and ammonia water (28%) were of analytical reagent grade and were purchased from Beijing Chemical Works (Beijing, China). Glutaraldehyde (50%) was also of analytical reagent grade and was obtained from Beijing Yili Fine Chemical Co., Ltd. (Beijing, China). All HPLC-purified DNA oligonucleotides were obtained from Sangon Inc. (Shanghai, China), and their sequences were listed in Table S1. All DNA oligonucleotides and SA were dissolved in Tris-HCl buffer (20 mM Tris, 140 nM NaCl, 5 mM $MgCl_2$, and 5 mM KCl; pH 7.4) and filtered by 0.45 μm filter membranes prior to use, then stored in the dark at 4 ^oC. All other chemicals employed were of analytical grade, and deionized water was used in all experiments.

Table S1. DNA oligonucleotide sequences.[a]

Name	Sequences (5' to 3')
Capture probe (CP)	5' HS-$(CH_2)_6$TTAAACACGCACCTCAAAGCTGTTC *CGTCCCAGT*GAACAGCTTTGAGGT
Capture probe*	5' NH_2-$(CH_2)_6$-TTAAACACGCACCTCAAAGCTGTTC *CGTCCCAGT*GAACAGCTTTGAGGT
Target p53	ACTGGGACGGAACAGCTTTGAGGTGCGTGTTT
T mutation (T)	ACTGGGACGGAACAGCTTTGAGGTGC<u>T</u>TGTTT
A mutation (A)	ACTGGGACGGAACAGCTTTGAGGTGC<u>A</u>TGTTT
C mutation (C)	ACTGGGACGGAACAGCTTTGAGGTGC<u>C</u>TGTTT
Direct mode	DNA1* Biotin-AACCTCAAAGCTGTTC DNA2* Biotin-AGAACAGCTTTGAGGT
Bridge mode	DNA1 Biotin-AACCTCAAAGCTGTTC DNA2 TATATCCCTGCCACTA-Biotin Linker AGTGGCAGGGATATAGAACAGCTTTGAGGT

[a] The loops are italicized in the capture probes. The mismatched bases in the mutant sequences are underlined.

Preparation of Building Blocks

The preparation of the two building blocks (DNA1-SA and DNA2-SA) was based on the fact that one streptavidin (SA) molecule has four binding sites for coupling with the biotinylated single strand DNA. DNA1-SA was obtained by successively incubating DNA1 (0.2 μM) with a linker DNA (0.1 μM) and SA (0.1 μM). DNA2-SA was prepared by directly incubating DNA2 (0.2 μM) with SA (0.1 μM). Each building block was preincubated for 30 min at room temperature before injection.

Quartz Crystal Microbalance Measurements

All binding processes were monitored on-line by using a Q-Sense E4 QCM-D instrument (Q-Sense AB, Västra Frölunda, Sweden) which has four chambers and can provide real-time responses of multiovertone frequencies. Prior to use, the crystal chips (5 MHz, AT-cut) (Dongwei Biotechnology Co., Ltd. Hangzhou, China) were

immersed in a newly prepared piranha solution (concentrated sulfuric and 30% H_2O_2 in a volume ratio of 3:1) for 5 min and rinsed with deionized water, then immersed in a boiling solution (30% H_2O_2, 28% ammonia water, and deionized water in a volume ratio of 1:1:5) for 15 min. Before loading to measuring cells, these cleaned chips were rinsed thoroughly with deionized water, and dried by nitrogen gas.

Caution: the piranha solution is extremely corrosive, reactive, and potentially explosive and should be careful to avoid skin contact.

The quartz crystal microbalance (QCM) measurements were performed at 20 ^oC in Tris-HCl buffer. In a typical experiment, a 0.1 μM capture probe (CP) solution was pretreated by 10 μM TCEP for 60 min, then injected into the QCM chambers for on-line self-assembly on the gold-coated crystal chip for 25 min. Subsequently, 1 mM MCH flowed through the chambers for 10 min to block the bare gold surface and remove the nonspecific CP adsorption. The running rate of CP and MCH was 20 μL min^{-1} (Ismatec IPC tubing pump (Glattbrugg, Switzerland)). After that, various concentration of target p53 was injected and well hybridized with the CP for 90 min. Sequentially, the amplified responses were obtained by alternately injecting two building blocks (DNA1-SA and DNA2-SA) for assembling the DNA-SA dendrimer nanostructure. Each building block solution was injected for 20 min. The running rate of target p53 and each building block was 10 μL min^{-1}. The frequency changes were obtained at 9 overtones.

Characterization of DNA-SA Dendrimers

The morphologies of DNA-SA dendrimers were characterized by scanning electron microscopy. The silicon wafers were immersed in 95% acetone containing 1% APTES for 5 min, then rinsed with acetone and dried by nitrogen gas. The capture probe* (1 μM, 100 μL) was immobilized on the silicon wafer surfaces through the amino-coupling using 5% glutaraldehyde as the linker, and subsequently blocked with 2.6% sodium borohydride. Target p53 (1 μM, 100 μL) was added to the dried surface and reacted overnight. Then, DNA1-SA and DNA2-SA (1 μM, 100 μL) were alternately added to the silicon surfaces and each building block was reacted for 2 h. Finally these silicon wafers were washed with deionized water and dried in a vacuum drying oven (Shanghai Yiheng Scientific Instrument Co., Ltd. Shanghai, China)

overnight for SEM characterization. The morphologies of DNA-SA dendrimers on the silicon wafers were observed and recorded using an S-4800 scanning electron microscope (Hitachi, Japan).

Preparation of HeLa Cell Lysates

HeLa cells were plated in 10 cm culture dishes (Corning, U.S.A.) in the high glucose (4.5 g L^{-1}) Dulbecco's Modified Essential Medium (DMEM, Solarbio, China) supplemented with 10% fetal bovine serum, incubated at 37 °C in 5% CO_2. Upon reaching confluence, the cells were harvested by treating with 0.25% trypsin, and resuspended with PBS. Then the incubated HeLa cells were pelleted at 3,000 rpm for 5 min at 4 °C and resuspended in RIPA cell lysis solution with a concentration of 5.0 × 10^6 cells mL^{-1}. After incubated for 30 min at −20 °C, the cells were centrifuged at 12,000 rpm for 30 min at 4 °C. The supernatant was collected and then filtered by 0.45 μm filter membrane prior to store at –20 °C.

Optimization of QCM Sensing Conditions

The QCM sensing conditions were optimized based on the procedures described in "Quartz Crystal Microbalance Measurements" section. The results were shown in Fig. S1−S6. The error bars represent the standard deviation of three measurements.

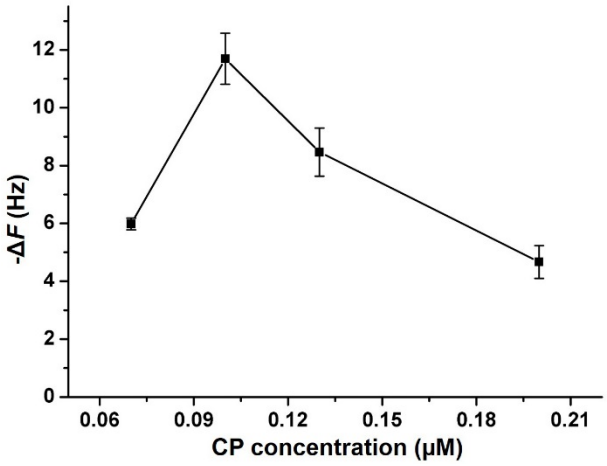

Fig. S1 Effect of capture probe (CP) concentration on frequency response of amplified QCM biosensor to 5 nM target p53. The highest Δ*F* response was observed at 0.1 μM CP concentration.

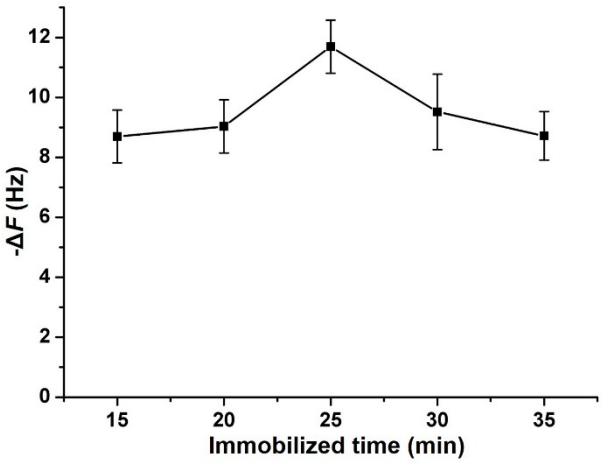

Fig. S2 Effect of immobilized time of capture probe on frequency response of amplified QCM biosensor to 5 nM target p53. The highest Δ*F* response was observed at the immobilized time of 25 min.

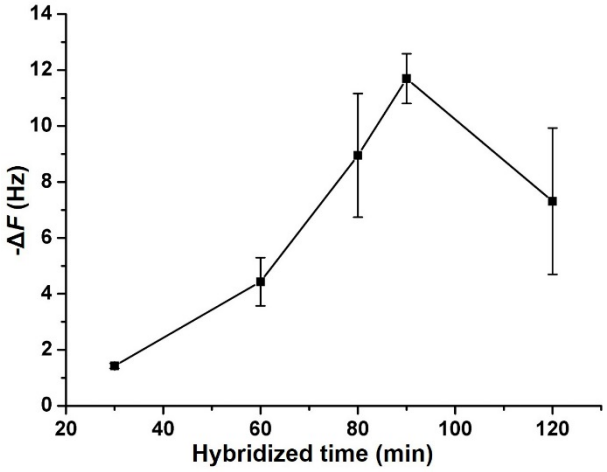

Fig. S3 Effect of hybridized time of target p53 with capture probe on frequency response of amplified QCM biosensor to 5 nM target p53. The highest Δ*F* response was observed at the hybridized time of 90 min.

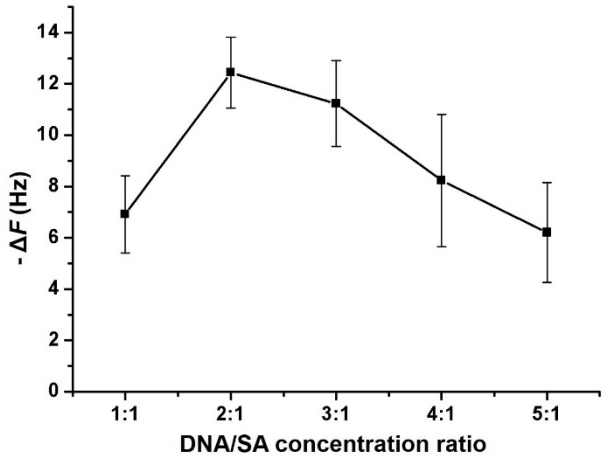

Fig. S4 Effect of DNA/SA concentration ratio of the building blocks on frequency response of amplified QCM biosensor to 5 nM target p53. The highest Δ*F* response was observed at the DNA/SA concentration ratio of 2:1.

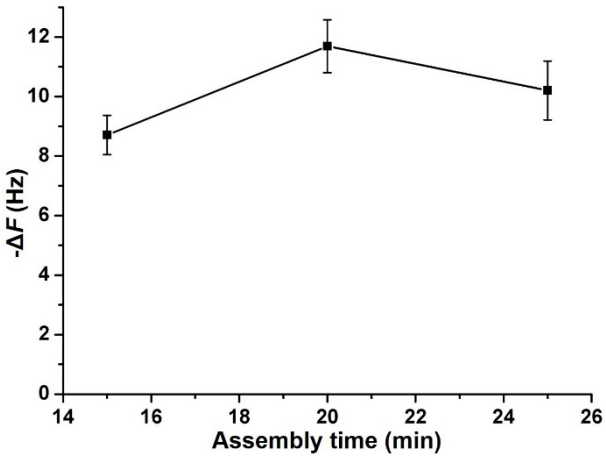

Fig. S5 Effect of assembly time of each DNA-SA dendrimer generation on frequency response of amplified QCM biosensor to 5 nM target p53. The highest Δ*F* response was observed at the assembly time of 20 min.

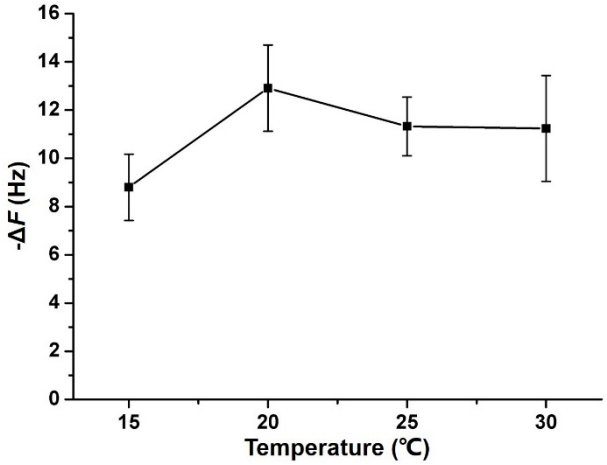

Fig. S6 Effect of temperature on frequency response of amplified QCM biosensor to 5 nM target p53. The highest ΔF response was observed at 20 ^{o}C.

Table S2. Comparison between the proposed QCM sensing platform with the QCM biosensors reported for nucleic acid detection.

Assay format	Fundamental frequency of QCM chip (MHz)	LOD	Reference
Assembled DNA-streptavidin dendrimer as a mass amplifier	5	23 pM	This work
Hybridization chain reaction	5	0.1 nM	1
Toehold-mediated strand displacement and streptavidin amplification	5	0.3 nM	2
Toehold-mediated strand displacement	5	0.8 nM	3
Direct hybridization assay	5	0.25 ng/μL	4
Thiolated pyrrolidinyl peptide nucleic acids as capture probes	5	5 μM	5
Thiol-derivatized peptide nucleic acid as a capture probe	5	1 μg/mL	6
DNA dendrimers as recognition elements	5	1 μg/mL	7
Multi-wall carbon nanotubes- doped polypyrrole matrices	5	4 pM	8
Gold-modified polystyrene spheres as carriers	8	10^{-12} M	9
Streptavidin conjugated Fe_3O_4 NPs as mass enhancers	8	10^{-12} M	10
Combination of hairpin probe with endonuclease/GNP signal amplification	9	2 pM	11
AuNPs as both surface modifier and signal amplifier	9	10^{-16} M	12
DNA ligase reaction and nano-Au amplification	9	2.6×10^{-9} M	13
DNA ligase reaction and biocatalyzed deposition amplification	9	0.1 nM	14
Loop-mediated isothermal amplification	9	0.8 pg/mL	15
Amplified detection by tagged liposomes	9	1×10^{-13} M	16
Dextran-streptavidin as a substrate modifier	9.5	0.12 μM	17
Lipid-based DNA immobilization method	10	1.6 nM	18
Combination of peptide nucleic acid probe with RecA protein amplification	10	8.6 pg/L	19
AuNPs for mass amplification	10	74 aM	20
MutS protein-based mutation recognition assay	10	1 nM	21

Au hollow balls with dendritic surface as a chip substrate	–	10^{-12} M	22

1 W. Tang, D. Wang, Y. Xu, N. Li and F. Liu, *Chem. Commun.*, 2012, **48**, 6678–6680.

2 D. Wang, W. Tang, X. Wu, X. Wang, G. Chen, Q. Chen, N. Li, and F. Liu, *Anal. Chem.*, 2012, **84**, 7008−7014.

3 D. Wang, G. Chen, H. Wang, W. Tang, W. Pan, N. Li and Feng Liu, *Biosens. Bioelectron*., 2013, **48**, 276–280.

4 İ. Karamollaoğlu, H. A. Öktem and M. Mutlu, *Biochem. Eng. J.*, 2009, **44**, 142–150.

5 C. Ananthanawat, T. Vilaivan and V. P. Hoven, *Sensors and Actuators B*, 2009, **137**, 215–221.

6 J. Wang, P. E. Nielsen, M. Jiang, X. Cai, J. R. Fernandes, D. H. Grant, M. Ozsoz, A. Beglieter and M. Mowat, *Anal. Chem.*, 1997, **69**, 5200–5202.

7 J. Wang and M. Jiang, *J. Am. Chem. Soc.*, 1998, **120**, 8281–8282.

8 T. T. N. Lien, T. D. Lam, V. T. H. An, T. V. Hoang, D. T. Quang, D. Q. Khieu, T. Tsukahara, Y. H. Lee and J. S. Kim, *Talanta*, 2010, **80**, 1164–1169.

9 S. Li, Y. Xia, J. Zhang, J. Han and L. Jiang, *Electrophoresis*, 2010, **31**, 3090–3096.

10 X. Mao, L. Yang, X. L. Su and Y. Li, *Biosens. Bioelectron*., 2006, **21**, 1178–1185.

11 Y. Fei, X. Y. Jin, Z. S. Wu, S. B. Zhang, G. Shen and R. Q. Yu, *Anal. Chim. Acta*, 2011, **691**, 95–102.

12 T. Liu, J. Tang and L. Jiang, *Biochem. Bioph. Res. Co.*, 2004, **313**, 3–7.

13 L. Pang, J. Li, J. Jiang, G. Shen and R. Yu, *Anal. Biochem.*, 2006, **358**, 99–103.

14 K. Feng, J. Li, J. H. Jiang, G. L. Shen and R. Q. Yu, *Biosens. Bioelectron*., 2007, **22**, 1651–1657.

15 P. Prakrankamanant, C. Leelayuwat, C. Promptmas, T. Limpaiboon, S. Wanram, P. Prasongdee, C. Pientong, J. Daduang and P. Jearanaikoon, *Biosens. Bioelectron*., 2013, **40**, 252–257.

16 F. Patolsky, A. Lichtenstein and I. Willner, *J. Am. Chem. Soc.*, 2001, **123**, 5194–5205.

17 D. Dell'Atti, S. Tombelli, M. Minunni and M. Mascini, *Biosens. Bioelectron*., 2006, **21**, 1876–1879.

18 S. Kim and S. J. Choi, *Anal. Biochem.*, 2014, **458**, 1–3.

19 C. Yao, T. Zhu, J. Tang, R. Wu, Q. Chen, M. Chen, B. Zhang, J. Huang and W. Fu, *Biosens. Bioelectron*., 2008, **23**, 879–885.

20 Z. Mo, H. Wang, Y. Liang, F. Liu and Y. Xue, *Analyst*, 2005, **130**, 1589–1594.

21 X. Su, R. Robelek, Y. Wu, G. Wang and W. Knoll, *Anal. Chem.*, 2004, **76**, 489–494.

22 W. Lu, L. Lin and L. Jiang, *Biosens. Bioelectron*., 2007, **22**, 1101–1105.