Electronic Supplementary Material (ESI) for Nanoscale.

An amplified impedimetric aptasensor combining target-induce DNA Hydrogel formation with pH-stimulated signal amplification for heparanase assay

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Materials

Tris(hydroxymethyl) aminomethane (Tris), acetic acid, sodium nitrate, ammonium persulfate (APS), magnesium nitrate, gold chloride ($HAuCl_4 \cdot 4H_2O$), N,N,N′,N′-tetramethylethylenediamine (TEMED), hexane thiol (HT) and acrylamide solution (40%) were purchased from Sigma–Aldrich (St. Louis, MO, USA). All Freeze-dried nucleic acid strands were purchased from Sangon Biotech (Shanghai) Co., Ltd, and the sequences are showed Table S1:

Table S1. Oligonucleotide sequences

name	sequences*(5′→3′)
aptamer	ACTTTTGAATGTGGCAACAAATTCGACAGG
S1	Acrydite-AAACCTGTCGAATTTGTTGCCACATTCAAAAGT

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S2	Acrydite-AAACCCCTAACCCC
S3	Acrydite-ACTTTTGAATGTGGC
S4	AACAAATTCGACAGG-$(CH_2)_6$-NH_2

Apparatus and Measurements

Morphologies and structures of the hydrogel samples were characterized by scanning electron microscope (SEM, S-4800, Hitachi, Japan) and atomic force microscopy (AFM, Dimension FastScan, Bruker, Germany). Briefly, after hydrogel sample was formed on a silicon slide surface, the sample was frozen at -95 °C for 6 h and then dried by sublimation of the generated ice crystals under vacuum, followed by coating with an Au film. A modified glassy carbon electrode (GCE), a platinum wire auxiliary electrode and a saturated calomel reference electrode (SCE) were used as a three-compartment electrochemical cell. Electrochemical measurement including cyclic voltammetry (CV) and electrochemical impedance spectra (EIS) were conducted by a CHI 660D electrochemical workstation (Shanghai Chenhua instrument, China). Circular dichroism (CD) spectra was recorded on a Chirascan Instrument (Applied Photophysics, UK). Ultraviolet-visible (UV-vis) spectra was performed by a UV-2501 PC Spectrometer (Shimadzu, Japan).

PAGE analysis. The notches of the freshly prepared non-denaturing polyacrylamide gel (16%) was used to load the samples, and electrophoresis was conducted at 120 V for 60 min in 1 × TBE buffer. Before loading, DNA samples were mixed with DNA loading buffer on a volume ratio of 5:1. As shown in Figure S2, the S4, S2, S1 and aptamer in lanes 1, 2, 3, 4 and 5 respectively, exhibited a different band (Figure S1B). Lane 5 showed the PAGE result for sequential hybridization of S2 with S1 and S4, and

Lane 6 showed the PAGE result for sequential hybridization of S2 with aptamer at the same concentration of 2 μM. As expected, bands with slower mobility could be observed (lane 6, 5 *vs*. 1, 2, 3, 4), suggesting successful hybridizations between single stand DNAs. Lane 7 showed the PAGE result for the mixture solution of 2 μM dsDNA between S2 and aptamer, 2 μM S2 and 2 μM S4. It could be seen that two obvious band including the dsDNA band and the single stand DNA (S4, S2) band were obtained. When addition target HPA into the mixture solution of dsDNA, S2 and S4, a new band with very slow mobility could be observed (lane 8), indicating that the target HPA successfully bound to the aptamer with release of S1 from S1/aptamer duplex.

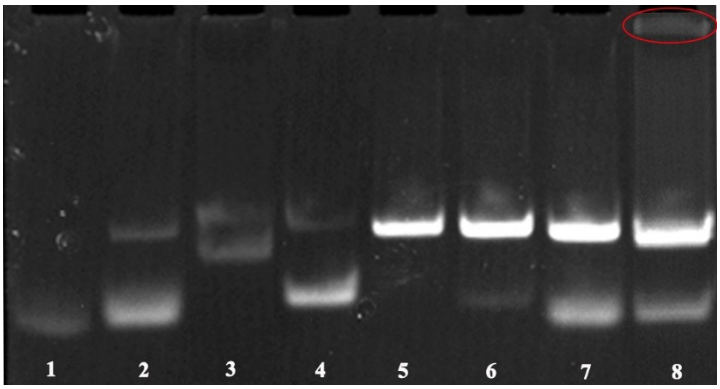

Figure S1 PAGE analysis of different samples, Line 1 to Line 8 correspond to S4, S2, S1, aptamer, S4+S2+S1, S1+aptamer, S1+aptamer+S2+S4, S1+aptamer+S2+S4+HPA.

Optimized Experimental Conditions. To obtain EIS response, the effect of acrydite-modified DNA ratio in the monomer mixture on the assay performance was studied. Firstly, S1 and S2 concentration was optimized by adding different volume S1 (100 μM) and S2 (100 μM) into the two mixture solution for synthesis of recognition

polymer and P2 respectively. As shown in Figure S2A, the EIS response when hydrogel formed on surface at 5 ng mL^{-1} HPA was increased with increasing the S1 and S2 volume and level off after 60 μL. Therefore, 60 μL S1 (100 μM) and S2 (100 μM) was chose for synthesis of recognition polymer and P2 respectively. Moreover, to obtain maximum amplification EIS signal, S3 concentration was also optimized by adding different volume of S3 (100 μM) into mixture solution for synthesis of recognition polymer and P2 (Figure S2B). The amplification EIS signal at 5 ng mL^{-1} HPA was increased with increasing the S3 volume and level off after 80 μL. Thus, 80 μL S3 (100 μM) was chose for synthesis of recognition polymer and P2. The effect of acrydite-modified DNA ratio in the monomer mixture on the assay performance was showed in revised supporting information.

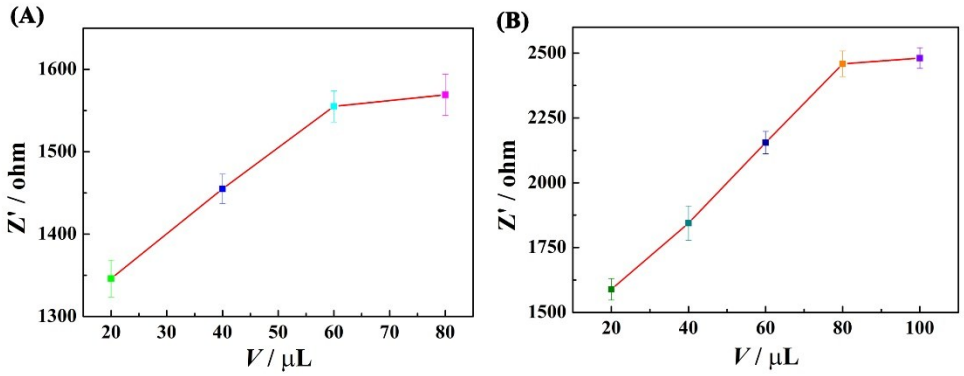

Figure S2 (A) Effect of S1 and S2 concentrations on EIS response. (B) Effect of S3 concentrations on EIS response.

In addition, pH-stimulated formation of i-motif structures of S3 played an important role on performance of signal amplification. To obtain the maximum value of EIS intensity signal, the pH value of tris-HAc buffer and the incubation time was optimized. Firstly, the proposed aptasensor was assessed by using EIS with three different pH

values (4.0, 5.0, 6.0) of tris-HAc buffer at 5 ng mL^{-1} HPA (Figure S3A). With decrease of the pH values of tris-HAc buffer, the EIS response increased. But a dramatic decrease in EIS response was observed at pH 4.0, which may be attributed to the hydrogel disintegration on electrode surface at pH 4.0. Thus, the optimal pH of tris-HAc buffer was 5.0. Based on intermittent detection (every 30 min) of the tris-HAc buffer-incubated aptasensor, the incubation time was optimized. The experimental results showed in Figure S3B. The EIS signal increased with the increment of Tris-HAc incubation time. However, the increase rate slowed between 120 min and 150 min. Thus, 120 min was chosen as the optimal time of the tris-HAc buffer-treated hydrogel film in this experiment.

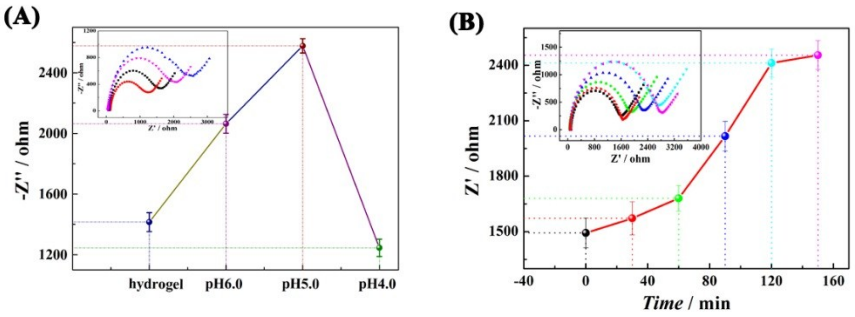

Figure S3 (A) Effect of pH value of tris-HAc buffer on EIS response. (B) Effect of pH-regulated time on EIS response.