Supporting information

A signal-on electrochemical biosensor for sensitive detection of microRNA-155 combining target recycling with cascade catalysis for amplification

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S1. Signal amplification properties of CHA.

To demonstrate the signal amplification capability of CHA in this approach, DPV was employed for signal measurements towards different modified electrodes. As shown in Fig. S1, the electrode modified H1 itself exhibited low peak current (Fig. S1, curve a). The peak current was scarcely influenced by the addition of only H2 (Fig. S1, curve b), indicating that H2 failed to react with the immobilized H1 in the absence of target. Upon the addition of target (10 pM) without H2, the peak current increased slightly owing to the formation of H1-T duplex (Fig. S1, curve c). Furthermore, the peak current had an obvious enhancement upon simultaneous addition of H2 and target (Fig. S1, curve d), demonstrating that the enhancement strategy (CHA) was realized.

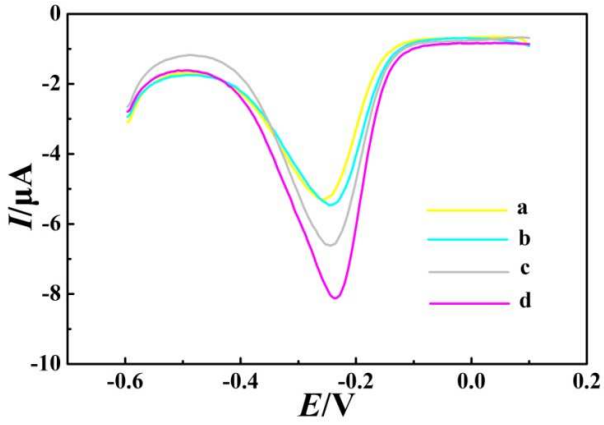

Fig. S1 DPVs of different modified electrode: the biosensor was constructed with (a) only H1; (b) H1+H2; (c) H1+T; (d) H1+H2+T (10 pM) in 0.1 M PBS (7.0).

S2. Mechanism of CHA

CHA reaction relies on hybridization and strand-exchange reactions, only requiring the ingenious design of base-pairing between strands. As shown in Fig. 2A, the target we detected composed of domain 1, 2, 3. Therefore, we designed the H1 containing five domains (0', 1', 2, 3, 4) and the H2 containing four domains (2, 3, 4, 5). The sequences in the toehold and stem of H1 (domain 1', 2', 3') are completely complementary to the target. Upon interaction of these domains with the target miRNA, the hairpin structure of H1 is opened, resulting in a T:H1 intermediate (Fig. 2B, reaction a). At this time, the domain 3 of H1 is no longer blocked and can bind to domain 3' of H2, initiating a branch migration reaction to form a H1:H2:T complex (Fig. 2B, reaction b). This complex is inherently unstable. Thus, target dissociates from the H1:H2 complex, which can then hybridize to another H1 and initiate the second cycle (Fig. 2B, reaction c). In this way, a single target can generate many H1:H2 complex and benefit for next steps. Therefore, it is promising for signal amplification.

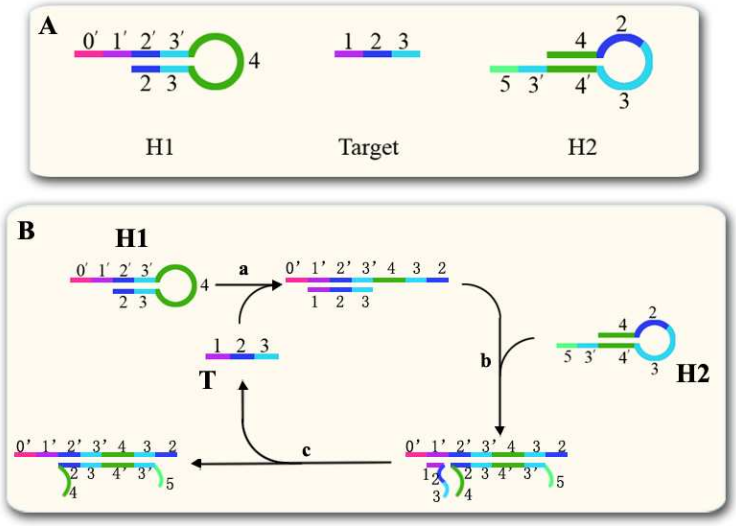

Fig. S2 (A) The sequence of DNA molecules in terms of numbered domains. Complementarity between numbered domains is denoted by an asterisk. (B) Scheme of CHA.

S3. Optimization of detection condition.

In order to enhance the bioelectrocatalytic efficiency and obtain a high sensitivity of the electrochemical miRNA biosensor, the effect of H1 concentration and ethanol concentration were investigated and the results are shown in Fig. S3A and Fig. S3B respectively.

10 μL of H1 with different concentrations ranging from 0.5 μM to 2.5 μM was dropped onto the electrode surface for 16 h and measured in 5 mM $[Fe(CN)_6]^{3-/4-}$ solution. Seen from Fig. S3A, the peak current decreased with the increase of H1 concentration and reached level at 2.0 μM, indicating the saturation of surface was reached. Thus, 2.0 μM of H1 is optimal for the proposed biosensor.

The concentration of ethanol was optimized by recording DPV responses when adding different volume of ethanol (from 30 μL to 150 μL) into the electrolyte of PBS

(1 mL, pH 7.0). As seen from Fig. S3B, the peak current increased with the increase of ethanol and then tended to reach saturation after adding 120 μL of ethanol, predicating the highest efficiency of bioelectrocatalysis at this point. Thus, 120 μL of ethanol was employed throughout the detection process.

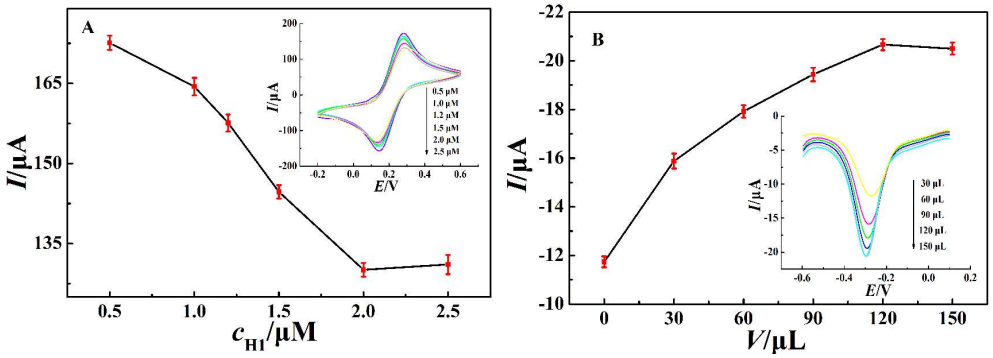

Fig. S3 The effect of (A) H1 concentration in 5 mM $[Fe(CN)_6]^{3-/4-}$ solution and the effect of (B) ethanol concentration in 1 mL PBS (pH 7.0).