DNA nanoflower blooms in nanochannel: a new strategy for miRNA detection

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Experimental section

Materials and reagents

DNA oligonucleotides were synthesised by Sangon Biotech Co., Ltd (Shanghai, China). They are listed in Table 1. PAA was purchased from Hefei Puyuan Nanotechnology Co., Ltd (Anhui, China). T4 DNA ligase and phi29 DNA polymerase were purchased from Beyotime Biotechnology (Shanghai, China). (3-aminopropyl)triethoxysilane (APTES), $K_3[Fe(CN)_6]$, KCl, benzaldehyde, hexaammineruthenium(III) chloride (RuHex) and other reagents of certified analytical grade were obtained from Sigma-Aldrich (Shanghai, China) or from Sigma-Aldrich Co., Ltd (St Louis, MO, USA). Electrochemical measurements were performed on a CHI660D electrochemical workstation (CH Instruments, Austin, TX, USA). All solutions were prepared with deionized water, which was purified with a Milli-Q purification system (Bedford, MA, USA) to a resistance of 18.2 MΩ cm.

PAA modification with DNA.

The morphology and size of PAA membranes were characterized by field-emission scanning electron microscopy (SEM, SU8020, Japan) with an acceleration voltage of 3 kV. PAA membranes were washed sequentially with ethanol and ultrapure water to remove impurities in the nanochannels. At room temperature, the PAA membranes were dried with nitrogen and then immersed in 1 mL of ethanol solution containing 5% APTES. Amino groups were generated on the inner wall of the PAA nanochannels after gentle shaking for 12 h. The PAA membranes were then washed with ethanol again to remove residual silylating reagents and dried with nitrogen. Then, 20 μL of 10 μM proDNA solution was dropped onto the surface of the PAA membrane and allowed to react for 24 h. It was important that the PAA membrane was hung in an airtight glass bottle with some water at the bottom. The saturated moisture in the bottle prevented the 20 μL proDNA solution from evaporating over 24 h. PAA membrane was immersed in 1 mL of ultrapure water containing 0.1% benzaldehyde and shaken gently for 12 h to block the remaining amino groups and then the PAA membranes were washed with ultrapure water to remove the unbound ssDNA and residual benzaldehyde. Then, the functionalized PAA membrane with ssDNA immobilized on the inner wall was obtained and stored in Tris buffer solution at 4 °C.

DNA nanoflower amplification synthesis.

To prepare the ligated DNA template, the following were added to a 200 μL centrifuge tube: 69.5 μL ultrapure water, 10 μL DNA ligation buffer (10×), 6 μL template (10 μM) and 12 μL primer (10 μM) in 100 μL system. The tube was vortexed, and the mixture was heated at 95 °C for 5 min. The mixture was cooled slowly to room temperature over a period of 3 h. Heating the DNA to 95 °C and subsequent gradual cooling to room temperature are important steps in achieving complete hybridization of cDNAs. T4 DNA ligase (2.5 μL, 40,000 U/mL) was then added to the annealed mixture, being mixed well with a pipette tip or by mild vortexing. Finally, the mixture circle-DNA was incubated at room temperature for 30 min. The ligated product could be stored at 4 °C for at least 1 month.

20 μL miR-21 solution of different concentrations, 1 μL 10× tris-HCL, and 100 mM NaCl (pH=7.4) were added at one side of PAA, followed by slowly shaking for 1 h to ensure sufficient hybridization of target miR-21 and ssDNA. The PAA was washed by ddH_20 to remove the uncombined miR-21 and dried by N_2. 100 μL mixture which contained 60 μL circle-DNA, 20 μL dNTP (10mM), 10 μl BSA (10×), 10 μL phi29 DNA polymerase buffer(10×), and 10 μl phi29 DNA polymerase(1,000U/ml) was added to PAA, and incubated at 30 °C for 6 h and then heat treated at 75 °C for 10 min to inactivate the phi29 DNA polymerase.

Electrochemical measurements.

All electrochemical devices were performed by refering to the study performed by the Wei group[19]. A self-made electrochemical detection device was used. A platinum sheet (1 cm × 1 cm) was the working electrode, and this was placed on top of a conductive copper base. A PAA membrane was placed on top of the platinum sheet. Then, a round insulated O-type silicone pad (PMMA) was placed at the top of the PAA membrane to prevent electrolyte leakage, and above this, there was an open electrolytic cell. Two electrodes were inserted into the electrode. A platinum electrode was used as a counter electrode and a saturated calomel electrode as a reference electrode, and this formed a three electrode electrochemical system with the platinum plate under the PAA membrane. A CHI 660D electrochemical workstation was used for all electrochemical measurements. The anodic current of 5 mM for $K_3[Fe(CN)_6]$ illustrated the steric hindrance changes in the PAA nanochannels with and without the miRNA triggering off the bloom of the DNA nanoflowers. Before electrochemical measurement, measures were taken to maintain a homogeneous $K_3[Fe(CN)_6]$ concentration inside and outside the nanochannels. Chronocoulometry was measured in Tris buffer solution containing 50 μm RuHex.

Table S1. Oligonucleotide sequences used in the experiments.

Oligonucleotide	**Sequences (5' to 3')**
proDNA	CHO-(CH2)6-T10-TCAACATCAGT
miR-21	UAGCUUAUCAGACUGAUGUUGA
primer	CTGATAAGCTATCCCTAGCTATGAGTTT
Circle-DNA	ATACTCAAAGAATGCGACTCATGAAGCTAATTCATT AGCTTCATGAGTCGCATTCAGGGATCGA

Table S2. Analysis data of the DNA nanoflowers-based sensors for miR-21 determination in human serum

Sample	**Added (fM)**	**Found (fM)**	**RSD (%)**	**Recovery（%）**
1	10	10.23, 10.92, 9.67	6.09	102.7
2	100	104.62, 97.18, 93.54	5.74	98.4
3	1000	989.97, 1026.54, 976.43	3.54	99.1

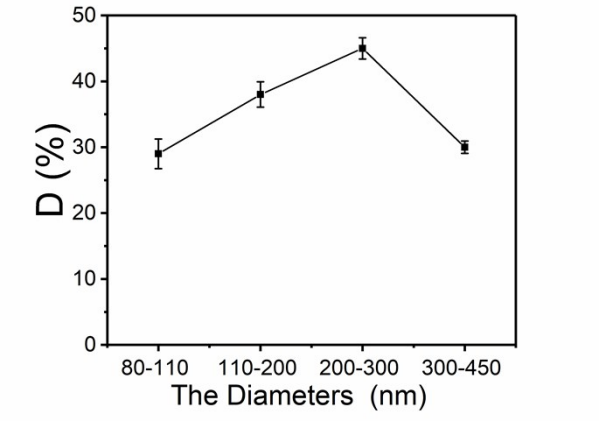

Figure S1. Impact of different pore diameters of PAA membrane on the current drop ratio. 1000 fM was used. Error bars showed the standard deviation of three experiments.

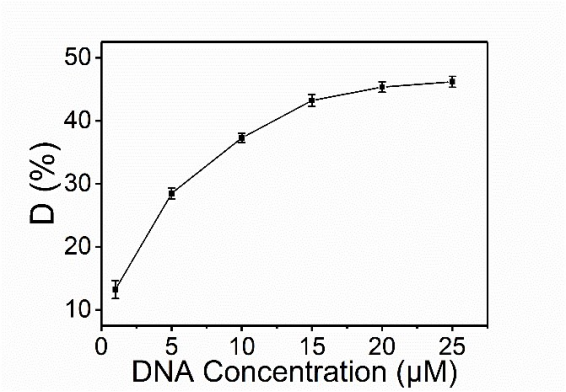

Figure S2. Impact of different DNA concentrations (1μL, 5μL, 10μL, 15μL, 20μL, 25μL) on the current drop ratio. 1000 fM was used. Error bars showed the standard deviation of three experiments.

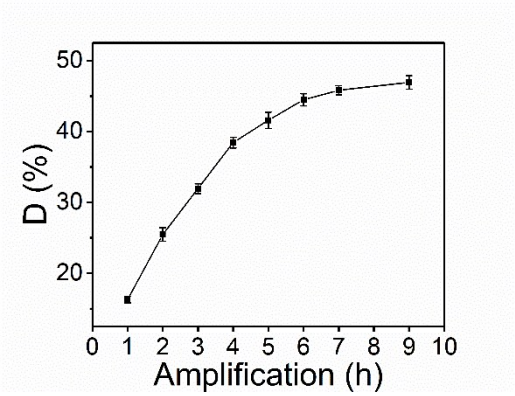

Figure S3. Impact of different RCA amplification time (1h, 2h, 3h, 4h, 5h, 6h, 7h, 10h) on the current drop ratio. 1000 fM was used. Error bars showed the standard deviation of three experiments.