

Supporting Information

Colorimetric Detection of MicroRNA and RNase H Activity in Homogeneous Solution with Cationic Polythiophene Derivative

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Experimental Section

Materials and Reagents

Cationic water-soluble poly [3-(3'-N, N, N-triethylamino-1-propyloxy)-4-methyl-2, 5-thiophene hydrochloride] (PMNT) was prepared from an oxidative polymerization in chloroform with FeCl₃ as the oxidizing agent according to the procedure in the literatures¹. RNase H and PAGE-purified DNA and HPLC-purified miRNA oligonucleotides were obtained from TaKaRa Biotechnology Co., Ltd (Dalian, China). The sequences are as follows:

miRNA(let-7a): 5'-UgAggUAgUAggUUgUAUAgUU-3'

complementary DNA: 5'-AACTATAACAACCTACTACCTCA-3'

one-base mismatch DNA: 5'-AACTATAACAACgTACTACCTCA-3'

three-base mismatch DNA: 5'-AACTACACACAACgTACTTCCCTCA-3'

non-complement DNA: 5'-CATgTgAgTgAATTgATgAATC-3'

The mismatched bases in the DNAs are underlined. The concentration of oligonucleotides was determined by measuring the absorbance at 260nm in 160 μ L quartz cuvette. All the DNA and RNA solutions were prepared with DEPC-treated water. The buffer used is purchased from TaKaRa Biotechnology Co., Ltd (Dalian, China). The reaction temperatures were controlled by a Biometra T1 Thermal Cycler (Germany). UV-vis absorption spectra were measured with a TU-1901 spectrophotometer (Purkjinie General Instrument Co., Ltd., Beijing, China).

Assay for miRNA: 1.2 μ L complementary DNA (1.0×10^{-4} M) and a certain amount of the miRNA were added into 100 μ L buffer (50 mM Tris-HCl, 75 mM KCl, 3 mM MgCl₂, 10 mM DTT,

pH 8.3) in a 200 μL PCR microtube. The mixture solution was then heated at 70°C for 3 min, 55°C for 1.5 h. After cooling to room temperature, 3.0 μL PMNT (1.0×10^{-3} M) was added and mixed. The UV-vis absorption spectra were measured in a 200 μL quartz cuvette at room temperature.

Digestion of miRNA in the DNA/miRNA hybrids by RNase H: 1.2 μL complementary DNA (1.0×10^{-4} M), miRNA (1.0×10^{-4} M) were added into 100 μL buffer (50 mM Tris-HCl, 75 mM KCl, 3 mM MgCl_2 , 10 mM DTT, pH 8.3) in a 200 μL PCR microtube. The mixture solution was then heated at 70°C for 3 min, 55°C for 1.5 h. After cooling to room temperature, RNase H was added, the mixed solution was incubated for 10 min at 30°C . After cooling, 3.0 μL PMNT (1.0×10^{-3} M) was added. The UV-vis absorption spectra were measured in a 200 μL quartz cuvette at room temperature.

Characteristics of the UV-vis absorption spectra of miRNA/PMNT and DNA/PMNT duplexes: As demonstrated in the text of this paper, after hybridization between miRNA and its complementary DNA and digestion by RNase H, the miRNA in the miRNA/DNA hybrids is specifically digested. The released single strand (ss) DNA combines with PMNT to form DNA/PMNT duplex. The absorbance of DNA/PMNT at 400 nm ($A_{400\text{ nm}}$) decreases and $A_{525\text{ nm}}$ increases compared to PMNT itself (Figure S1, a and b). When miRNA and PMNT form miRNA/PMNT duplex, as shown in Figure S1, d, the $A_{400\text{ nm}}$ of miRNA/PMNT duplex greatly increases and exhibits greater absorbance at longer wavelength than that of PMNT. When miRNA mixes with non-complementary DNA, the miRNA can not be digested by RNase H due to no hybridization between miRNA and non-complementary DNA. Therefore, the DNA/PMNT and miRNA/PMNT duplexes can be respectively formed in the solution. miRNA/PMNT duplexes show increased absorbance at 400 nm. As a result, the UV-vis absorption spectrum of the mixture of miRNA/PMNT and DNA/PMNT has much lower absorbance ratio of 525 nm to 400 nm than that of DNA/PMNT duplex.

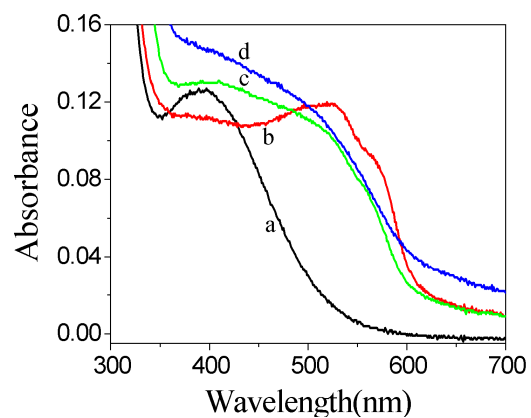


Figure S1. UV-vis absorption spectra of PMNT (a); (miRNA/complementary DNA+RNase H)/PMNT (b) ; (miRNA/non-complementary DNA+RNase H)/PMNT (c) and miRNA/PMNT. [PMNT]=30 μ M in RUs, [DNA]=[miRNA]=1.2 μ M, RNase H: 3Unit.

1 H. A. Ho, M. Boissinot, M. G. Bergeron, G. Corbeil, K. Dore, D. Boudreau, M. Leclerc, *Angew. Chem. Int. Ed.* 2002, **41**, 1548-1551.