Supporting Information

Detection of cofilin mRNA with hybridization-sensitive double-stranded fluorescent probes

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Experimental details

Synthesis of oligonucleotides

PyU- and DabU-modified oligonucleotides (ODNs) were synthesized on a CPG support (1 µmol scale, 1000 Å pore size) using standard phosphoramidite methods and an automated DNA synthesizer (POLYGEN DNA-Synthesizer). The synthesized ODNs were cleaved from the solid support upon treatment with 28–30% aqueous NH₄OH (1.0 mL) for 12 h at 55 °C. After filtration of the CPG, the crude products from the automated ODN synthesis were lyophilized and diluted with distilled water (1 mL). The ODNs were purified through reverse-phase HPLC (Merck LichoCART C18 column; 10 × 250 mm; 10 µm; pore size: 100 Å). The HPLC mobile phase was held isocratically for 10 min with 5% MeCN/0.1 M triethylammonium acetate (TEAA) (pH 7.2) at a flow rate of 2.5 mL/min. The gradient was then increased linearly over 10 min from 5 to 50% MeCN/0.1 M TEAA at the same flow rate. The fractions containing the purified ODNs were cooled and lyophilized. 80% Aqueous AcOH was added to the ODNs. After 1 h at ambient temperature, the AcOH was evaporated under reduced pressure. The residue was diluted with water (1 mL); this solution was then purified through HPLC using the same conditions as those described above. The ODNs were analyzed through reverse-phase HPLC using almost the same eluent system (detection: 254 nm). The products were characterized using MALDI-TOF mass spectrometry.

ODN sample preparation for experiments

For UV spectroscopy, fluorescence spectroscopy, and melting temperature (Tm) and circular dichroism (CD) measurements, 1.0 (UV and fluorescence spectroscopy), 2.0 (Tm), and 3.0 (CD) µM of the ODN was added to a solution of 1 M Tris-HCl buffer (pH 7.2, 100 µL), 1 M NaCl (100 µL), 200 mM MgCl₂ (50 µL) and water (in a 1.5-mL microtube) to give a total volume of 1 mL, followed by vortex-mixing. In the case of duplexes, the probe ODN and the target RNA and/or the quencher ODN were added to 1 M Tris-HCl buffer (pH 7.2, 100 µL), 1 M NaCl (100 µL), 200 mM MgCl₂ (50 µL) and water and then the sample subjected to vortex-mixing. To prepare annealed samples, the mixtures in a buffer solution were heated at 90 °C for 3 min, then slowly cooled under ambient conditions for 4 h.

UV and fluorescence spectra

UV and fluorescence spectra were recorded using Cary 100 and Eclipse spectrometers (Varian), respectively. Samples for UV and fluorescence spectroscopy were prepared in a quartz cell (path length: 1 cm). All samples were measured after baseline correction for UV spectra. Parameters for fluorescence spectra; excitation wavelength: 380 nm, scanning range: 390–700 nm, excitation and emission slits: 5 nm/5 nm; data interval: 1.0 nm.
Melting temperatures ($T_m$)

All values of $T_m$ were recorded at 260 nm in a quartz cell (path length: 1 cm) using a Cary 100 Conc UV–Vis spectrophotometer (Varian) equipped with a temperature controller. The values of $T_m$ were calculated from the maximum values of the first derivatives in plots of absorbance with respect to temperature.

CD spectroscopy

CD spectra of the ODNs were recorded using a J-810 apparatus (JASCO) equipped with a temperature controller. For each sample, five spectral scans were accumulated at 20 °C over the wavelength range from 220 to 340 nm.

20% Native polyacrylamide gel electrophoresis (PAGE)

5 mL of 40% acrylamide, 2 mL of 5X TBE buffer, and 3 mL of distilled water were mixed for 20% non-denaturing gel. 12 mg of ammonium persulfate was added to the mixed solution. For initiation of gel formation, 10 μL of $N, N', N', N$-tetramethylethylenediamine (TEMED) was added. 200 pmol of samples were used for PAGE. Dried samples were dissolved in 10 μL of buffer/formamide mixture (v/v = 1 : 1) for sample loading. PAGE was carried out at 90 V, 26 mA, 3 W, 25 °C for 2.5 h. After running, gels were mixed with stains all (Sigma-Aldrich) in formamide for 30 min. Gels were dried and exposed on light for visualization of DNA bands.
Table S1. Probe sequences, containing $^{3}T$ units, for each target sequence

Target RNA sequences

**T19** (19-mer): 5’-a agu ccc guc cua ggc acc-3’

**T21** (21-mer): 5’-uga cca cuc aug gaa gca gga-3’

Natural DNA sequence

**PN** (19-mer): 5’-GGT GCC TAG GAC GGG ACT T-3’

| Name | Sequence | Calculated MS ($m/z$) | Observed MS ($m/z$) |
|------|----------|----------------------|---------------------|
| P1   | 5’-GGT GCC $^{3}T$UAG GAC GGG ACT T-3’ | 6092.0637 | 6092.4612 |
| P2   | 5’-GGT GCC TAG GAC GGG AC$^{3}T$U T-3’ | 6092.0637 | 6092.3920 |
| P3   | 5’-GGT GCC $^{3}T$UAG GAC GGG AC$^{3}T$U T-3’ | 6302.1106 | 6301.6128 |
| P4   | 5’-TCC $^{3}T$UGC TTC CAT GAG TGG TCA-3’ | 6595.1380 | 6595.6727 |
| P5   | 5’-TCC TAG$^{3}T$UUC AT CAT GAG TGG TCA-3’ | 6595.1380 | 6594.1879 |
| P6   | 5’-TCC $^{3}T$UGC $^{3}T$UUC AT CAT GAG TGG TCA-3’ | 6805.1849 | 6805.3771 |

Figure S1. UV absorption spectra of P1–P6 in the absence and presence of the target RNA
Figure S2. Fluorescence emission spectra of P4–P6 in the absence and presence of the target T21

Table S2. Fluorescence enhancements of the probe sequences for the target RNA

|        | Wavelength (nm) | Fluorescence enhancement |
|--------|-----------------|--------------------------|
| P1     | 434             | 17.6                     |
| P2     | 435             | 1.8                      |
| P3     | 433             | 16.0                     |
| P4     | 431             | 13.6                     |
| P5     | 438             | 12.5                     |
| P6     | 439             | 16.2                     |
**Figure S3.** Melting curves of (A) P1–P3 in the presence of T19 and (B) P4–P6 in the presence of T21

(A) ![Melting curve graph](image)

(B) ![Melting curve graph](image)

**Table S3.** Melting temperature of each probe with its target RNA

| Duplex    | T<sub>m</sub> (°C) |
|-----------|-------------------|
| PN + T19  | 74.7              |
| P1 + T19  | 71.4              |
| P2 + T19  | 74.4              |
| P3 + T19  | 70.1              |
| P4 + T21  | 72.8              |
| P5 + T21  | 70.6              |
| P6 + T21  | 67.7              |
Table S4. Quencher sequences, containing a PyU or DabU unit, partially complementary to P1

Natural DNA sequences

N5: 3’-CA CGG ATC CTG-5’
N6: 3’-CCA CGG ATC CTG C-5’
N7: 3’-CCA CGG ATC CTG CCC-5’

| Name | Sequence | Calculated MS (m/z) | Observed MS (m/z) |
|------|----------|---------------------|------------------|
| U5   | 3’-CA CGG PyU TC CTG-5’ | 3516.6354 | 3516.9267 |
| U6   | 3’-CCA CGG PyU TC CTG C-5’ | 4094.7292 | 4093.9226 |
| U7   | 3’-CCA CGG PyU TC CTG CCC-5’ | 4672.8230 | 4672.1072 |
| Q5   | 3’-CA CGG DabU TC CTG-5’ | 3539.6838 | 3539.4791 |
| Q6   | 3’-CCA CGG DabU TC CTG C-5’ | 4117.7776 | 4118.6337 |
| Q7   | 3’-CCA CGG DabU TC CTG CCC-5’ | 4695.8714 | 4695.1693 |

Figure S4. Structure of the internal quencher DabU
Figure S5. (A) UV absorption spectra and (B) normalized UV absorption spectra of P1 in the presence of U5–U7

Figure S6. Temperature-dependent absorption spectra of (A) single-stranded P1 and (B, C) P1 in the presence of a quencher strand U5–U6
Figure S7. Fluorescence emission spectra of (A) single-stranded P1 and U5–U7 and (B–D) P1 in the presence of a quencher strand U5–U7 and T19.
Figure S8. UV absorption spectra of P1 in the presence of Q5–Q7

Figure S9. Fluorescence emission spectra of P1 in the presence of Q5–Q7 and T19
Figure S10. Melting curves of P1 in the presence of (A) U5–U7 and (B) Q5–Q7

Table S5. Melting temperature of P1 in the presence of U5–U7 and Q5–Q7

| Duplex | $T_m$ (°C) |
|--------|------------|
| P1 + U5 | 60.1       |
| P1 + U6 | 66.0       |
| P1 + U7 | 71.8       |
| P1 + Q5 | 51.5       |
| P1 + Q6 | 58.2       |
| P1 + Q7 | 65.3       |
| P1 + N5 | 46.8       |
| P1 + N6 | 56.0       |
| P1 + N7 | 63.0       |
| PN + N5 | 53.7       |
| PN + N6 | 59.2       |
| PN + N7 | 65.2       |
Figure S11. CD spectra of (A) P1 and T19 and (B–D) P1 in the presence of a quencher strand U5–U7 and T19.
Figure S12. Native polyacrylamide gel electrophoresis (PAGE) images of P1 with T19 in the presence of U5–U7 (A) stained with Stains-All and (B) under UV irradiation.
Figure S13. Time-dependent fluorescence intensity of P1 in the presence of U7 after the addition of T19.