Supporting information for the article:

A simple, label-free optical method for studies on G-quadruplex/duplex competition inside duplex DNAs using G-quadruplex-specific probe—TMPipEOPP

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1. Effects of NRAS, NRAS-c, and their mixture on the absorption spectrum of TMPipEOPP

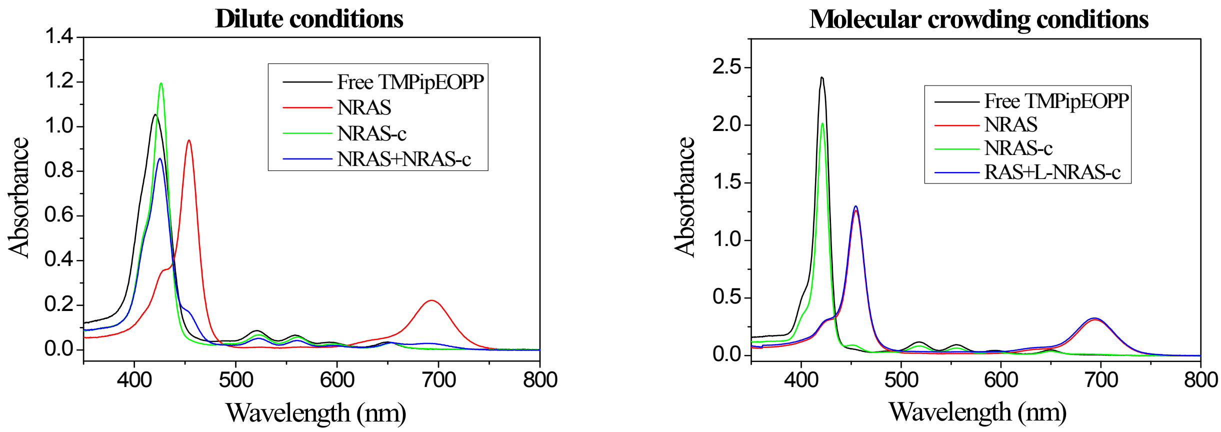


Fig. S1 Effects of NRAS, NRAS-c, and their mixture on the absorption spectrum of TMPipEOPP under dilute or molecular crowding conditions**.** Corresponding results for C-MYC are shown in Fig. 1.

2. Effects of PEG 200 concentration on the G-quadruplex/duplex competition

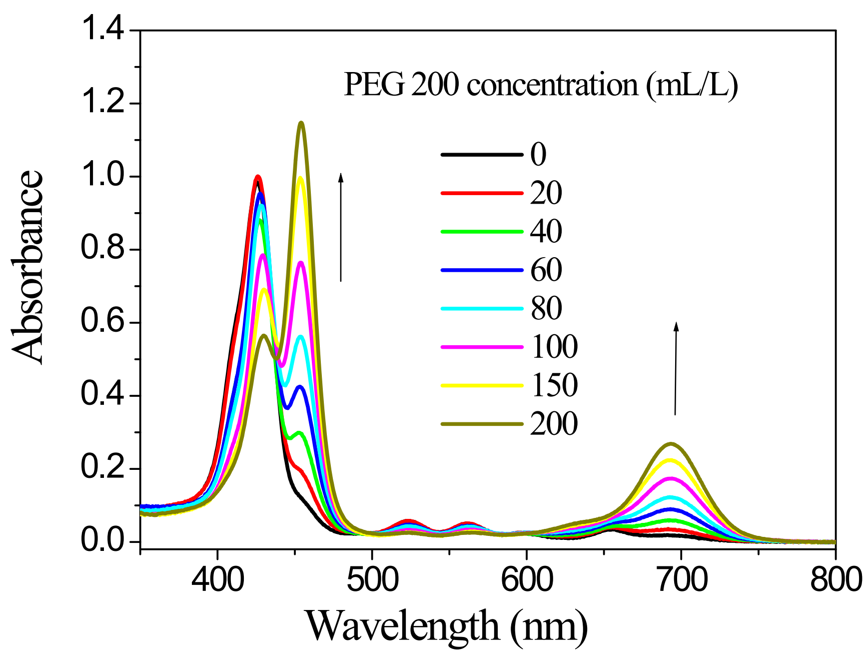


Fig. S2 Effects of PEG 200 concentration on the G-quadruplex/duplex competition in the C-MYC/C-MYC-c mixture.

3. **Effects of the mutants of NRAS, their complimentary sequences, and mixtures of the mutants and complementary sequences on the absorption spectrum of TMPipEOPP**

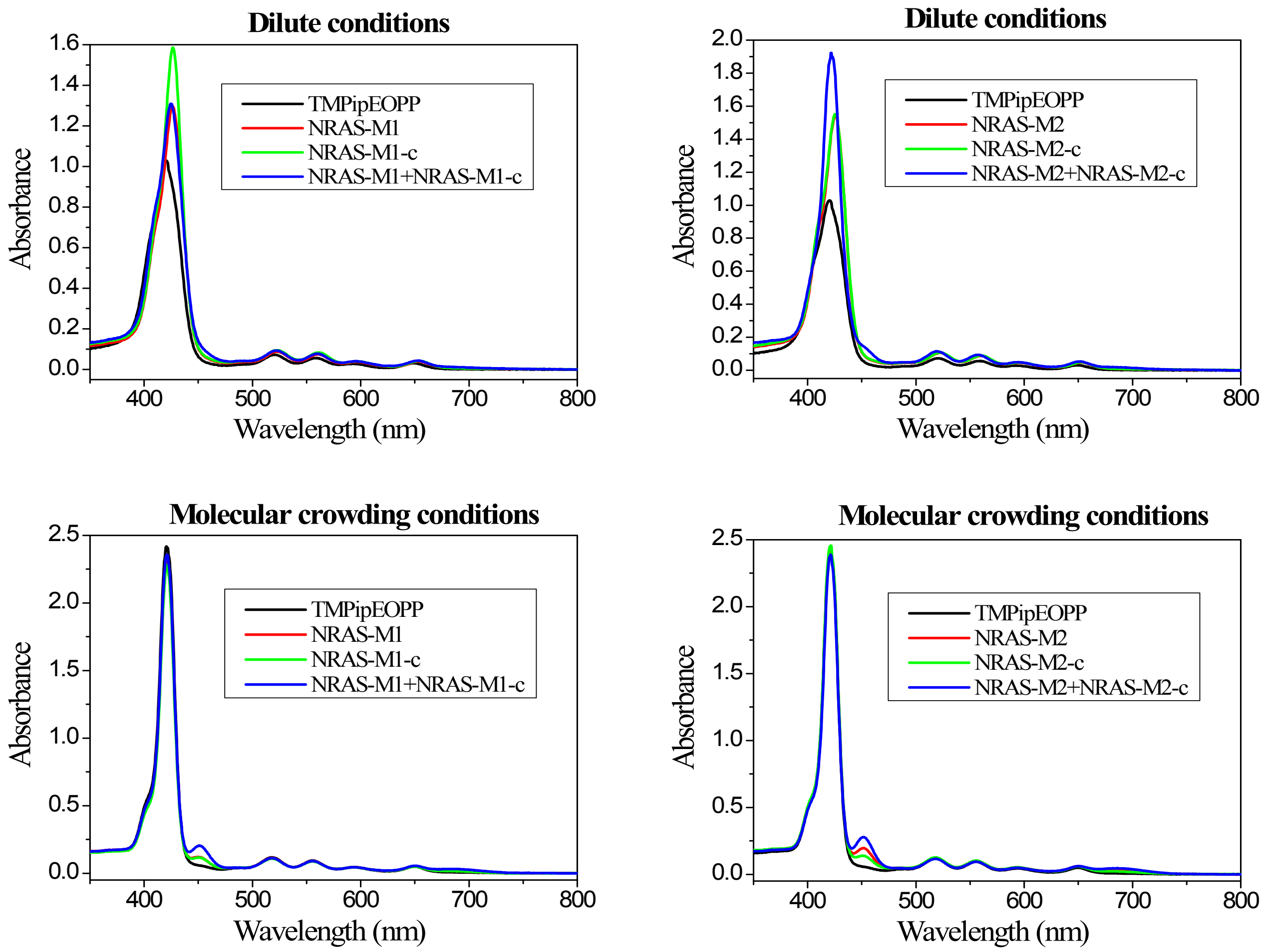


Fig. S3 Effects of the mutants of NRAS, their complimentary sequences, and mixtures of the mutants and complementary sequences on the absorption spectrum of TMPipEOPP under dilute or molecular crowding conditions. Corresponding results for the mutants of C-MYC are shown in Fig. 2.

4. Effects of NRAS, NRAS-c, and their mixture on the fluorescence spectrum of TMPipEOPP

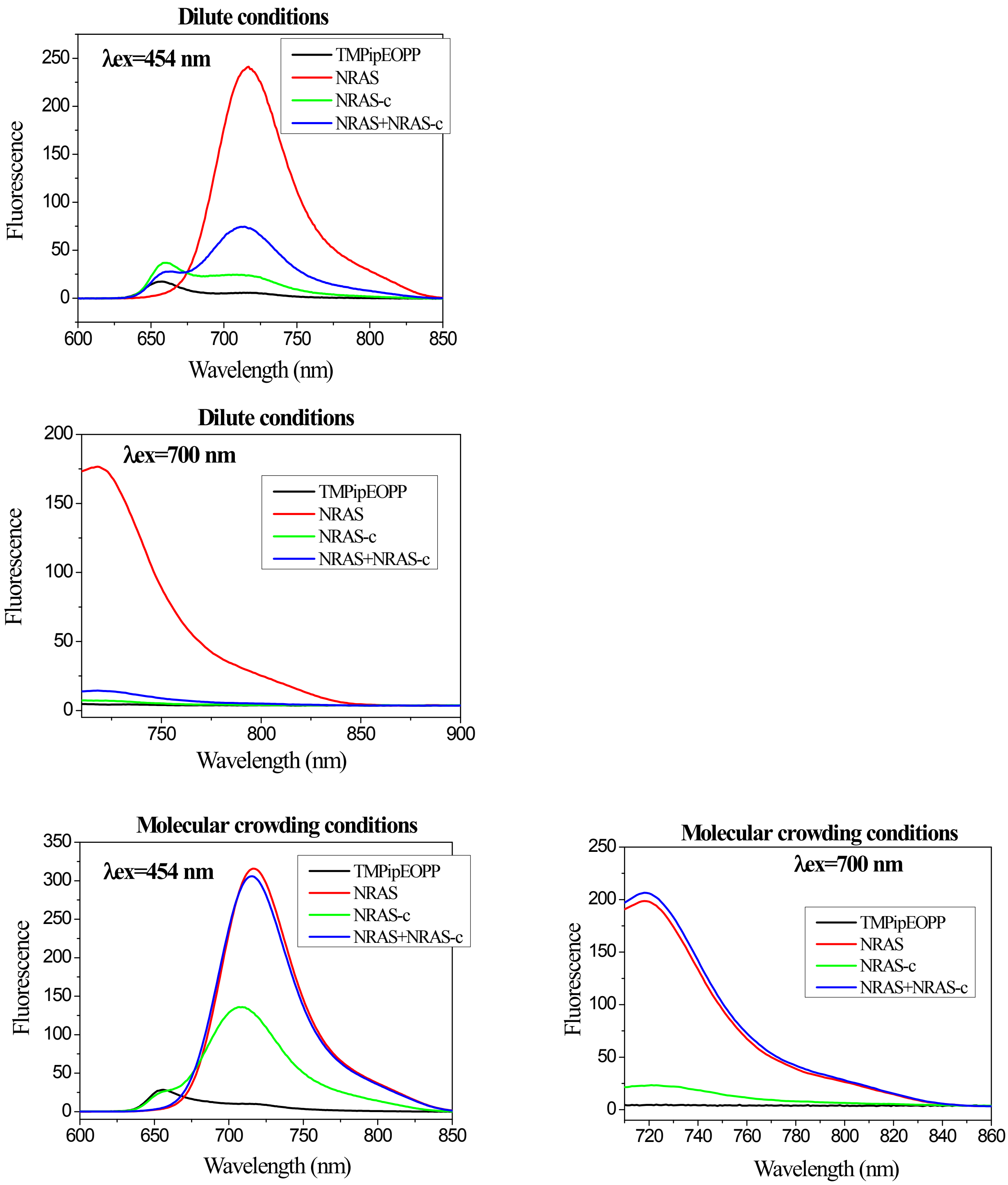


Fig. S4 Effects of NRAS, NRAS-c, and their mixture on the fluorescence spectrum of TMPipEOPP under dilute or molecular crowding conditions. Corresponding results for C-MYC are shown in Fig. 3.

5. Effects of the mutants of NRAS, their complimentary sequences, and mixtures of the mutants and complementary sequences on the fluorescence spectrum of TMPipEOPP

5.1 Under dilute conditions

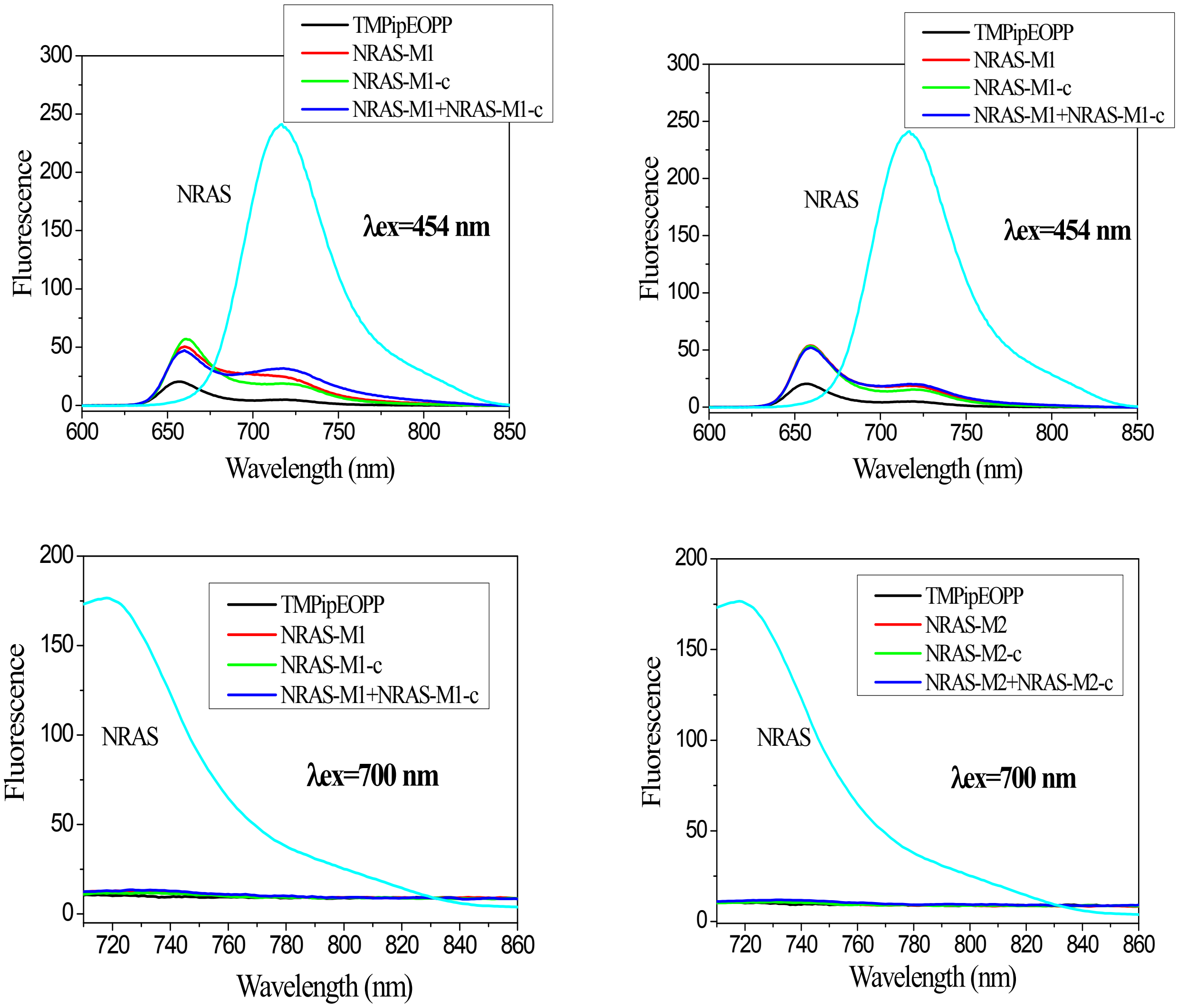


Fig. S5 Effects of the mutants of NRAS, their complimentary sequences, and mixtures of the mutants and complementary sequences on the fluorescence spectrum of TMPipEOPP under dilute conditions. The results for the mutants of C-MYC are shown in Fig. 4.

5.2 Under molecular crowding conditions

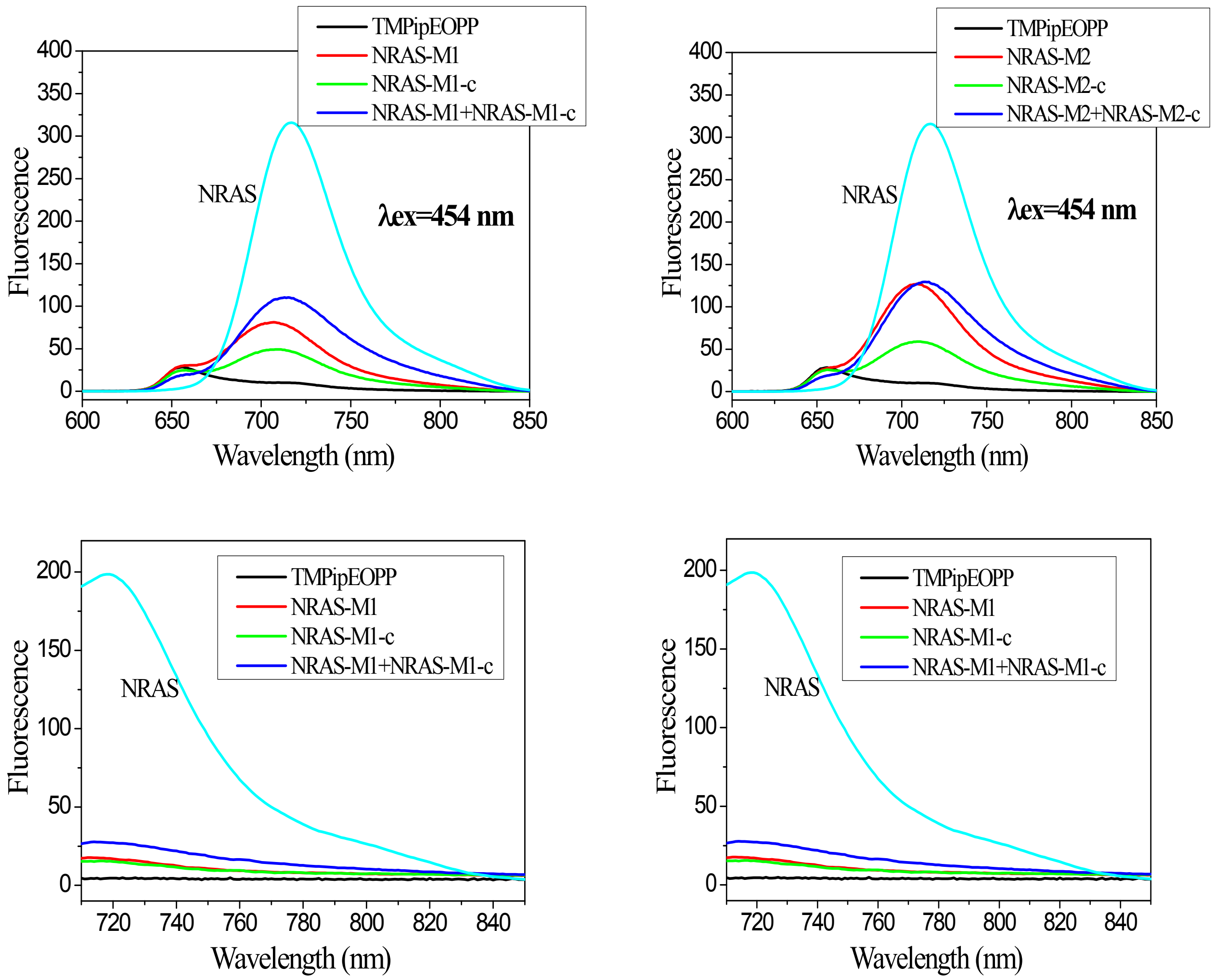


Fig. S6 Effects of the mutants of NRAS, their complimentary sequences, and mixtures of the mutants and complementary sequences on the fluorescence spectrum of TMPipEOPP under molecular crowding conditions. The results for the mutants of C-MYC are shown in Fig. 5.

6. NRAS-c concentration-dependent changes in absorption and fluorescence of TMPipEOPP/ NRAS mixture

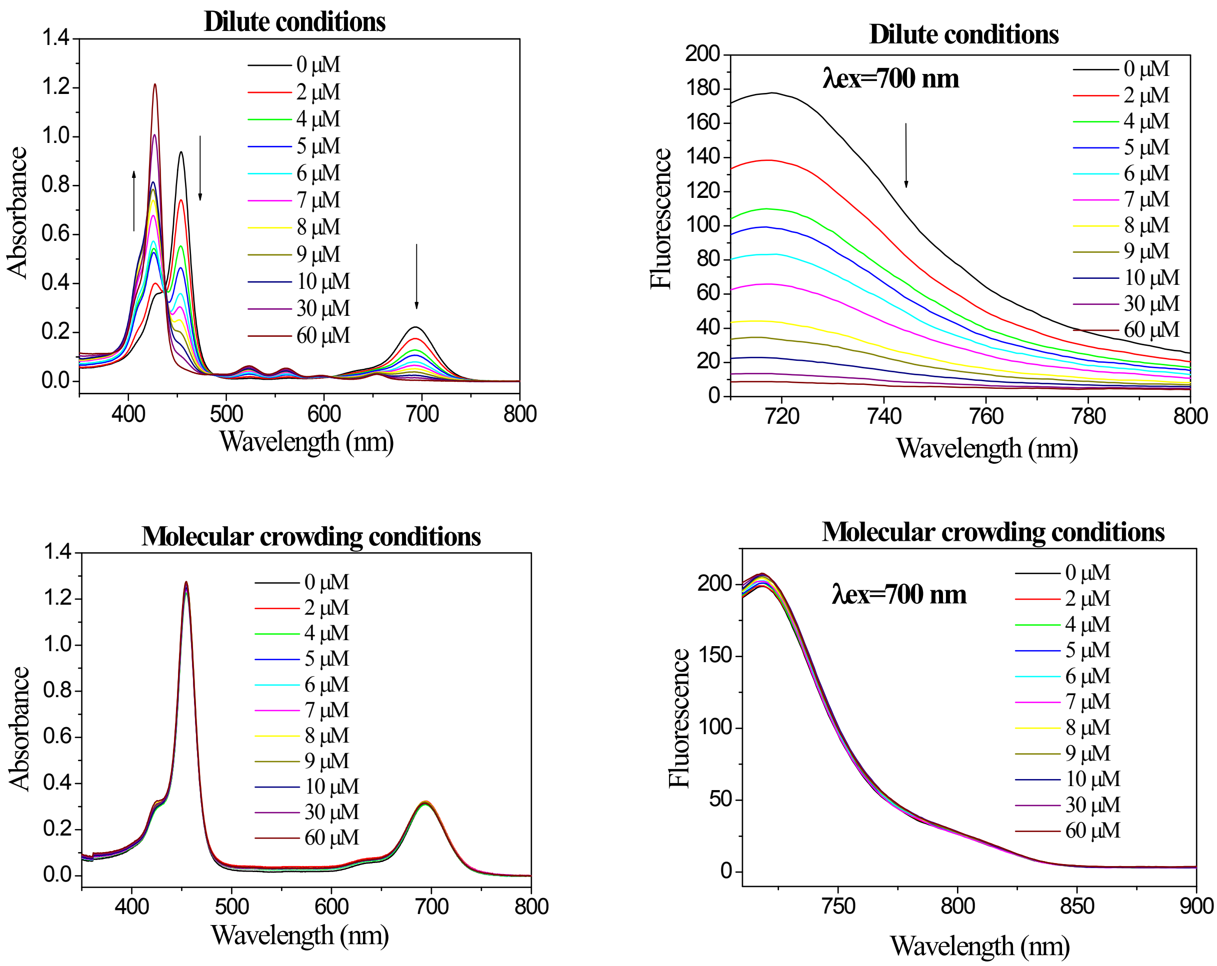


Fig. S7 NRAS-c concentration-dependent UV-vis absorption and fluorescence spectrum changes of TMPipEOPP/NRAS mixture under dilute or molecular crowding conditions. The concentrations of NRAS-c are shown in the figures. The results for C-MYC-c are shown in Fig. 6.

7. Effects of K^+ concentration on the G-quadruplex/duplex competition

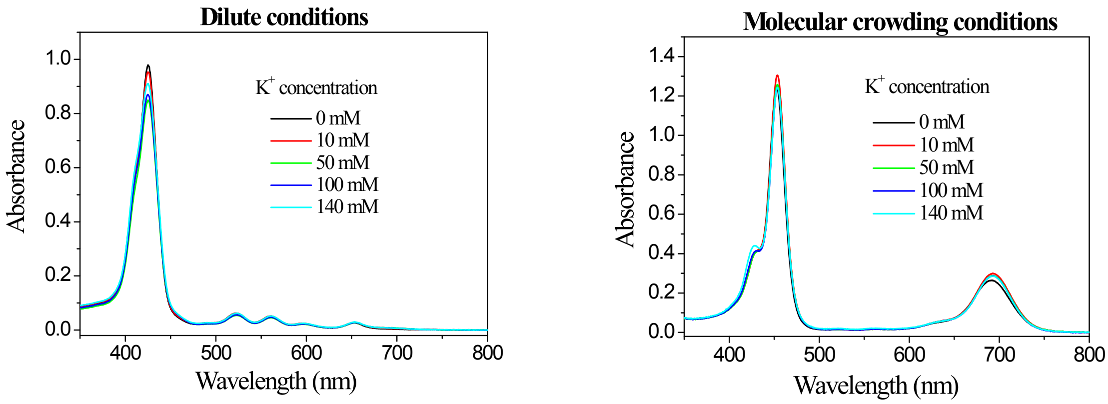
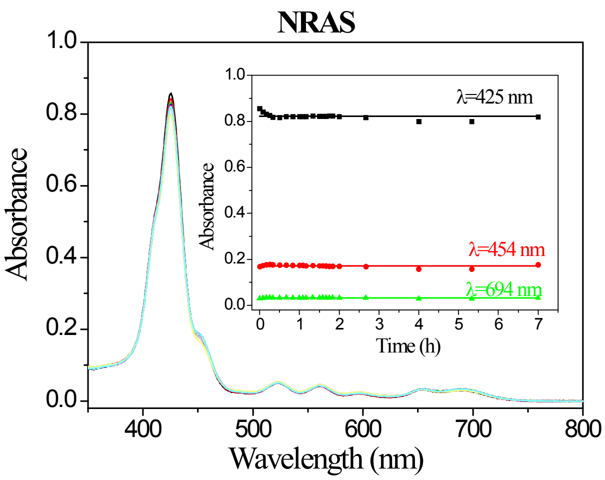


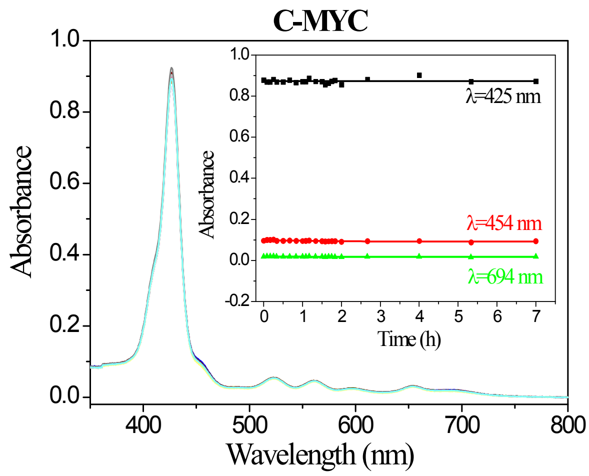
Fig. S8 Effects of K^+ concentration on the G-quadruplex/duplex competition in the C-MYC/C-MYC-c mixture under dilute and molecular crowding conditions.

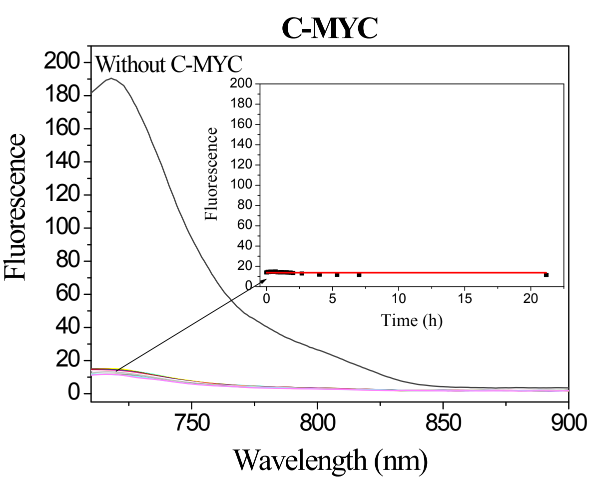
8. Monitoring G-quadruplex/duplex competition in real time

8.1 In the first condition

8.1.1 Under dilute conditions







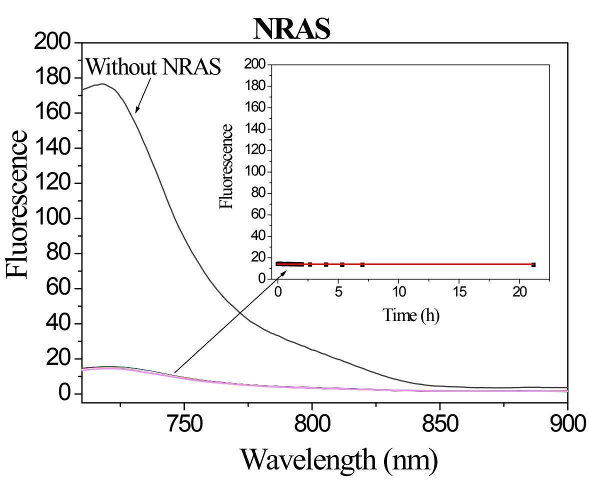


Fig. S9 Time-dependent G-quadruplex/duplex competition inside duplex DNAs under dilute conditions. In this experiment, G-rich sequences exist as single-stranded structures when mixing with their individual complimentary sequences. The inserts showed time-dependent absorption signal changes at 425, 454, 694 nm, and fluorescence signal changes at 720 nm.

8.1.2 Under molecular crowding conditions

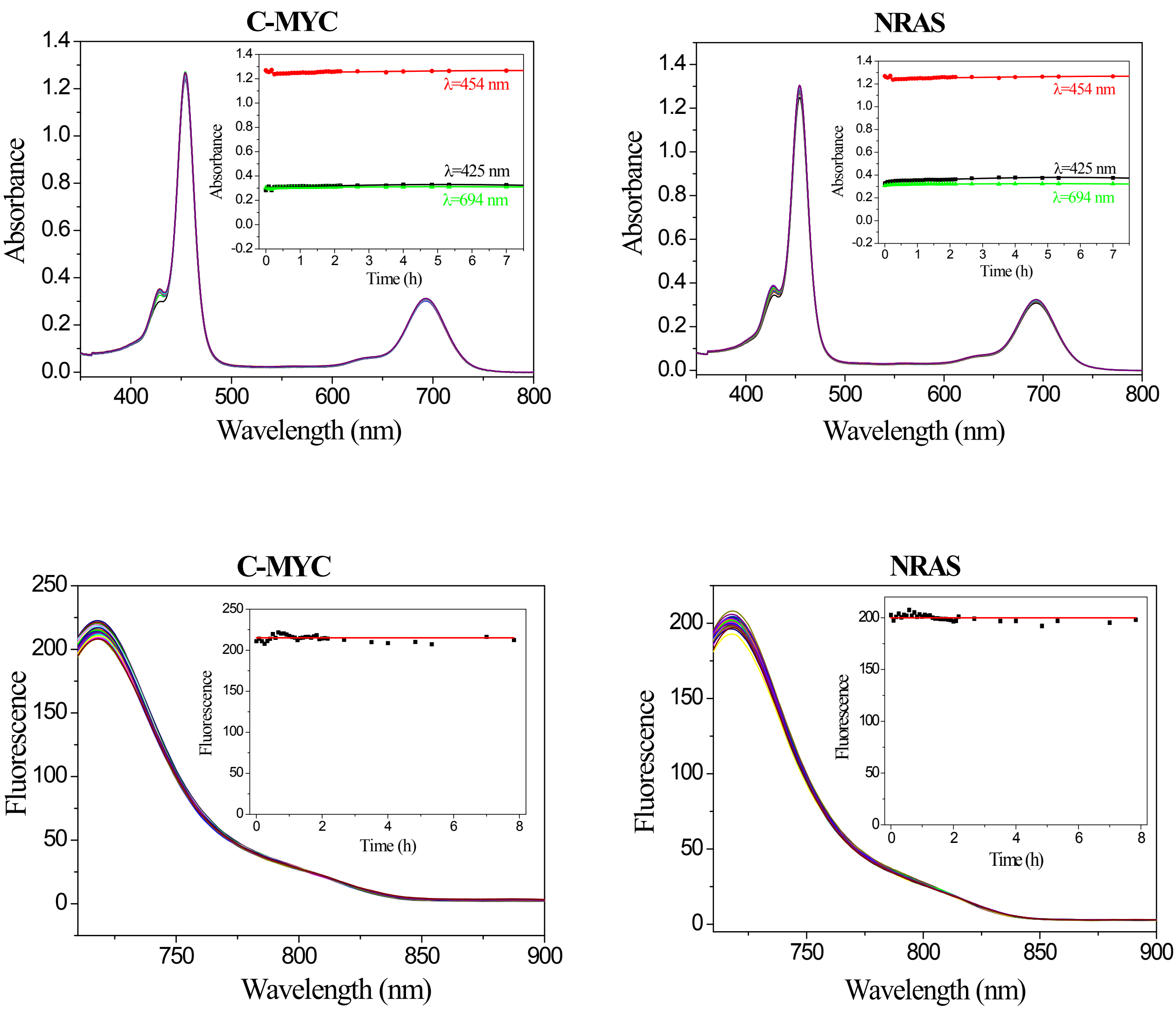


Fig. S10 Time-dependent G-quadruplex/duplex competition inside duplex DNAs under molecular crowding conditions. In this experiment, G-rich sequences exist as single-stranded structures when mixing with their individual complimentary sequences. The inserts showed time-dependent absorption signal changes at 425, 454, 694 nm, and fluorescence signal changes at 720 nm.

8.2 In the second condition

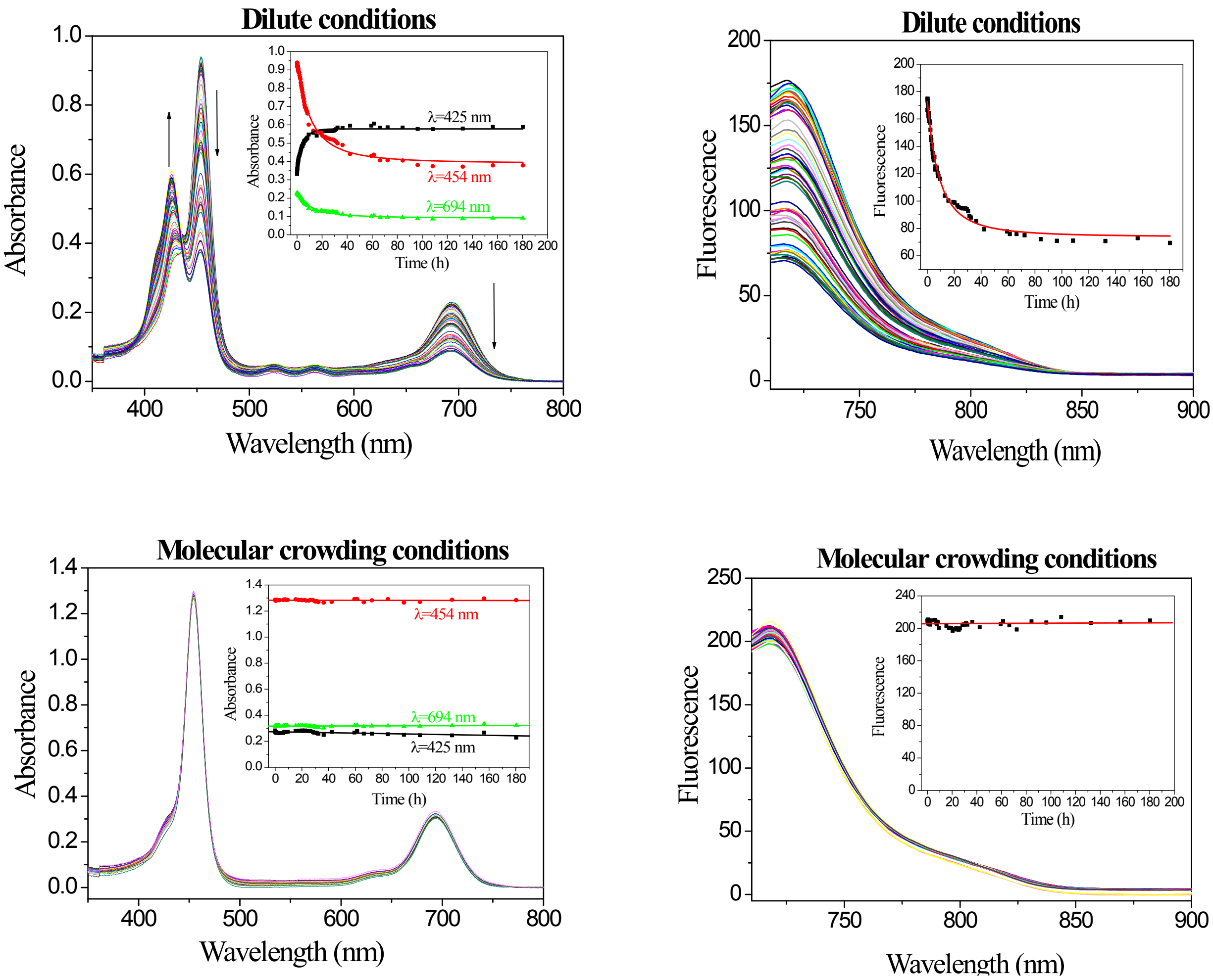
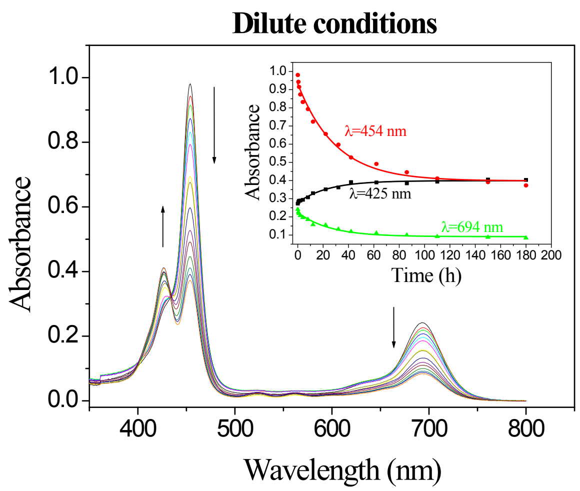
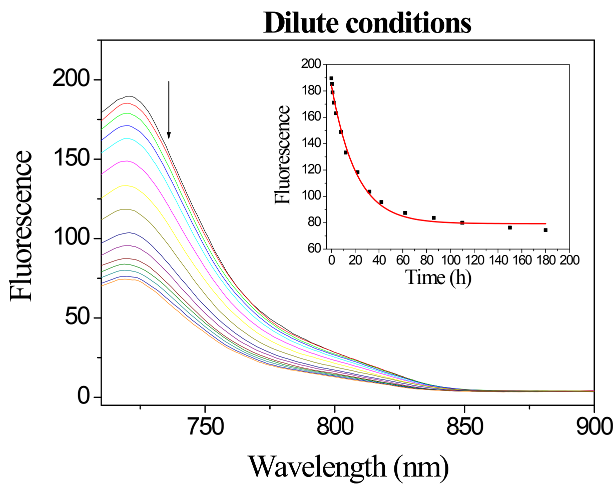


Fig. S11 Time-dependent G-quadruplex/duplex competition in flanking duplex DNAs under dilute or molecular crowding conditions. In this experiment, NRAS was allowed to form G-quadruplex structures before addition of NRAS-c. The inserts showed time-dependent absorption signal changes at 425, 454, 694 nm, and fluorescence signal changes at 720 nm. Corresponding results for C-MYC are shown in Fig. 7.





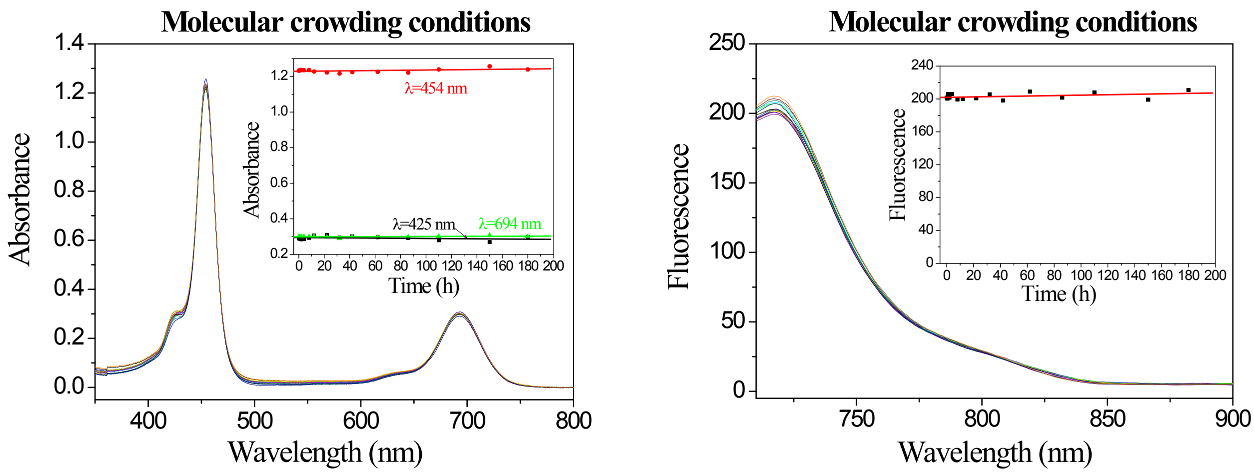


Fig. S12 Time-dependent G-quadruplex/duplex competition inside duplex DNAs under molecular crowding conditions. In this experiment, single-stranded G-rich sequence C-MYC was allowed to fold into G-quadruplex structure before addition of complimentary sequence C-MYC-c. TMPipEOPP was not included in the mixture of preformed G-quadruplex and complimentary sequence, but was added at different time points, and the absorption and fluorescence spectra of the mixture were recorded immediately after TMPipEOPP addition. The inserts showed time-dependent absorption signal changes at 425, 454, 694 nm, and fluorescence signal changes at 720 nm.

8.3 In the third condition

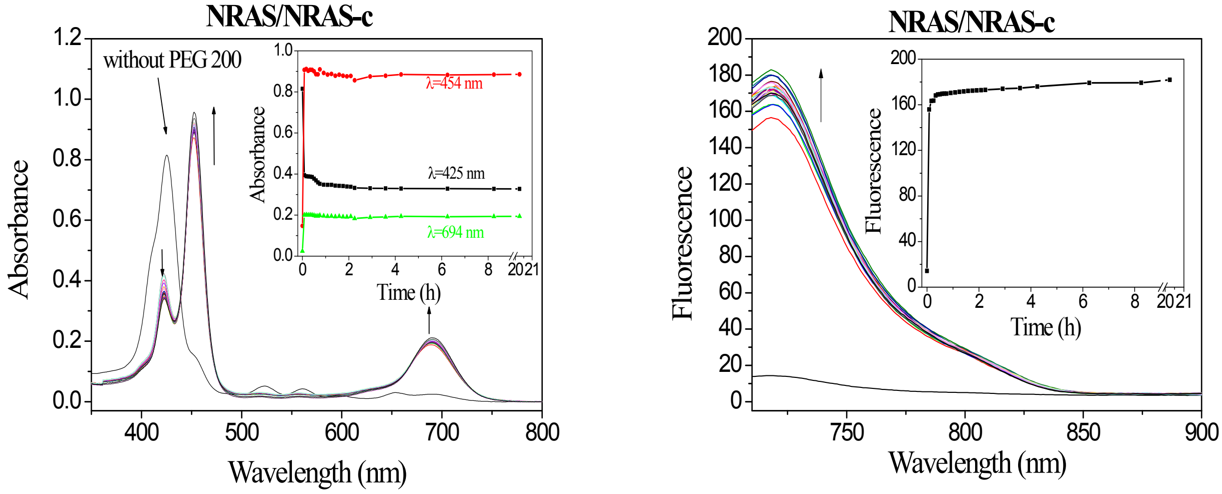


Fig. S13 Time-dependent G-quadruplex/duplex competition in NRAS/NRAS-c mixture after PEG 200 addition. In this experiment, duplex structures were formed under dilute conditions, then, PEG 200 was added to trigger G-quadruplex/duplex competition. The inserts showed time-dependent absorption signal changes at 425, 454, 694 nm, and fluorescence signal changes at 720 nm. The results for C-MYC/C-MYC-c mixture are shown in Fig. 8.

9. Circular dichroism spectroscopy was used for G-quadruplex/duplex competition

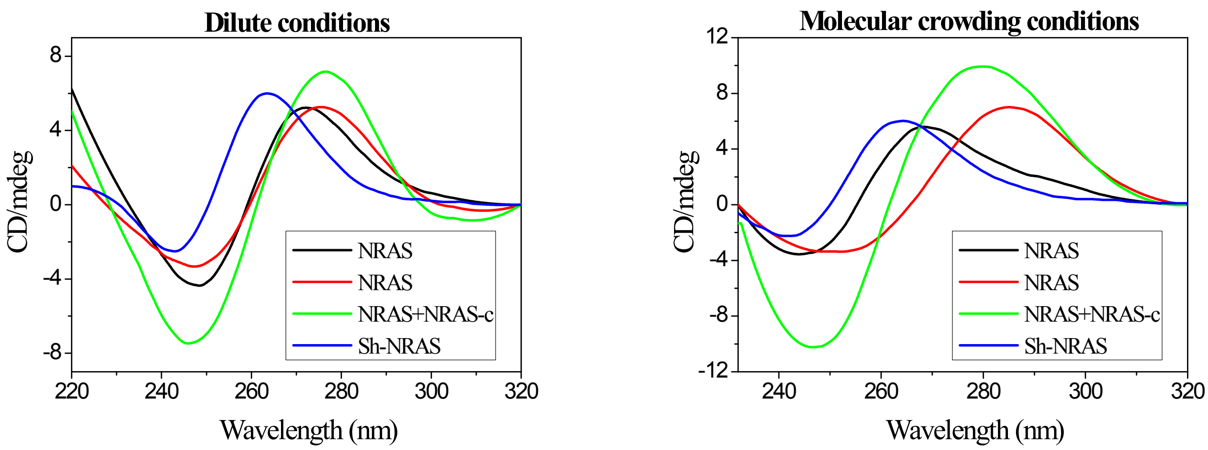


Fig. S14 CD spectra of NRAS, NRAS -c and their mixture under dilute or molecular crowding conditions. Sh- NRAS is used as a control. The results for C-MYC are shown in Fig. 9.