Supporting Information

Fluorescent Detection of Human Angiotensinogen Protein by G-quadruplex Aptamer

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Optimization of NMM conditions

The optimal conditions of NMM were determined by enzyme labeling instrument and fluorescence spectrometer. Red shift occurred with the increase of concentration. The excitation wavelength and emission wavelength of NMM / G-quadruplex are 399 nm and 610 nm respectively.[1] As shown in Figure S1 A, the absorption spectrum of NMM (0~100 μM) (OD is the optical density and represents the absorbance), NMM is 0~10 μM excitation spectrum (Figure S1 B). The concentration range with excitation wavelength of 399 nm and emission wavelength of 610 nm was selected. NMM at 0~4 μM fluorescence spectrum (Figure S1 C). The relationship between NMM concentration and fluorescence intensity (Figure S1 D). The NMM itself has weak fluorescence, 0.6 μM can be used as a reference standard for subsequent experimental conditions. The fluorescence spectrum of NMM / C20-4 min (Figure S1 E) increased significantly with the increase of G-quadruplex (C20-4 min) concentration. The fluorescence spectrum of NMM / NC (Figure S1 F) did not change significantly with the increase of nucleic acid concentration of non-G-quadruplex structure (NC). The nucleic acid concentrations are 0, 0.001 μM, 0.005 μM, 0.01 μM, 0.05 μM, 0.1 μM, 0.2 μM, 0.3 μM, 0.4 μM, 0.5 μM.

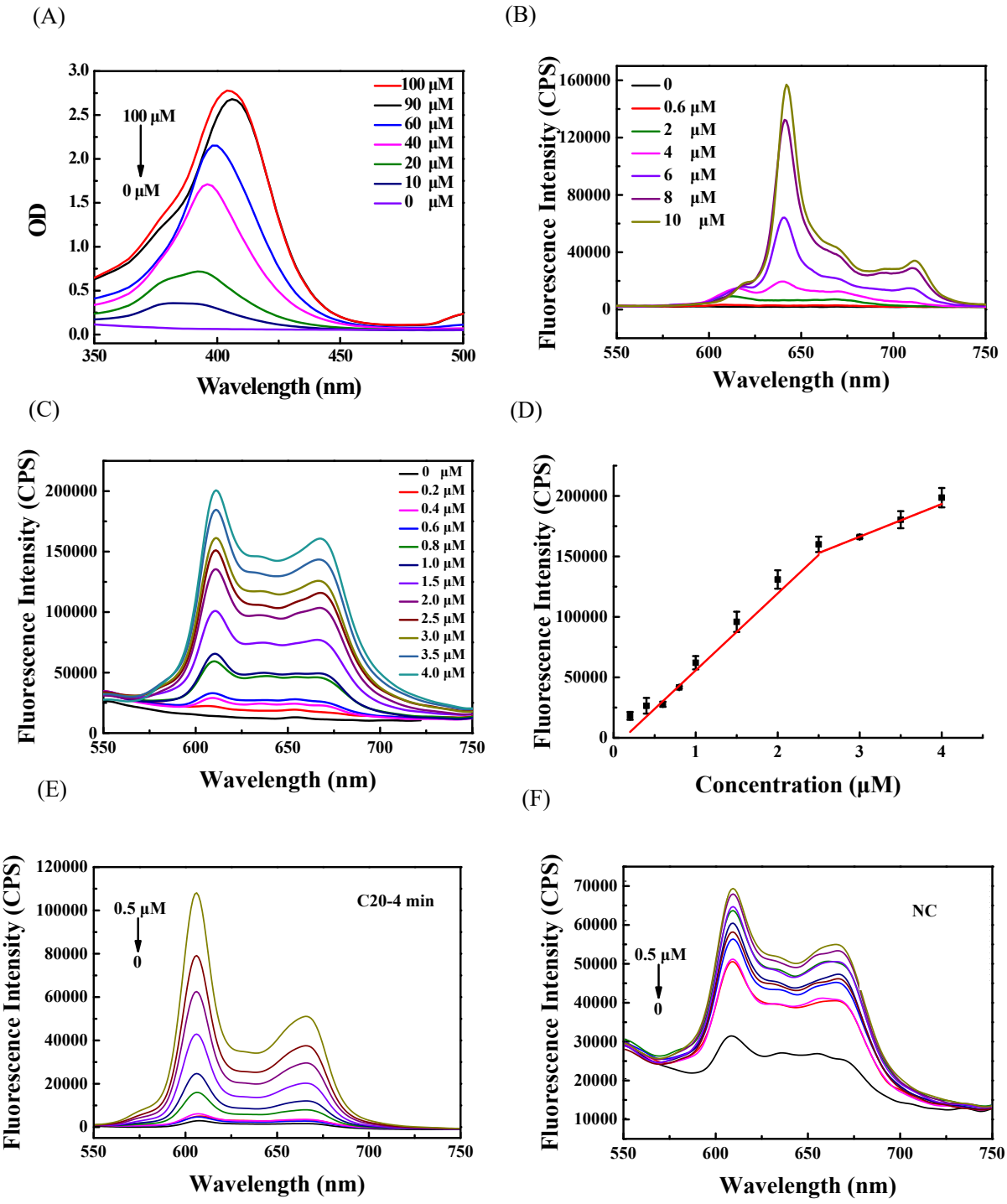

Figure S1. NMM conditions for NMM/G-quadruplex. (A) The absorption spectrum of NMM (0~100 μM), analysis of NMM excitation wavelength and concentration. (B) The excitation spectrum of NMM (0~10 μM), optimization of emission wavelength and concentration of NMM. (C) Fluorescence spectrum of NMM (0~4 μM). (D) The relationship between NMM concentration and fluorescence intensity (0~4 μM). (E) Fluorescence spectra of NMM / C20-4 min (0-0.5 μM). (F) Fluorescence spectra of NMM / NC (0-0.5 μM). To analyze the effect of nucleic acid concentration on NMM.

Effect of metal ions on G-quadruplex

The effect of different metal ion solutions on the fluorescence intensity of "NMM / C20-4 min" system was studied. The fluorescence intensity of the system increased significantly with the increase of K^+ concentration (Figure S2 A). Increased Li^+ concentration doesn't impact significantly the fluorescence intensity of the system (Figure S2 B). Metal ions affect the formation of G-quadruplex structure, K^+ promotes the formation of parallel G-quadruplex, and Li^+ is not conducive to the formation of G-quadruplex. The gradient concentration of metal ions is 100 μM, 50 μM, 10 μM, 5 μM, 1 μM, 0.5 μM, 0.1 μM, 5×10^{-2} μM, 10^{-2} μM, 5×10^{-3} μM, 10^{-3} μM, 5×10^{-4} μM, 10^{-4} μM, 5×10^{-5} μM.

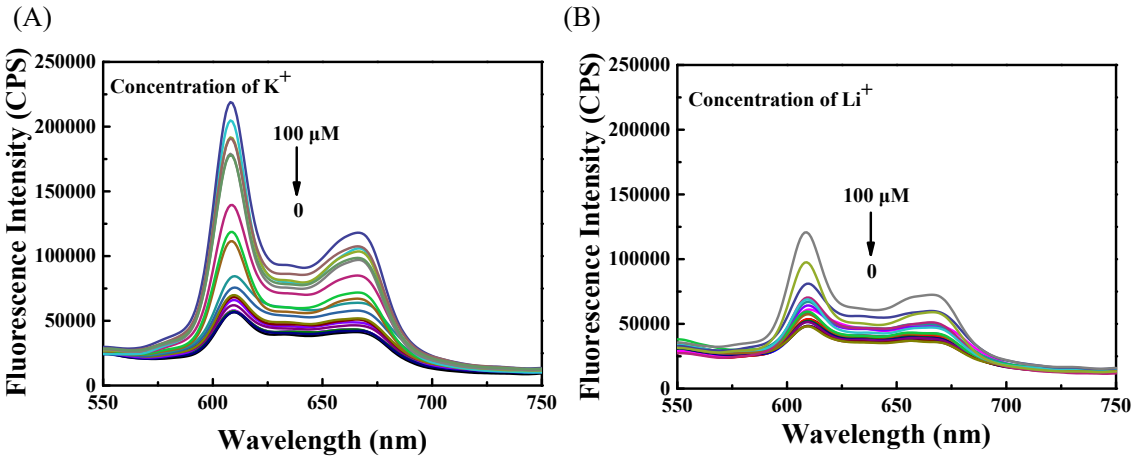

Figure S2. Analysis of the stability of G-quadruplex to different metal ions. (A) Fluorescence spectra of NMM / C20-4 min in K^+ (1~100 μM). (B) Fluorescence spectra of NMM / C20-4 min in Li^+ (1~100 μM).

References

1 A. Kreig, J. Calvert, J. Sanoica, E. Cullum, R. Tipanna and S. Myong, *Nucleic Acids Res.,* 2015, **43**, 7961-7970.