Supplementary information

for

Fluorescence lifetime probes for detection of RNA degradation

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Supplementary Results

Thermodynamics of electron transfer quenching of fluorescence by guanine

The fluorescence quenching of fluorescein can be explained by the electron transfer from the nucleobase, specifically guanine (G), to fluorescein in the singlet excited (S_1) state. The Gibbs energy of electron transfer (ΔG), a driving force, was calculated by the following equation (1):

$$\Delta G = e(E_{ox}{}^{G} - E_{ox}{}^{f}) - \frac{e^2}{8\pi\varepsilon_0}\left(\frac{1}{\varepsilon_w} - \frac{1}{\varepsilon_A}\right)\left(\frac{1}{r_G} + \frac{1}{r_f}\right) - \frac{e^2}{8\pi\varepsilon_0 r_d} \qquad (1)$$

where e is the elementary charge, $E_{ox}{}^{G}$ and $E_{ox}{}^{f}$ are the redox potentials of the one-electron oxidation of G (1.24 V vs. saturated calomel electrode (SCE) in acetonitrile)[1] and fluorescein (1.27 V vs. Ag/Ag^+ electrode in acetonitrile)[2], respectively, ε_0 is the permittivity of vacuum, ε_W and ε_A are dielectric constants of water and acetonitrile, respectively, r_G is the radius of G (about 5 Å), r_f is the radius of fluorescein (about 6 Å), and r_d is the distance between G and fluorescein (about 6 Å in the case of 5′-fluorescein-G -3′). These radii and distance were estimated from the molecular mechanics calculations (Fig. S1). The potential difference between SCE and Ag/Ag^+ is 0.042 V. The obtained value of ΔG was negative (−0.05 eV), supporting the notion about thermodynamical possibility of electron transfer quenching of fluorescein in the S_1 state by G. In contrast, the calculations using this equation showed positive values for other nucleobases (A: +0.40 eV, U: +0.61 eV, and C: +0.61 eV), suggesting that in those cases, electron transfer quenching by other nucleobases is unlikely.

Estimation of non-fluorescent species through static quenching

In general, the fluorescence quantum yield is proportional to the fluorescence lifetime of molecules. The average fluorescence lifetime of fluorescein after enzyme treatment (τ_f^{after}) can be expressed using the following equation:

$$\Phi_f^{after} = a \times \tau_f^{after} \qquad (2)$$

where Φ_f^{after} is the observed fluorescence quantum yield of the sample after degradation and a is the constant. If there are fluorescein-labeled but non-fluorescent, statically quenched species, this equation must be corrected. Under the assumption that non-fluorescent species exist in the sample before degradation, its average fluorescence lifetime (τ_f^{before}) can be determined using the relative amplitude of non-fluorescent species (r_{nf}) as follows:

$$\Phi_f^{before} = a \times \{(1 - r_{nf}) \times \tau_f^{before} + r_{nf} \times 0\} \quad (3)$$

where Φ_f^{before} is the observed fluorescence quantum yield of the sample before enzyme treatment. The following equation could be obtained from the equations (2) and (3):

$$r_{nf} = 1 - \frac{\Phi_f^{before}}{\Phi_f^{after}} \times \frac{\tau_f^{after}}{\tau_f^{before}}$$

The ratio of observed fluorescence quantum yields ($\Phi_f^{before}/\Phi_f^{after}$) is equal to the ratio of fluorescence intensities (F^{before}/F^{after}) when F^{before} and F^{after} were measured under the same conditions.

$$r_{nf} = 1 - \frac{F^{before}}{F^{after}} \times \frac{\tau_f^{after}}{\tau_f^{before}} \quad (4)$$

Under the assumption that non-fluorescent species exist before (but not after) degradation, this equation holds. F^{before}/F^{after} is always nearly equal to or less than $\tau_f^{before}/\tau_f^{after}$ in the present data (Figures 2-5), indicating that non-fluorescent species sometimes exist before degradation. In contrast, it is likely that non-fluorescent species do not exist after degradation, especially in the highly fluorescent molecules such as degraded fluUGU and fluUUG. From the equation (4), r_{nf} values of non-degraded fluUGU and fluUUG were estimated to be 0.78 and 0.59, respectively.

References

1. N. B. Salah and F. M. Mhalla, Mechanistic investigation of xanthene oxidation by heterogeneous and homogeneous electron transfers, J. Electroana. Chem., 2000, **485**, 42–48.
2. F. D. Lewis and Y. Wu, Dynamics of superexchange photoinduced electron transfer in duplex DNA. *J. Photochem. Photobiol. C: Photochem. Rev.*, 2001, **2**, 1–16.

Supplementary Figures

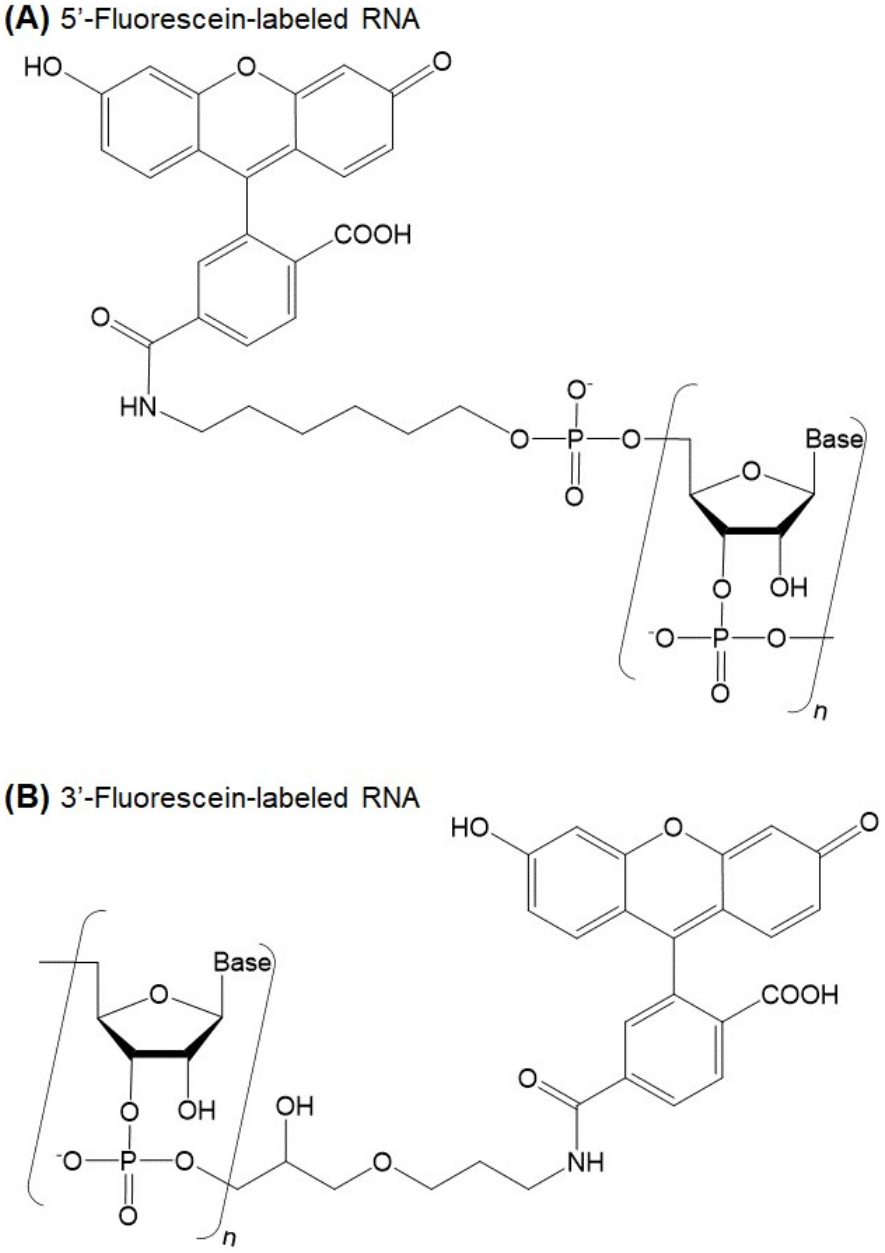

Figure S1. Chemical structures of fluorescein-labeled RNAs used in this study. (A) 5′-fluorescein-labeled RNA. **(B)** 3′-fluorescein-labeled RNA.

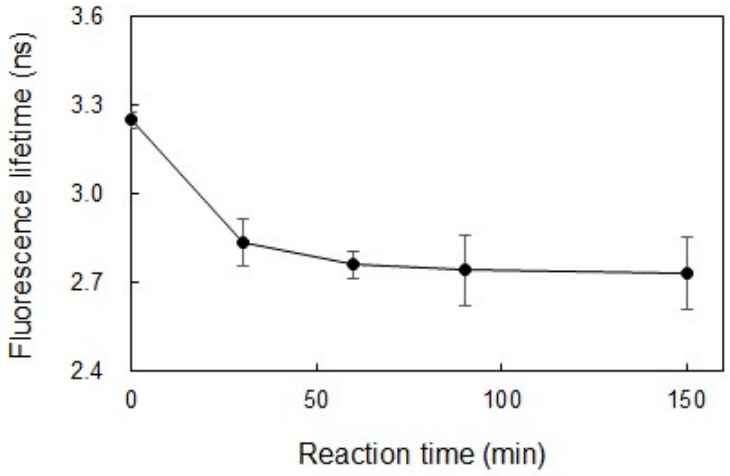

Figure S2. Monitoring of degradation of UCGflu by fluorescence lifetime. Fluorescence lifetime of the RNAs with RNase I_f treatment were measured over time. The RNA degradation was performed at 25 °C in a reaction solution containing 50 mM Tris-HCl (pH 7.9), 100 mM NaCl, 10 mM MgCl2, 1 mM DTT, 5 units/μL RNase I_f (New England Biolabs), and 8 μM of the RNA. Data are presented as the mean ± SD (n = 3).

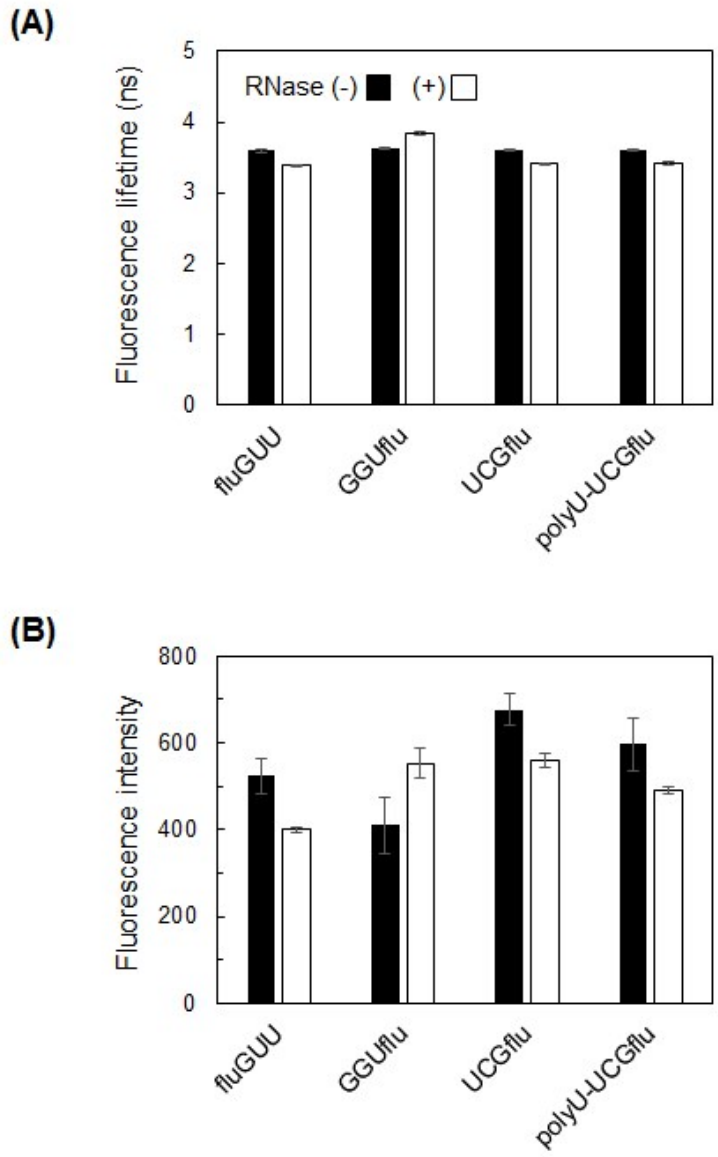

Figure S3. Fluorescence lifetime and fluorescence intensity of fluorescein-labeled RNAs measured in a molecular crowding condition. (A) Fluorescence lifetime of fluorescein-labeled RNAs. **(B)** Fluorescence intensity of fluorescein-labeled RNAs. The RNA degradation was performed as described in Materials and Methods in the main text. Before the fluorescence (lifetime and intensity) measurements, the degraded RNAs were dissolved in a buffer containing 10 mM HEPES-KOH (pH 7.6), 1 mM $MgCl_2$, 100 mM NaCl, and 20 % PEG200, to prepare 32 nM fluorescein-labeled RNA solution. Fluorescence lifetime values of the fluorescein-labeled RNA with and without RNase I_f treatment are shown by white and black bars, respectively. Data are presented as the mean ± SD (n = 3).

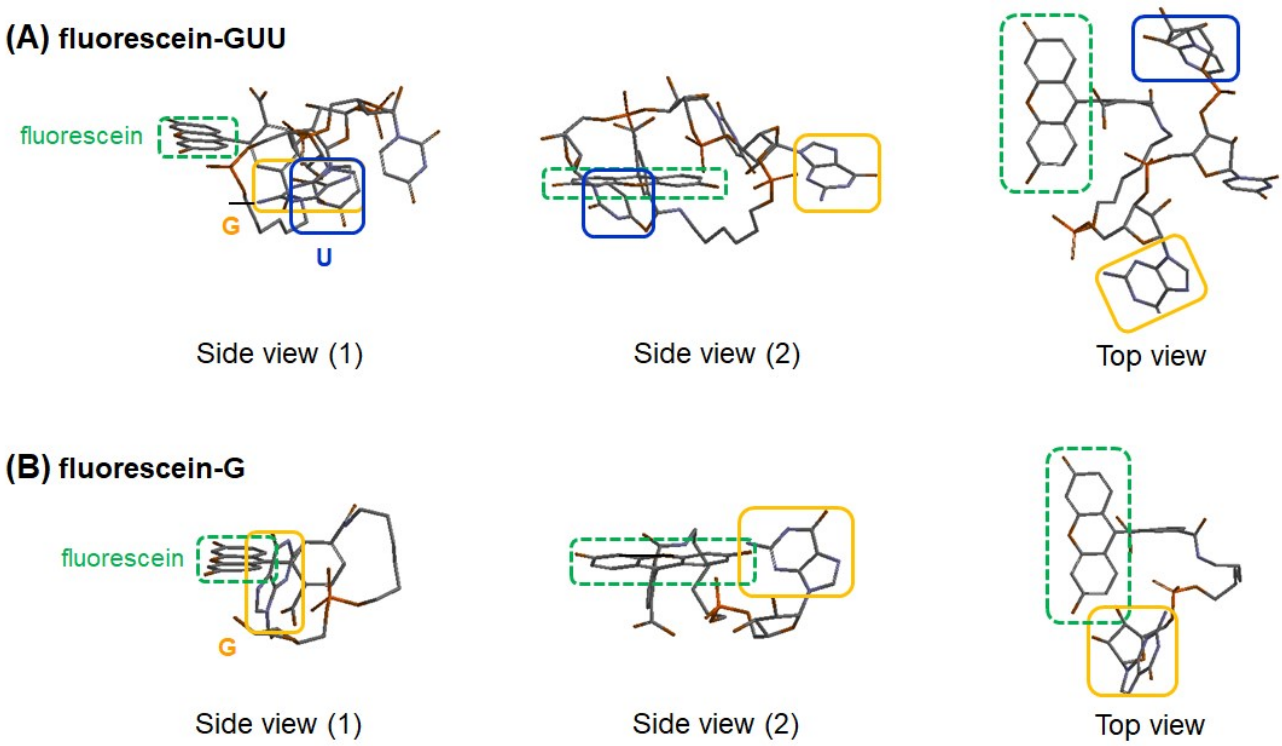

Figure S4. Optimized structures of 5′- fluorescein-GUU -3′ **(A)** and 5′- fluorescein-G -3′ **(B)** obtained by molecular mechanics calculation by using Spartan′18 (Wavefunction Inc., Irvine, CA, USA).

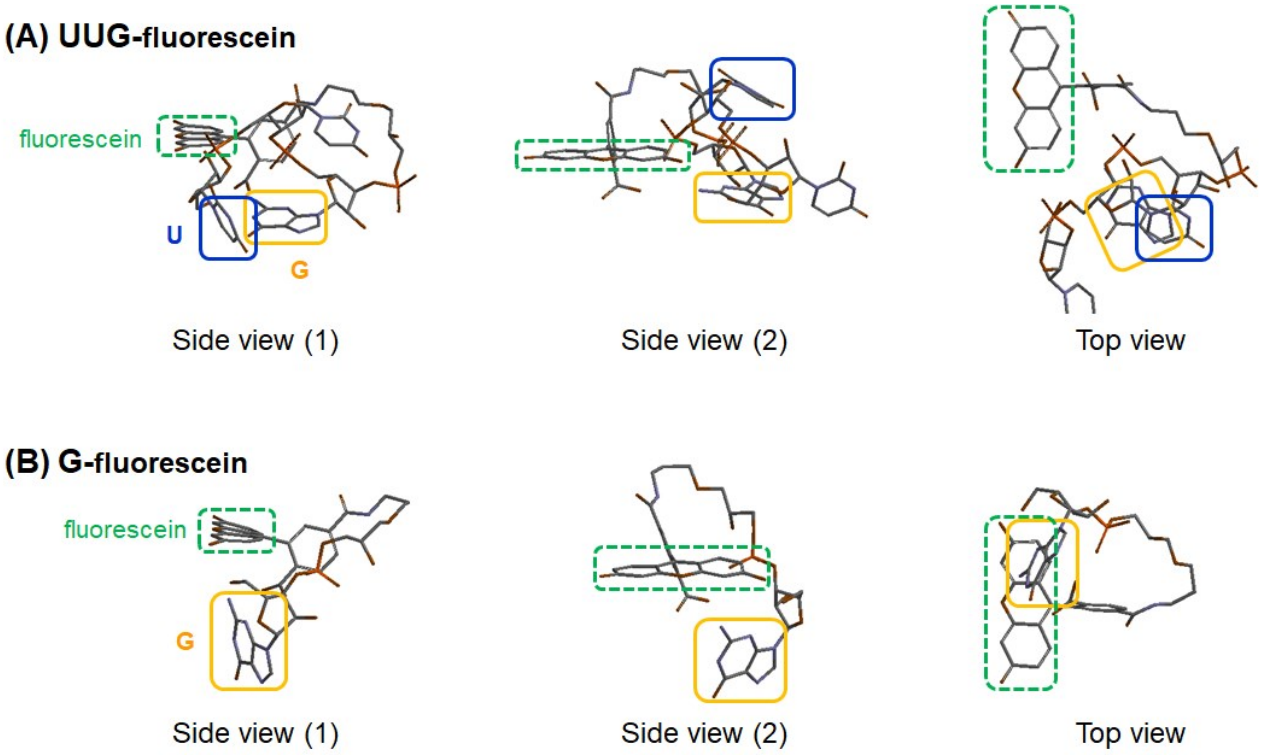

Figure S5. Optimized structures of 5'- UUG-fluorescein -3' **(A)** and 5'- G-fluorescein -3' **(B)** obtained by molecular mechanics calculation by using Spartan′18.