Electronic Supplementary Information

for

Direct Visualization and Real-time Monitoring of Dissipative Self-assembly by Synchronously Coupled Aggregation-induced Emission

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Table of Contents

1. Materials and methods

Chemicals. All the reagents and solvents were commercially available and used as received unless otherwise specified purification. 2-[4-(2-Hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES) were purchased from Meryer. Calcium chloride ($CaCl_2$) and magnesium sulphate ($MgSO_4$) were obtained from Tianjin Guangfu Fine Chemical Research Institute. Deoxyribonucleic acid from herring sperm (DNA) and deoxyribonuclease I (DNase I) were purchased from Sigma-Aldrich. N,N,N',N'-Tetramethylacridine-3,6-diamine (Acridine orange, AO) were obtained from Merck. 4,4′,4″,4‴-((Ethene-1,1,2,2-tetrayltetrakis(benzene-4,1-diyl))tetrakis(oxy))tetrakis(N,N,N-trimethylbutan-1-aminium) (TQA-TPE) was prepared according to the previous studies.[1]

Samples. The HEPES buffer solution of pH 7.4 was prepared by dissolving 2.38 g HEPES, 55.5 mg $CaCl_2$ and 301 mg $MgSO_4$ in approximate 900 mL double-distilled water. Titrate to pH 7.4 at the lab temperature of 25 °C with NaOH and make up volume to 1000 mL with double-distilled water. The pH value of the buffer solution was then verified on a pH-meter calibrated with two standard buffer solutions. The samples for Scanning electron microscopy (SEM) measurement were prepared by dropping the solution onto a silicon wafer which was then dried in air. Samples were coated with gold for two minutes using a gold sputter coater JFC-1600 prior to SEM experiments to improve surface conductivity. The sample for transmission electron microscopy (TEM) measurement was prepared by dropping the solution onto a copper grid without staining. The grid was then air-dried.

Apparatus. The sample solutions for dynamic light scattering (DLS) measurements were examined on a laser light scattering spectrometer (NanoBrook 173plus) equipped with a digital correlator at 659 nm at a scattering angle of 90°. SEM images were recorded on a JSM-7500F scanning electron microscope. The TEM sample was examined by a high-resolution TEM (Tecnai G2 F20 microscope, FEI) equipped with a CCD camera (Orius 832, Gatan). Steady-state and kinetic fluorescence measurements were recorded in a conventional quartz cell (light path 10 mm) on a Cary Eclipse equipped with a Cary single-cell peltier accessory. The fluorescence lifetimes were measured by time-correlated single photon counting on a FLS920 instrument (Edinburg Instruments Ltd., Livingstone, UK) with a H2 pulse lamp. Fluorescence microscopy images were observed by a confocal laser scanning microscope (Leica TCS SP8) with excitation at 405 nm. UV-Vis spectra were recorded in a quartz cell (light path 10 mm) on a Cary 100 UV-Vis spectrophotometer equipped with a Cary dual cell peltier accessory.

2. Steady-state fluorescence and lifetime measurements of TQA-TPE with DNA

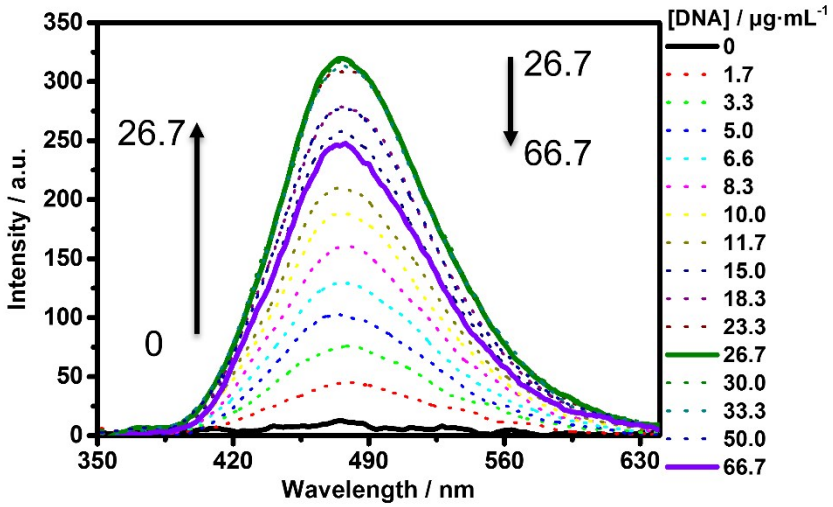

Figure S1. Fluorescence spectra (λ_{ex} = 330 nm) of TQA-TPE (10 μM) with different concentrations of DNA. Experimental conditions: [HEPES] = 10 mM, [$MgSO_4$] = 2.5 mM, [$CaCl_2$] = 0.5 mM, pH 7.4, T = 37 °C.

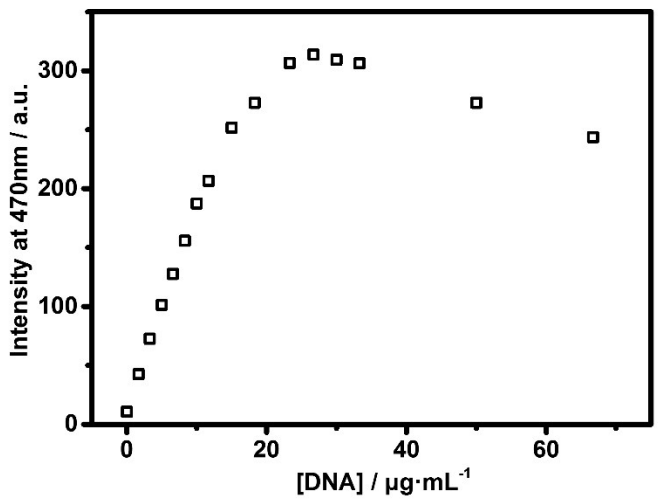

Figure S2. Fluorescence intensity at 470 nm (λ_{ex} = 330 nm) of TQA-TPE (10 μM) with different concentrations of DNA. Experimental conditions: [HEPES] = 10 mM, [$MgSO_4$] = 2.5 mM, [$CaCl_2$] = 0.5 mM, pH 7.4, T = 37 °C.

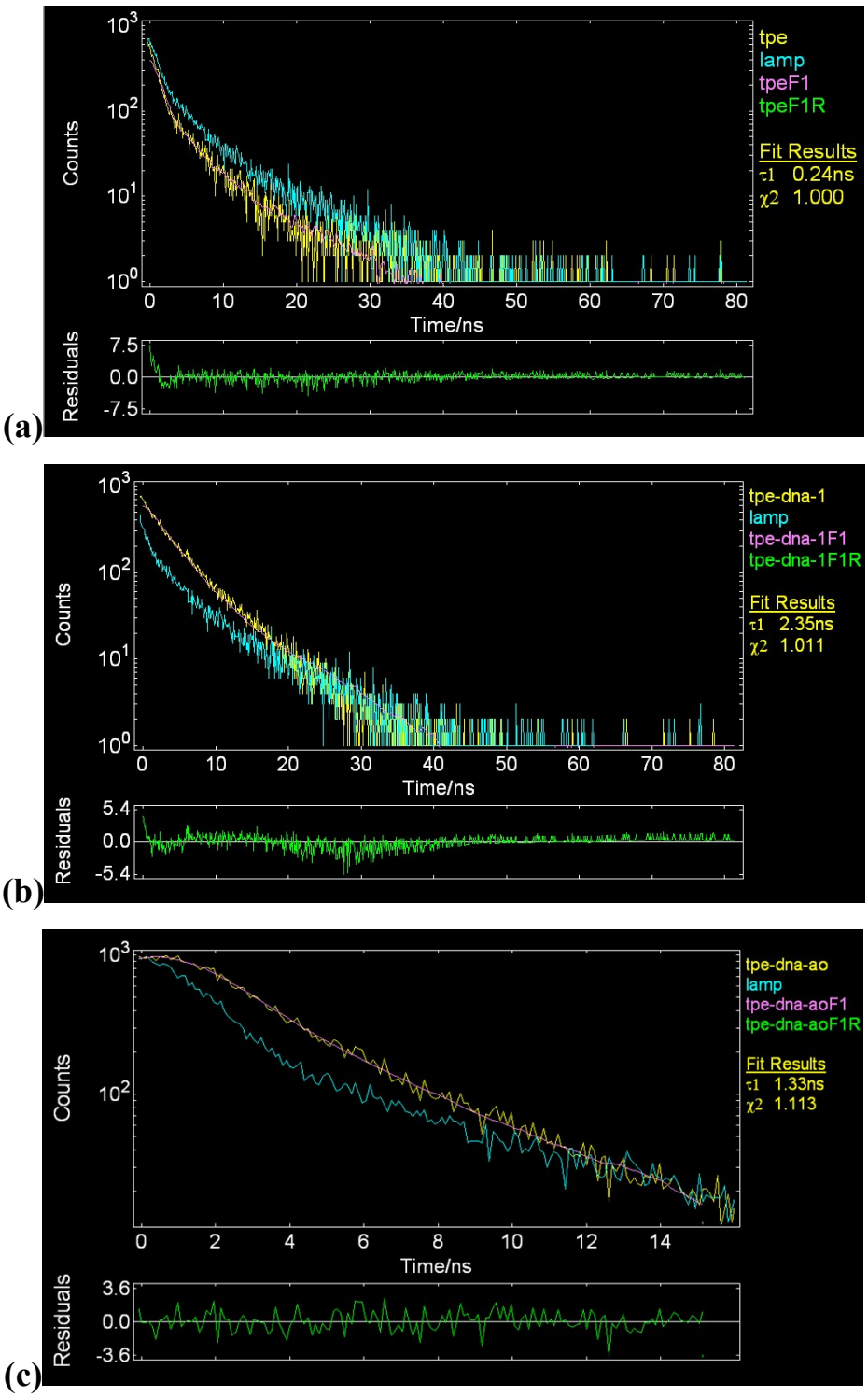

Figure S3. Fluorescence decay profiles of TQA-TPE (a), TQA-TPE&DNA (b) and TQA-TPE&DNA&AO (c). Experimental conditions: [HEPES] = 10 mM, [$MgSO_4$] = 2.5 mM, [$CaCl_2$] = 0.5 mM, T = 37 °C, [TQA-TPE] = 10 μM, [DNA] = 26.7 μg·mL^{-1}, [AO] = 5 μM, pH 7.4, λ_{ex} = 330 nm.

3. **Visualization and monitoring of dissipative assembly by fluorescence technique**

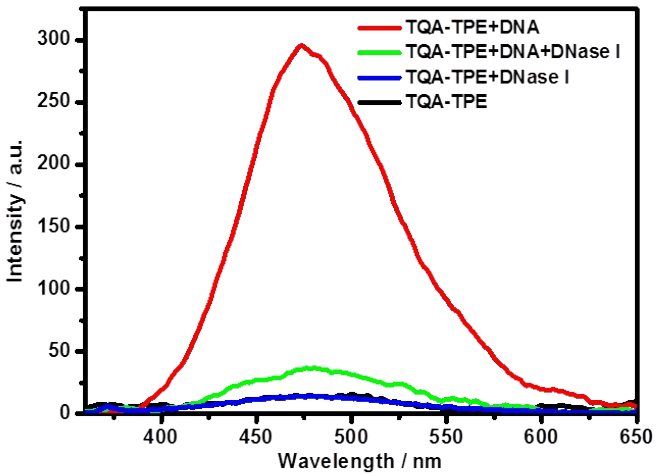

Figure S4. Fluorescence spectra of TQA-TPE, TQA-TPE&DNA, TQA-TPE&DNase I and TQA-TPE&DNA incubating with DNase I for 30 minutes. Experimental conditions: [HEPES] = 10 mM, [$MgSO_4$] = 2.5 mM, [$CaCl_2$] = 0.5 mM, pH 7.4, T = 37 °C, [TQA-TPE] = 10 μM, [DNA] = 26.7 μg·mL^{-1}, [DNase I] = 1.0 Kunitz units·mL^{-1}, λ_{ex} = 330 nm.

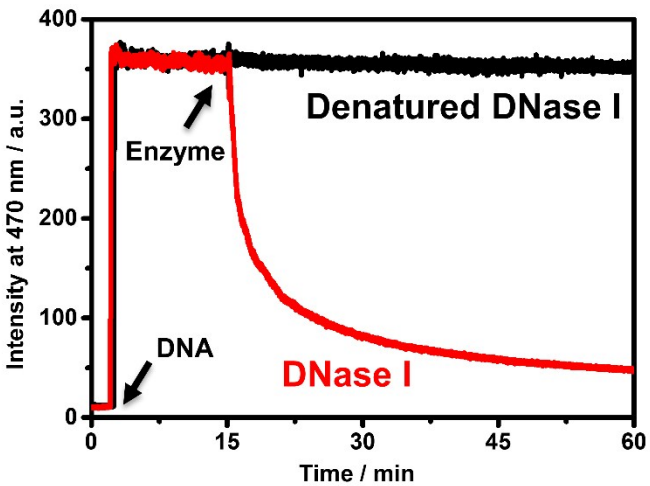

Figure S5. Time-dependent fluorescence intensity at 470 nm (λ_{ex} = 330 nm) of TQA-TPE (10 μM) with addition of DNA (26.7 μg·mL^{-1}) followed by DNase I (2.0 Kunitz units· mL^{-1}) or the same amount of denatured DNase I. Experimental conditions: [HEPES] = 10 mM, [$MgSO_4$] = 2.5 mM, [$CaCl_2$] = 0.5 mM, pH 7.4, T = 37 °C.

Fitting the dissipative kinetics. The kinetic data of fluorescence intensity and light scattering intensity were fitted with the DynaFit program,[2] according to the Michalis-Menton model presented in Scheme S1. The assembly and disassembly processes were ignored because they are much faster than enzymatic reactions. In the present dissipative system, the enzymatic reaction is the rate controlling process. As a result, we postulated that the kinetics of enzymatic reaction represents the dissipative kinetics.

Scheme S1. The Michalis-Menton model for the determination of dissipative kinetics.

The k_a for all fitting was fixed as a same arbitrary value to prevent over-parametrization. The initial enzyme concentration was set to an arbitrary value of 1 μM (0.1 Kunitz units·mL^{-1}). The script files were shown below.

Script file for fluorescence intensity

```
[task]
  data = progress
  task = fit
[mechanism]
  E + S <==> E.S    :  ka  kd
  E.S --- > E + P   :  kcat
[cons tan ts]
  ka = 1
  kd = {0.0001, 0.001, 0.01, 0.1, 1, 10} ?
  kcat = {0.0001, 0.001, 0.01, 0.1, 1, 10} ?
[concentrations]
  S = 26.7
[responses]
  S = 4 ?
  P = 0.2 ?
[data]
  directory  ./ gwc / dnase - flu / all / data
  extension txt
  offset 10

  file 0u  | conc E =  0
  file 0d1u | conc E =  1
  file 0d5u - 01 | conc E =  5
[output]
  directory ./ gwc / dnase - flu / all / output / notpe - 03
[end]
```

Script file for scattering intensity

```
[task]
  data = progress
  task = fit
[mechanism]
  E + S <==> E.S    :  ka  kd
  E.S --- > E + P   :  kcat
[cons tan ts]
  ka = 1
  kd = {0.0001, 0.001, 0.01, 0.1, 1, 10} ?
  kcat = {0.0001, 0.001, 0.01, 0.1, 1, 10} ?
[concentrations]
  S = 186.7
  E = 5
[responses]
  S = 0.33 ?
  P = 0.01 ?
[data]
  sheet  ./ gwc / dnase - dls / 0d5u.txt
  offset 20
  column 2
[output]
  directory ./ gwc / dnase - dls / output / notpe - 01
[end]
```

The fitting curves of fluorescence intensity and light scattering intensity are shown in Figures S6 and 2b, respectively. The dissipating rate constants were obtained as 2.86±0.05 min^{-1} by fluorescence and 19±4.9 min^{-1} by DLS, respectively.

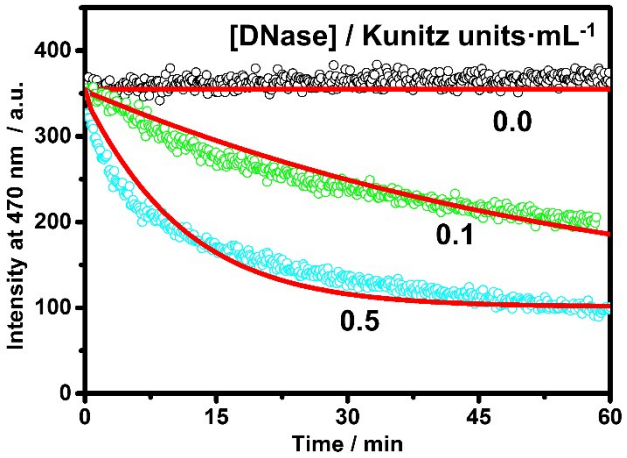

Figure S6. Time-depenent fluorescence intensity at 470 nm (λ_{ex} = 330 nm) of TQA-TPE (10 μM) following addition of DNA (26.7 μg·mL^{-1}) in the presence of different concentrations of DNase I and the corresponding fitting curves.

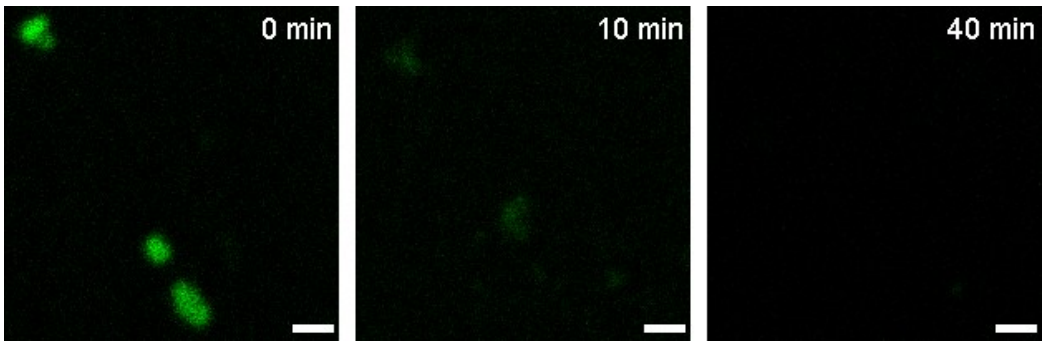

Figure S7. Fluorescence microscopy images of TQA-TPE (50 μM) at 0 min, 10 min and 40 min after addition of DNA in the presence of DNase I (0.5 Kunitz units·mL^{-1}). All images were taken with a 405 nm laser. Scale bar: 1.5 μm.

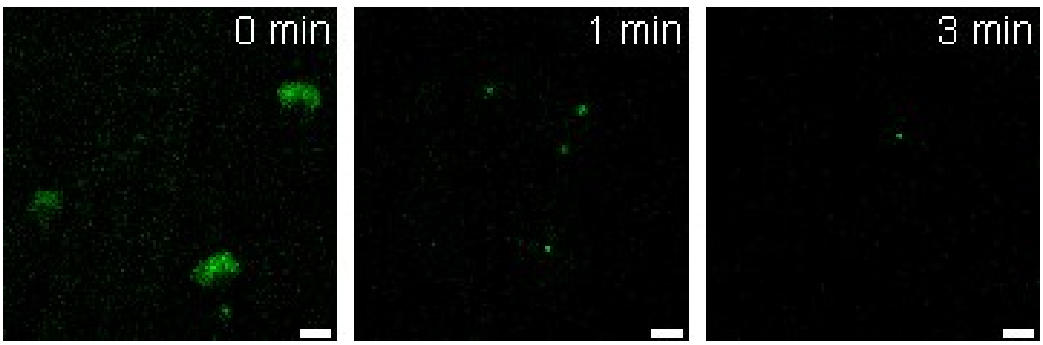

Figure S8. Fluorescence microscopy images of TQA-TPE (50 μM) at 0 min, 1 min and 3 min after addition of DNA in the presence of DNase I (5 Kunitz units·mL^{-1}). All images were taken with a 405 nm laser. Scale bar: 2.0 μm.

4. Transient Förster resonance energy transfer

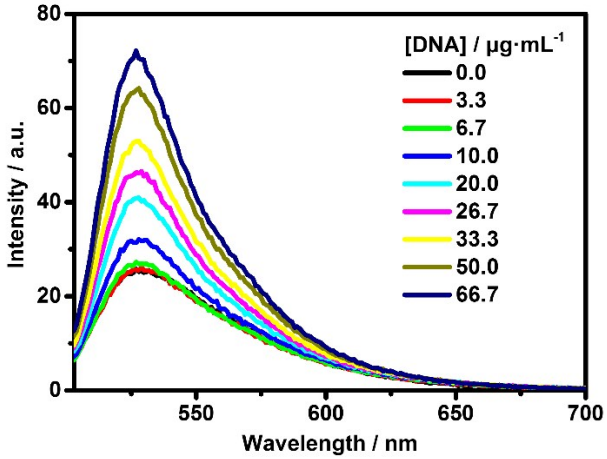

Figure S9. Fluorescence spectra of AO (5 μM) with different concentrations of DNA. Experimental conditions: [HEPES] = 10 mM, [$MgSO_4$] = 2.5 mM, [$CaCl_2$] = 0.5 mM, pH 7.4, T = 37 °C, λ_{ex} = 500 nm.

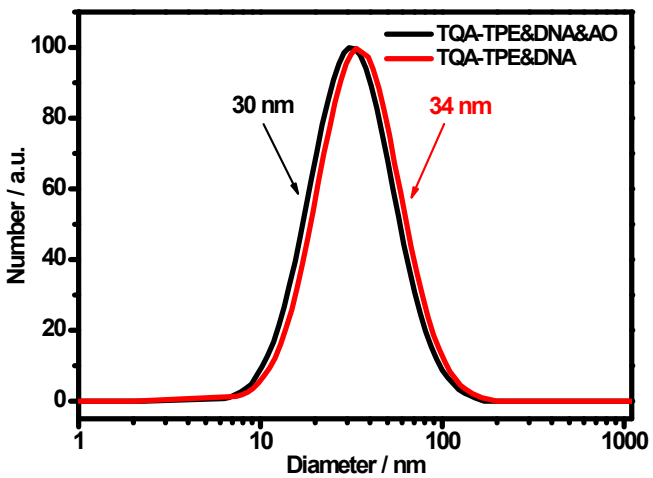

Figure S10. DLS data of TQA-TPE&DNA&AO (10 μM&26.7 μg·mL^{-1}&5 μM) and TQA-TPE&DNA (70 μM&186.9 μg·mL^{-1}). Experimental conditions: [HEPES] = 10 mM, [$MgSO_4$] = 2.5 mM, [$CaCl_2$] = 0.5 mM, pH 7.4, T = 37 °C.

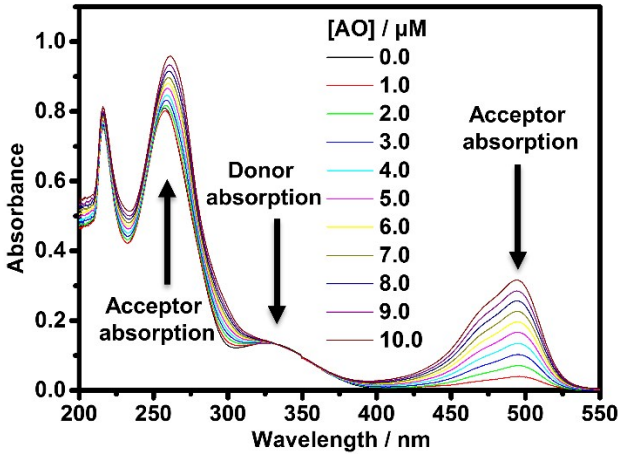

Figure S11. Absorption spectra of TQA-TPE&DNA with different concentrations of AO. Experimental conditions: [HEPES] = 10 mM, [$MgSO_4$] = 2.5 mM, [$CaCl_2$] = 0.5 mM, pH 7.4, T = 37 °C, [TQA-TPE] = 10 μM, [DNA] = 26.7 μg·mL^{-1}.

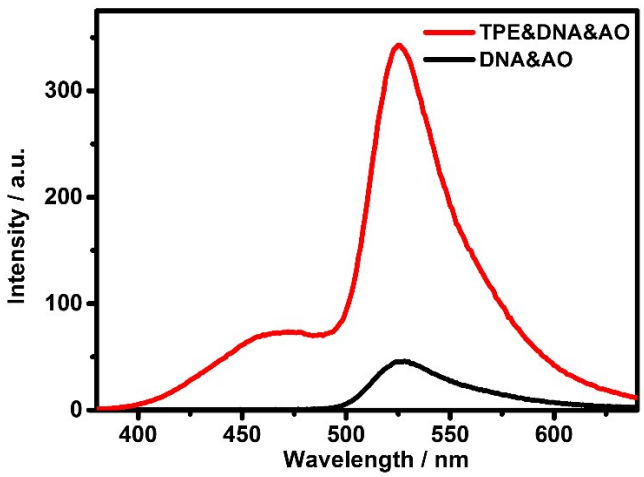

Figure S12. Fluorescence spectra of TQA-TPE&DNA&AO and DNA&AO. Experimental conditions: [HEPES] = 10 mM, [$MgSO_4$] = 2.5 mM, [$CaCl_2$] = 0.5 mM, pH 7.4, T = 37 °C, [TQA-TPE] = 10 μM, [DNA] = 26.7 μg·mL^{-1}, [AO] = 5 μM, λ_{ex} = 330 nm.

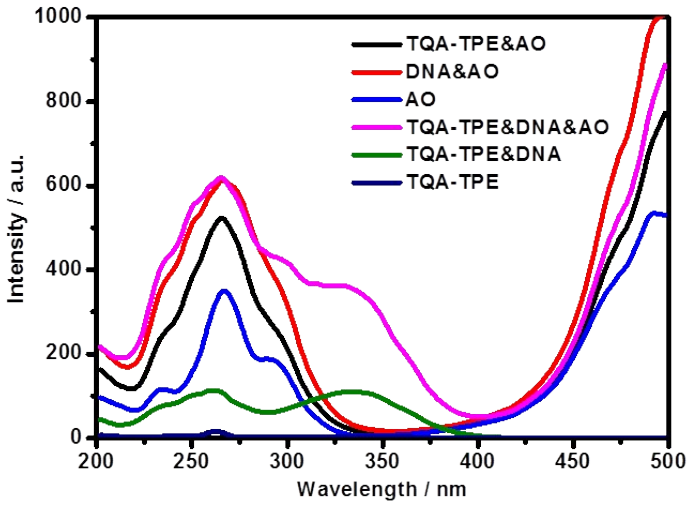

Figure S13. Fluorescence excitation spectra of TQA-TPE, TQA-TPE&DNA, AO, DNA&AO, TQA-TPE&AO and TQA-TPE&DNA&AO. Experimental conditions: [HEPES] = 10 mM, [$MgSO_4$] = 2.5 mM, [$CaCl_2$] = 0.5 mM, pH 7.4, T = 37 °C, [TQA-TPE] = 10 μM, [DNA] = 26.7 μg·mL^{-1}, [AO] = 5 μM, λ_{em} = 525 nm.

4. References

[1] a) Q. Zhang, Y. C. Liu, D. M. Kong, D. S. Guo, *Chem. Eur. J.* 2015, *21*, 13253-13260; b) Y.-C. Liu, Y.-Y. Wang, H.-W. Tian, Y. Liu, D.-S. Guo, *Org. Chem. Front.* 2016, *3*, 53-61.

[2] P. Kuzmič, *Anal. Biochem.* 1996, *237*, 260-273.