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Supporting Information for:

An AIE-based Metallo-Supramolecular Assembly Enabling an Indicator Displacement Assay inside Living Cells

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Experimental Details

Materials

DNAs were purchased from Takara Bio (Dalian) Inc., while deoxyadenosine triphosphate (dTMP) was purchased from Sangon Biotech (Shanghai) Co., Ltd. The selected DNA sequences are shown in **Table S1**. MitoTracker® Red FM was purchased from Thermo Fisher Scientific Inc. All the other reagents were commercially available and used without further purification.

Table S1. Sequences of the DNA used in this study

Abbreviatio	DNA sequences	Abbreviatio	DNA sequences
T2	5`-*TT*-3`	T20	5`-*TTTTTTTTTTTTTTTTTTTT*-3`
T3	5`-*TTT*-3`	G20	5`-GGGGGGGGGGGGGGGGGGGG-3`
T4	5`-*TTTT*-3`	A20	5`-AAAAAAAAAAAAAAAAAAAA-3
T5	5`-*TTTTT*-3`	C20	5`-CCCCCCCCCCCCCCCCCCCC-3`
G5	5`-GGGGG-3`	XA	5`-GG*T*GC*T*AAC*T*-3`
A5	5`-AAAAA-3`	YA	5`-AG*TT*AGCACC-3`
C5	5`-CCCCC-3`	YA1	5`-AGA*T*AGCACC-3`
T10	5`-*TTTTTTTTTT*-3`	YA2	5`-AGAGAGCACC-3`
G10	5`-GGGGGGGGGG-3`	NT	5`-CGGAAGCAGG-3`
A10	5`-AAAAAAAAAA-3`	TC1	5`-*TCTCTCTCTC*-3`
C10	5`-CCCCCCCCCC-3`	TC2	5`-*TCCTCCTCCTCCTCC*-3`
XB	5`-AA*T*ACC*T*GC*T*-3`	TC3	5`-*TCCCTCCCTCCCTCCCTCCC*-3`
YB	5`-AGCAGG*T*A*TT* -3`		

Characterization

^{1}H NMR and ^{13}C NMR spectra were collected on a MECUYRVX300 spectrometer. ESI-MS spectra were measured on a Bruker micrOTOF. Titration experiments were carried out at room temperature. Fluorescence spectra were recorded on a Hitachi F-4600 fluorescence spectrophotometer without waiting time. Cellular imaging was recorded on an Olympus Microscope (IX71) equipped with a high-speed camera in fluorescence modes.

Synthesis

The *Z*/*E*-1, 2-bis(4-(2-bromoethoxy)phenyl)-1, 2-diphenylethene (*Z*/*E*-TPE2Br) were

synthesized according to our previous report.[1]

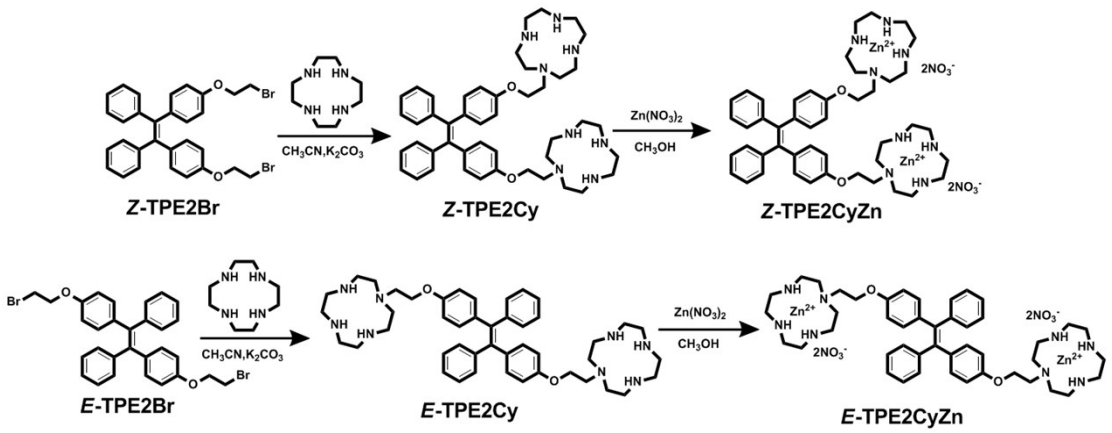
Synthesis of *Z*-TPE2Cy: 587 mg (1.0 mmol) *Z*-TPE2Br, 860 mg (5.0 mmol) cyclen, and 760 mg (5.5 mmol) anhydrous potassium carbonate were dissolved in 100 mL acetonitrile, and the mixture was refluxed at 90 °C for 12 h. The products were separated by the gradient eluent chloroform/methanol/ammonia (40:10:1) for several times, and the *Z*-TPE2Cy (yield: 24 %) was extracted, ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 7.07-7.00 (m, 10H), 6.86 (d, *J* = 8.7 Hz, 4H), 6.63 (d, *J* = 8.7 Hz, 4H), 3.94 (t, *J* = 4.8 Hz, 4H), 2.83 (t, *J* = 3.6 Hz, 4H), 2.72-2.43 (m, 32H)；^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 157.08, 144.25, 139.56, 136.21, 132.49, 132.31, 127.51, 125.99, 113.53, 66.53, 53.22, 51.88, 46.94, 46.00, 45.21. ESI-MS: *m/z* $[M+H]^+$: 761.55; $[M+2H]^{2+}$: 381.30.

Synthesis of *E*-TPE2Cy: Synthesis of *E*-TPE2Cy was similar to that of *Z*-TPE2Cy by using configurationally pure *E*-TPE2Br as the reactant (yield: 34 %). ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 7.07-7.00 (m, 10H), 6.90 (d, *J* = 8.7 Hz, 4H), 6.66 (d, *J* = 8.7 Hz, 4H), 3.96 (t, *J* = 7.2 Hz, 4H), 2.86 (t, *J* = 4.2 Hz, 4H), 2.80-2.52 (m, 32H)；^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 157.11, 144.11, 139.56, 136.31, 132.27, 129.28, 127.37, 125.98, 113.61, 66.51, 53.21, 51.88, 46.97, 46.07, 45.22. ESI-MS: *m/z* $[M+H]^+$: 761.40；$[M+2H]^{2+}$: 381.30.

Synthesis of *Z*-TPE2CyZn: 66 mg (0.22 mmol) methanol solution of zinc nitrate and 85 mg (0.11 mmol) *Z*-TPE2Cy were dissolved in 2 mL methanol, and the *Z*-TPE2CyZn (yield: 88 %) was obtained after filtration and drying. ^{1}H NMR (300 MHz, DMSO-d_6), δ (ppm): 7.13-6.98 (m, 10H), 6.85 (d, *J* = 7.2 Hz 4H), 6.70 (d, *J* = 7.5 Hz 2H), 4.57 (s, 2H), 4.42 (s, 2H), 4.13 (br, 4H), 3.03-2.74 (m, 32H)；^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 157.10, 144.37, 140.00, 136.74, 132.65, 131.42, 128.47, 126.68, 114.59, 63.56, 51.82, 50.64, 44.84, 44.05, 42.65. ESI-MS: *m/z* $[M-NO_3]^+$: 1078.33; $[M-2NO_3]^{2+}$:507.17.

Synthesis of *E*-TPE2CyZn: Synthesis of *E*-TPE2CyZn was similar to that of *Z*-TPE2CyZn by using configurationally pure *E*-TPE2Cy as the reactant (yield: 96 %). ^{1}H NMR (300 MHz, DMSO-d_6), δ (ppm): 7.10-6.92 (m, 14H), 6.74 (d, *J* = 8.7 Hz 4H), 4.58-4.43 (m, 4H), 4.15-4.08 (m, 4H), 3.04-2.74 (m, 32H)；^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 156.73, 144.07, 139.35, 136.35, 132.28, 131.04, 128.22, 126.80, 114.14, 63.26, 51.51, 50.35, 44.55, 43.76, 42.37. ESI-MS: *m/z* $[M-NO_3]^+$: 1078.33; $[M-2NO_3]^{2+}$:508.17.

The synthetic routes of *Z*-TPE2CyZn and *E*-TPE2CyZn are shown in **Scheme S1**.



Scheme S1. The synthetic routes for *Z*-TPE2CyZn and *E*-TPE2CyZn.

Determination of quantum yield

The quantum yields of fluorescence were determined by comparison of the integrated area of the emission spectrum of the samples with the reference of Quinine sulfate in 0.1M H_2SO_4 (Φ = 54%).[2] The quantum yields were calculated with the following expression:

$$\mathbf{\Phi}_x = \mathbf{\Phi}_{st} (\mathbf{I}_x/\mathbf{I}_{st}) (\mathbf{A}_{st}/\mathbf{A}_x) \quad \text{(S1)}$$

$\mathbf{\Phi}_{st}$ is the reported quantum yield of the standard, I is the area under the emission spectra, **A** is the absorbance at the excitation wavelength 330 nm. All the fluorescence spectra were measured on a Hitachi F-4600 spectrophotometer with same setting at room temperature.

In *Vitro* MTT Assay

B16-F10, HeLa and 3T3 cells were obtained from ATCC (USA). Cell viability of the *Z/E* -TPE2CyZn, PV, PR, and the supramolecular assembly were investigated by the microculture MTT reduction method. B16-F10 cells, a kind of tumor cell, was separately cultured with 20000 cells per well in a 96-well microtiter plate 24 h before the assay. Amount of compounds were 10 μM in the 96-well plate. The cells were incubated under 5% CO_2 at 37 °C for 24 h, followed by incubation with 10 μL MTT solution (5 mg/mL) for 4 h. The number of alive cells was expressed as follow (equation S2):

$$\textbf{Percent viability} = \frac{\mathbf{A_{570}(treated\ cells)\ -\ background}}{\mathbf{A_{570}\ (untreated\ cells)\ -\ background}} \times \mathbf{100\%} \quad \text{(S2)}$$

where A_{570} represents the absorbance at 570 nm, treated cells mean the cells were firstly treated with *Z/E* -TPE2CyZn, PV, and the supramolecular assembly, and then treated with MTT solution. Untreated cells represent that the cells were only treated with MTT solution.

Cellular Imaging

B16-F10 cells were separately cultured with 20000 cells per well in a 24-well microtiter plate 24 h before the assay. Cells were treated individually with 10 μM of *Z*-TPE2CyZn, *Z*-TPE2CyZn-PV, *Z*-TPE2CyZn-PR, *E*-TPE2CyZn, *E*-TPE2CyZn-PV, and *E*-TPE2CyZn-PR for 15 min. Subsequently, the cells in the 24-well microtiter plate were imaged on a fluorescence microscope, and the exposure time of all the samples was set as 1/145 s during the observation.Then, each well was treated with MitoTracker® Red FM (50 nM), and after 30 min, the cells were examined under a fluorescence microscope.

B16-F10 cells were separately cultured with 20000 cells per well in a 24-well microtiter plate with round glass sheets inside 24 h before the assay. Cells were treated individually with 10 μM of *Z*-TPE2CyZn, *Z*-TPE2CyZn-PV, *Z*-TPE2CyZn-PR for 30 min. The cellular imaging results were carried out on a Confocal Laser Scanning Microscope (CLSM).

Characterization

NMR spectra

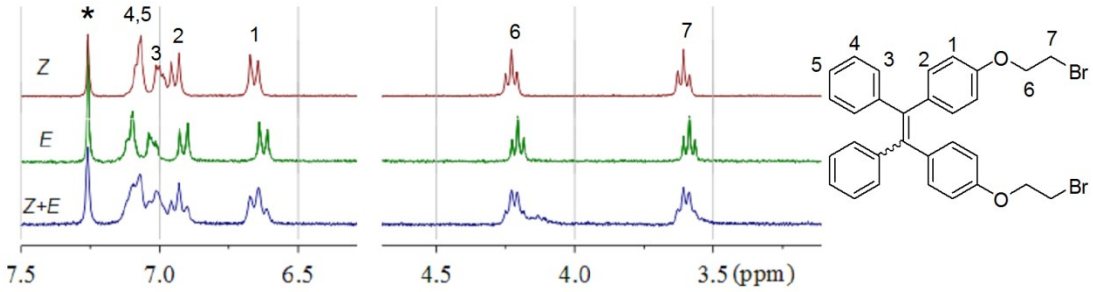


Figure. S1 [1]H-NMR spectra of TPEBr.

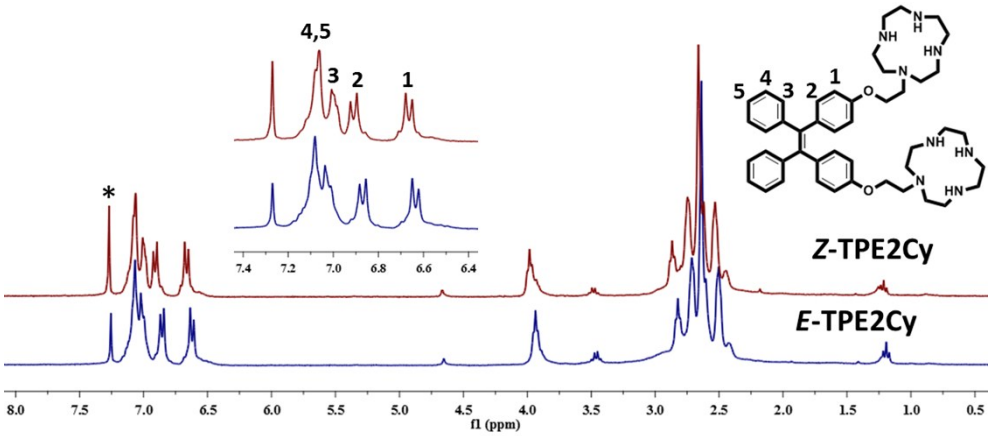


Figure S2. NMR [1]H of *Z*-TPE2Cy and *E*-TPE2Cy.

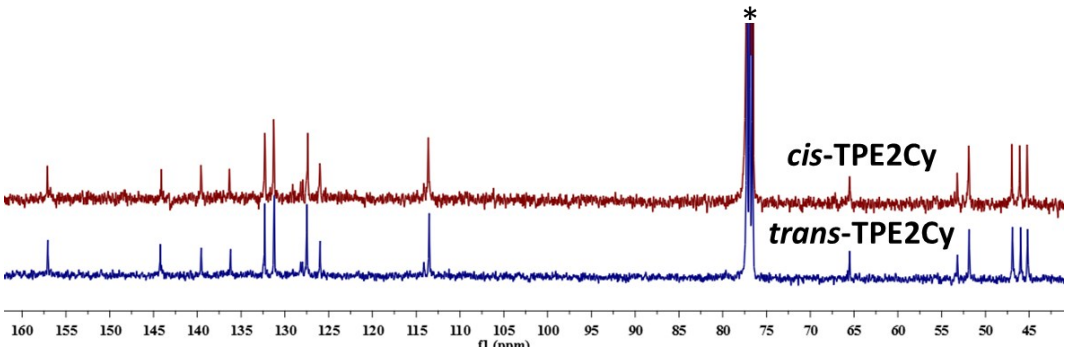


Figure S3. NMR ^{13}C of *Z*-TPE2Cy and *E*-TPE2Cy.

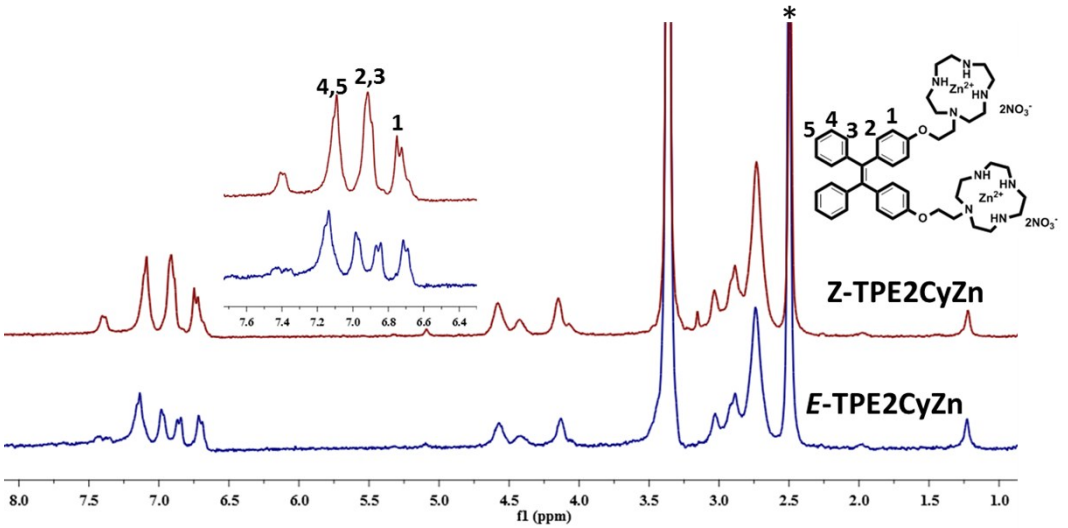


Figure S4. NMR ^{1}H of *Z*-TPE2CyZn and *E*-TPE2CyZn.

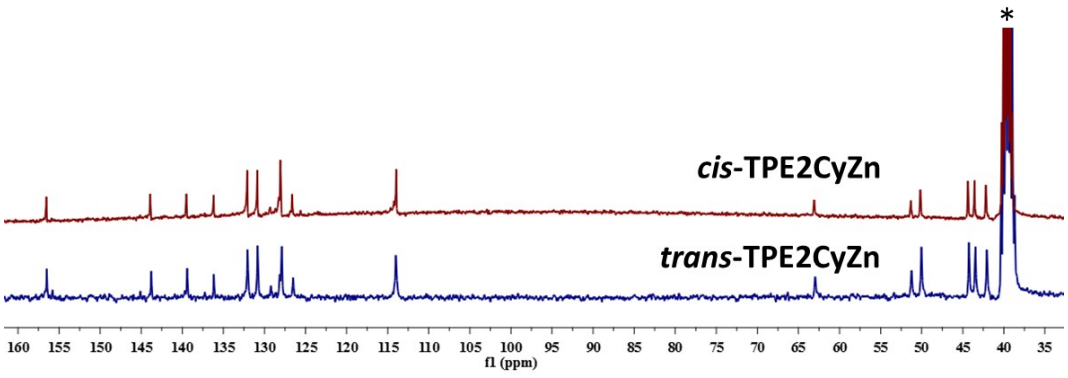


Figure S5. NMR ^{13}C of *Z*-TPE2CyZn and *E*-TPE2CyZn.

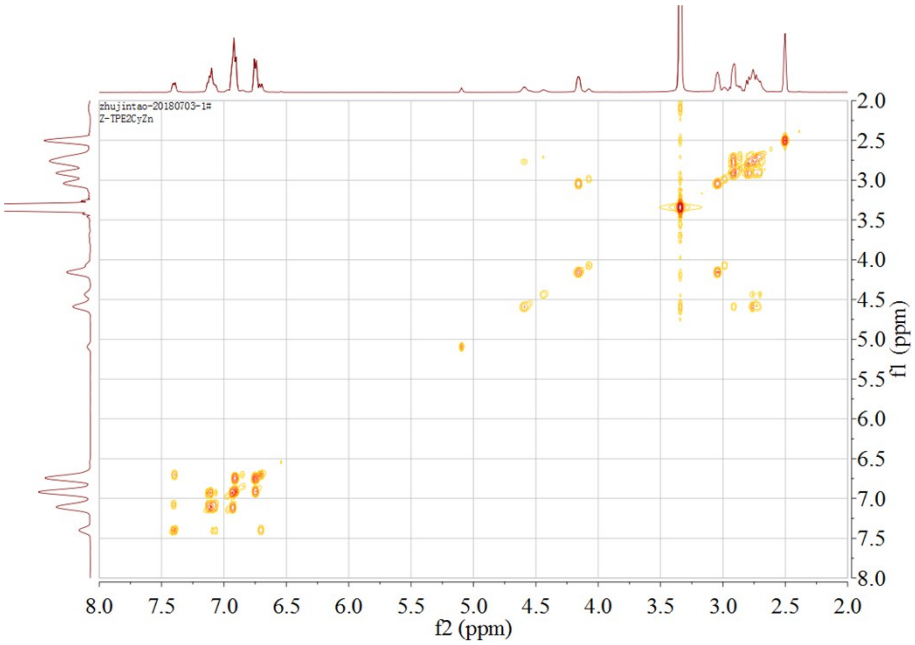


Figure S6. ^{1}H-^{1}H-COSEY spectrum of *Z*-TPE2CyZn.

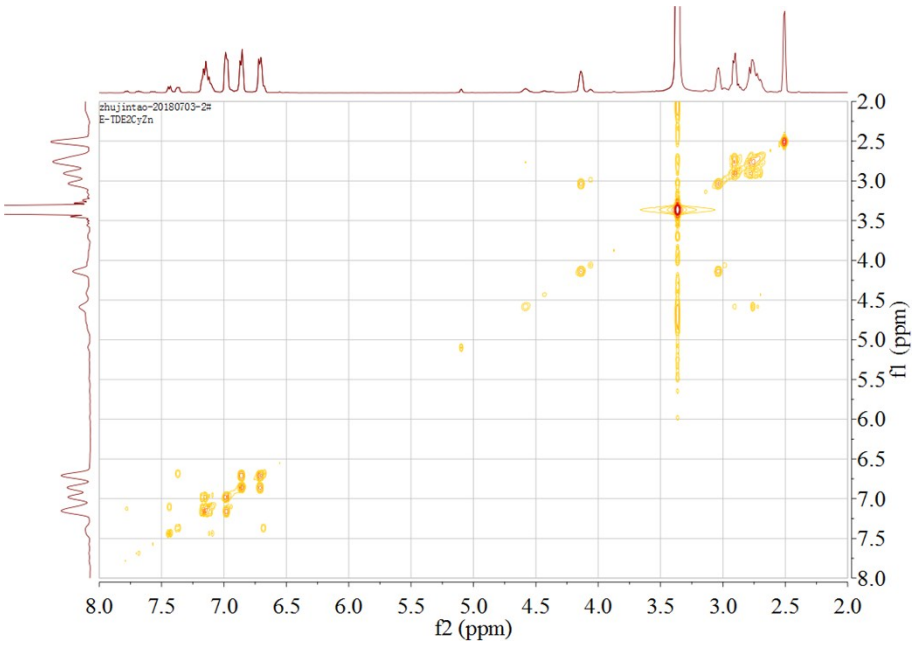


Figure S7. ^{1}H-^{1}H-COSEY spectrum of *E*-TPE2CyZn.

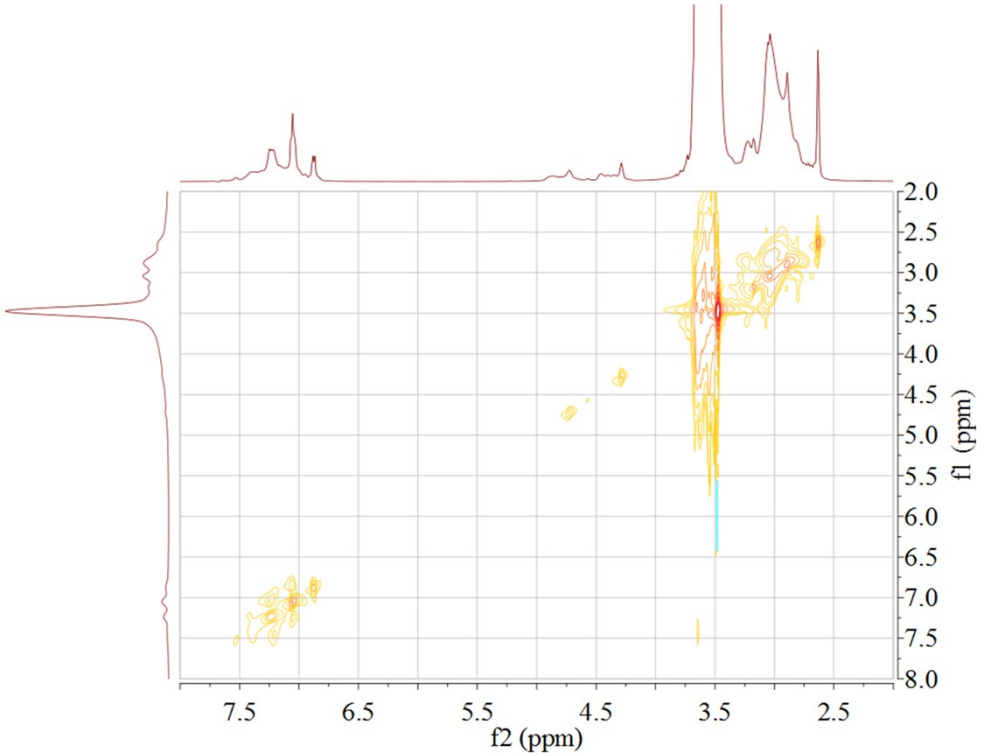


Figure S8. ^{1}H-^{1}H-NOESY spectrum of *Z*-TPE2CyZn.

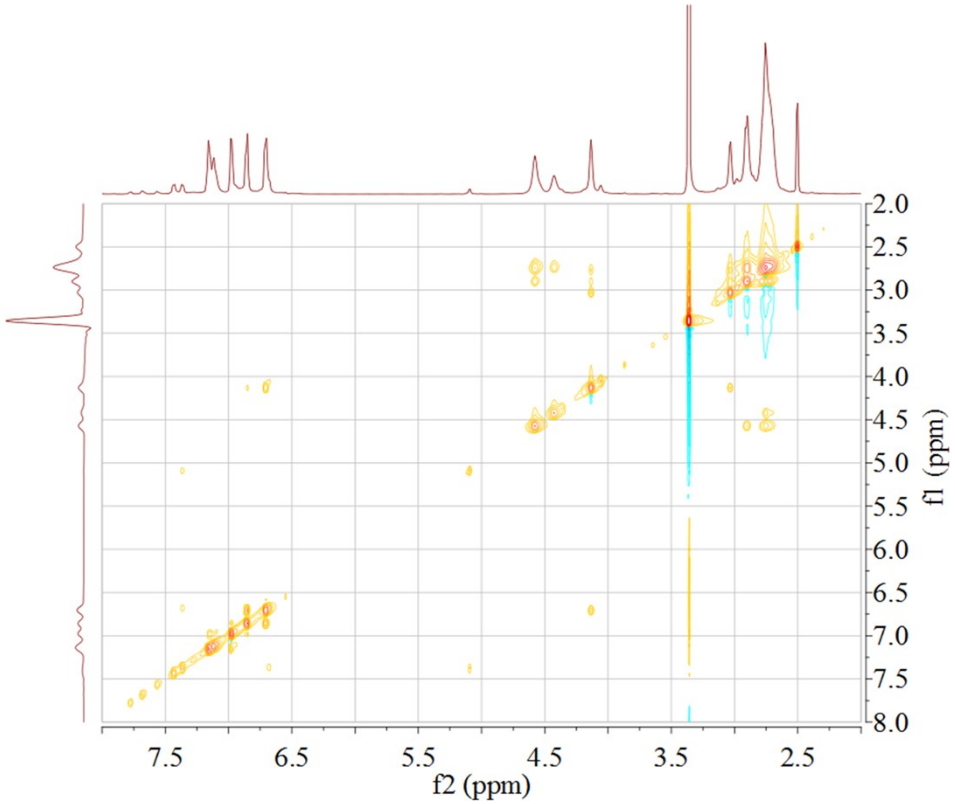


Figure S9. ^{1}H-^{1}H-NOESY spectrum of *E*-TPE2CyZn.

ESI-MS

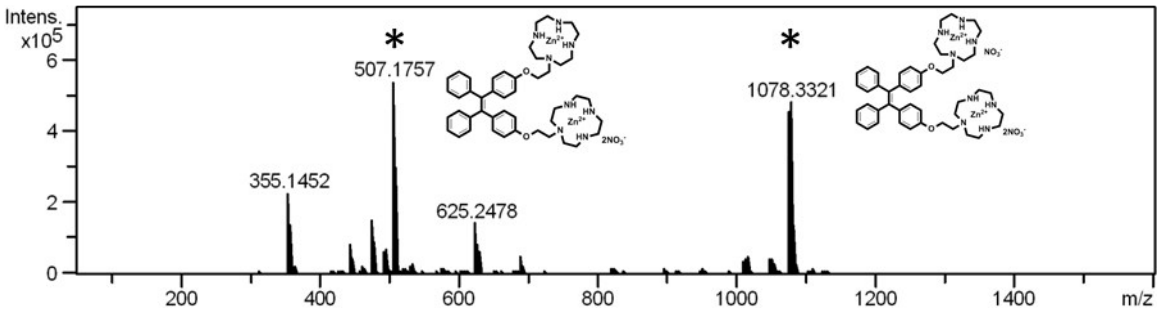


Figure S10. ESI-MS spectra of *Z*-TPE2CyZn.

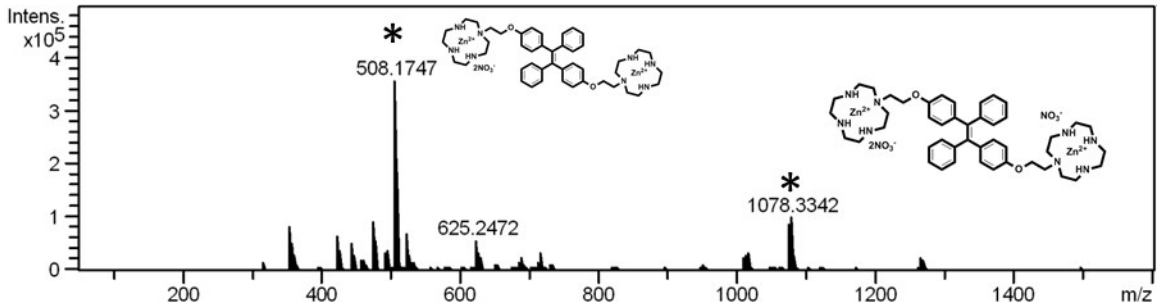


Figure S11. ESI-MS spectra of *E*-TPE2CyZn.

Determination of quantum yield

Table S2. Fluorescence quantum yield of TPE2CyZn in different solutions.

Addition	*Z*-TPE2CyZn	*E*-TPE2CyZn
None	0.64	0.048
PV	0.002	0.004
T5	0.25	0.095
T10	0.50	0.13
T20	0.51	0.25

[TPE2CyZn] =10 μM, [PV] = 5 μM，[NaCl] = 100 mM, [HEPES] = 10 mM, pH = 8.

UV−vis absorption spectra

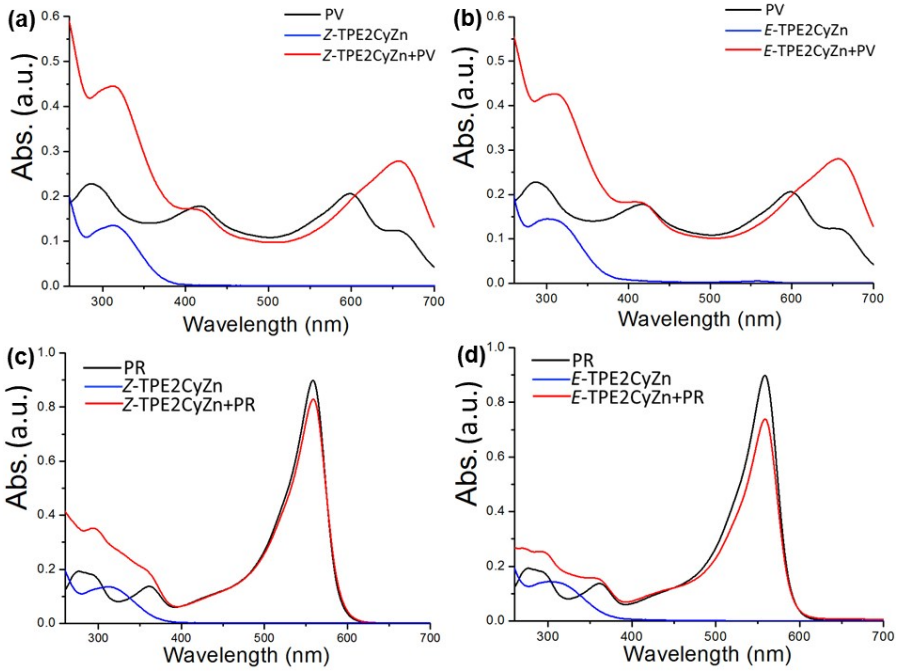


Figure S12. (a-b) Absorption spectra of PV (a-b) and PR (c-d) upon the addition of *Z*-TPE2CyZn and *E*-TPE2CyZn. [HEPES] = 10 mM, [NaCl] = 100 mM, pH = 8, [TPE2CyZn] =[PV] = [PR] = 20 μM.

Fluorescence Spectra

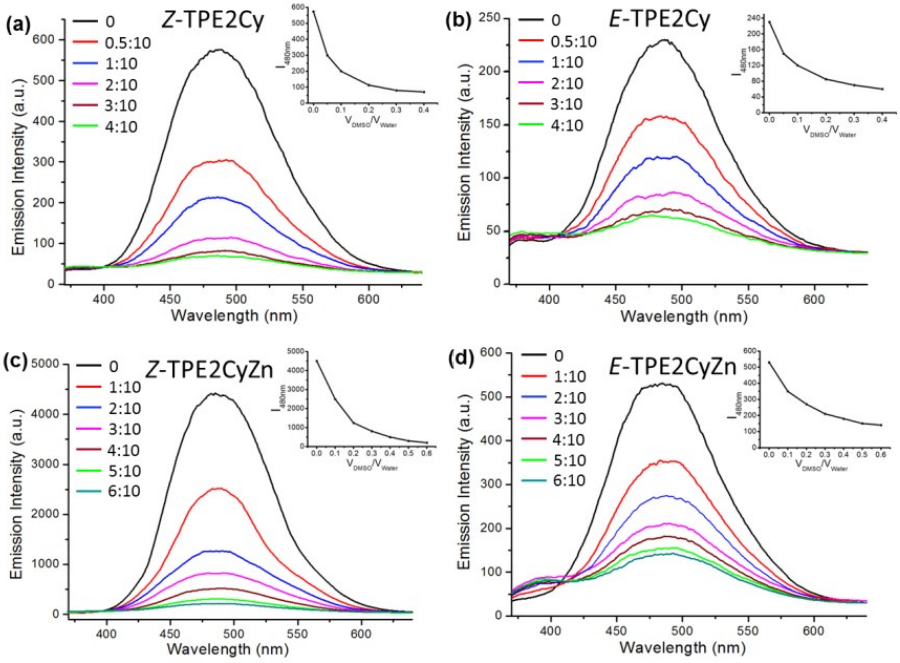


Figure S13. Fluorescence spectra of 100 μM *Z*-TPE2Cy (a) and *E*-TPE2CyZn (b), *Z*-TPE2CyZn (c) and *E*-TPE2CyZn (d) vs. the volume ratio of DMSO and water. λ_{ex} = 330 nm; λ_{em} = 470 nm.

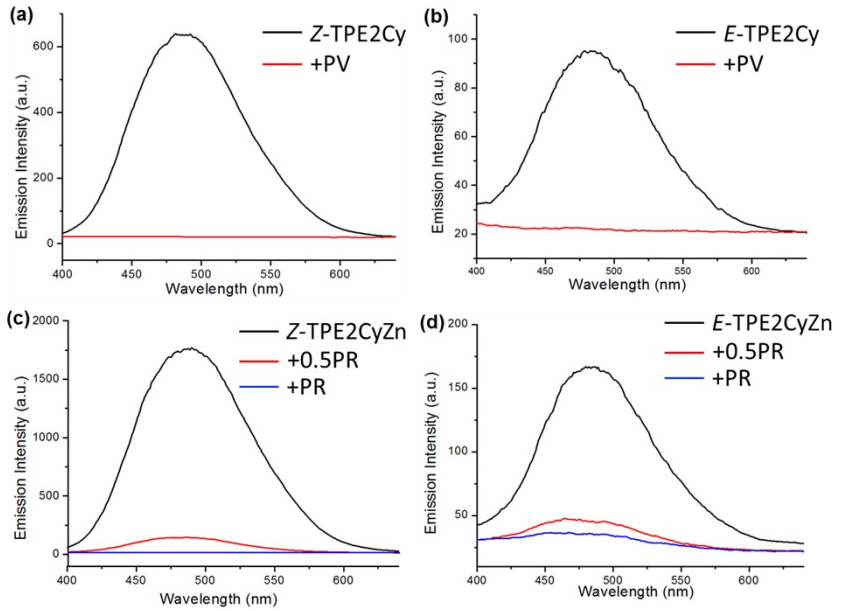


Figure S14. Fluorescence spectra of *Z*-TPE2Cy (a) and *E*-TPE2Cy (b) in aqueous buffers quenched with the addition of PV, [HEPES] = 10 mM, pH = 8.0, [*Z*-TPE2CyZn] = [*E*-TPE2CyZn] = 10 μM, [PV] = 5 μM, [NaCl] = 100 mM, λ_{ex} = 330 nm, λ_{em} = 470 nm. Fluorescence spectra of *Z*-TPE2Cy (c) and *E*-TPE2Cy (d) in aqueous buffers quenched with the addition of PR, [HEPES] = 10 mM, pH = 8.0, [*Z*-TPE2CyZn] = [*E*-TPE2CyZn] = 10 μM, [PR] = 5/10 μM, [NaCl] = 100 mM, λ_{ex} = 330 nm, λ_{em} = 470 nm.

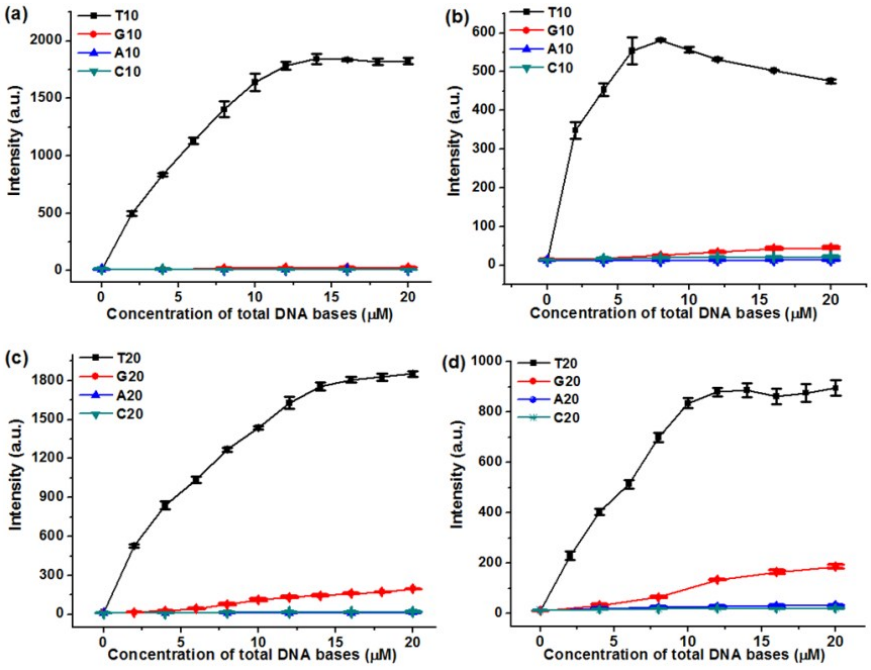


Figure S15. Plots showing fluorescence intensity of *Z*-TPE2CyZn-PV (a, c) and *E*-TPE2CyZn-PV (b, d) in aqueous buffers varied with the bases' concentration, [HEPES] = 10 mM, pH = 8.0, [*Z*-TPE2CyZn] = 10 μM, [PV] = 5 μM, [NaCl] = 100 mM, λ_{ex} = 330 nm, λ_{em} = 470 nm.

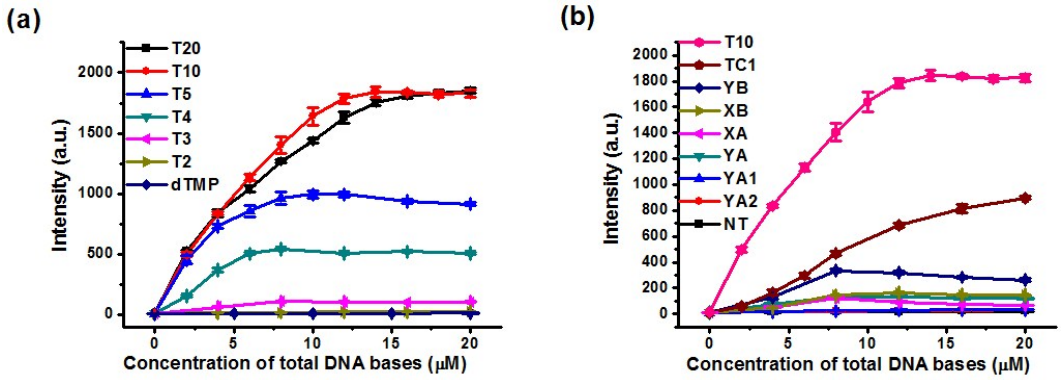


Figure S16. Plots showing the fluorescence intensity of *Z*-TPE2CyZn-PV in aqueous buffers as a function of ploy dT's concentration with different length, [HEPES] = 10 mM, pH= 8.0, [Z-TPE2CyZn] = 10 μM, [PV] = 5 μM, [NaCl] = 100 mM.

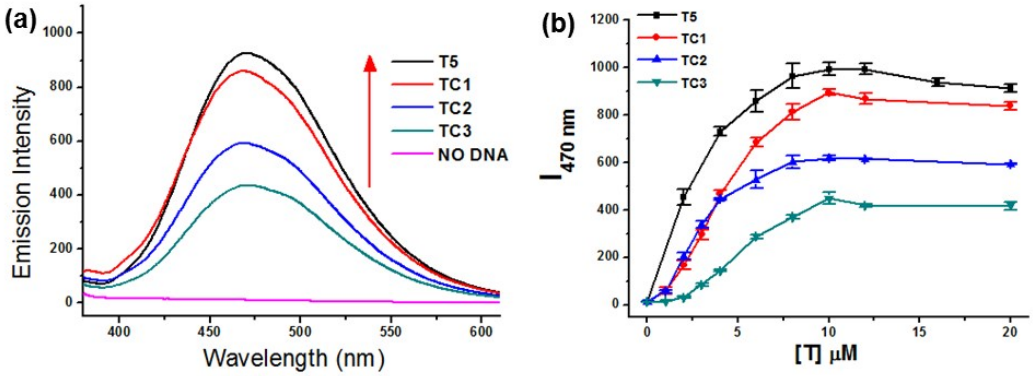


Figure S17. Plots showing the fluorescence intensity of *Z*-TPE2CyZn-PV in aqueous buffers as a function of ploy dT's concentration with different distance, [HEPES] = 10 mM, pH= 8.0, [Z-TPE2CyZn] = 10 μM, [PV] = 5 μM, [NaCl] = 100 mM.

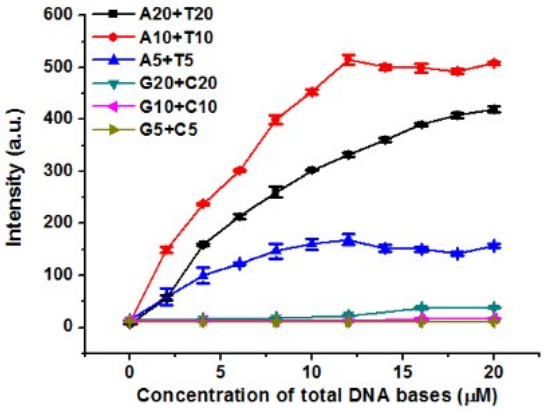


Figure S18. Plots showing the fluorescence intensity of *Z*-TPE2CyZn-PV in aqueous buffers as a function of the concentration of hybrid double-stranded DNA.

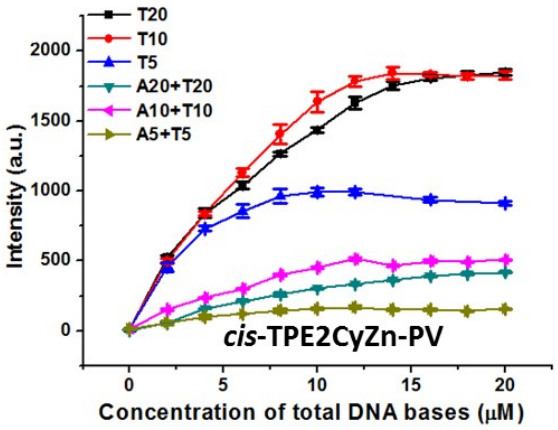


Fig. S19. Fluorescence intensity of *Z*-TPE2CyZn-PV in aqueous buffers as a function of the concentration of T-rich double-stranded DNA and single-stranded DNA. [HEPES] = 10 mM, pH = 8.0, [*Z*-TPE2CyZn] = 10 μM, [PV] = 5 μM, [NaCl] = 100 mM, λex = 330 nm, λem = 470 nm.

Histogram of Percentage Cell Viability

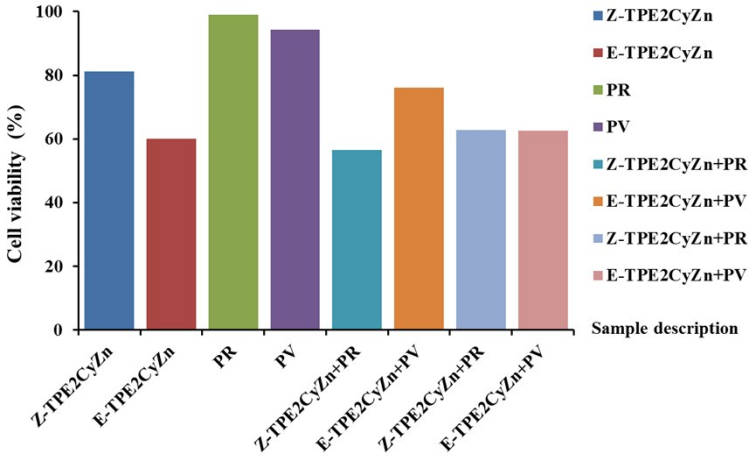


Figure S20. Percentage cell viability of B16-F10 and samples after 24 h incubation.

Concentration of all samples were 10 μM.

Cellular Fluorescence Imaging

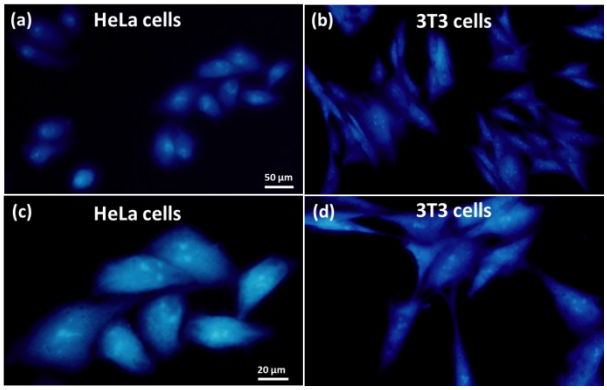


Figure S21. Fluorescence microscopy images of *Z*-TPE2CyZn (10 μM) incubated with HeLa cells (a, c), 3T3 cells (b, d) for 30 min. The scale bar in (a) can be applied to b, while the scale bar in c can be applied to d.

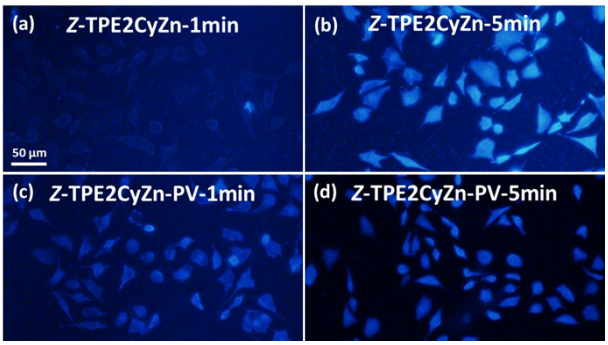
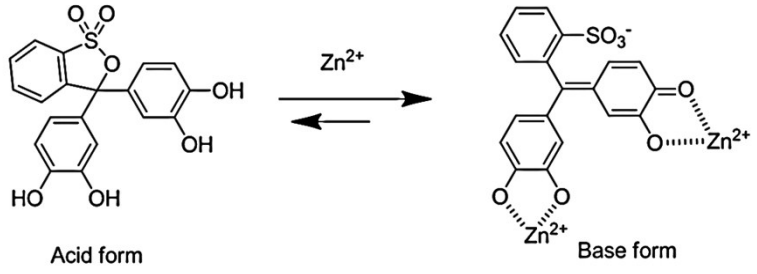
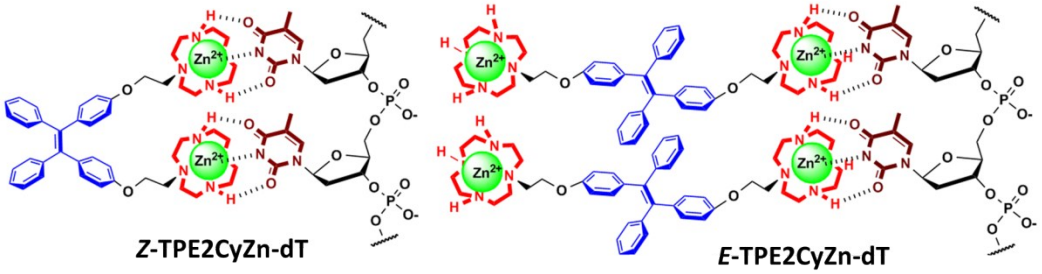


Figure S22. Fluorescence microscopy images of *Z*-TPE2CyZn (a,b) *Z*-TPE2CyZn-PV (c, d) contact with B16-F10 cells after 1 min and 5 min. The scale bar in (a) can be applied to (b, c, d).

Mechanism of Interactions



Scheme S2. The chemical equilibrium of PV between acid and base forms.



Scheme S3. The interactions between *Z*/*E*-TPE2CyZn and dT.

Notes and references

1 Z. C. Zhu, L. Xu, C. L. Yang, *Sens. Actuators B* 2015, **221**, 443-449.

2 W. H. Melhuish, *J. Phys. Chem.* 1961, **65**, 229-235.