Supplementary Information for

DNA embedding Schiff base molecule assemblies: An efficient biological detection approach based on clustering-triggered emission

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In this Supplementary Information, the following results are presented:

7. Optical analyses of the hybrid assemblies after thermal treatment (52°C).

8. Optical stability of the hybrid assemblies.

9. PL spectra of the hybrid assemblies incubation with various concentrations of tDNA and the limit of detection (LOD).

10. PL spectra of hybrid assemblies after incubation with tDNA molecules for 0-30 min and PL spectra of the pure DPIN assemblies after incubation with tDNA.

11. Detailed steps of the simulation.

1. General information:

NMR spectra were obtained with Bruker instruments (^{1}H: 300 MHz and ^{13}C: 125 MHz), respectively. XRD (Bruker, D8 Advance with DaVinci) patterns were obtained under Cu-Ka radiation (λ=1.540Å, 40kV, 40mA) in the range of 5~40°. Scanning electron microscope (SEM, Hitachi SU8010) was used to observe the morphology of hybrid assemblies. Confocal laser scanning microscope (CLSM, Carl Zeiss LSM700) was used to obtain the z-sectioning fluorescence images of the hybrid assemblies and trace the distribution of DNA molecules. Fluorescence spectrophotometer (Jasco F-4500PC) was used to observe photoluminescent signal, UV-Vis spectrophotometer (Jasco V-630) was used to record the absorption spectra, and dynamic light scattering (DLS) analyzer (Malvern ZS90) was used to evaluate zeta potential.

2. Chemicals and DNA sequences:

2-Hydroxy-1-naphthalenecarboxaldehyde (98%) was purchased from Alfa Aesar, N,N-dimethylbenzene-1,4-diamine (≥99%) and tetrahydrofuran (THF) (99%) were purchased from Aladdin, ethanol (99.7%) was purchased from Sinopharm Chemical Reagent. The DNA sequence was synthesized by Sangon Biotech (Shanghai) Co., Ltd, and the sequences were as follows:

Single strand DNA (ssDNA): 5′-AATAATAATAATAAT-3′

Complementary target DNA (tDNA): 5′-ATTATTATTATTATT-3′

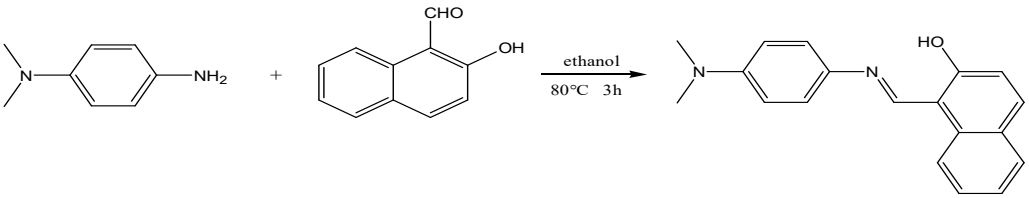
1-mer mismatched tDNA: 5′-ATTTTTATTATTATT-3′

Random mismatched tDNA: 5′-GGTTGGTGTGGTTGG-3′

Cy5-ssDNA: Cy5-5′-AATAATAATAATAAT-3'

Cy5-tDNA: Cy5-5′-ATTATTATTATTATT-3′

3. Synthesis and purification of DPIN molecules:



DPIN molecules were synthesized by the guidance of literature protocol [1]. 1.72 g of 2-Hydroxy-1-naphthalenecarbaldehyde, 1.36 g of N,N-dimethylbenzene-1,4-diamine and 40 mL of ethanol were mixed in a flask, the mixture was heated to 80 °C for 3 hours, cooled to room temperature (RT), then filtered and purified by column chromatography (silica gel, 1:2 petroleum ether/dichloromethane). After drying, we can obtain 1.93 g of orange-red solid DPIN molecules (yield: 67%).

^{1}H NMR (300 MHz, CD_2Cl_2): δ 15.93 (s, 1H), 9.41 (s, 1H), 8.19 (d, J= 9.1 Hz, 1H), 7.82-7.75 (m, 2H), 7.55 (m, 1H), 7.40-7.33 (m, 3H), 7.11 (d, J= 9.1 Hz, 1H), 6.83 (d, J = 9.1 Hz, 2H), 3.04 (s, 6H).

^{13}C{^{1}H} NMR (125 MHz, CD_2Cl_2): δ 167.89, 152.13, 149.681, 135.03, 134.62, 133.21, 129.07, 127.91, 127.21, 123.53, 123.23, 121.37, 119.10, 112.97, 109.16, 40.42.

MS (m/z): 290.02 $[M]^+$(calcd:290.19).

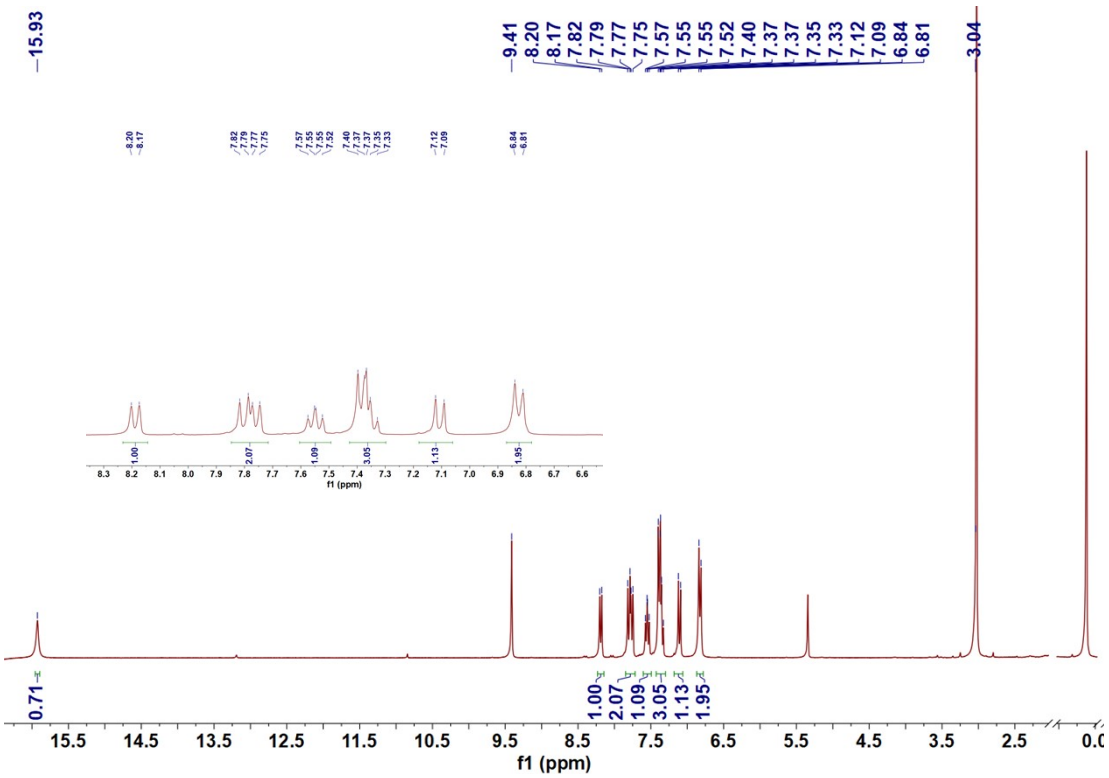


Figure S1. ^{1}H-NMR spectra of DPIN in CD_2Cl_2 (300 MHz).

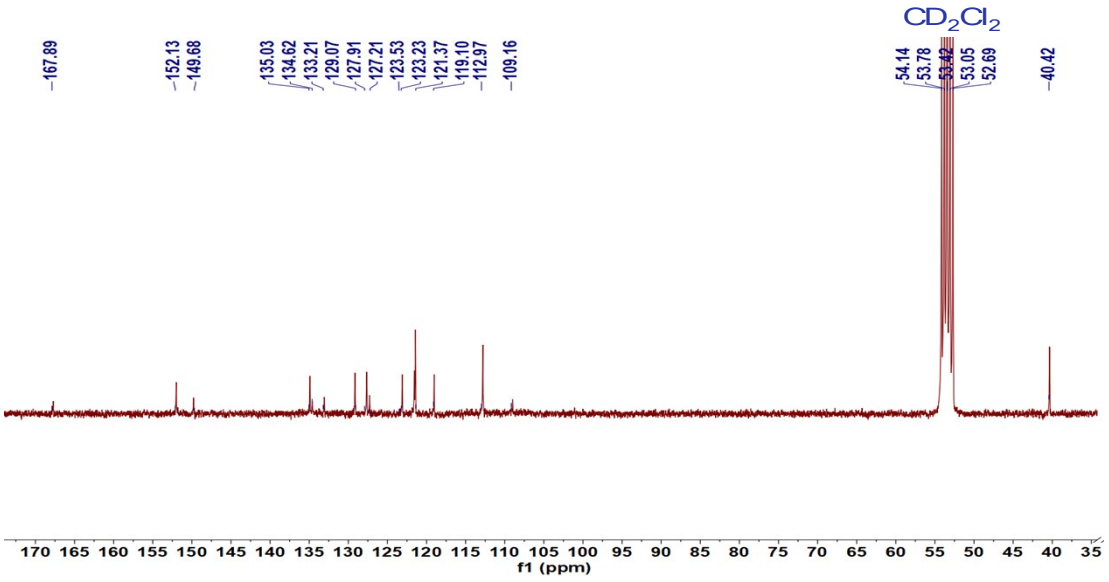


Figure S2. ^{13}C{^{1} H} NMR spectra of DPIN in CD_2Cl_2 (125 MHz).

4. Optical and morphological characterization of the pure DPIN assemblies.

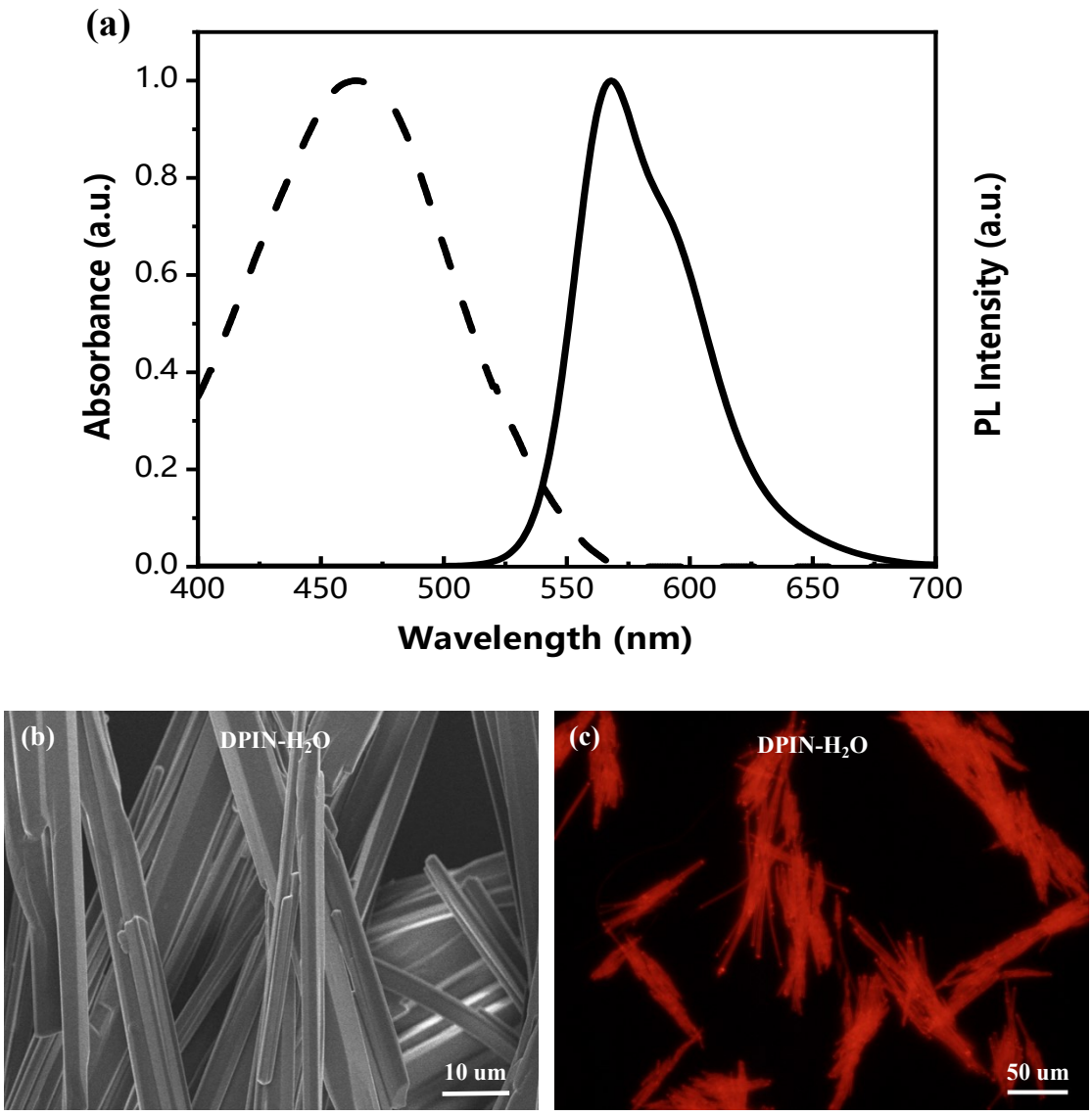


Figure S3. (a) Absorption (dotted line) and PL (black line) spectra of the pure DPIN assemblies. (b) SEM image of the pure DPIN assemblies. (c) Fluorescent image of the pure DPIN assemblies using the filter ranged from 540 to 580 nm.

5. Preparation of the hybrid assemblies and incubation with specific target DNA (tDNA) solution.

(1) Preparation of the hybrid assemblies:

The purified DPIN powders were dissolved in THF to prepare a stock solution with a concentration of 1 mg/mL, and 1 mL of the solution was poured into 4 mL of ssDNA solution (DNA concentration: 0.1 μM) and vigorously stirred for 2 minutes. Visible precipitates were formed as the prepared solution was kept at RT for 24 h.

(2) Incubation with specific tDNA solution:

An equal amount of tDNA solution was added to the prepared hybrid assemblies, stirred at 52 °C for 30 mins, and then slowly cooled to RT.

6. Photoluminescence (PL) spectra of the hybrid assemblies after incubation with various tDNA sequences.

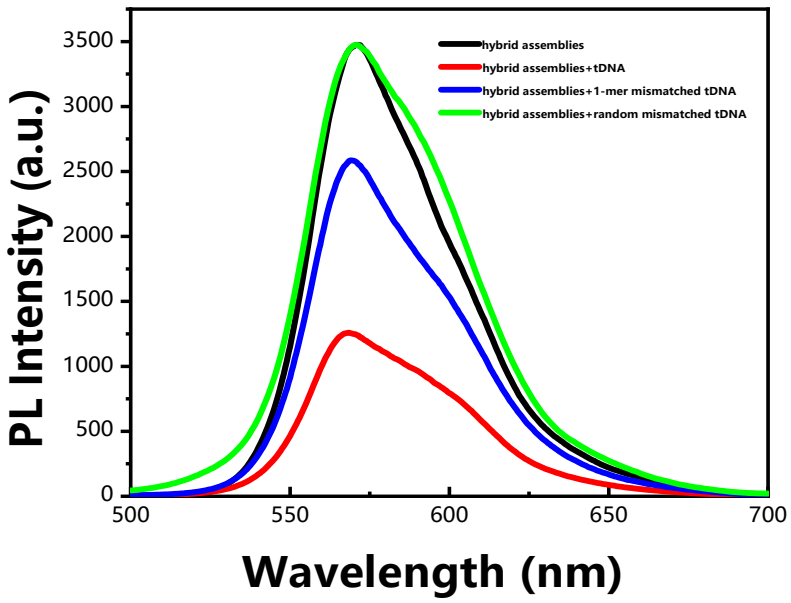


Figure S4. (a) PL spectra of the hybrid assemblies (black line), the ones after incubated with complementary tDNA (red line), 1-mer mismatched tDNA (blue line) and random mismatched tDNA (green line), respectively.

7. Optical analyses of the hybrid assemblies after thermal treatment (52°C).

Figure S5. The fluorescent image of the hybrid assemblies after thermal treatment.

8. Optical stability of the hybrid assemblies.

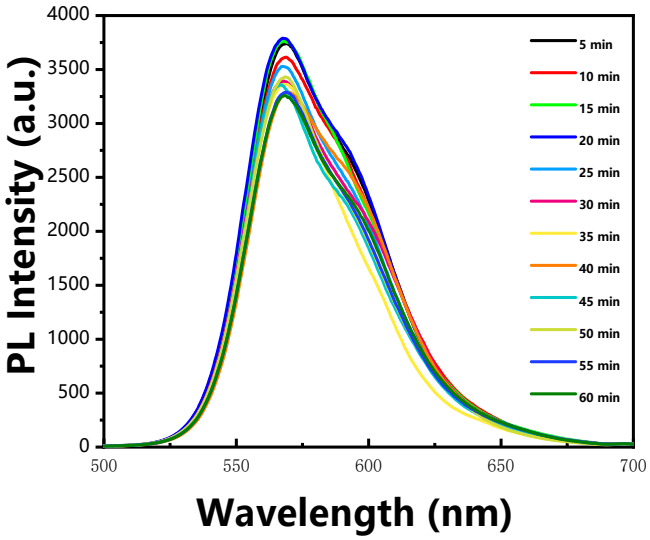


Figure S6. PL intensity of the hybrid assemblies within one hour.

9. PL spectra of the hybrid assemblies incubation with various concentrations of tDNA and the limit of detection (LOD).

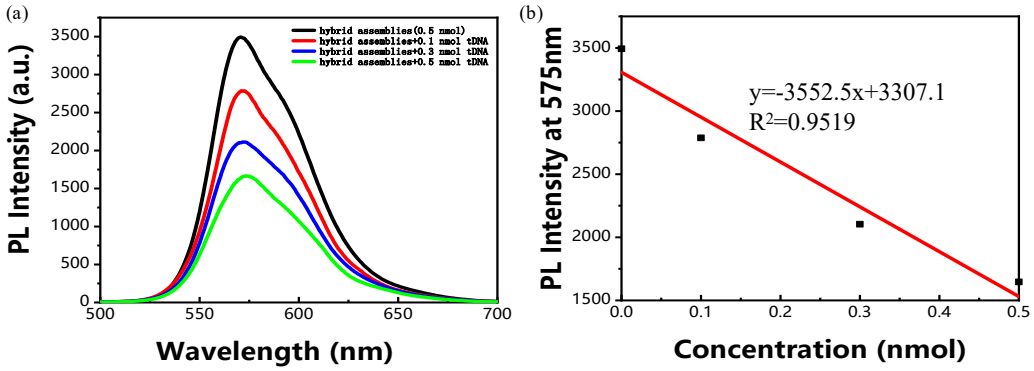


Figure S7. (a) PL spectra of the hybrid assemblies (5 mL) after incubation with various concentrations of tDNA. (b) The relationships between PL intensities of hybrid assemblies and various concentrations of tDNA. The limit of detection (LOD) is defined as $3S_0/S$, where S_0 is the standard deviation and S is the slope of the calibration curve. According to this formula, the LOD was calculated to be 3.8×10^{-8}M.

10. PL spectra of hybrid assemblies after incubation with tDNA molecules for 0-30 min and PL spectra of the pure DPIN assemblies after incubation with tDNA.

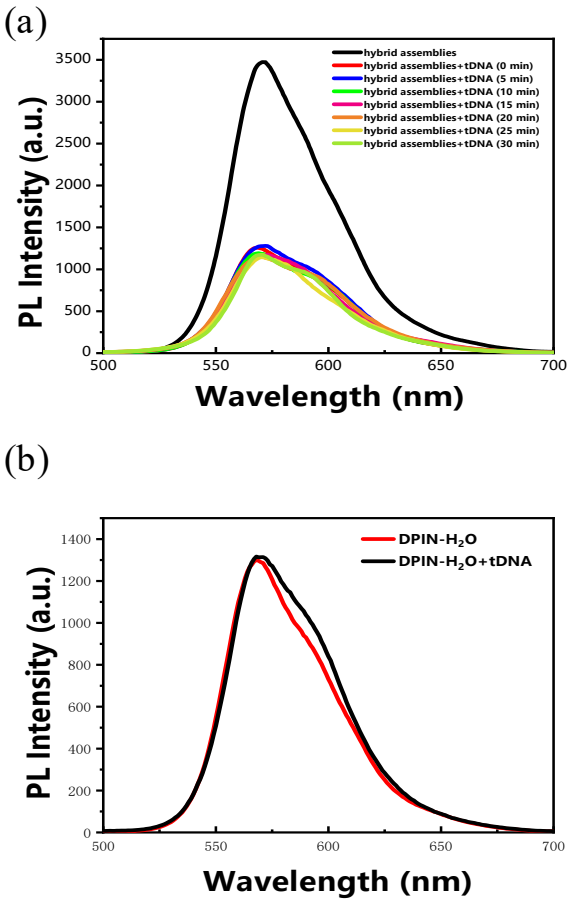


Figure S8. (a) PL spectra of hybrid assemblies after incubation with tDNA molecules for 0-30 min. (b) PL spectra of the pure DPIN assemblies after incubation with tDNA.

11. The detailed steps of the simulation:

First, Discovery studio 2019 was used to construct three-dimensional structures of single-stranded and double-stranded DNA. The structure of the small molecule was mapped by ChemDraw Professional 16.0 and optimized with Gaussian09. AutoDock 4.2 performs molecular docking. The docking mode of global docking was selected, docking box was set to wrap the whole DNA, 100 parameters were searched, and other parameters remained default. To improve the quality of the score, molecular dynamics simulations were performed at 100 ns and the binding free energy of the protein and ligand was calculated with g_mmpbsa. The conformation with the lowest energy in the molecular docking results was selected as the initial structure of molecular dynamics. The charge of the small molecule was calculated using the RESP charge, and the ESP charge was calculated using the HF/6-31G (d) base group of gaussian 09, and then converted to RESP charge using Ambertools22. Gromacs 2019.4 software was used for MD simulation under constant temperature and pressure and periodic boundary conditions. During simulation, Amber99sb-ildn was selected for force field, and TIP3P model was selected for water. The force field parameters of small molecules were generated by acpype.py scripts in AmberTools. In the MD simulation, the hydrogen bond involved was constrained by LINCS algorithm with an integral step size of 2 fs. The electrostatic interaction was calculated using the (Particle-mesh Ewald) PME method, with a cut-off value set to 1.2 nm. The non-bond interaction cut-off value was set to 10 Å and was updated every 10 steps. The V-rescale temperature coupling method was used to control the analog temperature to 300 K and the Berendsen method to

control the pressure to 1 bar. NVT and NPT balance simulations of 100 ps were performed at 300 K. Finally, the DNA-ligand complex system was simulated for 100 ns of finished MD, and the visualization of simulation results was done after using the Gromacs embedded program and Pymol 2.4.

Energy Type	DPIN-ssDNA (kJ/mol)	DPIN-dsDNA (kJ/mol)
van der Waal energy	-153.469 +/- 37.627	-113.525 +/- 50.796
Electrostattic energy	-19.610 +/- 14.969	5.968 +/- 6.552
Polar solvation energy	65.496 +/- 17.213	30.989 +/- 36.780
SASA energy	-13.794 +/- 2.880	-10.330 +/- 4.454
SAV energy	0	0
WCA energy	0	0
Binding energy	-121.377 +/- 36.784	-86.898 +/- 50.280

Table S1. Binding free energy calculated by molecular dynamics.

Reference

(1) H. Liu, Z. Lu, Z. Zhang, Y. Wang and H. Zhang, *Angew. Chem. Int. Edit.,* 2018, 57, 8448-8452.