

Supporting Information

Fluorescent Assay of DNA Hybridization with Label-free Molecular Switch: Reducing Background-Signal and Improving Specificity by Using Carbon Nanotubes

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General Procedure

The as grown SWNTs (HiPCo) were purchased from Carbon Nanotechnologies, Inc., Houston, TX). All PAGE-purified DNA synthesis reagents were from AuGCT Biotechnology Co., Beijing. Ethidium Bromide and proteins were purchased from Sigma-Aldrich Co., and were used without further purification. Bulk SWNTs (1mg) were primary dispersed in DMF (1mL) under sonication (JY92-II sonicator, Ningbo Scientz Biotechnology Co. LID) in an ice water bath for 30 min at a power of 40W to get a homogeneous black solution. Ethidium Bromide was dissolved in double deionized water to get a concentration of 25 M. DNA sequences were dissolved in DNA water as the stock solution, and the concentration were determined according to the UV absorption at 260 nm (Hitachi U-3010 Spectrophotometer).

Fluorescence measurements were performed on a Hitachi F-4500 fluorescence spectrofluorometer. The fluorescence titrations were carried in 0.1 M Tris/HCl buffer solution

(2.5mM MgCl₂, pH 7.5) by adding a few μ L of capturing DNA to 500 μ L aliquot of a Tris/HCl buffer solution containing 0.5 μ M EB and excess of SWNTs suspension, the volume of DMF in the mixture is less than 0.5% so that the influence of the solvent on the DNA activity is neglectable. The addition of capturing DNA was limited to 5 μ L so that the volume change was insignificant. The concentration of the capturing DNA is 1.0 μ M. The target hybrids were formed by adding target DNA to EB/SWNTs/ssDNA solution. After 15 seconds' stirring and 1 hour's incubation to allow the nanotubes being precipitated, the upper clear solution was collected. Then control solutions without target DNA and without all DNA were obtained by adding SWNTs to the EB working solution and EB/ssDNA complexes solution, separately. To compare the effect of SWNTs on the EB background fluorescence signal and selectivity, similar solutions were also prepared under the same procedure but without SWNTs.

To observe the time dependence of the fluorescence increasing, fluorescent measurements were performed using a 0.2 cm path length quartz cuvet with temperature fixed at 20°C. The background fluorescence of 0.5 ml of the Tris/HCl buffer containing was monitored for one minute. Then, 20 μ L of the mixture of EB, DNA P1 and SWNTs was added to the hybridization buffer and fluorescence was measured. After confirming that there was no change of fluorescence with time, 5-excess of target oligonucleotide was added, and the level of fluorescence was recorded. The fluorescence intensity was measured at 590 nm with an excitation wavelength of 510nm.

Spectroscopic Data

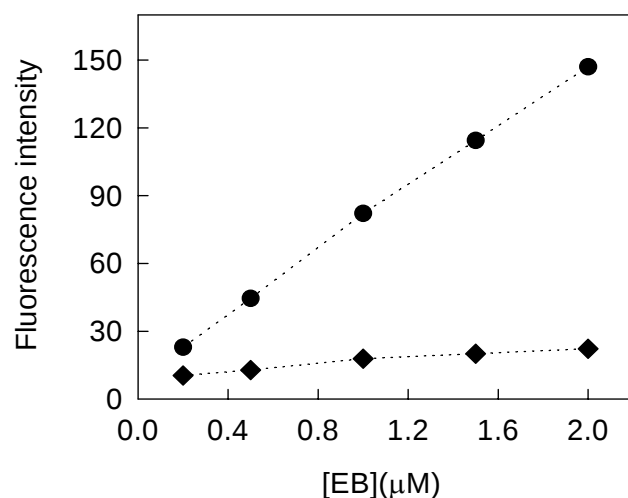


Figure S1. Fluorescence emission intensity enhancement of free EB (●) and EB/SWNTs (◆) as a function of the EB concentration. Excitation was at 510 nm, and emission was monitored at 600 nm.

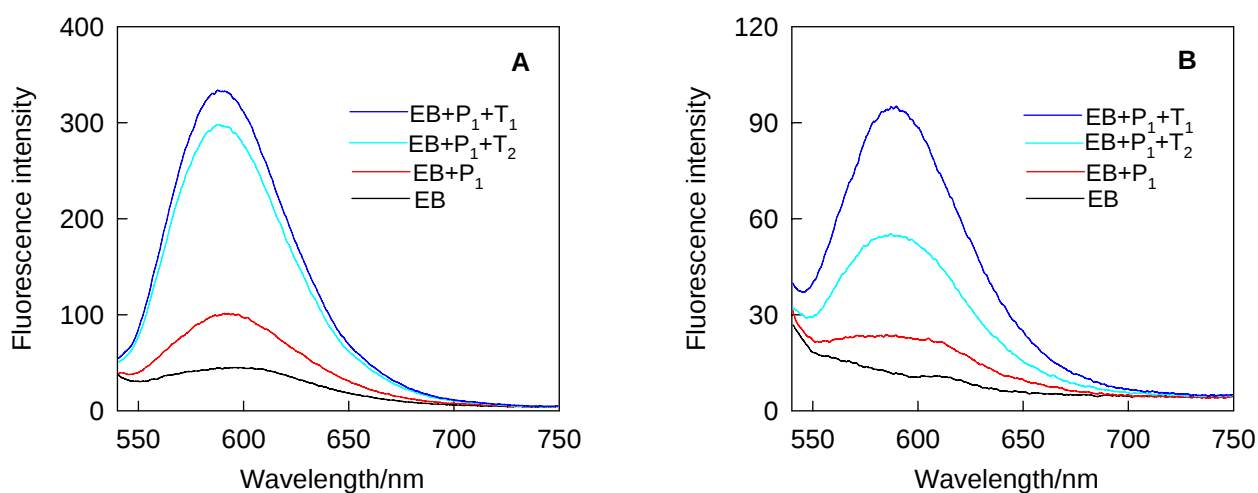


Figure S2. Fluorescence responses of (EB/P₁) toward T₁ and single-base mismatched target T₂ in the absence (A) and the presence of SWNTs (B). The concentrations of EB and the DNA are 0.5 μM and 1.0 μM, respectively. $\lambda_{\text{ex}} = 510$ nm.

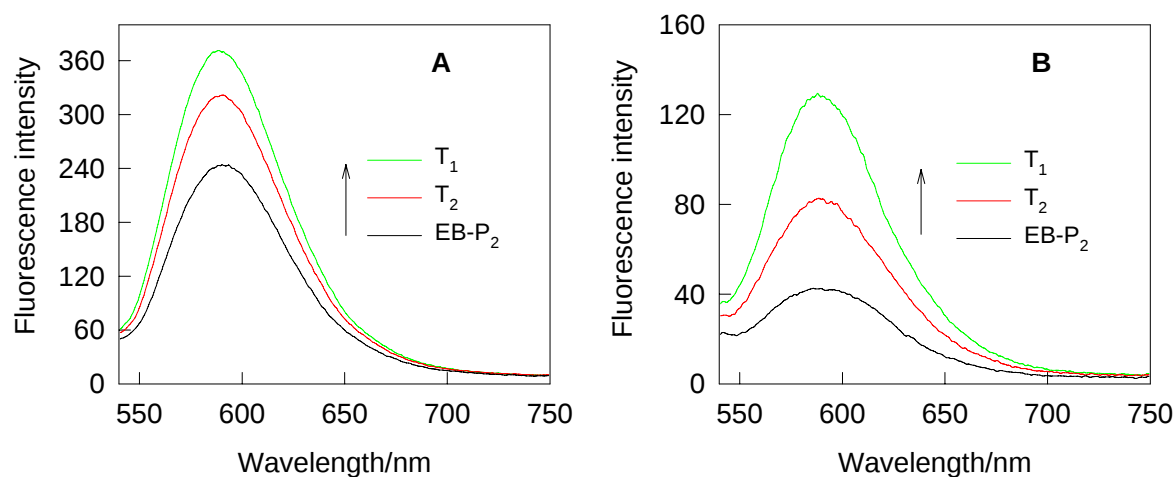


Figure S3. Fluorescence responses of (EB/P₂) toward T₁ and single-base mismatched target T₂ in the absence (A) and the presence of SWNTs (B). The concentrations of EB and the DNA are 0.5 μ M and 1.0 μ M, respectively. λ_{ex} = 510 nm.

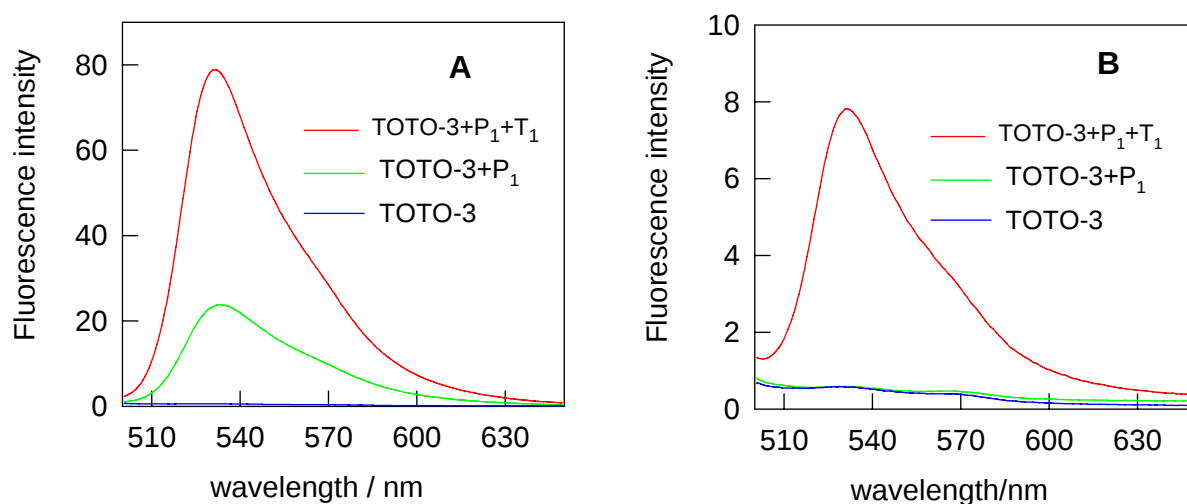


Figure S4. Fluorescence responses of TOTO-3/P₁ toward T₁ in the absence (A) and the presence of SWNTs (B). The concentrations of TOTO-3 and T1 are 0.5 μ M and 1.0 μ M, respectively. λ_{ex} = 480 nm

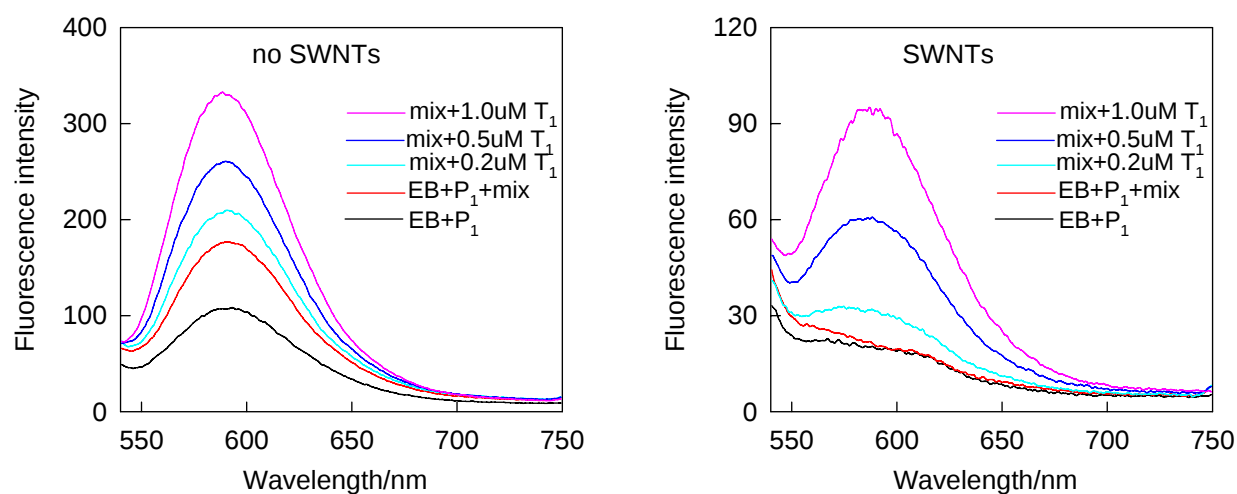


Figure S5. Fluorescence emission spectra of the Tris/HCl buffer solutions containing 1.0×10^{-6} M of (EB/P₁), (EB/P₁) + mixture, and (EB/P₁) + mixture + appropriate concentrations of T₁ in the absence (left) and the presence of SWNTs (right). $\lambda_{\text{ex}} = 510$ nm.