Supporting Information

Experimental Details

Reagents and Instruments

TOPO/TOP capped CdSe/ZnS core/shell QDs in decane were purchased from Invitrogen and were water soluble according to reported procedures[1]. 3-Mercaptoapropionic acid (MPA), 4-(dimethylaminopropyl) pyridine (DMAP) and N,N,-dimethylformamide (DMF) were from Sigma-Aldrich and used without further purification. Dithiothreitol (DTT), methanol, isopropanol, ethyl acetate were from Merck and used as received. Oligonucleotides were from MWG Biotech and used without further purification. Fluorescence spectra were measured using a Cary Eclipse Spectrofluorimeter equipped with an 80 Hz Xenon lamp.

Modified QD and DNA

TOPO/TOP stabilized CdSe/ZnS QDs in decane must be made water dispersible by direct ligand exchange with 3-mercaptopropeonic acid (MPA). In a typical procedure, 3.2 mL of the 75/25 anhydrous methanol/isopropanol mixture was added to 0.8 mL of QDs (1μM) and the solution was centrifuged for 8 min at 3000 rpm and the supernatant was discarded. The ligand exchange was accomplished by reaction of wet precipitate with an excess of 3-mercaptopropionic acid (0.1 mL, 1.15 mmol) in 1.0 mL of DMF. This solution was vortexed (1-2 min) and sonicated for 30 min and stored for 1-4 days at room temperature. In the next step, QDs (150 μL) were made water soluble by deporotonating the surface bonded MPA with DMAP (8.0 mg, 0.065 mmol) dissolved in 0.4 ml DMF. As a last step, the solution was centrifuged for 3 min at 3000 rpm and the precipitate was dried under argon. The oligonucleotides were dissolved in a mixture of 10 mM tris-hydrochloride buffer (pH 8) and 1mM EDTA and were stored at 0°C. Before each experiment, thiol modified oligonucleotides (2ml, 1-2 ODs/ml, 2.28μM) were redispersed in 2ml of a 4 mM DTT, 0.17 M phosphate buffer solution (pH 8) in a water bath (37 °C) for 16 h to cleave the mixed disulfide bonds. The unreacted DTT was removed using extraction with ethyl acetate (4ml) and this step was repeated two additional times before conjugation of oligonucleotides with QDs.

QD-DNA conjugates

QD-DNA conjugates were prepared by dissolving precipitate MPA-QDs in 1ml thiol modified oligonucleotides solution (1-2 ODs/ml, 2.28μM). The oligonucleotide sequences are listed in Table S1. In this case, 605QD-DNA as probe 1 (P605) and 705QD-DNA as probe 2 (P705) were prepared with modification of 605QD and 705QD with 1ml of 15-mer 3'propylthiol and 5' hexylthiol terminated oligonucleotide sequence, respectively. After 12 h, 0.15M NaCl was added to the QD-DNA solution and stored for an additional 12 h. Then, the concentration of NaCl was raised to twice and the mixture was aged for a further 40 h before QD assemblies.

QD assemblies

Solutions of 605QD-DNA (80μL, OD<1μM) was combined with a solution of 705QD-DNA (40 μL, OD<1μM) in a tube and then diluted with 500μL of PBS (0.3 M NaCl, 10 mM phosphate buffer (pH 7) and 0.01% sodium azide). After addition of target DNA as a linker (20μL, 0.6 μM), the solution was cooled to -78°C and then allowed to return to room temperature slowly.

Calculation of Förster distance

The Förster radius R_0, defined as the distance between the donor and acceptor that yields 50% energy-transfer efficiency, can be calculated as

$$R_0^6 = 8.79\times10^{-5}\kappa_p^2 n_D^{-4}\varphi_D J \quad (1)$$

Where n_D =1.33 is the refractive index of the medium, φ_D is the quantum yield of the donor and κ_p is an orientation factor, depending on the relative orientation of the donor and acceptor dipoles. $\kappa_p^2 = 2/3$ is the value for randomly oriented dipoles, which is suited for the systems studied here. The overlap integral J is a quantitative measure of donor-acceptor spectral overlap integrated over all wavelengths [2], which can be derived from the experimentally obtained absorption and emission spectra using Equation 2.

$$J = \frac{\int F_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda}{\int F_D(\lambda)\, d\lambda} \quad (2)$$

Where, F_D and ε_A represent the normalized donor emission spectrum and acceptor absorption spectrum, respectively. According to the Equation 2 the calculated overlap integral, J, for this donor-acceptor pair is 3.71×10^{10} nm^4. Quantum yield value (φ) for 605QD as a donor was determined relative to a reference fluorophore. Fluorescein dye in sodium borate buffer at pH 9.5 was

chosen as a reference that its quantum yield (φ_{ref}) under these conditions is known to be 0.93 [3]and the quantum yield, φ, of donor (605QD) was determined using Equation 3.

$$\varphi = \varphi_{ref} \frac{\int F d\lambda}{A} \frac{A_{ref}}{\int F_{ref} d\lambda} \quad (3)$$

Where F, A, F_{ref} and A_{ref} represent the donor emission, donor absorption at the wavelength of excitation (490 nm), Fluorescein emission and absorption, respectively. Using the obtained value 0.082 for φ and 3.71×10^{10} nm^4 for J, we find R_0 of 6.17 nm as an estimate for the Förster radius.

Table s1. Sequences of the target DNA capC (Anthrax-Related gene) and its associated oligonucleotide probes.

Denomination	Sequence
Target DNA **tDNA**	5'-ATG CCA TTT GAG ATT TTT GAA TTC CGT GGT-3'
605QD-DNA **Probe1**	5'-AAT CTC AAA TGG CAT-$(CH2)_3$ –SH-/ 605QD/-3'
705QD-DNA **Probe2**	5'-/705QD/-HS-$(CH2)_6$ -ACC ACG GAA TTC AAA-3'

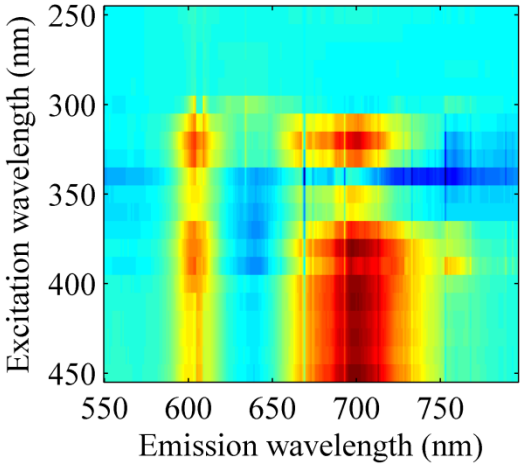


Figure S1: The 2D-PLE signals of sandwich nanoassembly which disturbing emission from directly excited P705 was subtracted.

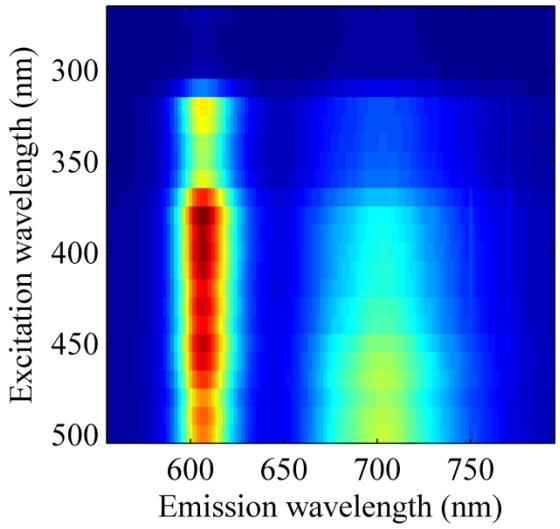


Figure S2: The 2D-PLE signals of two probes (P705 and P605) without target DNA (control experiment), which shows that the nonspecific adsorption is neglectable.

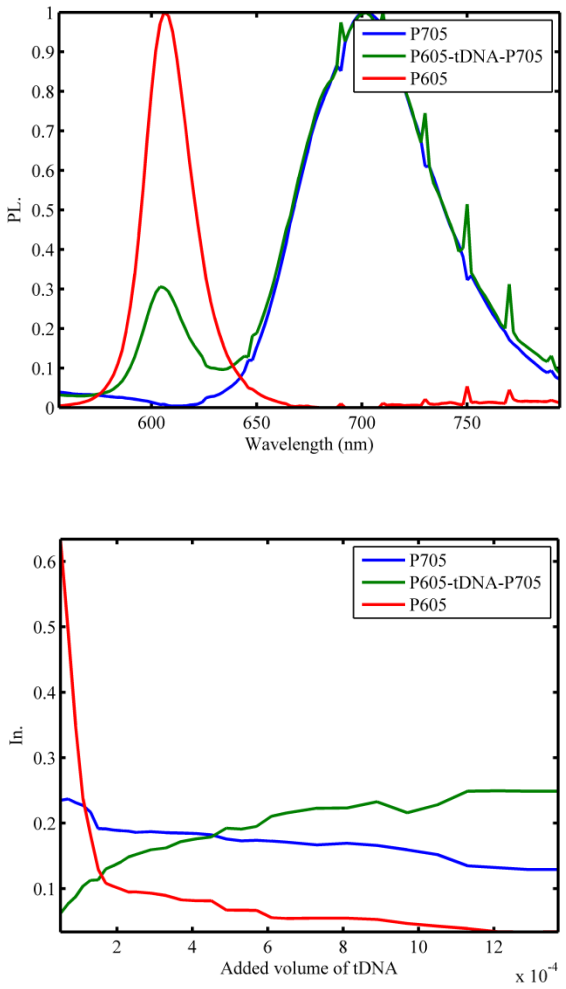
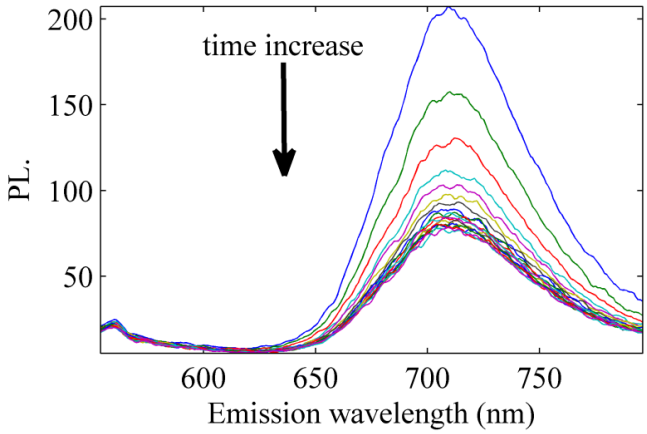


Figure S3. The calculated a) emission spectra and b) concentration profiles from applying PARAFAC on titration experiments data. Blue, green and red profiles are corresponding with P705, P605-tDNA-P705 and P605 species, respectively.

a)



b)

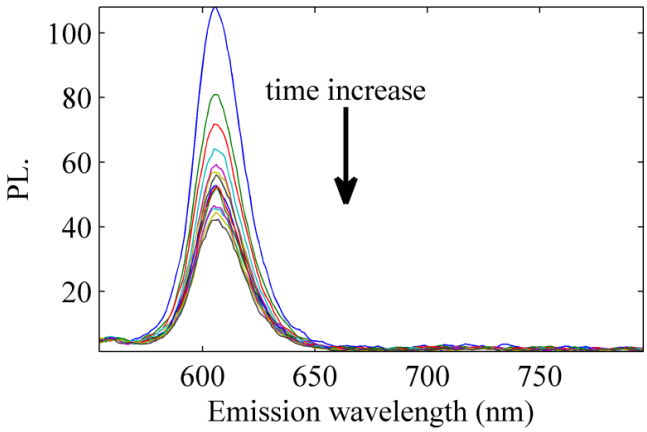


Figure S4. The obtained emission spectra during hybridization process from a) P705 b) P605, with target DNA(0.6μM) at time scale 45 min, which shows faster kinetics of (a) relative to (b).

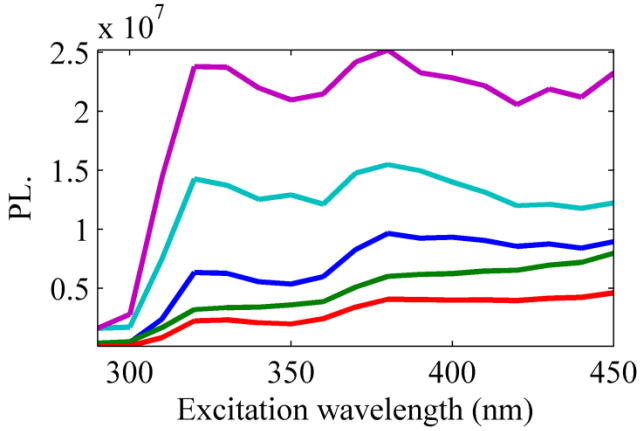


Figure S5. The resolved excitation profiles at fitted parameter for this nanoassembly.

1. G. P. Mitchell, C. A. Mirkin, R. L. Letsinger, Programmed Assembly of DNA Functionalized Quantum Dots. *Journal of the American Chemical Society* 1999, *121*. 8122-8123, DOI: 10.1021/ja991662v.
2. H. C. Cheung, *Topics in Fluorescence Spectroscopy*. Plenum Press: New York, 1991; Vol. 2.
3. R. Sjoback, J. Nygren, M. Kubista, Absorption and fluorescence properties of fluorescein. *Spectrochimica Acta A* 1995, *51*. L7-L21.