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Supplementary Information

Quantitative Detection of Single DNA Molecules on DNA Tetrahedron Decorated Substrates

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EXPERIMENTAL SECTION

Materials

All oligonucleotides were synthesized and purified by Sangon (Shanghai, China). The sequences were shown Table S1. 3-glycidyloxypropyltrimethoxysilane (GOPS) was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Microscope cover glasses (22 × 22 mm) were purchased from Cole-Parmer (Illinois, USA). QDs605-streptavidin conjugates (QDs) were obtained from Invitrogen Co. (Eugene, OK, USA). Tween-20 from Sigma (St. Louis, MO) was used in the work. All other chemicals were of analytical grade. All chemicals were used without further purification. Human serum was provided by Shandong University Hospital. All solutions were prepared with Milli-Q water (resistivity = 18 MΩ cm) from a Millipore system.

Self-Assemble of Functional DNA Tetrahedron onto Glass Substrates

The epoxy-functionalized glass slides were prepared according to literatures.[1] DNA tetrahedron was synthesized self-assembly. Four DNA strands (A, B, C and D) were diluted into a final concentration of 50 μM with TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0). A quantity of 2 μL each strand was mixed with 42 μL of TM buffer (20 mM Tris, 50 mM $MgCl_2$, pH 8.0), then the mixture was heated to 95 ℃ for 2 min and cooled in ice bath. Next, 30 μL of the functional DNA tetrahedron (diluted into proper concentration with TM buffer) was added to the epoxy-functionalized glass substrates and allowed to react overnight in 37 ℃. Finally, the substrate was rinsed

with phosphate buffered saline (PBS) containing 10 mM PB buffer and 0.1 M NaCl pH 7.4. Then, dried lightly with N_2 for subsequent use.

Electrophoretic Analysis of DNA Tetrahedron

The functional DNA tetrahedron was analyzed using polyacryamide gel electrophoresis (PAGE, 12.5%) in TAE-Mg buffer at a constant current of 5 mA at 4 °C.

Contact Angle Measurement of DTDS

Contact angle measurement was performed on Tensionmeter-K121 processor (Krüss Company, Germany). The concentration of DNA tetrahedron on the glass was 1.0 μM. The glass substrates were immersed into and retracted from ultra-pure water medium.

Characterization of Nonspecific Adsorption

To characterize the ability of inhibiting nonspecific adsorption on DTDS, different concentrations of functional DNA tetrahedron (0, 0.1, 2.5, 5.0, 1.0, 2.0 μM) were bound to glass. To prepare BSA blocking substrate, PBS buffer containing 5% BSA was incubated with epoxy-functionalized glass substrates for 2 h in 37 °C. All substrates were washed with PBS buffer. A quantity of 50 μL QDs (diluted into 0.2 nM with PBS buffer) was added to it and allowed to react 0.5 h in 37 °C, then washed 3 times with PBS-T (10 mM PBS contains 0.05% Tween-20), PBS , and ultra-pure water, dried lightly with N_2 for subsequent use. Before fluorescence imaging with epi-fluorescence microscopy, 50 μL borate buffer (pH 8.0) was added.

Single-Molecule Detection of target DNA on DTDS

Target DNA detection was performed in the sandwich format. Before use, target DNA and detection DNA were heated to 95 ℃ and cooled down to room temperature slowly. The target DNA was first mixed with the biotinylated detection DNA (0.1 μM) in hybridization buffer (1 M NaCl and 10 mM TE buffer pH 7.4). Then 50 μL of the above mixture was added to DTDS. After 80 min of incubation in 37 °C, the substrate was rinsed 3 times with PBS, ultra-pure water and incubated with 50 μL QDs (diluted into 0.2 nM with PBS buffer) for 30 min in 37 °C. Next, the substrate was washed 3 times with PBS-T, PBS, ultra-pure water, and dried lightly with N_2. Before fluorescence imaging with epi-fluorescence microscopy, 50 μL borate buffer (pH 8.0) were added.

Epi-Fluorescence Microscopy Imaging

SMD imaging was performed with an Olympus IX81 fluorescence microscope (Tokyo, Japan) equipped with a high-numerical-aperture 60×(1.45 NA) oil-immersion objective lens, a mercury lamp source, a mirror unit consisting of a 470 to 490 nm excitation filter (BP470-490), a 505 nm dichromatic mirror (DM 505), a > 580 nm emission filter (IF580), and a 16-bit thermoelectrically cooled EMCCD (Cascade 512B, Tucson, AZ, USA). The images corresponding to various locations were acquired by manually moving the XY sample stage. Imaging acquisition and data analysis were performed using the MetaMorph software (Universal Imaging, Downingtown, PA, USA). In order to increase the sensitivity of the assay, the detected target DNA number should be as high as possible.[2] Therefore, 10 images on the substrate were acquired by moving the microscope stage in the x and y direction.

The sum of the bright dots on the 10 images was counted. The fluorescence ‘‘blinking’’ between the ON and OFF state of single QDs affected the capturing of the QDs on the substrate to the images, to address this, repeating images for one location were obtained (three images for each location were acquired).[1a,3] Finally, those images were treated with the software and the bright dots on the images were counted.

Calculation of the Dots Number in Theory and Detection Efficiency

The dots number in theory was calculated according to following equation

$$N_{theory} = C_{target} \times V \times N_A \times \frac{S_{image}}{S_{well}} \quad (1)$$

C_{target} is the concentration of target DNA; V stands for the volume of target DNA; N_A is Avogadro's constant (6.02×10^{23}); S_{image} is the actual area of every images, S_{well} is the area of single chamber on DTDS.

The radius of single chamber on DTDS is 2 mm. So the S_{well} can be calculated easily. The EMCCD has an imaging region of 512×512 pixels with each pixel of 16 μm×16 μm in size. The light intensity gradually decreased from the center outward. A 150×150 pixels subregion was selected in the method with the 60×objective and each pixel imaged 0.267 μm×0.267 μm in the object plane.[1b] Thus, the image with an area of 40 μm×40 μm on DTDS was used for single-molecule detection. The volume of target DNA was 50 μL. The detection efficiency (η) was obtained by following equation:

$$\eta = \frac{N_{detection}}{N_{theory}} \times 100\% \quad (2)$$

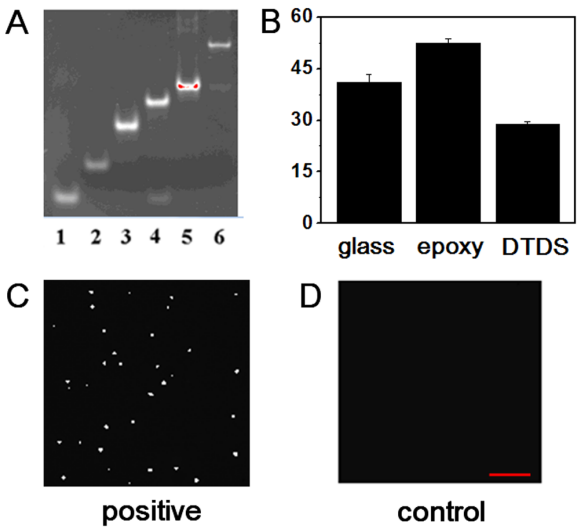

Fig. S1 Characterization of DNA tetrahedron and DTDS. (A) Electrophoretic analysis of DNA tetrahedron, 1-6 line stand for the electrophoretic image of sequence C (55 bp), A (80 bp), B+C (55bp + 55bp), A+C, B+C+D, A+B+C+D. (B) The contact angle measurement of different substrate, the Y axis stands for the contact angle value. (C) The fluorescence image of single-molecule DNA detection in the presence 100 fM target DNA. (D) The fluorescence image of single-molecule DNA detection in the absence of 100 fM target DNA, scale bar, 8 μm.

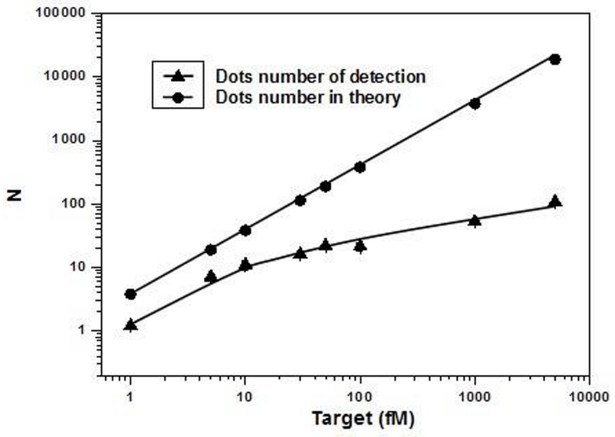

Fig. S2 Detection efficiencies of different target DNA concentrations. The data points (triangle) are actual target counts at various DNA concentrations, while the dotted line shows the theoretical numbers of target molecules. Condition: Each data point represents an average of 3 measurements (each error bar indicates the standard deviation).

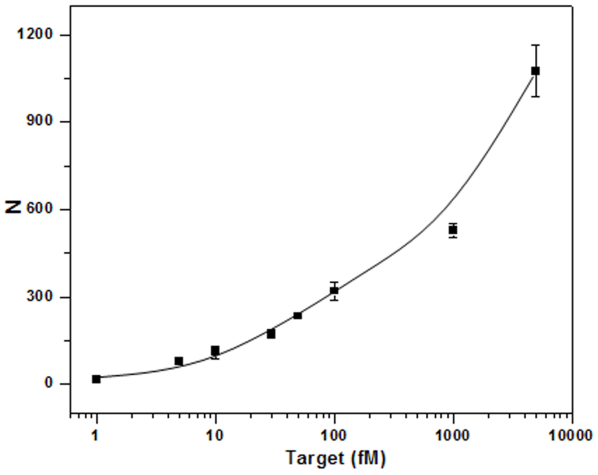

Fig. S3 The relationship between number of dots and concentrations of target DNA. Condition: Each data point represents an average of 3 measurements (each error bar indicates the standard deviation).

Table S1. Oligonucleotides Designed in the Present Study.

Name	sequence
A	5’-GTATCCAGTGGCTCATTTTTTTTTTACATTCCTAAGTCTGAA ACATTA CAGCTTGCTACACGAG AAGAGCCGCCATAGTA-3’
B	5’-NH2-C6-TATCACCAGGCAGTTGACAGTGTAGCAAGCTGTAA TAGAT GCG AGG GTCCA ATAC-3’
C	5’-NH2-C6-TCAACTGCCTGGTGATAAAACGACACTACGTGGGA ATCTAC TATGGCGGCTCTTC-3’
D	5’-NH2-C6-TTCAGACTTAGGAATGTGCTTCCCACGTAGTGTCGT TTGT ATTGGACCCTCGCAT-3’
target DNA	5’-TGAGCCACTGGATACCAAGAGCATTACTAGCATGC-3’
detection DNA	5’-biotin-GCATGCTAGTAATGCTCTTG -3’
1B-M-DNA	5’-TGAGCCTCTGGATACCAAGAGCATTACTAGCATGC-3’
3B-M-DNA	5’-TGAGCCTGAGATACCAAGAGCATTACTAGCATGC-3’

Table S2. Comparison of Methods for Single DNA molecules Detection.

method	strategy	detection limit
in solution		
ref 4	optical fiber probe excited	2.5 nM
ref 5	YOYO-1 specific labeled double strands DNA	5.0 pM
ref 6	dual-color fluorescence coincidence detection	20 fM
on substrate		
ref 7	adsorption on poly(L-lysine) coated glass substrate	16 fM
ref 2	electrochemical adsorption accumulation	3.0 fM
ref 8	capture by BSA and ethanolamine blocking substrate	10 fM
this work	immobilization on DTDS	1.0 fM

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