Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information for

Visualization of the Intracellular Location and Stability of DNA Origami with a Label-free Fluorescent Probe

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Experimental section

Materials. All oligonucleotides were purchased from Integrated DNA Technologies (Coralville, IA) or Invitrogen (Shanghai, China). The origami staple strands were stored in 96-well plates with concentrations normalized to 100μM, and were used without further purification. The concentration of each strand was estimated by measuring the UV absorbance at 260 nm. M13mp18 single-stranded DNA (N4040S) was purchased from New England Biolabs, Inc (Beijing, China). Cyanine fluorescent probe was synthesized following the protocol in the literature (X. J. Feng, et al *Org. Lett.* 2010, 12, 2194.).

Self-assembly of DNA origami. Tubular shaped DNA origami nanostructures were assembled according to Yan's methods (L. A. Stearns, H. Yan et al, *Angew. Chem. Int. Ed*., 2009, **45**, 8494.). A molar ratio of 1:10 between the long viral ssDNA M13mp18 (5nM) and the short helper strands (unpurified) was used. DNA origami was annealed and assembled in 1× TAE-Mg^{2+} buffer (Tris, 40 mM; Acetic acid, 20 mM; EDTA, 2 mM; and Magnesium acetate, 12.5 mM; pH 8.0) in an Eppendorf thermocycler (Eppendorf China) by slowly cooling from 90 °C to room temperature over 12 h for the origami..

Cyanine probes incorporation of DNA origami. Cyanine probe solution (25μM) was incubated with the DNA origami structures (tube, 5nM) for 24 hours. Before cellular incubation, excess helper strands and cyanine fluorophores were removed by using Amicon centrifuge filters YM-100 (Millipore, Beijing).

Characterization of DNA origami and origami-probes. Agarose gel electrophoreses was performed at room temperature (1%, pre-stained with ethidium bromide or not) and the images were collected by a gel imaging system (Yunyi, Beijing). AFM imaging of DNA nanostructures before and after cyanine probing molecules mixing is performed in tapping-in-buffer mode. Here, for both DNA origami structures and probe loaded DNA origami structures, 5μl of sample was deposited on mica and left to absorb to the surface for 20 min. The sample was subsequently washed with ddH_2O 3 times, and TAE/Mg^{2+} buffer was added for imaging (Technical Institute of Physics and Chemistry, CAS, Beijing, China).

Fluorescence detection of DNA origami by cyanine probe.

The solutions of cyanine probes (2.5μM) and tubular shaped DNA origami (0.5nM) were mixed thoroughly and incubated for 24hours at room temperature. Then the DNA origami-probe complex

were incubated with DNase I (20unit/mL), an endonuclease that nonspecifically cleaves DNA to release di-, tri- and oligonucleotide products with 5′-phosphorylated and 3′-hydroxylated ends, for 30min at 37°C. The free probe (2.5μM), origami-probe complex and DNase I digested origami-probes were detected the fluorescence emission by fluorescence spectrometry (HORIBA JOBIN YVON, FluoroMas-4 Spectrofluorometer) and imaged under blue light by a gel imaging system (CLiNX Science Instruments). Each sample was tested under the same conditions. The excitation wavelength is 460nm, resulting from excitation spectrum of cyanine probes. All the fluorescence spectral measurements were carried out at room temperature.

Cells. MCF 7, a human breast adenocarcinoma cancer cell line, was purchased from the Cell Center at the Institute of Basic Medical Sciences Chinese Academy of Medical Science. The cells were cultured in Dulbecco's Modified Eagle Medium, (Hyclone, Thermo Scientific), supplemented with 10% fetal bovine serum (Hyclone, Thermo Scientific), and with L-glutamine, penicillin, streptomycin (GIBICO, Invitrogen). Cells were cultured in 100 mm^2 dishes to ~ 80% confluency and incubated in an atmosphere of 5% CO2 at 37°C.

Intracellular location of DNA origami-probes. MCF 7 cells were seeded in confocal dishes and cultured over-night, then incubated with purified 0.5nM tube origami-probes for 12 hours. The living cells were then incubated with Hoechst 33342 (Dojindo, 25μg/ml) and LysoTracker Red (Invitrogen, 1μM) for 20 minutes at 37 °C for nuclei and lysosome labeling. After washing with PBS, all the cell samples were visualized by laser confocal fluorescent microscopy (Olympus). The excitation wavelengths were taken upon at 405 nm (for Hoechst 33342), 458nm (for cyanine probes incorporated with DNA origami) and 559nm (for LysoTracker Red), while the lasers power were 4%, 100% and 4%, respectively.

Intracellular stability of DNA origami-probes. MCF 7 cells were seeded in confocal dishes and cultured over-night, then incubated with purified 0.5nM DNA tube-probe complex. After 12 hours origami-probe administration, the cells were washed by PBS and then cultured for extra 0, 24, 48, 60 or 72 hours. After washing with PBS, the cells were visualized by laser confocal fluorescent microscopy (Olympus). The excitation wavelengths were taken upon at 458nm for DNA origami binding with cyanine probes, while the laser power was 100%. Quantitative analysis of the images was accomplished by using Image J.

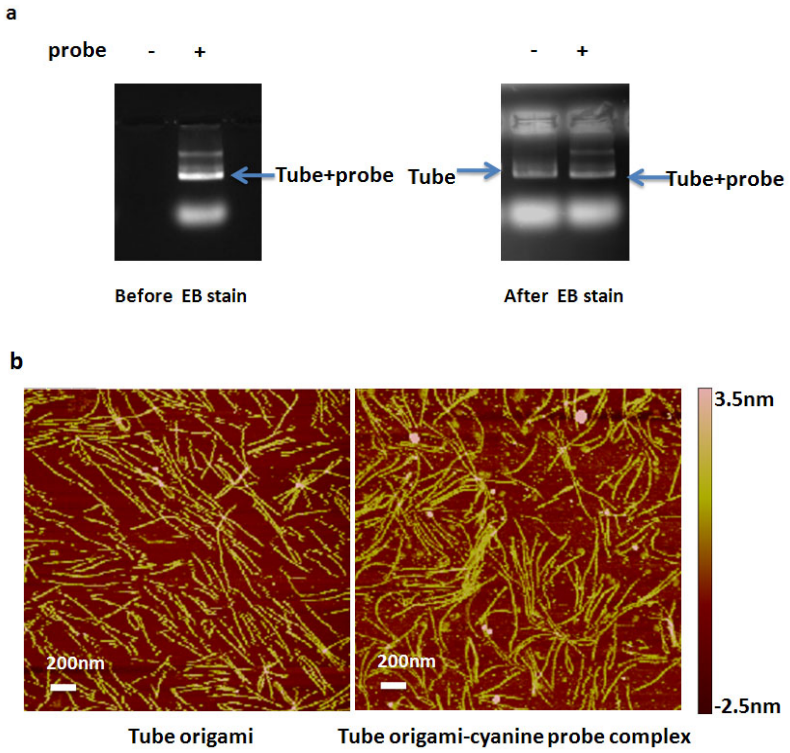

Figure S1. Cyanine probes incorporation of DNA origami. (a)After loading cyanine probes, the tubular DNA nanostructures exhibited strong fluorescence emission (EB-free gel, excitation wavelength at ~450nm) and slight shifts in mobility (EB stained gel) indicating that the probing molecules were incorporated in the DNA. Before and after mixing with probe, AFM images provided direct evidence that the morphology of the DNA nanostructures was retained after interaction of the cyanine molecules.

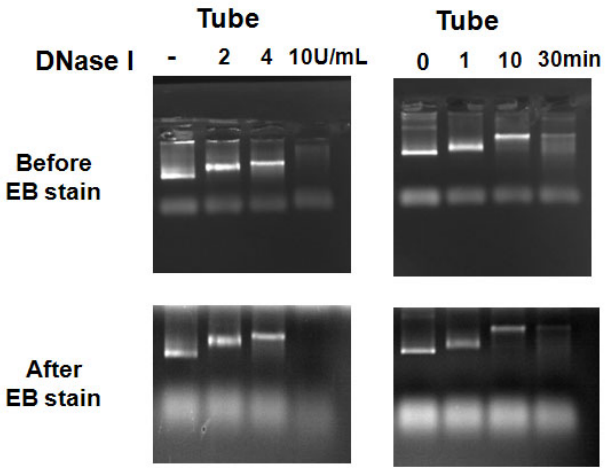

Figure S2. Gel electrophoresis of DNA origami after incubation with DNase I at different conditions. For DNase I digestion assay, different concentration from 0~10Unit/mL and incubation time from 0~30min were performed. The results confirmed the tubular origami nanostructures degradation, which lead to the cyanine probing molecules changed to more efficient intramolecular rotational motions states.

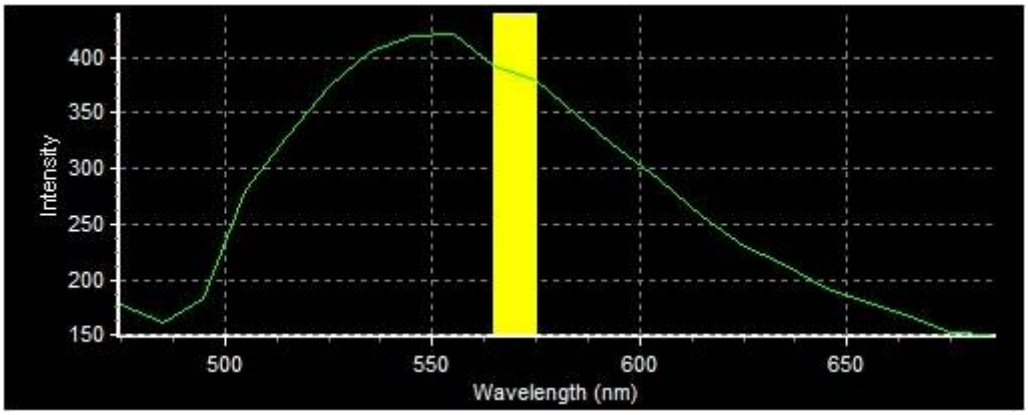

Figure S3. Intracellular fluorescence intensity of DNA origami- probe complex. The green-yellow fluorescence was visible inside cells treated with origami-cyanine probes, and the intracellular fluorescent spectrum was detected by confocal microscopy. The intracellular fluorescence emitted with maximum emission wavelength at about 560nm, as same as the fluorescence spectrum of DNA origami-probe in the figure 2.

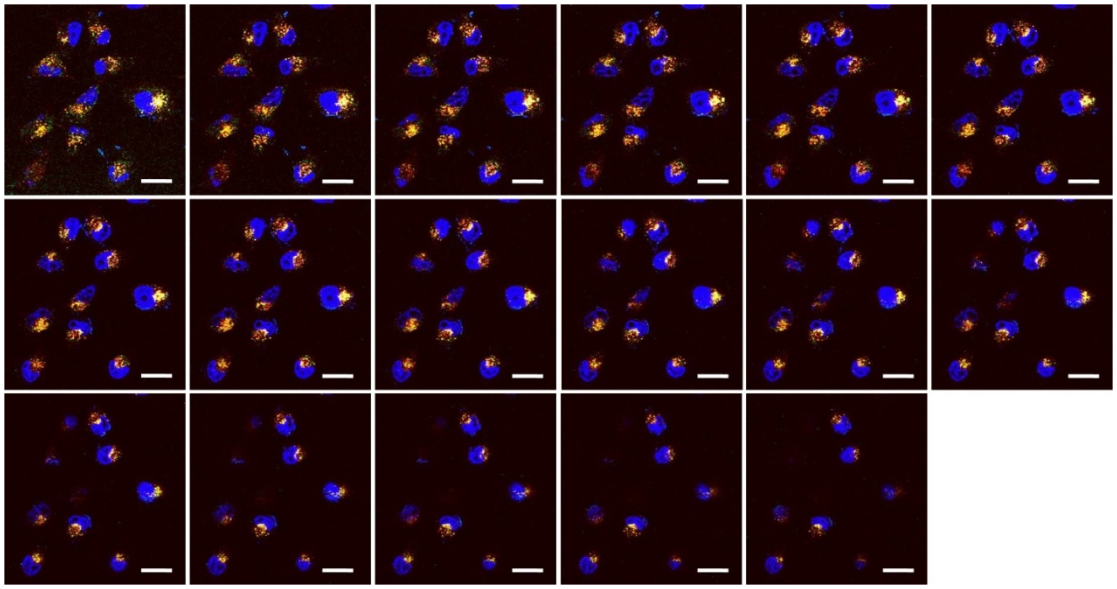

Figure S4. Z-scanning images of MCF 7 cells administrated with DNA origami-cyanine probes. The images were taken every 0.5μm section from the top to the bottom of intact cells. The results confirmed that the green-yellow fluorescence was emitted from the cyanine probing molecules incorporated within DNA origami nanostructures inside the MCF 7 cells.