Supporting information for

Observation of intracellular interactions of DNA origami and lysosomes by fluorescence localization microscopy

Meifang Fu, [+] Luru Dai, [+] Qiao Jiang, Yunqing Tang, Xiaoming Zhang, Baoquan Ding* and Junbai Li*

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 100 μM, 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). YOYO-1 was purchased from life technology. Methyl viologen, ascorbic acid, glucose oxidase and catalase were all purchased from Sigma Aldrich.

Self-assembly of DNA origami. Tube DNA origami structures were assembled according to Yan's methods.[1] A molar ratio of 1:10 between the long viral ssDNA M13mp18 (10nM) and the short helper strands 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.

Characterization of DNA origami. AFM imaging of DNA nanostructures was performed in tapping-in-buffer mode. 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).

Cell culture. NIH 3T3 cells, a mouse embryonic fibroblasts 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% newborn calf serum. NIH 3T3 cells were cultured in confocal culture dishes in an

atmosphere of 5% CO_2 at 37°C.

Confocal laser scanning microscopy. NIH 3T3 cells were seeded in confocal dishes. The living cells were incubated with LysoTracker Red (Beyotime Institute of Biotechnology, 10 μM) for 1 minutes at 37 °C for lysosome labeling. After washing with PBS, the cells were incubated with purified 10 nM YOYO-1 labeled tube origami for 4 hours. Then the cells were visualized by laser confocal fluorescent microscopy (Olympus) with a 100×1.3 NA oil immersion objective (PlanApoN, Olympus). The excitation wavelengths were taken upon at 488 nm (for YOYO-1) and 561nm (for LysoTracker Red).

TIRF fluorescence microscope. Our custom built setup is based on an Olympus IX-81 inverted microscope body (Olympus) with a 150×1.45 NA oil immersion objective (PlanApoN, Olympus). We use an AOTF (AOTF nC-VIS-TN 450–700 nm, AA Opto-Electronic Inc.) to control the throughput of two continuous wave laser sources, a 488 nm laser (Sapphire 488-150 CW, Coherent) and a 561 nm diode laser (Sapphire 561-200 CW; Coherent). TIRF was achieved by repositioning the laser beam from the center to the rim on the back aperture of the objective using a motorized mirror. Images were recorded with an EMCCD camera (iXon, ANDOR) cooled to − 80 °C using a pixel resolution of 512 × 512 pixels.

BALM measurement. For BALM measurements the 488 nm laser was used for excitation. Fluorescent emission is filtered with a dichroic mirror (FF509-FDi01, Semrock), in combination with a longpass filter (BLP01-488R, Semrock). Series of 2000 TIRF images (power of 488 nm laser: 100 mW, exposure time: 10 ms, pixel size: 66 nm) were recorded and subsequently analyzed for calculation of the fluorescence localization image.

The coverslips (24 × 60mm,Thermo scientific) were cleaned using Alconox detergent, sonicated in 1M NaOH, ethanol, and 1M NaOH sequentially before being rinsed with MilliQ water and flame dried. The coverslip was mounted in a home-made holder and the holder was mounted on the microscope.

YOYO-1 can pre-label the DNA structures or be added into the imaging buffer. The procedure to image the pre-labeled DNA structures is described as followed. 6 μL Tube DNA origami nanostructures (10 nM) were incubated with 3 μL YOYO-1 (10 μM) in 50 μL TAE-Mg for 2 hours at 40 ^{o}C.[2] Then the mixtures were ultrafiltrated to separate spare staple strands and dyes. YOYO-1 labeled origami nanostructures were allowed to adsorb on the cleaned cover slip. Then the

imaging buffer (Tris-HCL 50 mM, NaCl 50 mM, EDTA 1mM, Methyl viologen 1 mM, Ascorbic acid 10 mM) was added into the chamber. Image acquisition was started immediately thereafter.

YOYO-1 can also be added into the imaging buffer. 1 µL ultrafiltrated triangle DNA origami nanostructures (10 nM or 0.25 nM) were allowed to adsorb on the cleaned coverslip. Then 250 µL imaging buffer (YOYO-1 0.1 nM, Tris-HCL 50 mM, NaCl 50 mM, EDTA 1mM, Methyl viologen 1 mM, Ascorbic acid 10 mM) was added into the chamber. Image acquisition was started immediately thereafter.

Fluorescence localization imaging of DNA origami and TIRF imaging of lysosomes in 3T3 cells. 488 nm laser was used to excite YOYO-1 and 561 nm laser was used to excite LysoTracker Red. Fluorescent emission is filtered with a multiband dichroic mirror (Di01-R405/488/561/635-25x36, Semrock), in combination with a bandpass filter (FF01-446/523/600/677-25, Semrock). Series of 2000-2500 TIRF images of DNA origami were captured and subsequently analyzed for calculation of the fluorescence localization image. (Power of 488 nm laser: 60 mW, exposure time: 10 ms, Pixel size is 106 nm). TIRF images of lysosomes were acquired at the same time (Power of 561 nm laser: 20 mW, exposure time: 20 ms).

YOYO-1 labeled tube DNA origami was added into 3T3 cells and incubated for different times. 2 µL LysoTracker Red (Beyotime Institute of Biotechnology, 0.1 mM) was added to confocal culture dish that contained NIH 3T3 cells and 1 mL DMEM with 10% newborn calf serum. After incubation for 30 sec, the medium was replaced by 1 mL PBS buffer and washed 3 times every 2 minutes. Then the PBS buffer was replaced by 4% PFA (paraformaldehyde) and the immobilization time was 10 minutes. After fixation, the cells were rinsed with PBS for 3 times and the interval was 2 minutes. Then the PBS buffer was replaced by imaging buffer for fluorescence localization imaging (30 µL methyl viologen (50 mM), 75µL ascorbic acid (200 mM), 50 µL enzyme solution (20 µg/ml catalase, 4 mM Tris(2-carboxyelthyl) phosphine hydrochloride, 50% glycerin, 25 mM KCl, 20 mM Tris-HCl (pH 7.5), 1 mg/mL glucose oxidase), 400 µL glucose solution (100 mg/mL glucose, 10% glycerin) and 545 µL PBS). The oxygen scavenging system is not necessary in this experiment. The imaging buffer was added until the chamber was completely filled and sealed with a coverslip. Image acquisition was started immediately thereafter.

Image reconstruction. For each image stack a reconstructed image was both reconstructed

by SNSMIL[3] and FALCON[4]. The algorithm of SNSMIL is based on the principle of noise source, namely shot noise or Poisson noise of an image acquired with an EMCCD camera. SNSMIL introduces a newly defined quality metric, Q_{SNSMIL}. All other settings in SNSMIL are dictated by either the used equipment or the used fluorophore. FALCON is an algorithm for high-density super-resolution microscopy which combines a sparsity–promoting formulation with a Taylor series approximation of the PSF. The algorithm is designed to provide unbiased localization on continuous space and high recall rates for high-density imaging. The principle and the implementation of SNSMIL and FALCON were described in published paper[3, 4].

Quantitative analysis. The proportion of colocalization between tubes and lysosomes were analyzed by Image J. The particle analysis method is described online in detail.[5] We first run the whole analysis process on image of tubes on the glass reconstructed by SNSMIL to determine the analysis conditions.

Control experiment was carried out to confirm the value of Q_{SNSMIL} of the 488 nm channel to reduce the interference from background and the color crosstalk. Dual-color imaging was carried out without adding DNA origami. Make use of the fact that the signals from YOYO-1 was stronger than the intensity of auto fluorescence, Q_{SNSMIL} was raised to reduce interference signal and it was found that when Q_{SNSMIL} = 10 most of interference signals could be eliminated (Fig. S3a, b).

The image was first converted to binary image by automatically color threshold and type changing. To reduce the influence of discontinuous point in structures that will increase the numbers of tubes, the binary image was processed by “Close” (a function in Image J) to make the structures more intact (Fig. S4a, b). Then tubes were counted to work out numbers and areas of them. We defined an area threshold to remove dispersed signals. The area distribution of tubes was obtained without area limitation (Fig. S4c), which shows that the numbers of tubes increase significantly when area less than 64 pixels. Therefore, “64 pixels” was used as area threshold in this analysis system. Based on the former image processing, tubes were delineated and counted effectively (Fig. S4d, e).

The area threshold and processing procedure were used to analyze the combined dual-color images. In the combined images, colors of tubes (green) and lysosomes (red) were overlapped to generate yellow. The combined images of tubes and lysosomes were converted into binary images based on a selected colocalized sample (5a, b) and the standard was applied to all

images analyzed. Then the particle number in cell that indicated colocalization of DNA origami and lysosomes was obtained. The corresponding fluorescence localization images of tubes were processed in the same way to generate number of tubes in cell. Finally, the proportions of colocalization were obtained and averaged to give the final result (Table. S1).

The colocalization analyze applied to FALCON images was almost the same except two changes. Firstly, we adjusted the area threshold based on image of tube origami on the glass reconstructed by FALCON (Fig. 2g); secondly, we did not process FALCON images by processing method of "Close" because relatively high density structures were reconstructed. Although FALCON and SNSMIL use different data processing methods, the colocalization ratio obey the same trend. The corresponding colocalization ratio of FALCON images was obtained (Table. S1) and both results reconstructed by SNSMIL and FALCON show that tubes were captured as well as degraded by cells with time.

References:

1 L. A. Stearns, H. Yan et al, *Angew. Chem. Int. Ed.,* 2009, **45**, 8494.

2 C. Carisson, M. Johnson and B. Åkerman, *Nucleic Acids Research*, 1995, **23**, 2413.

3 Y. Tang, L. Dai, X. Zhang, J. Li, J. Hendriks, X. Fan, N. Gruteser, A. Meisenberg, A. Baumann, A. Katranidis and T. Gensch, *Sci. Rep.,* 2015, **5**, 11073.

4 J. Min, C. Vonesch, H. Kirshner, L. Carlini, N. Olivier, S. Holden, S. Manley, J. C. Ye and M. Unser, *Sci. Rep.,* 2014, **4**, 4577.

5 Online Manual for the WCIF, http://www.uhnresearch.ca/facilities/wcif/imagej/particle-_analysis.htm#particle_auto, (accessed December 2015).

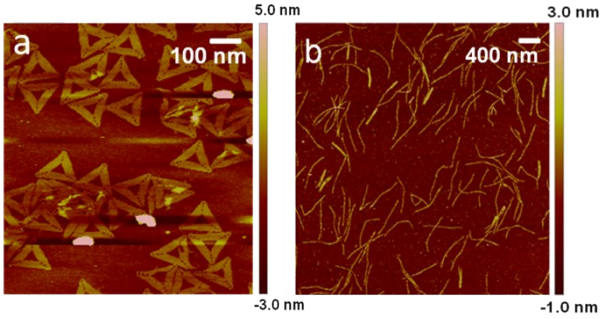

Fig. S1 (a) AFM image of triangle DNA origami nanostructures; (b) AFM image of tube origami nanostructures.

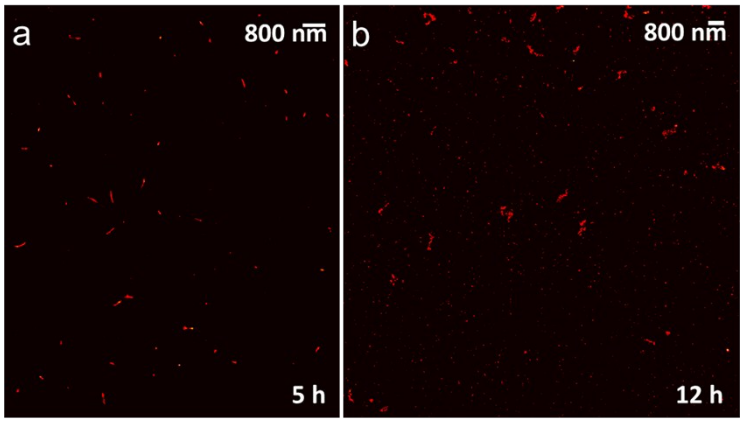

Fig. S2 Corresponding fluorescence localization images of the tube origami in cell culture medium reconstructed by FALCON. (a) Tube origami in cell culture medium for 5 hours. (b) Tube origami in cell culture medium for 12 hours.

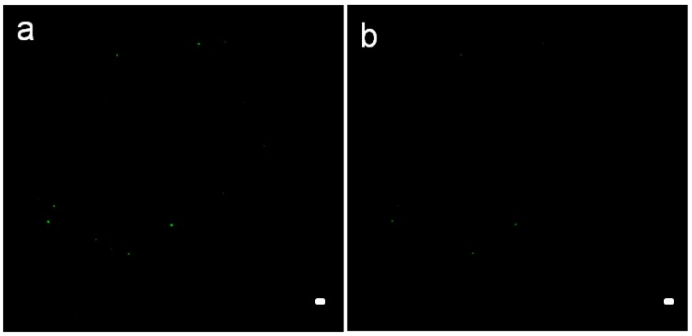

Fig. S3 Control experiments to confirm the value of Q_{SNSMI}. To confirm the value of Q_{SNSMI} of 488 nm channel, dual-color imaging was carried out without adding DNA origami.(a) Q_{SNSMI} = 10; (b) Q_{SNSMI} = 12. When Q_{SNSMI} = 10 most of interference signals could be eliminated. Scale bars represent 800 nm.

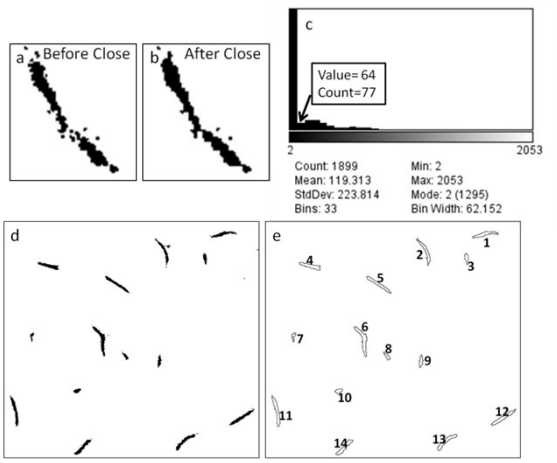

Fig. S4 Data processing of the fluorescence localization image of tube DNA origami to generate the numbers of tubes. (a, b) Single tube before and after the processing of “close”. After close, the structure is more contact and make the particle analyzing process more reliable; (c) The area distribution of tubes without area threshold. When area less than 64 pixels, tube number increase significantly due to scatter plots. So we set the area threshold (64 pixels) to remove those scatter plots; (d) Fluorescence localization image of tube DNA origami after being processed; (e) Reconstructed outlines and numbers of tubes shown in (d) by Image J.

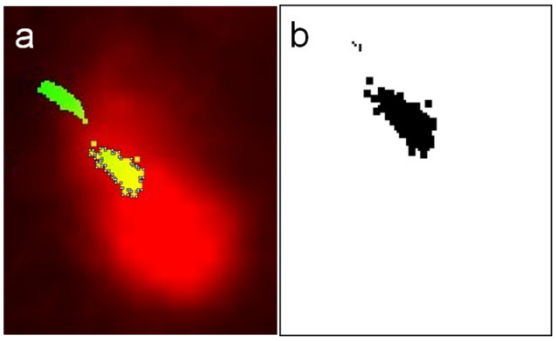

Fig. S5 Selected sample to extract colocalization information. (a) Combined image of fluorescence localization image of DNA origami (green) and TIRF image of lysosomes (red). The overlap of green and red was selected by the polygon (white square); (b) The extracted binary image of colocalization location.

24 h	SNSMIL			FALCON		
samples	overlap	tube	Ratio	overlap	tube	Ratio
1	12	157	7.64%	23	459	5.01%
2	81	145	55.86%	92	134	68.66%
3	29	82	35.37%	95	115	82.61%
4	146	269	54.28%	172	205	83.90%
5	22	116	18.97%	25	397	6.30%
Average	58	153.8	34.42%	81.4	262	49.30%
4 h						
samples	overlap	tube	Ratio	overlap	tube	Ratio
1	29	309	9.38%	25	567	4.41%
2	32	145	22.07%	20	224	8.93%
3	74	520	14.23%	22	408	5.39%
4	23	531	4.33%	34	283	12.01%
5	9	560	1.61%	16	552	2.90%
Average	33.4	413	10.32%	23.4	406.8	6.73%

Table. S1 Proportions of colocalization of tube DNA origami and lysosomes in different cells and different incubation times. The table showed results of both SNSMIL images and FALCON images. It is shown that the ratios vary in different cells in the same incubation time because of different conditions of cells. The proportions of colocalization of SNSMIL images in 4 hours and 24 hours are 10.32% and 34.42% respectively. Correspondingly, the colocalization ratio of FALCON images in 4 hours and 24 hours are 6.73% and 49.30%. Although different conditions of cells, it is interesting to notice that average tube number of 4 h is more than that of 24 h. In the condition of SNSMIL, the average tube number of 4 h is 2.68 times of the average value of 24 h and the ratio turns to 1.5 in the condition of FALCON. Although applying different algorithm, SNSMIL and FALCON both show that tubes were captured as well as degraded by cells with time.

Supplementary Movies:

Mov. S1 The blinking of YOYO-1 when the free dyes binding triangle DNA origami nanostructures

Mov. S2 The blinking of YOYO-1 when reducing the concentration of triangle DNA origami nanostructures.

Mov. S3 The blinking of YOYO-1 labeling tube DNA origami nanostructures in vitro for fluorescence localization imaging

Mov. S4 The blinking of YOYO-1 labeling tube DNA origami nanostructures in NIH 3T3 cells for 24 h (488 nm)