Electronic Supplementary Material (ESI) for Nanoscale.
This journal is © The Royal Society of Chemistry 2014

Supporting Information

Tempo-spatially Resolved Cellular Dynamics of Human Immunodeficiency Virus Transacting Activator of Transcription (Tat) Peptide-modified Nanocargos in Living Cells

Lin Wei, Qiaoyu Yang, and Lehui Xiao*

Key Laboratory of Chemical Biology & Traditional Chinese Medicine Research, Ministry of Education, College of Chemistry and Chemical Engineering, Hunan Normal University, Changsha, Hunan, 410081, P. R. China,

*Corresponding author

E-mail: lehuixiao@gmail.com

EXPERIMENTAL SECTION

Chemicals and Materials.

$HAuCl_4 \cdot 3H_2O$, cetyltrimethylammonium bromide (CTAB), ascorbic acid (AA), $NaBH_4$, and NaCl were purchased from Sinopharm Chemical (Shanghai, China).

Dynasore, genistein, Bis-(p-sulfonatophenyl)phenylphosphine dehydrate dipotassium (BSPP), 4, 7, 10, 13, 16, 19, 22, 25, 32, 35, 38, 41, 44, 47, 50, 53-Hexadecaoxa-28, 29-dithiahexapentacontanedioic acid di-N-succinimidyl ester (SH-PEG-NHS, $C_{46}H_{80}N_2O_{24}S_2$, MW:1109) and O-[2-(3-Mercaptopropionylamino)ethyl]-O′-methylpolyethylene glycol (SH-PEG-CH_3, $CH_3O(CH_2CH_2O)_nCH_2CH_2SH$, MW: 5000) were obtained from Sigma-Aldrich (St. Louis, MO, U.S.A.). NH_3-Tat peptide (sequence, YGRKKRRQRRR) and NH_3-Tat-FITC were purchased from AnaSpec. (San Jose, CA, U.S.A.). All other chemicals not mentioned here were purchased from Sigma-Aldrich.

Gold Nanoparticles Fabrication and Characterization.

The spherical gold nanoparticles used in this experiment were synthesized based on a seed-mediated growth method.[1] The seed gold nanoparticles were fabricated as follow. In brief, 1.066 mL of 2.346×10^{-2} M $HAuCl_4$ was mixed with 98.934 mL of DI H_2O, and then the mixture was heated to 110 °C for 5 min. 10 mL of 1.455×10^{-2} M trisodium citrate was rapidly injected to the boiling solution. The mixture was vigorously stirred and refluxed for 20 min. The color of the mixture would gradually change after 5 min from colorless to pale red, pale purple, and finally to the color of wine red. After the color change process was complete, the colloidal solution was kept stirring at room temperature for another 15 min until the solution was cooled down. The size of gold nanoparticles made by this method is around 18.6 ± 1.2 nm. To synthesize larger size gold nanoparticles (60 ± 2.5 nm), the reduction process was repeated again with 18 nm gold nanoparticles as the seeds. Typically, 10 mL of the seed gold nanoparticle solution was diluted to 120 mL with the final trisodium citrate concentration of 3.74×10^{-3} M. The mixture was then heated to 110 °C and followed by gradually adding 24 mL of 2.346 ×

10^{-3} M $HAuCl_4$ to the hot solution. After the reaction was complete (around 25 min), the solution was gradually cooled down to room temperature and stored at 4 °C before usage. The size of these gold nanoparticles were characterized by UV-vis absorption spectrum and transmission electron microscopy (TEM) (JEM 1230, JEOL, Japan), Figure S6.

Preparation of Tat Peptide-modified Gold Nanoparticles (TGNPs).

The conjugation protocol for Tat peptides is similar to previous procedures.[1] Briefly, 2 mL of gold nanoparticle stock solution was centrifuged at 6000 rpm for 10 min to remove the extra chemicals in the solution. The pellet was dispersed in 150 μL of 1 mg/mL BSPP solution. To graft Tat peptides onto the nanocargo surface, 40 μL of borate buffer (50 mM, pH 8.2) and 1.2 μL of SH-PEG-NHS (20 mM) were firstly mixed together with 0.8 μg of Tat peptides (0.2 mg/mL) with gentle stirred for 3 h. After that, the mixture was introduced to the nanocargo solution and left to react for additional 3 h. In order to increase the stability of the nanocargo in salt solution, 5 μL of 20 mM SH-PEG-CH_3 was added to the mixture and kept stirred for another 5-8 h. Those unreacted chemicals were removed by centrifugation for three times. The TGNPs were suspended in 200 μL of deionized water and stored at 4 ^{o}C prior to usage.

The density of Tat peptides on the surface of gold nanoparticles was quantified by the fluorescence-based assay. In this assay, gold nanoparticles were modified by FITC conjugated Tat peptides with other conditions been the same. After the modification process, the FITC labeled Tat peptides were stripped from the surface of gold nanoparticles by dithiothreitol (DTT, 0.1M) in 0.5× PBS buffer. The concentration of FITC labeled Tat peptides in the solution was estimated based on the concentration calibration curve. The concentration of gold nanoparticles was determined by UV-vis

absorbance (the molar extinction coefficient of 60 nm gold nanoparticles is 3.96×10^{10} $M^{-1}cm^{-1}$). Thus the density of Tat peptides on the surface of gold nanoparticle can be readily quantified through dividing the amount of Tat peptides by the number of gold nanoparticles in the solution. Herein, the density was estimated to be 0.35±0.06 per nm^2.

Cell Culture.

Cervical cancer HeLa cells were obtained from American Type Culture Collection (ATCC, USA). HeLa cells were cultured on a cleaned glass slide in a plastic cell culture dish. Before the uptake and biochemical inhibitor studies, the cells were maintained in Dulbecco's modified Eagle's medium (DMEM, GIBCO) supplemented with 10% fatal bovine serum (GIBCO) at 37 °C/5% CO_2 in a humidified atmosphere.

TGNPs Uptake and Biochemical Inhibitor Studies.

In a typical uptake experiment, 20 μL of freshly prepared TGNPs stock solution was mixed with 200 μL of serum-free DMEM and then incubated together with HeLa cells. It should be noted that before the uptake experiments, the culture medium were replaced with fresh serum-free DMEM.

For the biochemical inhibitor studies, HeLa cells were firstly treated with Dynasore (80 μM), or genistein (200 μM), or both of them in serum-free DMEM at 37 °C/5% CO_2 for 1 h. After this treatment, the cells were supplemented with fresh serum-free DMEM and co-incubated with TGNPs and drugs for additional 2 h.

Imaging of TGNPs with Dual Wavelength View Darkfield Microscope (DWVD).

The darkfield imaging experiments were performed on a Nikon Eclipse Ni-U upright optical microscope (Japan). The halogen lamp was replaced by a Y-shaped optical fiber coupled with two DPSS lasers (445 nm and 532 nm, CNI laser Changchun,

China). Two continuously variable neutral density filters were mounted in front of those two lasers to precisely adjust the intensity ratio between those two channels. The mixed laser line was focused onto the sample obliquely via an oil immersion darkfield condenser (NA 1.43-1.20). Scattered light from the sample was collected using a 60× objective and then split by a dual wavelength view modality with a dichroic mirror (Optosplit II, Andor, Northern Ireland). This system is more convenient than our previously used spectral channel splitting device. There is no image distortion between these two channels. More importantly, with this device, there is no limitation for the choice of light source. One can readily select two different wavelengths with a suitable dichroic mirror. The images from 445 nm and 532 nm channels were simultaneously captured by an sCMOS camera (Orcaflash 4.0, Hamamatusu, Japan, the data acquisition rate in our experiments is set to 100 Hz for dynamic tracking) within a single frame and then the images from different channels could be extracted by splitting the whole image into two parts. The images were processed and analyzed with Image J (http://rsb.info.nih.gov/ij/) and Matalab.

The diffusion trajectories of TGNPs on cell membrane were analyzed with feature point tracking algorithm.[2] The tracking process requires no a priori mathematical modeling of the motion. The detail procedures for image processing can be found in supporting information ref. 2. With this imaging setup (the pixel size of the sCMOS camera is 6.5 μm × 6.5 μm, that is in the image plane each pixel represents an area of 108 nm × 108 nm), the temporal localization accuracy is comparable to results in our previous work (better than 3 nm), ref. 3.

Determination of Gold Content.

HeLa cells were incubated with TGNPs in serum-free cell culture medium for 1 h. The content of nanoparticles remained in the culture medium was then determined by digesting them with aqua regia for 6 h. Then the solution was heated at 80 ^{o}C for half hour, and the gold content was determined by atomic absorption spectrum. Those TGNPs loaded HeLa cells were supplemented with fresh DMEM. The metabolic kinetics of TGNPs by HeLa cells could then be determined by monitoring the content of gold remained inside HeLa cells and excreted into the culture medium separately.

SUPPORTING FIGURES

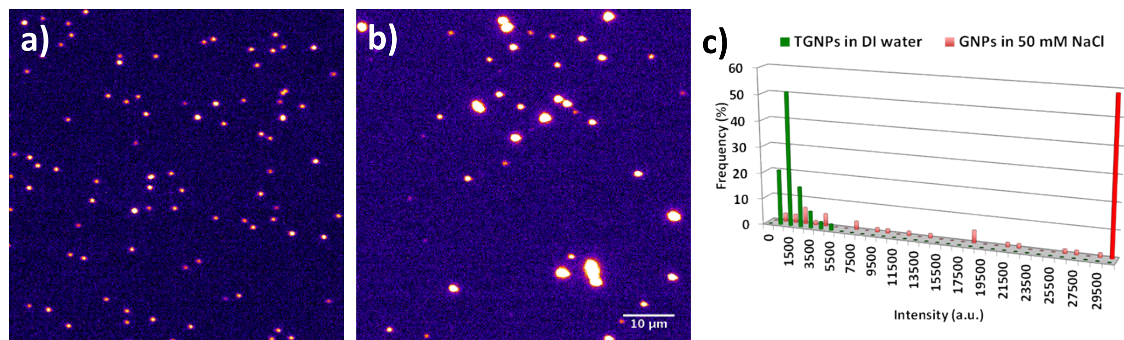

Figure S1. Typical darkfield images of TGNPs in DI water a) GNPs in 50 mM NaCl b). c) the corresponding intensity distributions of TGNPs in DI water and GNPs in 50 mM NaCl solution.

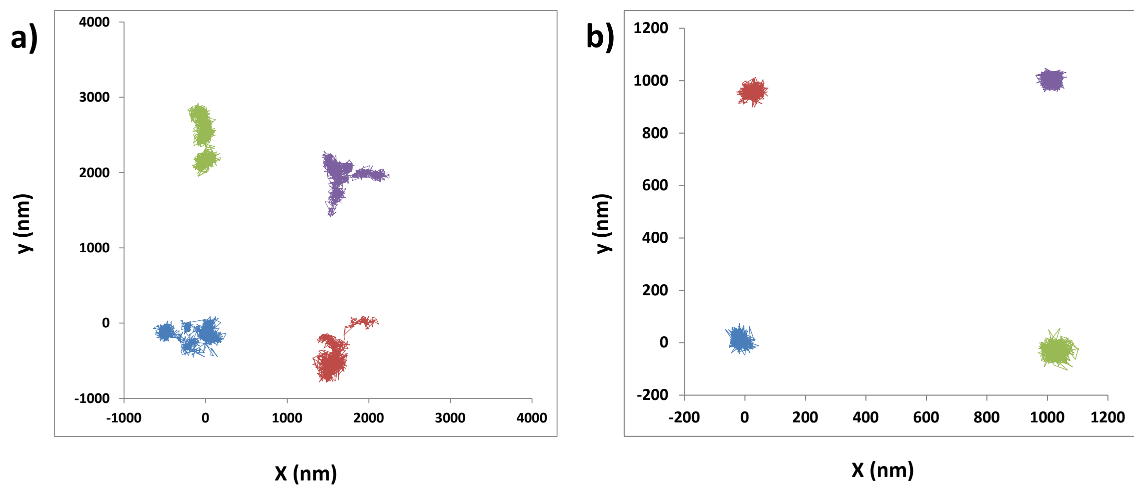

Figure S2. Representative trajectories of TGNPs (a) and CTAB-modified GNPs (b) on cell membrane.

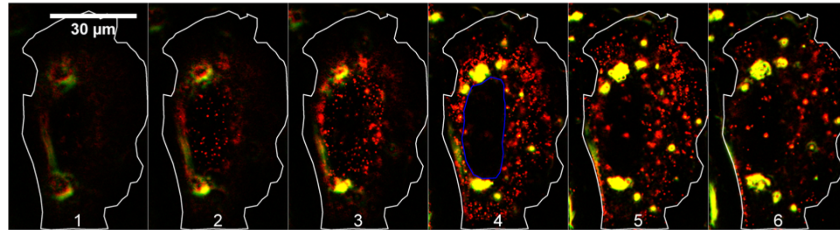

Figure S3. Color-coded three dimensional section images of HeLa cell co-incubated with TGNPs at 37 ^{o}C for 1 h. The stepping size from top to down is around 300 nm.

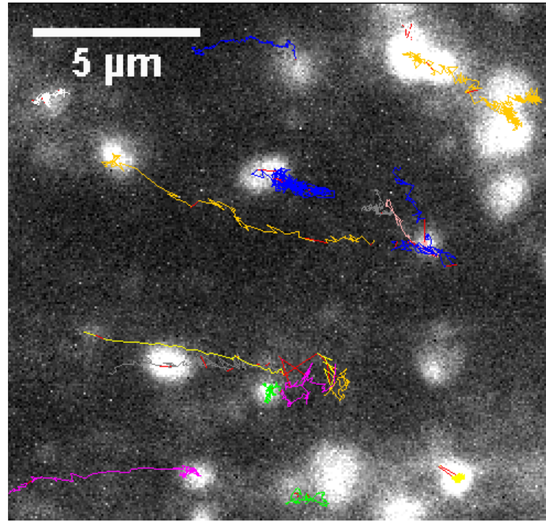

Figure S4. Confined and directed movements of TGNPs inside HeLa cell. The trajectories of TGNPs within 30 s are marked with color lines.

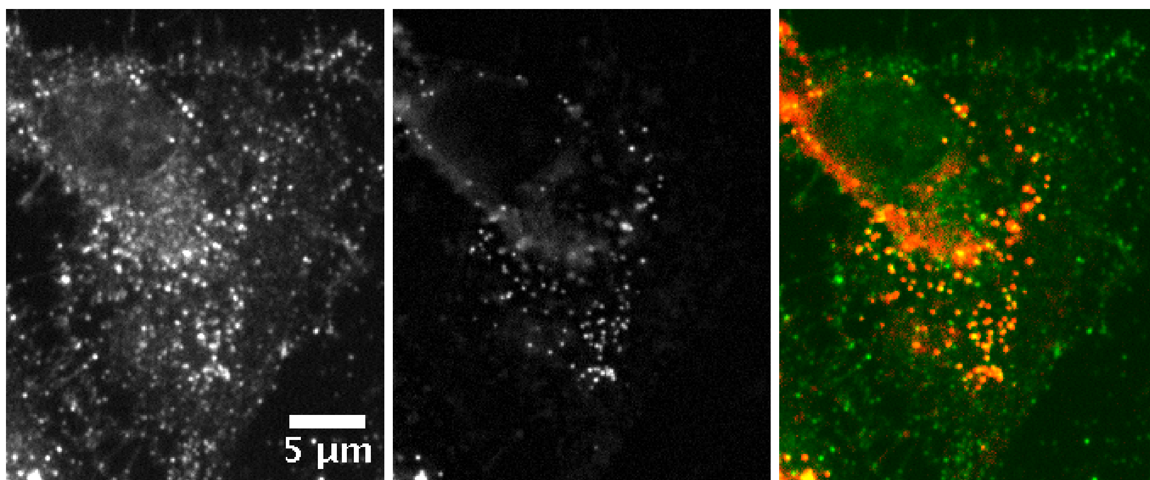

Figure S5. From left to right are the darkfield image of TGNPs loaded HeLa cell (co-incubated with TGNPs for 1 h), the fluorescence image of acridine orange labeled acidic vesicles, and the merged darkfield (green) and fluorescence (red) image.

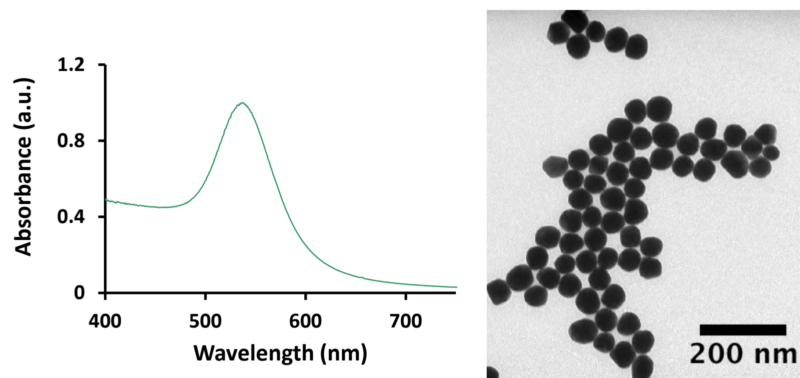

Figure S6. UV-vis absorption spectrum and TEM image of 60 ± 2.5 nm nm GNPs.

REFERENCES:

(1) Wei, L.; Zhao, X.; Chen, B.; Li, H.; Xiao, L.; Yeung, E. S. *Anal. Chem.* **2013**, *85*, 5169–5175.

(2) Sbalzarini, I. F.; Koumoutsakos, P. *J. Struct. Biol.* **2005**, *151*, 182–195.

(3) Xiao, L.; Wei, L.; Liu, C.; He, Y.; Yeung, E. S. Angew. Chem. Int. Ed. **2012**, 51, 4181-4184.