Supporting information for

Two GPSes in a ball: deciphering the endosomal tug-of-war using plasmonic dark-field STORM

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CONTENTS

1. Supporting Methods ........................................................................................................... 2
2. Supporting Notes ..............................................................................................................10
3. Supporting Figures ...........................................................................................................12
4. Supporting Movie Legends ..............................................................................................26
5. Supporting References .....................................................................................................27
1. Supporting Methods

**Reagents** Deionised water and all buffers were filtered using filters with 0.2 μm pore size before use, and all chemical reagents below were purchased and used without further purification.

*For general experiments.* Acetic acid (99.7%; Sigma-Aldrich, St. Louis, MO, USA), acetone (99%; Alfa Aesar, Ward Hill, MA, USA), agarose powder (low EEO; Sigma-Aldrich), alconox detergent (Sigma-Aldrich), 1,2-dihexadecanoyl-sn-glycerol-3-phosphoethanolamine triethylammonium salt (Texas Red DMSO; Thermo Fisher Scientific, Waltham, MA, USA), ethanol (EtOH, 96%; Alfa Aesar), 10x phosphate buffered saline (PBS; Sigma-Aldrich), sodium chloride (NaCl, ≥ 99.0%; Sigma-Aldrich), sodium hydroxide beads (NaOH, ≥ 97%; Sigma-Aldrich), Tris/Borate/EDTA buffer (TBE; Sigma-Aldrich), methanol (MeOH, 99%; Alfa Aesar), deionised water (DI water, 18.2 MΩ)

*For synthesis of gold (Au) nanoparticles (NPs).* Ascorbic acid (99%; Sigma-Aldrich), ammonium hydroxide solution (NH₄OH, 28%; Sigma-Aldrich), bis(p-sulphonatophenyl)phenylphosphine dihydrate dipotassium salt (BSPP, ≥ 97%; Strem Chemicals, Newburyport, MA, USA), 5-bromosalicylic acid (90%; Sigma-Aldrich), carboxyethylsilanetriol (25% in water, disodium salt (Sigma-Aldrich), hexadecyltrimethylammonium bromide (CTAB, 95%, Sigma-Aldrich), iso-propanol (i-PrOH, ≥ 98%; Sigma-Aldrich), sodium borohydride (NaBH₄, 98%; Sigma-Aldrich), sodium citrate tribasic dihydrate (≥ 99%; Sigma-Aldrich), sodium hydroxide beads (NaOH, ≥ 97%; Sigma-Aldrich), tetrachloroaurate trihydrate (99.9 %, HAuCl₄; Sigma-Aldrich), tetraethyl orthosilicate (TEOS, ≥ 99%; Sigma-Aldrich), HS-(CH₂)₁₁-(OCH₂CH₂)₆-OCH₂CO₂H (HS-C₁₁-EG₆-COOH; ProChimia, Sopot, Poland)

*For synthesis of quantum dots (QDs).* Acetone (99.5%; Sigma-Aldrich), cadmium oxide (CdO, 99.99%; Sigma-Aldrich), chloroform (99.5%; Sigma-Aldrich), 3-mercaptopropionic acid (MPA, ≥ 99%; Sigma-Aldrich), methanol (99.5%; Sigma-Aldrich), n-hexane (96%; Sigma-Aldrich), 1-octadecene (ODE, 90%; Sigma-Aldrich), octadecylamine (ODA, 99%; Sigma-Aldrich), oleic acid (OA, 90%; Sigma-Aldrich), selenium powder (99.99%; Alfa Aesar), stearic acid (95%; Sigma-Aldrich), sulphur (99.98%, powder; Sigma-Aldrich), tertramethylenammonium hydroxide pentahydrate (TMAH((CH₃)₄N(OH))·5H₂O, 97%; Sigma-Aldrich), tributylphosphine (TBP, 97%; Sigma-Aldrich), trioctylphosphine oxide (TOPO, 90%; Sigma-Aldrich), zinc oxide (99.99%, powder; Alfa Aesar), HS-(CH₂)₂-(OCH₂CH₂)₆-OCH₃ (HS-C₂-EG₆-OCH₃; ProChimia)

For surface immobilisation of monovalent DNA-conjugated Au nanorods (NRs). Anhydrous dimethyl sulfoxide (DMSO, 99.9%, Sigma-Aldrich), EZ-Link NHS-Biotin (Thermo Scientific), streptavidin from streptomyces avidinii (Sigma-Aldrich), thiolated DNA [HS-(ACTG)]₁₀-biotin (Bioneer, Daejeon, Republic of Korea), sequence: 5’ [Thiol]ACTG ACTG ACTG ACTG ACTG ACTG ACTG ACTG ACTG ACTG[ADT][Biotin]₃’

*For cell culture and transferrin-mediated endocytosis.* Collagen I (3.6 mg/mL, rat tail; Santa Cruz
Biotechnology, Dallas, TX, USA), Dulbecco’s modified Eagle’s medium (DMEM, high glucose; Biowest, Nuaillé, France), foetal bovine serum (FBS; Biowest), holo-transferrin human (Tf, ≥97%; Sigma-Aldrich), 2-N-(N-morpholino)ethanesulfonic acid buffer (MES; Thermo scientific), penicillin-streptomycin solution 100X (Biowest), 0.25% trypsin-EDTA (Biowest), U2OS cells (Korean Cell Line Bank)

For imaging of AuNRs in endosomes using electron microscopy. Embed-812 kit (Electron Microscopy Sciences, Hatfield, PA, USA), ethyl alcohol (99.5%; Sigma-Aldrich), glutaraldehyde (50% in H₂O; Sigma-Aldrich), osmium tetroxide (99.8%; Sigma-Aldrich), propylene oxide (99%; Sigma-Aldrich), formalin solution (10%, neutral buffered; Sigma-Aldrich), sodium cacodylate (98%; Sigma-Aldrich), uranyl acetate (98.0-102.0%; Electron Microscopy Sciences)

**Synthesis of transferrin-coated silica AuNRs (Au NR@Tf)** A four-step sequential synthetic strategy was used to prepare Au NR@Tf.

**a. AuNRs:** The AuNRs were synthesised following a published method with slight modifications¹. Briefly, the processes were divided into preparation of the seed and growth solutions. For preparation of the seed solution, 5 mL of 0.5 mM HAuCl₄ was added to 5 mL of 0.2 M CTAB in a 20 mL vial. Next, we injected 1 mL of 6.0 mM fresh NaBH₄ into the seed solution with vigorous stirring for 2 min. The colour of the seed solution immediately turned yellowish brown, and it was left to stand for 30 min. To prepare the growth solution, 0.55 g of 5-bromosalicylic acid and 4.5 g of CTAB were dissolved in 150 mL of DI water using a sonicator for 2 h. The mixture solution was cooled to 40 °C, and then 6 mL of 4.0 mM AgNO₃ was added. The reaction mixture was incubated undisturbed for 15 min, and 125 mL of 1.0 mM HAuCl₄ was then mixed in by stirring at 400 rpm. After 15 min, 1 mL of 64 mM ascorbic acid solution was added and stirred until the solution became transparent. Finally, 30 µL of aged seed solution was injected into to the growth solution. After stirring at 400 rpm for 5 min, the solution was left undisturbed for 12 h. The NPs were purified via centrifugation at 7,000 rpm for 25 min and then concentrated to 20 nM by re-dispersion in 36 mM CTAB solution (extinction coefficient: 5.30 × 10^5 M⁻¹cm⁻¹ at 633 nm for 68 × 32 nm).

**b. Au NR@SiO₂:** The Au NR@SiO₂ were synthesised following a published method with slight modifications². Two hundred microlitres of the as-synthesised NRs at 20 nM was injected into 8.8 mL of DI water in a 10 mL vial with stirring at 70 rpm. Subsequently, 88 µL of 0.1 M NaOH was added to the mixture. Twenty-five microlitres of 20% (v/v) TEOS in MeOH were injected to the reaction mixture and the mixture incubated for another 30 min. After two additional injections and incubations, the reaction mixture was left undisturbed for 3 d. Subsequently, 10 µL of 28% NH₃·H₂O was added and the mixture incubated for 1 d. The product was purified via centrifugation at 7,000 rpm for 5 min. The pellet was re-dispersed in ETOH.

**c. Carboxyl-functionalised silica AuNRs (Au NR-CO₂H):** The Au NR-CO₂H were synthesised by following a published method with slight modifications³. We mixed 500 µL of the as-synthesised Au NR@SiO₂ at 0.4 nM with 1 µL of 28% NH₃·H₂O in 1.5 mL epi-tube. After 10 min, 1 µL of carboxyethylsilanetriol (2.5% in water) was added to the solution, which was left undisturbed for 2 d. The reaction mixture was purified via centrifugation at 7,000 rpm for 5 min. The pellet was re-dispersed in DI water.
d. **Transferrin-coated silica AuNRs (Au NR@Ti):** The Au NR@Ti were synthesised using a slightly modified version of a published method\(^6\). Ten microlitres of 1 mM holo-transferrin solution and 10 µL of the as-synthesised 20 nM AuNRs-CO₂:H were mixed with 10 µL of MES buffer (pH 5.7) in a 1.5 mL eppendorf. The reaction solution was shaken for 12 h. After purification via centrifugation at 7,000 rpm for 5 min, the reaction mixture was dissolved in DMEM and immediately used.

**Synthesis of silica-coated Au NPs (Au@SiO\(_2\)):** A two-step sequential synthetic strategy was used to prepare Au NP@SiO\(_2\).

a. **Au NPs:** The Au NPs were synthesised following published method with slight modifications\(^5\). For seed synthesis, 1 mL of 25 mM HAuCl\(_4\) was added into 150 mL of 2.2 mM sodium citrate aqueous solution at 80 °C. The solution turned the colour of red wine under vigorous stirring for 2 h. An additional 1 mL of 25 mM HAuCl\(_4\) was added to the solution and stirred for 30 min. After repeating the injection and incubation twice more, the solution was diluted by removing 99 mL of the sample and adding 53 mL of DI water and 2 mL of 60 mM sodium citrate. The reaction solution was heated to 80 °C. The additional injection and incubation steps were repeated twice, and then the solution was allowed to cool to room temperature. The reaction product was used immediately without further purification (extinction coefficient: 8.42 × 10\(^8\) M\(^{-1}\)cm\(^{-1}\) at 529 nm for 40 nm Au NPs)

b. **Silica-coated Au NPs (Au@SiO\(_2\)):** Au@SiO\(_2\) were synthesised following a published method with slight modifications\(^6\). One and a half millilitres of 0.2 nM as-synthesised Au NPs were mixed with the 5 mL of i-PrOH in a 50 mL conical tube. One hundred and twenty-five microlitres of 28% NH\(_2\)OH was added to the solution under vigorous shaking. Subsequently, 80 µL of 10 mM TEOS in i-PrOH solution was added to the reaction solution and the mixture incubated for 2 h. After three additional injections and incubations, the reaction products were shaken for 18 h, followed by two more injections and incubations and shaking for 18 h. The reaction products were purified via centrifugation at 5,000 rpm for 15 min and re-dispersed in i-PrOH. After two additional washes with i-PrOH, the pellet was re-dispersed in DI water.

**Surface immobilisation of monovalent DNA-conjugated AuNRs** A three-step sequential synthetic strategy was used to prepare surface immobilisation of monovalent DNA-conjugated AuNRs. To test the performance of pdf-STORM using randomly rotating AuNRs with a fixed polariser, the AuNRs were newly synthesised at 25 °C (extinction coefficient: 7.35 × 10\(^8\) M\(^{-1}\)cm\(^{-1}\) at 643 nm for 80 × 34 nm).

a. **Ligand exchange of AuNRs with BSPP:** The surface exchange of AuNRs was conducted according to a published method with slight modifications\(^7, 8\). Five hundred microlitres of 20 nM as-synthesised AuNRs was washed via centrifugation at 7,000 rpm for 5 min and re-dispersed in 1 mL of DI water. After an additional washing process, the pellet was re-suspended in 0.5 mL of BSPP aqueous solution (10 mg/mL). The solution was mixed with 0.5 mL of phenol:chloroform:isoamyl alcohol (25:24:1) and stirred for 12 h in a 2 mL vial. To obtain BSPP-coated AuNRs from the milky emulsion, the solution was left undisturbed for 1 h. The aqueous layer was extracted via a pipette and filtered through a PTFE filter (0.2 µm pore size). The reaction products were purified via centrifugation at 7,000 rpm for 10 min and re-dispersed in 1 mg/mL BSPP solution.

b. **Single DNA-conjugated (monovalent) AuNRs:** The monovalent DNA-conjugated AuNRs were synthesised using a slightly modified published method\(^6\). Forty microlitres of 3 nM AuNRs, 10 µL of
buffer (10× Tris and 300 mM NaCl), 10 µL of 50 mg/mL BSPP solution, and 40 µL of 30 nM thiolated DNA were mixed in a 1.5 mL epi-tube and shaken overnight. The DNA-conjugated AuNRs were separated via centrifugation at 7,000 rpm for 5 min and re-dispersed in 1 mL of deionised water. Five microlitres of 10 mM HS-C11-EG6-COOH was added and the mixture incubated for 3 min. After three washes to remove excess oligonucleotides, the reaction product was used immediately.

c. Surface immobilisation of monovalent DNA-conjugated AuNRs: NHS-biotin (151 µL, 10 mM) in anhydrous DMSO and 1 mg of bovine serum albumin (BSA) were mixed with 1 mL of PBS (pH 7.2) in a 1.5 mL epi-tube. After 1 h, the mixture was purified using a NAP 10 desalting column (GE Healthcare) to remove excess NHS-biotin. The purified product was used immediately. For microscopy experiments, 150 µL of 0.1 wt% biotinylated BSA solution was plated on a 35 mm glass-bottom dish (MatTek, P35G-0-10-C) for 1 h. After washing excess biotinylated BSA with the buffer, 100 µL of 100 pM streptavidin in PBS buffer was incubated on the dish for 20 min. To remove excess streptavidin, the dish was washed three times with the buffer. Seventy microlitres of the as-synthesised 500 pM DNA-conjugated AuNRs was added to the dish for 5 min and washed several times with the buffer. When the biotinylated DNA-conjugated AuNRs bound to the streptavidin dish, the particles were confined to a very small region.

Synthesis of QDs QDs were synthesised using the hot injection method\(^\text{10}\). We vacuumed and heated 0.026 g of CdO, 0.23 g of stearic acid, and 2.0 g of ODE to about 250 °C in a three-necked flask under Ar gas flow. After cooling the reaction mixture to room temperature, 1.5 g of ODA and 0.5 g of TOPO were added into the reaction mixture. The temperature of the solution was raised to 280 °C under Ar gas flow and 1.6 mmol of Se dissolved in 0.74 mL of TBP under Ar was loaded in the flask at 280 °C. The temperature was lowered to 250 °C, and the flask was stirred for 5 min to grow the core. The reaction mixture was cooled to room temperature and washed with hexane and methanol to remove by-products from the CdSe core nanocrystal. After precipitation of the CdSe core in acetone via centrifugation, the nanocrystal, 10 g of ODE, and 3.0 g of ODA in a three-necked flask were degassed and heated to 220 °C under Ar gas flow.

Cadmium precursor solution (0.1 M) was prepared by heating 0.5 mmol of CdO, 4 mmol of oleic acid, and 3.7 mL of ODE at 250 °C under Ar gas flow. Zinc precursor solution (0.1 M) was prepared by heating 0.5 mmol of ZnO, 4 mmol of oleic acid, and 3.7 mL of ODE at 320 °C under Ar gas flow. Sulphur precursor solution (0.1 M) was prepared by heating the 0.5 mmol of sulphur and 5 mL of ODE at 150 °C under Ar gas flow. To grow the shell layer, 0.9 mL of the cadmium precursor solution and 0.9 mL of sulphur precursor solution were added into the flask at 10 min intervals. The reaction mixture was heated to 240 °C, and then zinc, cadmium, and sulphur precursors were added in 10 min intervals in the following order: 0.45 mL of Zn, 0.45 mL of Cd, 0.9 mL of S, 0.9 mL of Zn, 0.9 mL of S, and 0.9 mL of Zn solution. The reaction mixture was cooled to room temperature and precipitated with acetone. After hexane extraction, CdSe/CdS/ZnS QDs were purified several times using acetone or methanol and the QDs were re-dispersed in chloroform.

Surface ligands of the synthesised QDs were exchanged with HS-C2-EG6-OCH\(_3\) via two steps. One hundred micrograms of TMAH and 50 µL of MPA were mixed with 1 mL of chloroform. After 15 min, a clear aqueous layer formed. After the biphasic solution was shaken for 1 h, 100 µL of 0.1 µM TOPO-capped QDs dissolved in chloroform was added to the solution and shaken for 40 h at room temperature.
The aqueous layer was collected and purified by a NAP 10 desalting column (GE Healthcare) using 10 mM Tris containing 30 mM NaCl (pH 8) as an eluent. A 1.8 µL volume of HS-C_{12}-EG_{6}-OCH_{3} was added and the solution was shaken for 1 d. The solution was purified again in the same way.

**Nanoparticle Characterisation** Absorption spectra were obtained using UV-vis spectrophotometer (Agilent, Cary8454). The effective surface charges of the AuNRs were measured using Zetasizer nanoseries (Malvern, MAL1160456). Agarose gel electrophoresis was conducted using Mini-Sub Cell GT Cell (Bio-Rad) in TBE buffer (pH 7.6) at 150 V for 15 min.

**Electro microscopic characterisation** The NPs were characterised using field-emission transmission electron microscopy (FE-TEM, Hitachi, HF-3300). To prepare samples, a TEM grid (Formvar/Carbon on 300 mesh, TED PELLA) was subjected to the O₂ plasma process to create hydrophilic conditions using an advanced plasma cleaner (Gatan/950M). AuNRs in the endosomes were identified via Bio-TEM (FEI, Tecnai G2 F20 TWIN TMP) at 80 kV. To confirm the performance of pdf-STORM, the AuNRs loaded on grided coverslips (Ibidi, grid-50) were analysed with pdf-STORM and the image was compared at the same location with field-emission scanning electron microscopy (FE-SEM, Hitachi, SU8020, operated at 10 kV voltage and 10 µA current under BSE mode).

**TEM analysis to confirm monovalent AuNR** Since conjugating DNA to AuNPs increases their hydrodynamic size, DNA-conjugated Au NPs can appear as a discrete band in gel electrophoresis, depending on their valency. However, the DNA-conjugated Au NR did not exhibit a discrete band during gel electrophoresis because of its larger size. We confirmed the monovalent DNA conjugation with AuNR by hybridizing complementary DNA-conjugated Au NPs and visualizing them with TEM. (Figure S10)

For monovalent DNA-conjugated Au NPs, 25 nm Au NPs were synthesised by controlling additional injection. Subsequently, 40 µL of 100 nM Au NPs, 10 µL of buffer (10× Tris and 300 mM NaCl), 10 µL of 50 mg/mL BSPP solution, and 40 µL of 30 nM thiolated DNA were mixed in a 1.5 mL epi-tube and mixed overnight. The presence of monovalent DNA-conjugated Au NPs was confirmed by performing 3% agarose gel electrophoresis in TBE (pH 7.6) at 150 V for 15 min. The product was isolated using a clean razor blade (smartSlicer Gel Cutting Tool & RaZor, Sigma-Aldrich) and dialysis tubing (SnakeSkin dialysis tubing, MWKO 10K, Thermo Scientific). The extracted product was centrifuged at 10,000 rpm for 5 min. Subsequently, 10 µL of 10 mM HS-C_{11}-EG_{6}-COOH was added to the solution and incubated for 10 min to prevent aggregation during hybridisation. Finally, the as-synthesised DNA-conjugated AuNRs and the extracted Au NPs were mixed at a 1:10 ratio in PBS buffer for 2 h. After centrifugation at 7,000 rpm for 5 min, the product was confirmed by TEM.

**Optical microscopy** Total internal reflection fluorescence (TIRF) and single molecular dark-field spectral optical microscopy were performed with an inverted microscope (Nikon, ECLIPSE Ti2-E) equipped with a perfect focus system (PFS, Ti2-N-ND-P), a motorised stage (Ti2-S-SE-E), a stage-top-incubator with controlled temperature and CO₂ concentration (Okolab, UNO-T-H-PREMIXED), and an electron multiplying charge-coupled device (EM CCD, Andor, iXon Ultra 897). Super-resolution radial fluctuation (SSRF) images of microtubules were obtained by processing 100 frames of TIRF images using the super-resolution radial fluctuations (SRRF) algorithm (Andor). The dark-field images were acquired using an ellipsoidal dot mirror installed at a filter cube set and a mercury lamp (Nikon, C-HGFIE Intensilight) as a light source. The polarised dark-field images were acquired under a linear
polariser (Thorlabs, with N-BK7 windows, LPVISE50-A) equipped with a motorised rotation stage (Thorlabs, DDR25/M) connected to a DC Servo Motor Controller (KBD101). A laser (Nikon, LU-N4 Laser Unit, 488/561 wavelength) was used as a light source for TIRF microscopy. All dark-field, SRRF, and pdf-STORM images were observed under a 100× objective lens (Nikon, 1.49 NA, oil immersion, CFI SR HP Apochromat TIRF). For spectroscopy, a beam splitter, a spectroscopic detector (Andor, Newton DU-971), and a spectrometer (Andor, Shamrock 193i-A) equipped at the emission part of the microscope were used.

**Fabrication of the dot mirror** To fabricate the dot patterned mirror, an optical window white plate (N-BK7 or B270 SCHOTT, Shibuya Optical co.) was used as a substrate. An elliptical pattern (Dimension: 26 mm × 36 mm) was created using photolithography and an AZ 4330 positive photoresist (AZ Electronic Materials). For the formation of the dot mirror, a 300 nm-thick aluminium (Al) or silver (Ag) film was deposited on the patterned substrate using the thermal evaporator (TENG). Finally, the photoresist residue was removed using an acetone solution.

**Development of pdf-STORM** The plasmonic properties AuNRs exhibit angle dependency on polarised light. To resolve two AuNRs within the diffraction limit, we fluctuated the intensity using two methods. For tethered AuNRs, the image was observed using a fixed polariser and acquired with 200 fps because the motion of rotation was very fast. For AuNRs fixed on glass, the image was taken using a rotating polariser. After subtracting background signals with Fiji11, the temporally separated point spread function was fitted with integrated Gaussian fit to locate their centres and the point maps were reconstructed using the ImageJ plug-in of the ThunderSTORM algorithm12. In the filtering process, the x and y coordinates, frame, and intensity of resolved particles were exported. When rotating the polariser, the traces of scattering intensity were fitted with the sine wavelet function.

**Evaluation of STORM algorithm using synthetic dark-field images** To validate the performance of the STORM algorithm, a synthetic image was used. For synthetic image generation, the optical properties of Au NR and the point spread function of the dark-field images were determined. The full width at half maximum (FWHM) of point source function was 400 nm. Finite-difference time domain (FDTD) calculations were performed to obtain the theoretical variation in the scattering intensity depending on the polariser angle ($\theta_{pol}$). When the $\theta_{pol}$ is parallel to Au NR, the signal proportional to $\cos(2\theta_{pol})$ has maximum intensity ($I_0$).

The synthetic image was generated using MATLAB R2020a. It shows the maximum intensity at the centre of the particle and a Gaussian distribution with standard deviation 170 nm (FWHM 400 nm). To match the conditions same as of our microscope image, the synthetic image was binned to have a resolution of 110 nm per pixel.

To simulate fixed Au NR with the rotating polariser, the image was generated by increasing $\theta_{pol}$ from 0° to 358.5°. By varying the orientation and distance between the two AuNRs, the accuracy of angular and spatial information of the pdf-STORM for fixed Au NR was measured. Assuming no plasmonic coupling of the two particles, the intensity at each point was calculated as the simple sum of the two Gaussian distributions. To simulate randomly fluctuating AuNRs with the fixed polariser, the image sequences were generated by assuming rotational Brownian motion of the Au NR. By varying the distance between the two AuNRs, the accuracy of the spatial information of the pdf-STORM for fluctuating Au NR was
FDTD simulations FDTD (8.7.1 version, Lumerical solutions Inc.) simulations were performed to predict the plasmon wavelength of a single Au NR. Based on the TEM images, a single Au NR was modelled as two hemispheres of 16 nm radius each side and a total z-span of 68 nm, and the dielectric function of Au was taken from the experimental value of Johnson and Christy\textsuperscript{13}. For 20 nm thick-silica shell of Au NR@SiO\textsubscript{2} nanostructures, the silica shell was modelled with two hemispheres with a 26 nm radius each side and a total z-span of 108 nm; the dielectric function of silica was taken from the model of Palik\textsuperscript{14}. The mesh order of Au and silica were set as 1 and 2, respectively. The electromagnetic wavelength ranged from 300 nm to 800 nm, and the radiated to a 1,300 nm FDTD box containing the nanostructures. The mesh inside the box was divided into 0.5 nm and the mesh accuracy was set to 4, considering memory requirements and simulation time. Perfectly matched layer (PML) absorbing boundary was used in all directions. The surrounding medium was set to 1.33, which is the reflective index of water. As a light source, total-field scattered field (TFSF) was used to study the scattering behaviour of the nanostructures by isolating the scattered field from the incident field. To identify the plasmon wavelength of a single Au NR as a function of linearly polarised light, the incident light was propagated to the nanostructures and its electrical field wave plane was changed from parallel to perpendicular to the long axis of the NR. After identifying the maximum plasmon wavelength, the local electric field distribution of the nanostructure was calculated using a frequency-domain field monitor. The field enhancement was mapped in y and z-axes with a colour bar range from 0 to 25.

Cell lines U2OS cells were cultured in a T25 flask (Corning), grown in DMEM with 10% FBS and were passaged every 3–4 d. The cells were maintained at 37 °C and in 5% CO\textsubscript{2} in a humidified incubator (Binder, Model C 170). The pAcGFP1-Tubulin plasmid (PT3836-5, Clontech) was transfected into the U2OS cell line using the Neon Transfection system [Shock Conditions, cell number: 2.5 × 10\textsuperscript{5}, plasmid: 1 \mu g, voltage, width, and number: 1,230 V, 10 ms, 4, respectively] (MPK5000, Thermo Fisher). After transfection, the cells were plated and washed with media several times before use.

Specific labelling of Au NR@Tf NPs in live cells For microscopic observation, appropriate numbers of cells were plated on collagen I-coated 35 mm glass-bottom dishes (MatTek, P35G-0-10-C) to achieve 70% confluence. Seventy microlitres of the as-synthesised Au NR@Tf (0.2 nM) was added to cells and incubated for 20 min at 37 °C and washed five times with DMEM containing 10% FBS. Live cell images were acquired using dark-field microscopy. The image sequences were acquired at 85 Hz without delay.

Fixation of cells for transmission electron microscopy (TEM) To observe endosomes containing AuNRs using TEM without deforming the cells, we prepared samples using the following steps: 1\textsuperscript{st} and 2\textsuperscript{nd} fixations, dehydration, embedding, sectioning, and negative staining, all conducted per a published method with slight modifications \textsuperscript{15}. Briefly, the live cells were washed with sodium cacodylate buffer (0.2 M, pH 7.4) at 37 °C. Cells were fixed with a mixture of 2.5% (v/v) glutaraldehyde and 2.0% (v/v) formaldehyde in the buffer for 20 min at room temperature and left undisturbed at 4 °C overnight before being washed with the buffer. The cells were incubated in the 2\textsuperscript{nd} fixative solution containing 1% (v/v) osmium tetroxide for 40 min, and washed with buffer and water, sequentially. Next, the samples were dehydrated in a graded series of EtOH (30, 50, 70, 80, 90, 95, and 100%). Following removal of the EtOH, the solution was replaced with propylene oxide and samples were incubated for 2 h. For the
embedding process, graded mixtures of 3:1, 1:1, and 1:3 propylene oxide and resin, were incubated for 2 h. Next, the samples were incubated in pure resin for 2 h. After removing resin from the sample, it was left to solidify at 68 °C in an oven for 48 h.

The sample was cooled down using liquid N₂ to remove glass from the resin. Subsequently, the embedded cells were sectioned into ultrathin layers (~100 nm) using a cryo ultrathin microtome (Leica, EM UC7), and the sectioned film was collected with a TEM grid (TED PELLA, Formvar on 200 mesh, Cu). For negative staining, the grid was incubated in the dark in filtered 1% uranyl acetate for 20 min and rinsed with water. After drying, the sample was observed in Bio-TEM.

**Single particle tracking algorithm** To separate conventional pause and directional motion of endocytic vesicles, Au NR@Tf tracks were obtained manually using TrackNTrace in MATLAB¹⁶. MSD for the trajectory was calculated with segmented 50 frames, and temporally analysed by sliding frames. The MSD plot was fitted with the linear equation and used to calculate the diffusion coefficient. Detailed criteria used for the comparison are listed in Note S1.

**Distribution of step angle for endosomal rotation** We evaluated the distributions of the endosomal step angle to describe the rotational dynamics of endosomes under the pause state. According to general formulations of free-spreading behaviour, translational and angular displacement of Brownian particles show Gaussian distribution¹⁷. Thus, we considered Gaussian distribution as a null model for the endosomal step angle. Despite this theoretical presumption, several direct observations have revealed that random fluctuations in some biological systems follow non-Gaussian distributions with exponential tails¹⁸⁻²⁰. We used MATLAB's built-in Maximum Likelihood Estimation (MLE) algorithm for distribution fitting. We also examined Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) as measurements of goodness of fit.
2. Supporting Notes

Note S1. Calculation of dwell time for conventional pause, pause (super-resolution), and subtle rotating period during transport of endocytic vesicles.

Criteria for determining the conventional pause. To evaluate relative movements of immobilised objects inside a live cell and define conventional pauses in the trajectory of endocytic vesicles, we evaluated two cases of diffusion coefficients of immobilised AuNRs as control experiments: non-specifically attached NRs i) on a glass surface ($D_{\text{glass}}$), and ii) on live cells ($D_{\text{cell}}$). The dark-field sequences were acquired at 85 fps, and the individual particle tracking was conducted to determine the particle position and diffusion coefficient. For two-dimensional diffusion, the mean square displacement (MSD) was calculated as follows:

$$\langle (r(t) - r_0)^2 \rangle = 4Dt$$

where $r(t)$ is the position of the particle at time $t$, $r_0$ is the initial position, and $D$ is the diffusion coefficient. In the trajectory, the first 10 spots in the MSD plot were fitted with a linear equation to calculate $D$. The Note Figure 1 show the histogram of measured $D$ ($D_{\text{glass}} = 0.00006 \, \mu m^2/s$ and $D_{\text{cell}} = 0.0021 \, \mu m^2/s$, mean). Our optical and imaging equipment and single particle tracking algorithm show negligible perturbation in the imaging and tracking of endosomes. The immobile probes on the cell membrane moved approximately 0.002 $\mu m^2/s$ due to the movement of live cells or membrane fluidity. Considering the inherent cell fluctuation, we set reference $D$ to determine the pause as 0.004 $\mu m^2/s$, which was calculated by mean + SD of $D_{\text{cell}}$. To detect the change in motion during transport, the trajectory of endocytic vesicles was segmented to the length of 50 frames, and the $D$ was calculated by sliding the frames. By applying reference $D$, we determined the conventional pause (Figure 3).

Criteria for determining the pause (SR). To analyse the pause states more precisely, we imaged an endosome using the pdf-STORM for a defined period (sequence of dark-field images while changing the $\theta$, at 360°/s for 1 s). To detect the pause between the subtle rotating periods in pdf-STORM, we acquired the sequential pdf-STORM images by sliding the reconstruction range. By comparing completely resolved two spots based on the image before and after rotation (Figure S13), we found the frame in which the change started and ended, and four clear spots appeared between them. The resolved spots are reliable ranges when considering the spatial resolution of pdf-STORM (Figure 2), and the evaluation of the Thunder STORM algorithm (Figure S6).
Note S2. Rotation formalisms of endocytic vesicles in 3D (Euler angle) In our study, the pause and directional motion repeated in the trajectory of endocytic vesicles. To analyse the angular displacement in 3D (e.g., rotation along x, y, or z-axis) of microtubules, the path of directional motion ahead of the pause was set as the + x-axis. The 2D coordinates of the pdf-STORM stacks were converted to Euler coordinates using two assumptions. i) The straight line connecting the centre of two AuNRs passes through the origin (or centre) of the endosome, and ii) the longest projected distance (maximum d(t) in Figure 1c) is the moment when the centres of both the Au nanorods (NRs) exist in a horizontal plane (xy plane). Since the size of the early stage of the endosome was 100 nm to 200 nm, which is consistent with our TEM observation (Figure 1d), we assumed that they were close to both ends due to the steric hindrance. In addition, we monitored the motion of endocytic vesicles over a long period to observe most of the rotation at pause.

As described previously, the (x, y) coordinates for the resolved NRs were directly derived from the pdf-STORM image and z coordinates were calculated as follows:

\[ r^2 = (x_2 - x_1)^2 + (y_2 - y_1)^2 + (z_2 - z_1)^2, y_2 < y_1, z_2 < z_1 \]

\[ r = d = \sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2}, z_2 - z_1 = 0 \]

where, the coordinates (x, y, z), r, and d denote the 3D position of the particle, interparticle distance between the two AuNRs, and projected distance, respectively.

To analyse the angular displacement of the vesicle with the Euler axis and angle, the three-dimensional coordinates were converted to vector form:

\[ \vec{V}_N = (l_N, m_N, n_N) = (x_2 - x_1, y_2 - y_1, z_2 - z_1) \]

where, V and N respectively denote the straight line connecting the two AuNRs and the number of pdf-STORM images.

According to the hypothesis i), the Nth and (N+1)th vectors were not parallel. The rotation axis \( \phi(x, y, z) \) between two vectors were calculated by the outer product:

\[ \phi(x, y, z) = \vec{V}_N \times \vec{V}_{N+1} = \begin{vmatrix} \hat{x} & \hat{y} & \hat{z} \\ l_N & m_N & n_N \\ l_{N+1} & m_{N+1} & n_{N+1} \end{vmatrix} \]

\[ = \begin{vmatrix} m_N & n_N \\ m_{N+1} & n_{N+1} \end{vmatrix} \hat{x} - \begin{vmatrix} l_N & n_N \\ l_{N+1} & n_{N+1} \end{vmatrix} \hat{y} + \begin{vmatrix} l_N & m_N \\ l_{N+1} & m_{N+1} \end{vmatrix} \hat{z} \]

\[ = (m_Nn_{N+1} - n_Nm_{N+1}, n_Nl_{N+1} - l_Nn_{N+1}, l_Nm_{N+1} - m_Nl_{N+1}) \]

The included angle \( \phi \) between two vectors were measured by following equation,

\[ \sin(\phi) \hat{n} = \frac{\vec{V}_N \times \vec{V}_{N+1}}{|\vec{V}_N| |\vec{V}_{N+1}|} \]

where, \( \hat{n} \) denotes the unit vector perpendicular to the two vectors.

The rotational motion of endocytic vesicles was described with the combination of the rotation axis \( \phi(x, y, z) \) and included angle \( \phi \). (Figure 4)
3. Supporting Figures

Figure S1. Fabrication of an aluminium patterned dot mirror for the development of an inverted dark-field microscope. (a-d) To fabricate the aluminium patterned dot mirror, photolithography processes were followed: (a) coating of positive photoresist on glass, photomask placed on the upper layer and irradiated by UV light, (b) photoresist developing and stripping, (c) aluminium film deposited by thermal evaporator on the patterned substrate, (d) final structure of aluminium patterned mirror after washing away residue. (e) Photograph of aluminium patterned mirror equipped in cube box. (f) Schematic illustrating the principles of dark-field microscopy. The light source was reflected from the dot mirror to the sample. Light scattered by particles came back through the objective and were visualised by CCD. The light moving to the aluminium pattern was blocked, resulting in the dark-field area in the image.
Figure S2. Optical properties of gold nanorods (AuNRs) compared with those of conventional fluorescence probes. Scattering intensity time traces for Au NR@SiO$_2$ under (a) non-polarised and (b) rotating linearly polarised light. Compared to fluorescent molecules, the plasmonic nanoparticles were advantageous for long-time observation, as they showed high signal-to-noise ratios without undergoing photobleaching. Additionally, the Au NR depends on the angle of polarised light, which was fitted to a sine wavelet function with a period of 180°. (c) Single particle spectrum of Au NR@SiO$_2$. The relative intensities were high and low when the polarised light was parallel and perpendicular to the plasmonic nanoparticle, respectively, which have similar tendencies with (d) theoretical finite-difference time-domain (FDTD) calculation of surface charge density of Au NR. All images were acquired at 10 Hz with no interval. (e-f) Representative fluorescence time traces (right) for a single (e) Texas Red and (f) quantum dots (inset: TEM) were observed using TIRF microscopy. The blinking/bleaching of fluorescent molecules was achieved under laser irradiation. Hidden Markov modelling (HMM) was used to separate the bright and dark states of fluorescent molecules.
Figure S3. Acquisition strategies of pdf-STORM for immobilised or tethered AuNRs within the diffraction limit. (a) Schematic illustration of the microscope setup used for the pdf-STORM imaging. In conventional dark-field microscopy, all plasmonic nanoparticles were visualised simultaneously, which revealed that the two nanoparticles within the diffraction limit were not resolved. In pdf-STORM, the plasmonic nanoparticles were observed with a polarised dark-field microscope, thereafter they were located by the Gaussian fit of individual PSF and the centroids of each particle were reconstructed. (b-d) Explanation of pdf-STORM on immobilised rods. (b) Schematic illustration showing the immobilised AuNRs non-specifically attached to the glass surface. Under the rotating polariser condition, (c) the dark-field image sequences of two AuNRs were acquired, and the particles were not distinguished with intensity time traces. Using the STORM algorithm, the particles were resolved, and the intensity of each particle was fitted with a sine wavelet function. Using this process, the coordinates of x, y, and orientation ($\theta_{\text{NR}}$) were introduced. (e-g) Explanation for the pdf-STORM using tethered rods. (e) Schematic illustration showing the tethered AuNRs conjugated to glass surface via biotin-streptavidin interaction. Under the fixed polariser condition, (f) the tethered two AuNRs were imaged at 200 fps for 1 s since the rotational Brownian motion was too fast. (g) Using the STORM algorithm, the particles were resolved, and the fluctuating intensity of each particle was analysed. In this way, a higher spatial resolution was obtained, since the two AuNRs within the diffraction limit could be distinguished.
Figure S4. Preparation and characterisation of transferrin decorated gold nanorods (Au NR@Tf) for the clathrin-mediated endocytosis in live cells. (a) Schematic overview of synthesis, surface modifications, and cellular uptake of Au NR@Tf. The Au NR was synthesised using a modified seed-mediated synthesis method. To avoid the plasmonic coupling between two or more AuNRs, the silica shell thickness was optimised to 20 nm (exceeding half-length of Au NR). Transferrin conjugated to Au NR was used to facilitate clathrin-mediated endocytosis. After the cellular uptake, the endocytic vesicles were transported by motor proteins along the cytoskeletal structure. (b-g) Characterisation of surface functionalised Au NR. (b) Transmission electron microscopy (TEM) images and (c) histogram showing size distribution. The measured sizes of particles were fitted to Gaussian function (68 ± 6 nm, 32 ± 4, and 20 ± 2 nm, length, thickness, and SiO$_2$ shell thickness, respectively). (d) UV-Vis absorption spectra. The absorbance peak was positioned at 633 nm for Au NR and showed red shift to 642–643 nm for silica-coated AuNRs. (e) Zeta potential measurements in DI water. The average zeta potentials of Au NR, Au NR@SiO$_2$, Au NR-CO$_2$H, Au NR@Tf were 14.9 mV, -33.2 mV, -49.8 mV, and -10.5 mV, respectively. (f) 1.5% agarose gel electrophoresis using TBE buffer (pH 7.6) and (g) photograph acquired in PBS buffer (pH 7.4). The positions of discrete bands were different due to size and surface charges. For Au NR and Au NR@SiO$_2$, the particles were aggregated at the starting line, indicating that the surface of nanoparticles was unstable compared with that of carboxyl-functionalised AuNRs. The Au NR-CO$_2$H exhibits faster mobility than the Au NR@Tf, which suggests that the surface of Au NR-CO$_2$H was neutralised due to electrostatic interaction between transferrin molecules. (h) The dark-field images showing specific binding of Au NR-CO$_2$H and Au NR@Tf on the cell membrane. (i) Comparison of the number of bound Au NR. The Au NR@Tf bound to cells with 10-fold better affinity than Au NR-
Au NR@Tf do not show random Brownian motion inside endosomes as well as on the surface of the cell membrane. (a) Schematic illustration of Au NR@Tf bound to the plasma membrane. When the AuNRs were attached to the plasma membrane, they lost translational motion. To investigate the binding state of the Au NR in lamellipodium, (b) the Au NR was monitored with fixed (left) and rotating polariser (right). Sequential scattering image was captured for the initial duration (inset). The graphs of scattering intensity were fitted with linear and sine wavelet function, indicating that multiple Au NR bound to the plasma membrane without rotational motion. (c) Schematic illustration of the Au NR bound to endocytic vesicles. After cellular uptake, (d) scattering intensity of the single Au NR moving along the (e) microtubules (SRRF image) at pause was measured. Compared to binding on the plasma membrane, the fluctuating intensity of the Au NR in endocytic vesicles was negligible, suggesting that the multiple binding sites were maintained after cellular uptake.
Figure S6. Evaluation of the Thunder STORM algorithm using synthetic dark-field images. (a) Workflow to assess the performance of the pdf-STORM. The simulated image sequences were generated with the information of x and y position and orientation (ΩNR), and the reconstructed images were compared with simulation value. Simulated images of (b) the immobilised and (c) tethered two AuNRs within the diffraction limit (top). The pdf-STORM images were obtained with varying interparticle distance and orientation (middle). The positional information was acquired from the PSF localisation, and the angular information was derived from fitting of intensity fluctuation with sine wavelet function. Error estimation for positional information is shown at the bottom. The error rate was calculated below 0.15 and decreased with increasing interparticle distance. In addition, the error for the orientation followed similar tendencies with positional information.
Figure S7. pdf-STORM showed comparable performance to electron microscopy on 2D plane. (a-c) Three representative pdf-STORM results based on the relative angle between two AuNRs. Interparticle angles were approximately 30°, 60°, and 90°. The conventional dark-field image of two AuNRs was observed without a polariser (left). By combining the positional information (middle-1, (x,y)) for the white box in DF and angular information (middle-2, θNR), the spatio-angular map was reconstructed (middle-3) and compared with the results of scanning electron microscopy (SEM, right) in the same region. The angular information was acquired by rotating 180°, while the scattering intensity, including duplicated signal (dotted circle), was fitted with the sine wavelet function. (d) Table showing a comparison between the interparticle distance and angle measured with pdf-STORM and SEM. Our results confirmed that pdf-STORM can resolve two particles within the diffraction limit, regardless of the interparticle angle when the relative angle exceeds 30°. Related to Figure 2.
Figure S8. Negative control experiments for pdf-STORM to show the importance of the alternating intensity fluctuation of the two AuNRs; when two NRs are parallel or when plasmonic coupling occurs. Representative pdf-STORM results based on interparticle angle and distance are shown; (a) AuNRs aligned in parallel within the diffraction limit, (b) Plasmon-coupled bare AuNRs. The conventional dark-field image of two AuNRs was observed without polariser (left). The pdf-STORM image was compared with that acquired using scanning electron microscopy (SEM) for the same region, which is outlined by the white box in DF (middle, right). When interparticle angle was below 20°, the two particles were not resolved. Additionally, the contacted AuNRs were not resolved in pdf-STORM regardless of interparticle distance and angle. Our results confirmed that sequential intensity fluctuation of two AuNRs is essential for the development of the pdf-STORM.
Figure S9. Negative control experiments for pdf-STORM to show the importance of the angle dependency of the probes; spherical Au nanoparticle. Transmission electron microscope (TEM) images of (a) Au NPs and (b) Au NP@SiO\(_2\). The measured diameter and SiO\(_2\) shell thickness were 40 ± 4 nm and 50 ± 4 nm, respectively. (c) Scattering intensity time trace for the Au NP and Au NP@SiO\(_2\) observed under rotating linear polariser. The intensity fluctuation was not observed due to the isotropy in spherical shape. (d) The conventional dark-field image of Au NP@SiO\(_2\) without polariser. (e) Scanning electron microscopy (SEM) and (f) pdf-STORM images show the same position as in panel (d). Compared to SEM, the pdf-STORM showed the same positional information for single particles, but the two particles within the diffraction limit were not resolved and marked in the midpoint of the particles.
Figure S10. Brownian motion of monovalent DNA-conjugated Au NR in tethered models. (a) Scheme illustration of microscope setup used for (b) observing Brownian fluctuations of AuNRs with linear polarised light. (c) To generate the randomly fluctuating Au NR without lateral movement, single-strand DNA was conjugated to the AuNRs as flexible linker. (d) To measure very fast rotational motion of monovalent Au NR (mRod), the dark-field images were acquired at 200 Hz under fixed polariser. (e) Schematic illustration of the method used to confirm the valency of the AuNRs hybridised with excess complementary DNA-conjugated Au NPs (top). Transmission electron microscope (TEM) images showing the hybridised AuNRs (bottom-left). Statistical analysis of the number of hybridised AuNRs (bottom right). The monovalent DNA-conjugated AuNRs were approximately 30%. (f) Scheme of the experimental setup (left). Scattering images of the glass surface labelled with mRod under various conditions (right). The glass surface was coated with biotinylated bovine serum albumin (BSA) to prevent non-specific binding. Streptavidin, with four binding sites for biotin, was used to connect the biotinylated BSA and DNA. Specific conjugation was observed only in positive control. (g) Scattering intensity time traces of the mRod. The bright and dark state were measured when linearly polarised light was parallel or perpendicular to Au NR, respectively. The mRods exhibit high fluctuation frequency without photobleaching, which was advantageous to develop pdf-STORM for the temporal resolution and long-duration observation.
Figure S11. pdf-STORM resolved tethered AuNRs. We used the pdf-STORM to resolve tethered NRs. The randomly rotating Au NR exhibited changes in plasmonic length, thereby fluctuating the intensity of linearly polarised light. The polarised dark-field image sequences were acquired with fixed polariser and analysed using the STORM algorithm. (a-b) Representative pdf-STORM images of resolved particles within the diffraction limit. Dark-field images of two AuNRs without polariser (left). Point spread function (PSF) and pdf-STORM image for the same region, outlined by a white box in DF (middle, right). Based on the binding state between ssDNA and AuNR, the localisation range varied for resolved particles. Our results confirmed that pdf-STORM resolved two randomly fluctuating particles within the diffraction limit under fixed polariser.
Figure S12. Pause of an endocytic vesicle at the microtubule intersection. (a) The behaviour of the endocytic vesicle on the microtubule. The trajectory of an endocytic vesicle was colour coded to show the travelling time and was overlaid with the super-resolution radial fluctuations (SRRF) image of the microtubules. The SRRF image was obtained by reconstructing 100 frames for 15 s. (b) Eight frames derived from a time-lapse movie of the endocytic vesicle, which were the small regions outlined by the white box in (a). The endocytic vesicles encounter several intersections of microtubules, which had three pause states. The pass and pause at microtubule intersections are marked with orange and red dotted circles, respectively. Our results confirmed that the pause states did not always occur at microtubule intersection, but almost all observed pause states were noted at intersections.
Figure S13. Measurement of the subtle rotating periods by sliding reconstruction range.
Examples of the measurement of the subtle rotating period by moving the reconstruction range. The polarised dark-field image sequences were acquired at 85 Hz with a rotating polariser at 360°/s and reconstructed with 85 frames. The pdf-STORM image sequences were obtained while sliding reconstruction range one by one. When centre of reconstruction range was near the discontinuous and rapid displacement, the four well-separated spots could be simultaneously observed (image 5). Based on the pdf-STORM images before and after rotation (images 1–3 and 7–8, respectively), we found completely altered images, and measure the subtle rotating periods. (a) is related to Figure 3c.
Figure S14. Statistical model fitting for the step angle distribution of endosomes. (a-e) Probability density function (PDF) and cumulative density function (CDF) for the step angle distribution of endocytic vesicles. The measured PDF and CDF were fitted to (a) Gaussian, (b) exponential, and a combined model of (c) two Gaussian components, (d) two exponential components, and (e) Gaussian and exponential components using maximum likelihood estimation (MLE). (f) Tables for goodness of fit for the various models. The combined model of two Gaussian components was the best fit to explain the experimental step angle distribution of the endocytic vesicle. Related information can be found in Figure 4d.
4. Supporting Movie Legends

**Movie S1.** The process of pdf-STORM acquisition from synthetic image sequences that simulate immobilised two Au nanorods (NRs). Intended positions of the NRs with x and y coordinates and orientation (top-left). Simulated time-lapse movie of the NRs with sequential intensity fluctuation for rotating linearly polarised light (top right). To ascertain the locations of each particle, the point spread function (PSF) of image sequences were fitted to a Gaussian function (bottom-left). The intensity of each particle was plotted depending on the polarisation angle and fitted with the sine wavelet function (bottom right). After the reconstruction process, the pdf-STORM image was acquired.

**Movie S2.** The process of pdf-STORM acquisition from synthetic image sequences that simulate two tethered Au nanorods (NRs). Intended position for the NRs with x and y coordinates (top-left). Simulated time-lapse movie of the randomly fluctuating (Brownian motion) NRs under linearly polarised light (top right). To ascertain the locations of each particle, the point spread function (PSF) of image sequences were fitted with a Gaussian function (bottom-left). Intensity for each particle was plotted depending on time (bottom right). After the reconstruction process, the pdf-STORM image was acquired.

**Movie S3.** Time-lapse movie of a single endocytic vesicle in a conventional dark-field microscope, and the pdf-STORM sequences at pause. The endocytic vesicle containing the two Au NR@Tf was transported. At 0–11.75 s and 24–35.25 s of the movie, the vesicle was observed by dark-field microscopy, and the footage is played at double speed. During 11.75–24.00 s, the pdf-STORM images were acquired. The corresponding analytical data are shown in Figure 3.
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