Electronic Supplementary Material (ESI) for Chemical Communications

Supplementary Information

Differential Interference Contrast Polarization Anisotropy for Tracking Rotational Dynamics of Gold Nanorods

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This document contains the experimental details and a movie showing the circular translocation of an AuNR on a kinesin-driven microtubule.

MOVIE

Movie 1: The circular translocation of an AuNR on a kinesin-driven microtubule.

EXPERIMENTAL SECTION

Differential Interference Contrast (DIC) Microscope. An upright Nikon Eclipse 80i microscope was used in this study. The DIC mode used a pair of Nomarski prisms, two polarizers, a quarter-wave plate, a Plan Apo oil-immersion objective (100×, N.A. = 1.40) to collect the signals from the samples, and an oil-immersion condenser (N.A. = 1.40). A Photometrics CoolSnap ES CCD camera (1392 × 1040 imaging array, 6.45 μm × 6.45 μm pixel size) was employed to record highly detailed DIC images of gold nanorods (AuNRs). An Andor iXon^{EM}+ CCD camera (512 × 512 imaging array, 16 μm × 16 μm pixel size) was used to capture fast dynamics of AuNRs on kinesin-driven microtubules. The DIC imaging at 720 nm close to the longitudinal surface plasmon resonance (SPR) of AuNRs was carried out by inserting a 720 nm band-pass filter into the light path in the microscope. The band-pass filter was obtained from Thorlabs (Newton, NJ) and it has central wavelength of 720 nm and a full width at half-maximum (FWHM) of 10 nm.

Sample preparation of immobilized AuNRs. AuNRs (25 × 73 nm) were purchased from Nanopartz (Salt Lake, UT). The AuNR colloid solution was first diluted with 18.2-MΩ pure water to a proper concentration. Then the diluted solution was sonicated for 15 min at room temperature. After sonication, 6 μL of the diluted solution was placed onto a pre-cleaned slide which is then covered with a 22 × 22 mm No. 1.5 coverslip (Corning, NY). The

positively charged AuNRs were immobilized onto the negatively charged surface of the coverslip by electrostatic forces.

Polarization-dependent DIC imaging of immobilized AuNRs. The sample glass slide was placed on a 360° rotating mirror holder affixed onto the microscope stge. By rotating the mirror holder 5° or 10° per step, the nanorods were positioned in different orientations, and DIC images at 720 nm were taken with the Photometrics CoolSnap ES CCD camera. The collected images were analyzed by MATLAB and NIH ImageJ.

Preparation of kinesin motor proteins and microtubules. BL21 (DE3) *E. coli* bacteria with the full-length His-tagged kinesin plasmid were obtained from Dr. William O. Hancock at Pennsylvania State University. The *E. coli* bacteria were induced to express kinesin with isopropyl β-D-1-thiogalactopyranoside (IPTG). Then, the kinesin was purified on a Ni-nitrilotriacetic acid (NTA) agarose column according to published protocol.[1] All the tubulin proteins, GTP and taxol were purchased from Cytoskeleton (Denver, CO). Tubulin proteins were mixed and aliquoted with ratio as following: 86 % unlabeled tubulin, 7 % Rhodamine labeled tubulin and 7 % biotinylated tubulin. Microtubules were prepared by applying published protocols.[2,3] 10 μL BRB80 buffer supplemented with 9 μM tubulin, 4 mM $MgCl_2$, 0.5 mM GTP and 10 μM taxol inside was incubated at 37 °C for 3 hrs; microtubules were then diluted and stabilized in 100 μL BRB80 buffer supplemented by 10 μM taxol.

Microtubules gliding on the kinesin-coated substrate. Two strips of double-sided tape were placed on a clean glass slide to serve as the spacers, and one clean coverslip was then placed on top to form a chamber. Penta-His antibodies in BRB80 was allowed to flow into

the chamber. After 5 min a 0.5 mg/mL casein (Sigma, St. Louis, MO) in BRB80 was introduced to prevent nonspecific protein binding. After 5 min BRB80 solution containing 0.2 mg/mL casein, 0.2 mM MgATP and kinesin (with a His-tag) was introduced into the chamber to replace the previous liquid. The kinesin motors bind specifically to the antibodies by their His-tags. A futher 5 min later BRB80 solution containing 0.2 mg/mL casein, 0.2 mM MgATP, 10 μM Taxol and microtubules was flowed into the chamber and the microtubules were allowed to bind to the motors for 5 min. Subsequently, BRB80 solution containing 0.2 mg/mL casein, 0.2 mM MgATP, 10 μM Taxol and neutravidin-modified AuNR (10 × 35 nm, Nanopartz) was flowed into the chamber and was kept at room temperature for 7 min. The neutravidin-modified AuNR was firmly attached to the microtubule surface through multiple strong biotin-neutravidin linkages. Finally, the chamber was filled with a motility solution containing BRB80, 0.2 mg/mL casein, 1 mM MgATP, 10 μM Taxol, and an oxygen scavenger mixture [50 μg/mL glucose oxidase (Sigma), 4 μg/mL catalase (Sigma), 1% (w/v) glucose (Sigma) and 0.1% (v/v) β-mercaptoethanol (Fluka)]. DIC imaging of AuNRs attached to microtubules was performed by observing the sample slide under a DIC microscope. A video (32 frames per second) was captured with an Andor iXon^{EM}+ CCD camera. MATLAB and NIH ImageJ were used to analyze and process the collected video.

Calculation of the orientation angle θ from the polarization anisotropy P. Two orthogonal intensities from bright and dark polarization directions are obtained in DIC microscopy. We have shown that the DIC bright intensity of an AuNR is proportional to the fourth power of the sine of the orientation angle θ between the long axis of a NR and the dark axis.[4] In addition, the DIC dark intensity is proportional to the fourth power of the cosine of the orientation angle θ. Therefore, the normalized bright and dark intensities ($I_{B,N}$, $I_{D,N}$) as a

function of the orientation angle θ can be written as

$$I_{\mathrm{B,N}}(\theta) = \sin^4(\theta)$$

$$I_{\mathrm{D,N}}(\theta) = \cos^4(\theta)$$

DIC polarization anisotropy P is defined as

$$P = \frac{I_{\mathrm{B,N}} - I_{\mathrm{D,N}}}{I_{\mathrm{B,N}} + I_{\mathrm{D,N}}}$$

Therefore, the polarization anisotropy P can be rewritten as

$$P = \frac{\sin^4(\theta) - \cos^4(\theta)}{\sin^4(\theta) + \cos^4(\theta)}$$

The orientation angle θ can be expressed in terms of P and the following relationship for the orientation angle θ as a function of P was finally obtained.

$$\theta = \mathrm{acos}\left(\sqrt{\frac{A - \sqrt{A^2 - 2A}}{2}}\right), \qquad P < 0$$

$$\theta = \mathrm{acos}\left(\sqrt{\frac{A + \sqrt{A^2 - 2A}}{2}}\right), \qquad P > 0$$

where A is defined as (P-1)/P.

References

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