Tunable Elliptical Cylinders for Rotational Mechanical Studies of Single DNA Molecules

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Primary CNF Tools Used: ASML DUV Stepper, Oxford 81 Etcher, Oxford 82 Etcher, Oxford PECVD, SC4500 Odd-Hour Evaporator, CVC SC4500 Evaporators, Zeiss Supra SEM, Zeiss Ultra SEM

Abstract:

The angular optical trap (AOT) is a powerful technique for measuring the DNA topology and rotational mechanics of fundamental biological processes. Realizing the full potential of the AOT requires rapid torsional control of these processes. However, existing AOT quartz cylinders are limited in their ability to meet the high rotation rate requirement while minimizing laser-induced photodamage. In this work, we present a trapping particle design to meet this challenge by creating small metamaterial elliptical cylinders with tunable trapping force and torque properties. The optical torque of these cylinders arises from their shape anisotropy, with their optical properties tuned via multilayered SiO2 and Si3N4 deposition. We demonstrate that these cylinders can be rotated at about three times the rate of quartz cylinders without slippage while enhancing the torque measurement resolution during DNA torsional elasticity studies. This approach opens opportunities for previously inaccessible rotational studies of DNA processing.

Summary of Research:

We have achieved our goal by creating smaller elliptical cylinders with tunable trapping force and torque properties[1]. Instead of using optical birefringence for optical torque generation as with the quartz cylinders, these elliptical cylinders experience an optical torque via their shape anisotropy because the major axis of their elliptical cross-section tends to align with the laser's linear polarization (Fig. 1, A and B). These cylinders are made of a metamaterial that affords an effective index of refraction higher than quartz via alternating layers of SiO2 and Si3N4 (Fig. 1C).

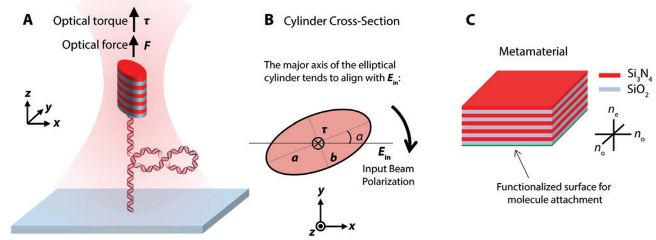

Figure 1: Operational principle of a metamaterial elliptical cylinder in an AOT. (A) experimental configuration of DNA torsional mechanics measurements using a meta-material elliptical cylinder in an AOT. (B) Optical torque generation of a dielectric elliptical cylinder. (C) The metamaterial.

We optimized the optical force, torque, and trapping stability by tuning the size, shape, and composition of these particles via an established COMSOL simulation platform[1,2]. Then, we nanofabricated the metamaterial elliptical cylinders with the targeted dimensions obtained from simulations via a top-down, DUV lithography-based process (Fig. 2A). The liftoff method for cylinder collection resulted in more uniform metamaterial elliptical cylinders with ~ 4-times volume

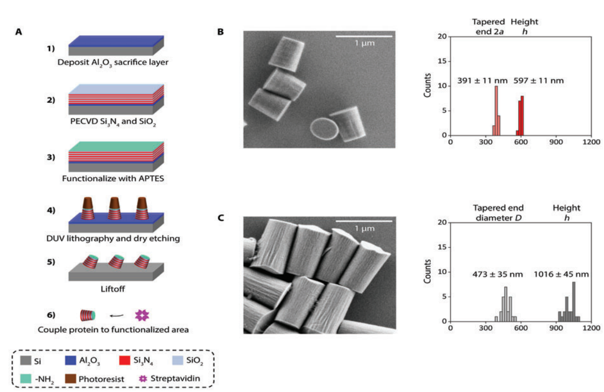

Figure 2: Nanofabrication of metamaterial elliptical cylinders. (A) Fabrication process flow of metamaterial elliptical cylinders. (B) An SEM image of nanofabricated metamaterial elliptical cylinders. The size and uniformity characterization is also shown. (C) An SEM image of nanofabricated quartz cylinders. The size and uniformity characterization is also shown.

reduction compared with the conventional quartz cylinders (Fig. 2, B and C).

We validated the trapping properties of these nanofabricated metamaterial elliptical cylinders with the AOT. We showed that our metamaterial elliptical cylinders can generate a maximum force and a maximum torque comparable to the quartz cylinders (Fig. 3, A and B). In addition, we measured the rotational motion of metamaterial elliptical cylinders and found they show a threefold reduction in γθ: 3.2 ± 0.3 pN·nm·s/turn (mean ± SD, n = 17), in comparison to 9.4 ± 1.7 pN·nm·s/turn (mean ± SD, n = 14) of the quartz cylinder (Fig. 3A). The reduced γθ of the metamaterial elliptical cylinders resulted in ~ 3- times faster cylinder rotation rate without slippage (Fig. 3B).

Moreover, the reduced γ_θ of the metamaterial elliptical cylinders also had an extra benefit of a greater signal-to-noise ratio (SNR) in the torque measurement of a DNA molecule. This threefold reduction in γθ should provide a 1.7-fold reduction in the noise of the measured torque of a DNA molecule, which was experimentally validated via the DNA torsional measurements (Fig. 4, A, B, and C).

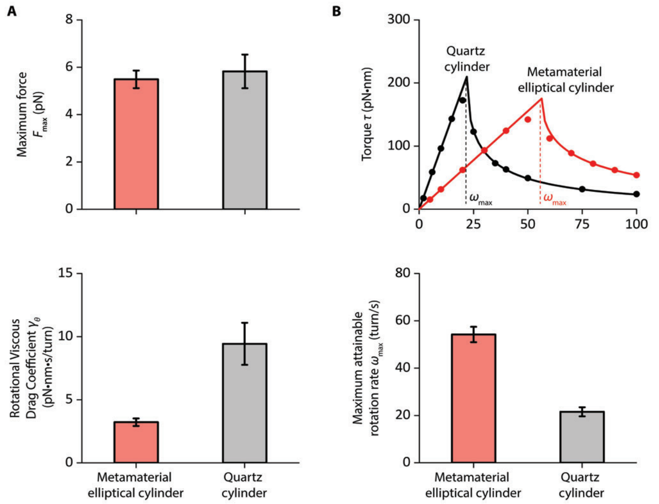

Figure 3: Trapping properties and maximum rotation rate of metamaterial elliptical cylinders. (A) Measurements of the maximum trapping force Fmax (top panel) at 30 mW laser power before the objective and rotational viscous drag coefficient γθ (bottom panel). (B) Method to determine the maximum trapping torque τmax (top panel) and the maximum rotation rate ωmax (bottom panel) without slippage at 30 mW laser power before the objective.

Conclusions and Future Steps:

We demonstrated, both theoretically and experimentally, that our small-size biocompatible metamaterial elliptical cylinders can permit cylinder rotation about three times the rate of the quartz cylinders while providing high force and torque for DNA torsional mechanics studies with enhanced torque resolution. Moreover, our methodology offers versatility in tuning the refractive index, shape anisotropy, and cylinder size to optimize the trapping properties. We anticipate that using these cylinders can enable previously inaccessible rotational studies of DNA-based biological processes.

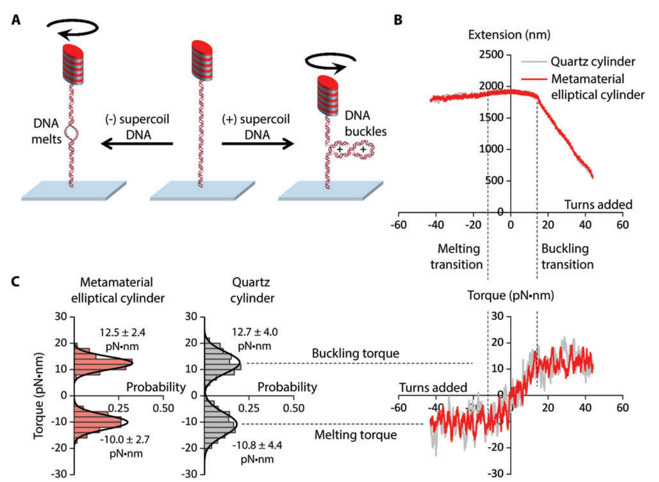

Figure 4: DNA torque measurements using a metamaterial elliptical cylinder, in comparison with those from a quartz cylinder. (A) experimental configuration for the measurements. (B) Measured DNA extension and torque as a function of turns added to DNA under 1 pN force. (C) Histograms of measured torque upon (+) DNA buckling and (−) DNA melting. Each histogram is fit by a Gaussian, with the mean and the SD of the fit shown.

References:

[1] Hong, Y.; Ye, F.; Gao, X.; Inman, J. T.; Wang, M. D. Tunable Elliptical Cylinders for Rotational Mechanical Studies of Single DNA Molecules. Sci. Adv. 10 (50), eadr4519. 2024 https://doi.org/10.1126/sciadv.adr4519.

[2] Hong, Y.; Ye, F.; Qian, J.; Gao, X.; Inman, J. T.; Wang, M. D. Optical Torque Calculations and Measurements for DNA Torsional Studies. Biophys. J. 2024. https://doi.org/10.1016/j.bpj.2024.07.005.