

Biocompatible Cylinders for Studying Protein Torsional Dynamics

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Primary CNF Tools Used: ASML Deep Ultraviolet Stepper, Oxford 81 Etcher, Oxford 82 Etcher, Oxford PECVD, SC4500 Odd-Hour Evaporator, SC4500 Even-Hour Evaporator, Zeiss Supra SEM, Zeiss Ultra SEM

Abstract:

The angular optical trap (AOT) is a powerful instrument for measuring rotational mechanics of fundamental biological processes. The AOT can simultaneously measure the force and torque exerted to a DNA substrate, while applying rotational motion to it. One of the key components of the AOT is its trapping particle, which is designed with optical torque generation. So far, we have developed two types of particles that can generate torque, namely the metamaterial elliptical cylinder (torque generated via shape anisotropy) and the quartz cylinder (torque generated via birefringence).

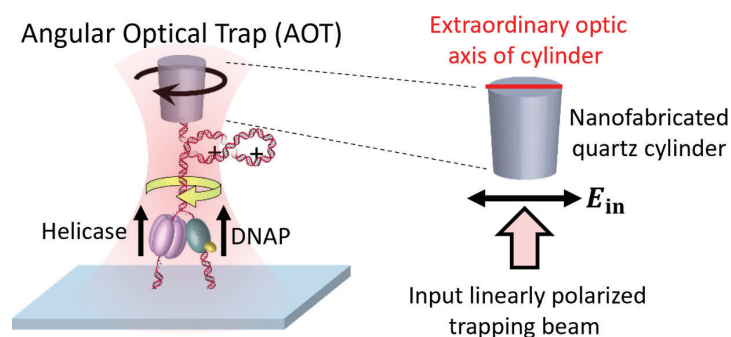


Figure 1: An experimental configuration to track replisome rotation relative to DNA using the AOT.

Summary of Research:

We have applied the metamaterial elliptical cylinders and the birefringent quartz cylinders to many fundamental biological studies, including studying the DNA torsional mechanics (1, 2), DNA replication (3), and transcription (4). The optical force and torque generation capacity of those cylinders were optimized via COMSOL simulations (1, 2, 5). Those cylinders were nanofabricated via a deep-ultraviolet-lithography-based process (6) and functionalized through a chemical reaction with (3-Aminopropyl)triethoxysilane (7). In the past year, we have used those cylinders to reveal the mechanisms of torsion-mediated replication process (Fig. 1) (3) and transcription-through-nucleosome process (Fig. 2) (4).

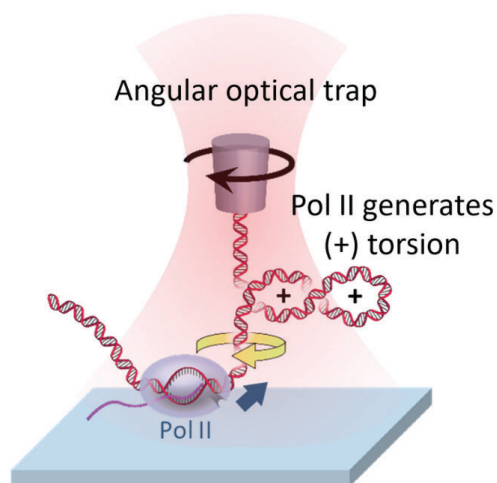


Figure 2: An experimental configuration to track RNA polymerase rotation relative to nucleosomal DNA using the AOT.

Conclusions and Future Steps:

During DNA replication, the replisome can rotate rapidly relative to the DNA substrate. For example, 50 – 100 turns/s in bacteria (8). In such a system, the existing cylinders may be challenging to keep up with such a fast rotational speed (which is determined by the maximum achievable optical torque and the rotational viscous drag coefficient). To enable the detection of faster rotational motion of a replisome, we are working on developing metamaterial elliptical cylinder with a higher cross-section eccentricity to further increase the maximum optical torque while remaining the rotational viscous drag coefficient.

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