Nanofabricated quartz cylinders for angular trapping: DNA supercoiling torque detection

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We designed and created nanofabricated quartz cylinders well suited for torque application and detection in an angular optical trap. We made the cylinder axis perpendicular to the extraordinary axis of the quartz crystal and chemically functionalized only one end of each cylinder for attachment to a DNA molecule. We directly measured the torque on a single DNA molecule as it underwent a phase transition from B-form to supercoiled P-form.

DNA molecules experience substantial torsional strain during many cellular processes such as DNA packing and unpacking, transcription, replication, DNA recombination and DNA repair. Molecular motors such as RNA polymerase can exert torque on their DNA substrate as they translocate and thereby twist DNA into a supercoiled state. To fully understand such processes and the mechanisms of the enzymes involved, it would be ideal to have an instrument that could generate and measure both torque and force on microscopic structures. Single-molecule techniques have made considerable contributions in these areas. Magnetic tweezers have been widely used to investigate DNA supercoiling and the action of various enzymes on supercoiled DNA, albeit without a direct measurement of torque (for a review, see ref. 1). Viscous drag force and/or the angular Brownian motion of a bead have provided measurements of DNA torsional elasticity as well as torque during DNA structural transitions[2,3]. We and others have developed angular optical trapping instruments capable of direct application and detection of torque on optically anisotropic, birefringent microparticles[4–6]. Torque is measured by detecting a change in angular momentum of the transmitted trapping beam. In this work we show that nanofabricated quartz cylinders, when used with an angular trapping instrument, allow direct and simultaneous measurement of torque, angle, force and position with high resolution and bandwidth as demonstrated by measurements of DNA supercoiling.

In most biophysical single-molecule studies using optical tweezers, a micrometer-sized particle serves as a handle to facilitate the manipulation, calibration and measurement of a chemically attached molecule of interest (for example, DNA). Conventional trapping particles are optically isotropic microspheres, which are only adequate for applying force. More specialized handles are needed to generate torque, and require either shape or optical anisotropy. Micrometer-sized quartz fragments have been previously used for torque generation[6], but large heterogeneities in shape, size and optical properties of such fragments complicate precise measurements on biological molecules. More regularly shaped particles, such as vaterite[7] or lysozyme[8] crystals, and compressed polystyrene beads[3], have also been used to generate torque. However, biochemical coupling of these particles to biological structures either has yet to be shown[7,8] or was nonspecific[3].

Ideally, angular trapping handles used to generate both torque and force should have the following attributes: (i) positive optical anisotropy for generation of torques well suited for biological applications, (ii) confinement of all three rotational degrees of

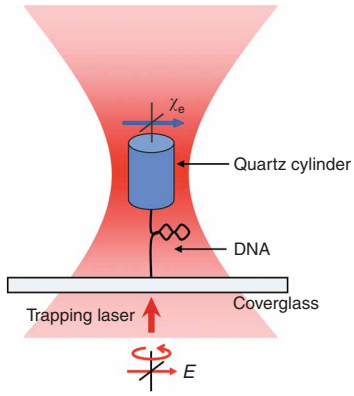

Figure 1 | Illustration of the angular optical trapping configuration. A cylinder fabricated from pure crystalline quartz is designed to trap with its extraordinary axis perpendicular to the propagation direction of the trapping laser. Its bottom surface is chemically functionalized for attachment to DNA. The height of the cylinder is greater than its diameter, causing the particle to align its cylinder axis with the laser propagation direction. A DNA molecule can be attached at one end to the bottom face of the cylinder via multiple biotin-streptavidin connections and at the other end to the surface of a coverglass via multiple digoxygenin–anti-digoxygenin connections. During a typical supercoiling experiment, the DNA is first stretched in the axial direction. The cylinder is then rotated via controlled rotation of the linear polarization of the laser to generate twist in the DNA. *E*, electric field of the trapping laser; χ_e, electric susceptability of the extraordinary axis of the quartz crystal.

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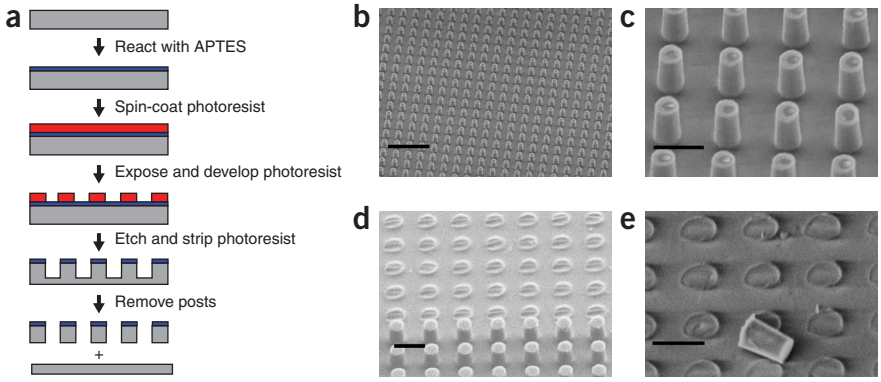

Figure 2 | Nanofabrication of quartz cylinders. (**a**) Schematic outline of the nanofabrication protocol. (**b**–**e**) Scanning electron micrographs of nanofabricated cylinders. Nanofabricated cylindrical posts on the wafer (**b**,**c**). The cylinders were 1.1 μm high and 0.53 μm in diameter. Quartz substrate after a portion of the posts was removed from the wafer (**d**). The quartz posts fractured evenly at their bases in a consistent manner. A single quartz cylinder after mechanical removal (**e**). Scale bars, 5 μm in **b** and 1 μm in **c**–**e**.

freedom to achieve a true angular trap, (iii) specific chemical derivatization at a well-defined location on the handle for attachment to a molecule of interest, (iv) independent control of the application of force and torque and (v) uniform size, shape and optical properties for ease of calibration and reproducibility.

A crystalline quartz cylinder with its extraordinary axis perpendicular to its cylinder axis and one of its ends chemically derivatized has these attributes (**Fig. 1**). As a cylinder with a height greater than its diameter will have a tendency to align with the laser propagation direction, provided that the cylinder diameter is comparable to the beam diameter[8,9], a cylindrical quartz particle can be rotated about its long axis by rotation of the linear polarization of the trapping laser. In this design, the optical anisotropy confines two of the three rotational degrees of freedom[6], and the cylindrical shape anisotropy also confines the third degree of freedom to achieve a true angular trap. Attaching a biological molecule to one end of a cylinder allows the application of force to the molecule along the laser propagation direction with minimal tilting of the trapped cylinder. This ensures that the extraordinary axis is maintained perpendicular to the laser propagation direction even when the attached molecule is under tension. This is desirable because tilting of the cylinder would result in suboptimal application of torque, and loss of independent control of torque and force. Nanofabrication techniques (**Fig. 2**) allow for the mass production of cylinders of uniform size, shape and optical properties as well as specific chemical derivatization of only one end of each cylinder.

The nanofabrication protocol (**Supplementary Methods** online) is outlined in **Figure 2a**. Briefly, we surface-derivatized an x-cut, single crystal quartz wafer with 3-aminopropyltriethoxysilane (APTES)[10]. We then layered, patterned and developed OIR-620-7i photoresist. We dry-etched the patterned wafer and stripped residual photoresist. At this point, the wafer contained ~1 billion functionalized quartz posts of nearly uniform height (1.1 ± 0.1 μm), diameter (0.53 ± 0.05 μm) and vertical sidewall angle (87 ± 2 degrees; **Fig. 2b**,**c**). The homogeneity in size was limited by nanofabrication processing, primarily by the focusing and leveling capabilities of the instrument used to pattern photoresist. Some of these procedures for nanofabricating posts are similar to those previously described[11]. We applied mechanical pressure from a microtome blade to remove the cylindrical quartz posts from the wafer substrate. The quartz posts fractured evenly at their bases (**Fig. 2d**,**e**). Finally we covalently coupled the cylinder's amino-functionalized surface to streptavidin.

We investigated the trapping properties of the quartz cylinders using an angular optical trap (**Supplementary Methods**). The angular stiffness[6] of a trapped cylinder was 11.4 ± 1.6 nN nm rad^{-1} (mean ± s.d.) for each watt of laser power entering the objective; nearly 3,000 pN nm of torque could be exerted on a quartz cylinder with 0.5 W of laser power. The axial linear stiffness[12] of a trapped cylinder was 0.59 ± 0.04 pN nm^{-1} for each Watt of laser power entering the objective. Over 100 pN of force could be exerted on a quartz cylinder with 0.5 W of laser power. These torques and forces are well suited for studies of biological molecules.

We tested the performance of the quartz cylinders in a DNA supercoiling assay (**Fig. 1**). When placed in an optical trap, a single quartz cylinder naturally oriented with its functionalized and slightly smaller end toward the coverglass, and therefore assumed the proper orientation for DNA attachment. When a DNA molecule is positively supercoiled under moderate constant tension (~4–28 pN), the DNA is expected to undergo a phase transition from B-form to supercoiled P-DNA (scP-DNA)[2,13]. The onset of the phase transition should be marked by an abrupt plateauing of torque. We ligated a linear 2.1-kbp dsDNA segment to a 62-bp, 6-biotin–tagged oligomer at one end and a 62-bp, 6-digoxygenin–tagged oligomer at the other end. The multiple tags at each end ensured that the ends of the DNA were torsionally constrained at both the streptavidin-coated end of the quartz cylinder and the anti-digoxygenin–coated coverglass. We tethered the dsDNA

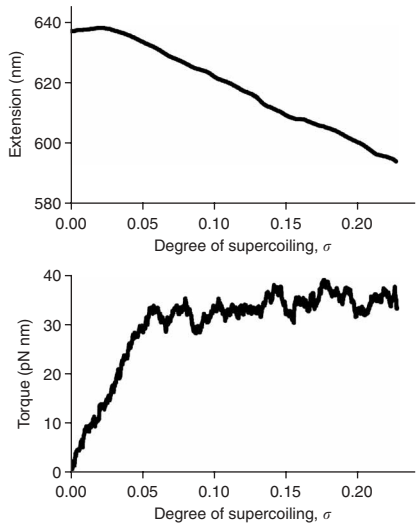

Figure 3 | Measurements during DNA supercoiling. A 2.2-kbp dsDNA molecule was held at 10 pN, and positive supercoils were added at a rate of 2 turns per second. The torque versus σ and the corresponding extension versus σ data were plotted.

molecule in PBS and then held it under 10 pN of tension. We added positive twist to the dsDNA molecule at a rate of 2 turns per second while a computer-controlled servo loop feeding back on a piezoelectric stage maintained constant tension in the dsDNA molecule. We simultaneously recorded five signals: axial force, axial displacement of the cylinder from the trap center, the axial position of the piezo, torque and the angular displacement of the extraordinary axis of the cylinder from the angular trap center. Data were antialias filtered at 1 kHz, digitized at 2 kHz and averaged with a 1.5-s moving window to reduce Brownian noise.

We measured both the torque and DNA extension as functions of the degree of supercoiling (σ), defined as the number of turns added to dsDNA divided by the number of naturally occurring helical turns in the given dsDNA (**Fig. 3**). At low σ values (0.00–0.05), the DNA exhibited a nearly linear increase in torque with σ. Over this range of σ, the DNA is expected to adopt the canonical B-DNA form. Once σ reached 0.05, the torque began to plateau at $\sim$33 pN nm, indicating the beginning of a phase transition. These critical σ and critical torque values are consistent with previous measurements, which have been interpreted as indicative of the B-DNA to scP-DNA transition[2]. We also observed a slight increase in extension for σ values in the range of 0.00–0.03. This has been attributed to a negative twist-stretch coupling[14,15].

These results demonstrate that nanofabricated quartz cylinders are well suited for precision measurements in an angular optical trap. For the first time, torque, angle, force and DNA extension during DNA supercoiling can be simultaneously monitored at kilohertz rates. This capability will allow future detection of rapid events and concurrent observation of the linear and angular behaviors of DNA. The cylinders should provide a powerful tool for the investigation of torsional properties of biopolymers and rotational motions of biological molecular motors.

Note: Supplementary information is available on the Nature Methods website.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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