## Supplementary Information

# **Tunable Top-Down Fabrication and Functional Surface Coating of Single-Crystal Titanium Dioxide Nanostructures and Nanoparticles**

Seungkyu Ha, Richard Janissen, Yera Ye. Ussembayev, Maarten M. van Oene, Belen Solano and Nynke H. Dekker\*

Department of Bionanoscience, Kavli Institute of Nanoscience, Faculty of Applied Sciences, Delft University of Technology, Lorentzweg 1, 2628 CJ Delft, The Netherlands.

\*E-mail: n.h.dekker@tudelft.nl

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## **Supplementary References**

#### **Supplementary Methods**

#### Orientation of the optic axis in single-crystal rutile TiO<sub>2</sub> nanocylinder for OTW experiment

To control polarization-based rotation of optically trapped nanoparticles in an OTW, birefringent positive uniaxial single-crystals are desirable substrate materials. Single-crystal rutile TiO<sub>2</sub> is such a material, and it has an exceptionally high birefringence that is advantageous for effective torque transfer in an OTW. In particular, cylindrically shaped nanoparticles align their long axis with the direction of laser beam propagation, fixing two of the three rotational degrees of freedom (DOF). The remaining rotational DOF is controllable via the polarization of the laser beam provided that the optic axis is perpendicular to the nanocylinder's long axis (**Fig. 5a**). To appropriately control the orientation of this optic axis within the nanocylinders, it is necessary to etch into (100)-cut single-crystal rutile TiO<sub>2</sub> substrates. A similar approach has been employed for the case of X-cut single-crystal quartz SiO<sub>2</sub> substrates.<sup>1–5</sup>

#### Considerations for optimal fabrication of the Cr etch mask for single-crystal TiO<sub>2</sub> etching

We consider the optimal fabrication of the Cr etch mask for the desired size of single-crystal TiO<sub>2</sub> nanostructures. The deposited Cr layer should be sufficiently thick for the mask to remain functional until the end of etching process, taking into account the fact that the mask top surface will not be perfectly flat. Also, the overall thickness of the Cr mask is limited by that of the used PMMA layer. The thickness of the PMMA should be 2–3 times larger than that of the Cr mask to facilitate complete lift-off, but it has an upper limit determined by its concentration. In practice, Cr layers thicker than ~150 nm tend to cause more severe deformation in patterned PMMA layers, resulting in higher nonuniformity. This deformation is presumably due to the built-up stress in Cr layers during physical vapor deposition.<sup>6</sup> A further consideration in mask fabrication is that mask shapes tend to be more cone-like when thicker Cr layers and/or smaller patterned apertures are used<sup>7</sup> (**Fig. 1**, **step 4**, **inset illustration**). Such masks are not suitable for anisotropic etching for vertical sidewall due to more rapid erosion of their thinner edges.

#### Surface functionalization procedure of single-crystal TiO<sub>2</sub>

For the surface functionalization of single-crystal TiO<sub>2</sub> substrates, we have compared four different surface linker molecules (**Fig. S5**†): ETA (Sigma-Aldrich, The Netherlands), GPDMES (Sigma-Aldrich, The Netherlands), APDMES (Sigma-Aldrich, The Netherlands), and BADMSCP (abcr GmbH, Germany). For the binding of ETA linker, we dissolve ETA in anhydrous dimethyl sulfoxide (DMSO) (Sigma-Aldrich, The Netherlands) to a final concentration of 5 M. We use this ETA/DMSO solution to incubate the substrates for 12 h at room temperature, followed by washing the substrates with DI water. For the binding of epoxysilane linker (GPDMES), we incubate the substrates for 15 min at 75 °C using non-diluted GPDMES solution, followed by chloroform washing. For the binding of the APDMES and BADMSCP linkers, we incubate the substrates in ethanol containing either 5% (v/v) of APDMES or BADMSCP. We carry out the silanization reaction for 12 h at 70 °C and then wash with chloroform (or ethanol). Additionally, we perform PEGylation of ETA-coated surfaces with heterobifunctional NHS-PEG-COOH (MW 5,000, LaysanBio, USA).<sup>8</sup> We incubate the surfaces with 2 mM PEG dissolved in 100 mM 2-(N-morpholino)ethanesulfonic acid (MES) buffer (pH 4.7, Sigma-Aldrich, The Netherlands) for 1 h at room

temperature. Afterwards, we wash the PEGylated surfaces with DI water. For every washing step involved, we wash three times for 15 s each, and then dry the substrates under a N<sub>2</sub> stream. The above protocols can also be applied to other oxidized surfaces, e.g. quartz (SiO<sub>2</sub>) (**Fig. S5**†), silicon (Si), silicon nitride (Si<sub>3</sub>N<sub>4</sub>), and non-noble metals.

#### Evaluation of single-crystal TiO<sub>2</sub> surface functionalization efficiency via fluorescence microscopy

We fabricate 25 × 25  $\mu$ m<sup>2</sup> micro-patterns of PMMA on single-crystal rutile TiO<sub>2</sub> and quartz SiO<sub>2</sub> substrates for quantitative evaluation of the surface linker functionalization efficiencies via fluorescence microscopy. To fabricate the PMMA micro-patterns, we utilize a similar protocol as described for TiO<sub>2</sub> nanocylinders (**Methods**). The main differences include spin-coating PMMA 950k A11 to achieve a ~1.9  $\mu$ m-thick layer and altered ebeam settings (a current of 312 nA, a diameter of 300 nm through defocusing the beam, and a dose of 1000  $\mu$ C/cm<sup>2</sup>). For the quartz SiO<sub>2</sub> substrates (X-cut, University Wafer, USA) alone, we sputter a 30 nm-thick gold (Au) layer (EM ACE600, Leica, The Netherlands) onto the spin-coated PMMA layer to prevent charging. Following ebeam patterning, we remove the Au layer by a wet etchant (TFA, Transene, USA). As described in **Methods**, we treat the micro-patterned substrates with O<sub>2</sub> plasma (Plasma-PREEN I) prior to the functionalization process.

For the evaluation of the surface functionalization efficiencies of the different linkers to single-crystal TiO<sub>2</sub> (and SiO<sub>2</sub>) substrates, we use amino and NHS-ester modified fluorophores (ATTO 647N, ATTO-TEC GmbH, Germany). They are covalently added to the organic functional groups of the surface linkers employed. For the substrates coated with ETA, APDMES, and BADMSCP, we dissolve NHS-ester labeled fluorophores in PBS buffer (pH 8.4, Sigma-Aldrich, The Netherlands) to a final concentration of 10 µM and add to the functionalized surfaces. After the reaction time of 1 h, we wash the substrates three times each with PBS/TWEEN® buffer (pH 7.4) and DI water to remove residual physisorbed molecules, and dry under a N<sub>2</sub> stream. For GPDMES-coated and PEGylated surfaces, we add 10 µM of amino-labeled fluorophores to PBS buffer (pH 7.4) and MES/EDC buffer (100 mM MES (pH 4.7) containing 50 mM EDC (1-Ethyl-3-(3-dimethylaminopropyl)carbodiimid, Sigma-Aldrich, The Netherlands)), respectively, and the fluorophore coupling reactions take place for 1 h. We wash the substrates with PBS/TWEEN® buffer (pH 7.4) and DI water, followed by drying under a N<sub>2</sub> stream, as stated before.

We perform the fluorescence measurements of the functionalized surfaces and nanocylinders using an epifluorescence microscope (IX-81, Olympus, The Netherlands) equipped with a Peltier-cooled back-illuminated electron multiplying charge coupled device (EMCCD) camera (IXON, 512 × 512 pixels, Andor, Ireland), in combination with an oil-immersion objective lens (100×/NA1.3, UPLNFLN, Olympus, The Netherlands). The fluorophores are excited using a diode laser ( $\lambda$  = 640 nm, Cell Laser System, Olympus, The Netherlands). For quantitative measurements, we measure the fluorescence intensity (in photon counts per second) of an area of 12.5 × 12.5 µm<sup>2</sup> for different square micro-patterns on each sample. We calculate the average intensity – normalized to the area of 1 µm<sup>2</sup> – and the corresponding standard deviations to compare the different linker molecule coverages (**Fig. S5**†).

#### Preparation of DNA construct for OTW experiments

We carry out the DNA extension and supercoiling measurements (**Fig. 5**) on a linear 21.8 kbp DNA that contains biotin and digoxigenin modified nucleotides (biotin-16-dUTP and digoxigenin-11-dUTP, respectively, Roche Diagnostics, The Netherlands) at the opposite extremities (600 bp each). We prepare the DNA by ligating the biotin- and digoxigenin-enriched handles to a 20.6 kbp DNA fragment that is obtained via a *Notl/Xhol* digestion of Supercos1-lambda 1,2 plasmid (Agilent Technologies, USA). We create the DNA handles by PCR amplification of a 1.2 kbp fragment from pBlueScript II SK+ (Agilent Technologies, USA) using the primers – 5'-GACCGAGATAGGGTTGAGTG and 5'-CAGGGTCGGAACAGGAGAGC – in the presence of either biotin-16-dUTP or digoxigenin-11-dUTP. Prior to ligation using T4 DNA ligase (New England Biolabs, UK), the biotin and digoxigenin containing handles are digested with *Notl* and *Xhol*, respectively.<sup>9</sup>

#### Preparation of flow cell for OTW experiments

We perform OTW experiments (**Fig. 5**) in a custom-made flow cell assembled from two  $24 \times 60 \text{ mm}^2$  borosilicate coverslips (#1.5, ~170 µm thickness, Menzel GmbH, Germany) separated by a single-layer Parafilm® (Sigma-Aldrich, The Netherlands) spacer.<sup>1,8,10</sup> We drill two holes of ~1 mm diameter in the top coverslips using a sand blaster, to connect with inlet and outlet tubings. Prior to flow cell assembly, we clean the coverslips using a 4% (v/v) aqueous Hellmanex® III (Hellma GmbH, Germany) solution and then DI water, in both cases by sonication for 20 min at 40 °C. We dry the cleaned coverslips under a N<sub>2</sub> stream. For the bottom coverslips, we perform surface functionalization to attach biomolecules. To increase the density of surface hydroxyl groups, which allows for denser, more homogeneous functionalization, we treat the bottom coverslips with O<sub>2</sub> plasma (Plasma-PREEN I) for 1 min with O<sub>2</sub> flow rate of 3 scfh and RF power of 200 W. We incubate these coverslips in DMSO solution containing 5 M ETA for 12 h at room temperature. Afterwards, we wash the functionalized coverslips thoroughly with DI water and dry them under a N<sub>2</sub> stream. Single-layer Parafilm® spacers are prepared by cutting out the desired flow cell channel shape, which is properly aligned to the holes in the top coverslips. Finally, we assemble the flow cells and seal the channels by melting the Parafilm® spacers between the coverslips on a hotplate for 30 s at 90 °C.

#### Bioconjugation of DNA to single-crystal TiO<sub>2</sub> nanocylinders for OTW experiments

In an OTW, we are able to carry out the extension and coiling measurements on individual, torsionally constrained DNA molecules.<sup>1–5</sup> We tether the DNA molecules to the bottom surface of the flow cell channel via digoxigenin:anti-digoxigenin coupling and to the functionalized single-crystal rutile TiO<sub>2</sub> nanocylinders via biotin:streptavidin coupling (**Fig. 5a**). To do so, we perform three steps. First, we PEGylate the ETA-coated flow cell channel both to covalently attach digoxigenin antibodies and to ensure an effective surface passivation against non-specific physisorption of streptavidin-coated TiO<sub>2</sub> nanocylinders. We achieve this by incubating the channel with 2 mM PEG dissolved in 100 mM MES buffer (pH 4.7) for 1 h. After washing with DI water, we incubate for 1 h with 8  $\mu$ M digoxigenin IgG antibodies (Roche Diagnostics, The Netherlands) dissolved in MES/EDC buffer (pH 4.7). We wash the channel with PBS buffer (pH 7.4) and incubate BlockAid<sup>TM</sup> (Life

Technologies, USA) for 1 h for additional surface passivation. Subsequently, we wash the channel with PBS buffer (pH 7.4). Second, we attach individual DNA molecules via the digoxigenin handles to the digoxigenin antibody-covered flow cell channel by incubating 5 pM of DNA for 1 h. We remove non-specifically adhered DNA molecules by washing the channel with PBS buffer (pH 7.4). Third, we attach the DNA via the biotinylated handles to the streptavidin-coated TiO<sub>2</sub> nanocylinders (**Methods**) by incubating them in the flow cell channel for ~30 min. We remove non-attached nanocylinders by flushing 1:1 diluted (v/v) BlockAid<sup>TM</sup> in PBS/Triton<sup>TM</sup> buffer (pH 7.4). We perform all DNA extension and coiling measurements in PBS/Triton<sup>TM</sup> buffer.

#### OTW instrumentation and DNA measurements with single-crystal TiO<sub>2</sub> nanocylinders

For optical trapping of single-crystal TiO<sub>2</sub> nanocylinders in our OTW setup (**Fig. 5b,c**), we expand and collimate a linearly polarized laser ( $\lambda$  = 1064 nm, Compass CW 1064-4000M, Coherent, The Netherlands) beam using a beam expander (4401-181-000-20, LINOS, Germany) to fill properly the input aperture of an objective lens (60×/NA1.2, CFI-PLAN-APO-VC-60XA-WI, Nikon, The Netherlands). The power entering the objective lens is always set to 100 mW during experiments. The objective lens focuses the laser beam into a flow cell, generating an optical trap.

To calibrate the optical trap, we monitor the fluctuations of an isolated, optically trapped  $TiO_2$  nanocylinder using a position-sensitive detector (PSD) (DL100-7PCBA3, Pacific Silicon Sensor, The Netherlands) that acquires at 100 kHz (**Fig. S7**†). The nanocylinder trapping position is ~7 µm above from the bottom surface of the flow cell channel, to conserve the similar measurement conditions with the case of DNA-tethered nanocylinders.<sup>11</sup> We can calculate the restoring force acting on a nanocylinder displaced from the trap center, by multiplying the measured displacement with the calibrated trap stiffness. Precise linear translation of the flow cell is possible using a piezo-actuator (P-517.3CD, Physik Instrumente, Germany) in x, y, and z directions.

For the force-induced DNA-extension experiments, we move the flow cell along the axis of laser beam propagation (axial direction) at a constant speed (~1 µm/s) while maintaining the laser beam at a fixed position. As the flow cell moves away from the laser beam focus, the increased DNA-tethered nanocylinder's displacement from the trap center induces higher axial force to the DNA molecule. For the DNA coiling experiments, a constant axial force should be applied to the trapped, DNA-tethered nanocylinder since whether the DNA twists or forms plectonemic supercoils strongly influenced by the tension applied to the molecule.<sup>12</sup> We use a feedback loop between the piezo-actuator and PSD to clamp the axial force at the specified setpoint. By simultaneously rotating the linear input polarization of the laser beam, we apply torque to the TiO<sub>2</sub> nanocylinder, coiling the DNA molecule. The polarization can be rotated either by manual rotation of a half-wave plate (PWPS-1064-10-2, CVI Melles Griot, Germany), or by a fast electro-optical modulator (EOM) (LM 0202-LT, LINOS, Germany) in combination with quarter-wave plates (PWPS-1064-10-4, CVI Melles Griot, Germany).

## **Supplementary Tables**

Sample	CHF₃ flow	O₂ flow	Ar flow	RF power	Chamber pressure	TiO₂ etch rate	Cr etch rate	Etch selectivity
	[sccm]	[sccm]	[sccm]	[W]	[µbar]	[nm/min]	[nm/min]	[TiO <sub>2</sub> :Cr]
Ra1	50	5	30	200	50	68	3.7	18
Ra2	50	5	30	165	50	58	3.2	18
Ra3	50	5	30	100	50	30	1.7	18
Ra4	50	5	30	200	10	30	8.3	4
Rb1	50	5	30	200	50	77	4.1	19
Rh2	50	5	0	200	50	73	35	21

Table S1. Dry etching conditions for single-crystal TiO<sub>2</sub> during optimization of the CHF<sub>3</sub>-RIE process.<sup>a)</sup>

<sup>a)</sup> The bold red numbers denote altered process parameters compared to the reference samples (Ra1 and Rb1).

#### Dry etching conditions for single-crystal TiO<sub>2</sub> during optimization of the CHF<sub>3</sub>-RIE process

We show the representative etching conditions (**Table S1**<sup>†</sup>) used during optimization of the CHF<sub>3</sub>-based plasma etching of single-crystal TiO<sub>2</sub> in an RIE system. Throughout the optimization, we fix the flow rate of CHF<sub>3</sub> gas, the main etchant, at the maximum value of 50 sccm, and set the flow rate of O<sub>2</sub> gas at 5 sccm which is the median value of our target range (0–10 sccm). More accurate comparisons are possible within the same batches of the applied Cr etch mask, designated as Ra (H: 100 nm, D: 535 nm) and Rb (H: 100 nm, D: 345 nm). The samples Ra1 and Rb1 are the reference samples, while the other samples differ by a single process parameter (designated as bold red numbers in **Table S1**<sup>†</sup>).

As an elevated RF power increases the etch rates of TiO<sub>2</sub> while maintaining nearly constant etch selectivity (compare samples Ra1, Ra2, and Ra3 in **Table S1**†), we select the highest available power (200 W). We find that an increase in the chamber pressure (from 10 to 50 µbar) enhances the etch selectivity by increasing the TiO<sub>2</sub> etch rates and decreasing the Cr etch rates (compare samples Ra1 and Ra4 in **Table S1**†). However, we do not utilize chamber pressures exceeding 50 µbar, as higher values result in excessive deposition of fluorocarbon passivation layers<sup>13</sup> that are sufficiently thick to reduce the TiO<sub>2</sub> etch rates again. With these parameters fixed, we vary the gas composition. The addition of Ar gas, which results in harsher physical etching by heavy ions, generally increases etch rates.<sup>14</sup> Indeed, upon its addition (at 30 sccm) both the Cr and TiO<sub>2</sub> etch rates are increased; however, as the increase in the Cr etch rate surpasses that of TiO<sub>2</sub>, a deteriorated etch selectivity results (compare samples Rb1 and Rb2 in **Table S1**†). Our optimized process condition thus consists of CHF<sub>3</sub> at 50 sccm, a chamber pressure of 50 µbar, and an RF power of 200 W. With these fixed parameters, we vary O<sub>2</sub> gas flow rate from 0 sccm to 10 sccm to control sidewall profiles (**Fig. S3**†), and vertical sidewall can be obtained with 0.5 sccm of O<sub>2</sub>.

Table S2. Dry etching conditions for single-crystal TiO<sub>2</sub> nanofabrication.<sup>a)</sup>

Figure Etching System		Etching conditions				
Fig. 2a	F1	Cr etch mask (H: 45 nm, D: 175 nm), CHF <sub>3</sub> :O <sub>2</sub> = 50:0.5 sccm, RF 200 W, chamber pressure 50 $\mu$ bar, DC bias -950 V, etch time 11 min ( <b>optimized</b> ).				
Fig. 2b	F2	Cr etch mask (H: 100 nm, D: 255 nm), CHF <sub>3</sub> :O <sub>2</sub> = 50:8 sccm, RF 200 W, chamber pressure 50 $\mu$ bar, DC bias -1100 V, etch time 15 min ( <b>optimized</b> ).				
Fig. 2c	F1	Cr etch mask (H: 100 nm, D: 535 nm), CHF₃:O₂:Ar = 50:5:30 sccm, RF 165 W, chamber pressure 50 µbar, DC bias -855 V, etch time 15 min.				
Fig. 2d	F3	Cr etch mask (H: 100 nm, D: 535 nm), <b>SF</b> 6:CH4:Ar = 15:30:50 sccm, ICP:RF = 2500:300 W, chamber pressure 30 µbar, DC bias -50 V, etch time 10 min, sample holder temperature 0 °C, chamber temperature 200 °C.				
Fig. 2e	F4	Cr etch mask (H: 130 nm, D: 185 nm), SF <sub>6</sub> :He = 50:100 sccm, ICP:RF = 800:200 W, chamber pressure 50 $\mu$ bar, DC bias -475 V, etch time 12 min, chamber temperature 25 °C.				
Fig. 2f	F4	Cr etch mask (H: 120 nm, D: 205 nm), SF <sub>6</sub> :He = 20:100 sccm, ICP:RF = 300:300 W, chamber pressure 100 $\mu$ bar, DC bias -835 V, etch time 4 min, chamber temperature 25 °C (optimized).				
Fig. 3	F2	Cr etch mask (H: 60 nm, D: 220 nm), CHF <sub>3</sub> :O <sub>2</sub> = 50:4 sccm, RF 200 W, chamber pressure 50 $\mu$ bar, DC bias -1100 V, etch time 15 min ( <b>optimized</b> ).				
Fig. 5	F4	Cr etch mask, the same etching condition as <b>Fig. 2f (SF</b> 6).				
Fig. S2†	F3	Various etch mask materials, <b>SF</b> <sub>6</sub> :CH₄:Ar = 15:30:50 sccm, ICP:RF = 1500:250 W, chamber pressure 30 µbar, DC bias -55 V, etch time 10 min, sample holder temperature 0 °C, chamber temperature 200 °C.				
Fig. S3†	F1	Cr etch mask (H: 100 nm, D: 345 nm), the same etching condition as <b>Fig. 2a (CHF</b> <sub>3</sub> ), except for the etch time (15 min) and the O <sub>2</sub> gas flow rate (0, 0.5, 1, 5, or 10 sccm).				
Fig. S4†	F2	Cr etch mask (H: 30 nm, D: 190 nm), the same etching condition as <b>Fig. 3 (CHF</b> <sub>3</sub> ), except for the etch time (8 min).				
Fig. S7†	F4	Cr etch mask, the same etching condition as <b>Fig. 2f (SF</b> <sub>6</sub> ).				

<sup>a)</sup> The bold red letters denote the main etchant gas in each condition.

#### Dry etching conditions for single-crystal TiO<sub>2</sub> nanofabrication

We use etching systems that are denoted as F1 (Fluor Z401S, Leybold Heraeus, Germany), F2 (Fluor Z401S, Leybold Heraeus, Germany), F3 (AMS100 I-speeder, Adixen, France), and F4 (Plasmalab System 100, Oxford Instr., UK). The F1 and F2 are two nominally identical RIE systems, while F3 and F4 are two distinct ICP-RIE systems. We summarize the etching conditions for each batch of TiO<sub>2</sub> nanocylinders in **Table S2**<sup>+</sup>.

Sample	Statistical parameter	Top diameter	Bottom diameter	Height	Top roundness	Bottom roundness	Volume
<b>Fig. 3</b> (15 min etch)	Average	151 nm	215 nm	652 nm	0.97	0.98	0.017 μm³
	Local RSD	4.4%	2.3%	0.6%	1.0%	0.5%	4.5%
	Global RSD	4.7%	2.7%	1.4%	1.1%	0.5%	5.2%
<b>Fig. S4</b> † (8 min etch)	Average	149 nm	187 nm	273 nm	0.98	0.99	0.006 µm³
	Local RSD	1.5%	0.9%	1.3%	0.5%	0.3%	2.1%
	Global RSD	2.4%	1.7%	3.1%	0.6%	0.4%	4.1%

Table S3. Dimensional analysis of high and low aspect-ratio  $TiO_2$  nanocylinders.

## Dimensional analysis of high and low aspect-ratio $\text{TiO}_2$ nanocylinders

We have analyzed SEM images of high and low aspect-ratio nanocylinder batches to quantify both the local and global structural uniformity. We summarize the obtained statistical parameters for each batch of  $TiO_2$  nanocylinders in **Table S3**<sup>†</sup>, and present the corresponding graphs in **Fig. 3d-f** (high aspect-ratio: 3.6) and **Fig. S4d-i**<sup>†</sup> (low aspect-ratio: 1.6).

## **Supplementary Figures**



**Fig. S1.** Diverse applications of TiO<sub>2</sub> at the nanoscale. **(a)** Overview of the chemical, electrical, and optical properties that make TiO<sub>2</sub> a versatile material for different applications. **(b)** Illustrations of nanoscale applications of TiO<sub>2</sub> (yellow). Top, left to right: a TiO<sub>2</sub> nanoparticle serves as a photocatalyst for water splitting into oxygen and hydrogen gases (*hv*: input radiation energy, CB: conduction band, VB: valence band, e: electron, h<sup>+</sup>: hole); a TiO<sub>2</sub> nanopillar array acts as a gas sensor (gray: metal electrodes, green: gas molecules, A: current as a sensing signal); a TiO<sub>2</sub> thin film acts as a tunable resistance material in a resistive random access memory device (gray: metal electrodes, orange: TiO<sub>2-x</sub>, V: voltage as a read/write signal); a TiO<sub>2</sub> thin film can be used as a channel layer in a transparent transistor (gray: metal electrodes, blue: gate insulator, purple: transparent electrode, V<sub>5</sub>, V<sub>0</sub>, and V<sub>6</sub> denote voltages at source, drain, and gate terminals, respectively). Bottom, left to right: a TiO<sub>2</sub> nanoord array acts as a photoanode in a solar cell (*hv*: radiation energy from the sun, gray: metal electrode, purple: transparent electrode, magenta: sensitization layer e.g. dye molecules or quantum dots, brown: electrolyte, A: current as converted energy); a nanostructured TiO<sub>2</sub> forms the core of a strip waveguide to support lightwave propagation (blue: cladding layers, red: confined lightwave); a two-dimensional TiO<sub>2</sub> photonic crystal slab used to manipulate the flow of lightwave (blue: cladding layers, red: confined lightwave); an optically trapped TiO<sub>2</sub> nanoparticle used as a force transducer (red: focused laser beam, dotted circle: trap center, F: force induced by the displacement of the nanoparticle).



**Fig. S2.** The etch rates and etch selectivities of different mask materials. **(a)** The etch rates for substrate (blue bars) materials (TiO<sub>2</sub>: (100)-cut rutile single crystal, ~140 nm/min; SiO<sub>2</sub>: X-cut quartz single crystal, ~220 nm/min) and mask (red bars) materials (a-Si: amorphous silicon layer deposited by a plasma-enhanced chemical vapor deposition system (Plasmalab 80 Plus, Oxford Instr., UK), ~300 nm/min; ER: spin-coated and baked e-beam resist layer (NEB-22A2E, Sumitomo Chemical, Belgium), ~110 nm/min; W: tungsten layer deposited by an e-beam evaporator (Temescal FC-2000, Ferrotec, Germany), ~60 nm/min; Cr: chromium layer deposited by the same evaporator, ~5 nm/min). All results are obtained under the same dry etching conditions (using the F3 etching system; etching conditions in **Table S2**†). **(b)** Etch selectivity of each mask material for single-crystal TiO<sub>2</sub> dry etching, which is the etch rate of TiO<sub>2</sub> divided by that of the mask material (a-Si: 0.5, ER: 1.3, W: 2.3, Cr: 28).



Fig. S3. Control of sidewall profiles and etch characteristics in single-crystal TiO<sub>2</sub> nanocylinders by variation of O<sub>2</sub> flow rate in the CHF<sub>3</sub>-RIE process. (a-e) SEM images of single-crystal TiO<sub>2</sub> nanocylinders produced under identical etching conditions (using the F1 etching system; etching conditions in **Table S2**<sup> $\dagger$ </sup>) apart from the O<sub>2</sub> flow rate (values shown at the bottom of each image). Scale bars denote 500 nm. Top surfaces of the nanocylinders are marked with yellow lines because the remaining Cr masks are also visible in these images. The insets in top-right corner illustrate (to scale) the corresponding three-dimensional shapes of the nanocylinder SEM images. The types of obtained nanocylinder shapes include (a) positive sidewall angles, (b) vertical sidewall angles, (c) negative sidewall angles, (d) symmetric hourglass shapes, and (e) asymmetric hourglass shapes. In (b) and (e), the insets in top-left corner (scale bars denote 500 nm) show top-view SEM images of nanocylinders cut at their middle, displaying cross-sections that are (b) circular or (e) diamond-shaped. (f) For the analysis of sidewall angles ( $\theta$ ), we use two-dimensional models as defined here. The definition of sidewall angles is illustrated for the cases of positive, vertical, and negative angles, using the measured heights (H) and diameters of top (Dt) and bottom (Db). The hourglass-shaped nanocylinders possess two sidewall angles and heights for both top ( $\theta_t$ ,  $H_t$ ) and bottom ( $\theta_b$ ,  $H_b$ ) sides, and an additional waist diameter ( $D_w$ ). (g-i) The etch characteristics are shown as a function of the  $O_2$  flow rate: the etch rates of (g) TiO<sub>2</sub>, (h) Cr, and (i) the resulting etch selectivities (TiO<sub>2</sub>:Cr). The measured dimensions extracted from the SEM images (a-e) are as follows: (a)  $D_t$ : 285 nm,  $D_b$ : 395 nm, H: 500 nm,  $\theta$ : 84°; **(b)**  $D_t$  =  $D_b$ : 275 nm, H: 545,  $\theta$ : 90°; **(c)**  $D_t$ : 305 nm,  $D_b$ : 260 nm, H: 520 nm, θ: -88°; (d) Dt = Db: 260 nm, Dw: 175 nm, Ht = Hb: 555 nm, -θt = θb: 86°; (e) Dt: 245 nm, Db: 370 nm, Dw: 135 nm, Ht: 385 nm, H<sub>b</sub>: 1085 nm, θ<sub>t</sub>: -82°, θ<sub>b</sub>: 84°.

#### Control mechanism for sidewall profiles in CHF3-etched single-crystal TiO2 nanocylinders

We observe that tuning the  $O_2$  flow rate during the single-crystal TiO<sub>2</sub> etching process allows us to control the sidewall profile of the nanostructures. We attribute the formation of different sidewall profiles (positive, vertical, negative, and hourglass-shaped) to underlying changes in the thickness of a sidewall passivation layer that result from the interplay between CHF<sub>3</sub> and O<sub>2</sub> plasma. The thickness variation permits both the formation

of positive sidewall angles in the absence of  $O_2$  flow and the formation of vertical (or negative) sidewall angles at an  $O_2$  flow of 0.5 (1.0) sccm (**Fig. S3a-c**<sup>+</sup>). The hourglass-shaped etch profiles likely result from substantially reduced TiO<sub>2</sub> surface passivation combined with the random trajectories of reactive ions<sup>15,16</sup> (**Fig. S3d,e**<sup>+</sup>).

# Control mechanism for cross-sectional shapes and etch selectivity in CHF<sub>3</sub>-etched single-crystal TiO<sub>2</sub> nanocylinders

We find that the single-crystal TiO<sub>2</sub> nanocylinders etched at low O<sub>2</sub> flow rates (0–1 sccm) exhibit circular crosssections (**Fig. S3b**†, **inset** in top-left corner), while those etched at high O<sub>2</sub> flow rates (5–10 sccm) feature diamond-shaped cross-sections (**Fig. S3e**†, **inset** in top-left corner). These differences may result from changes in the predominant etching mode: at low O<sub>2</sub> flow rates, the initial shape of the etch masks (circular in the case of **Fig. S3**†) should be directly transferred to the etched nanostructures, as the dominance of physical etching by ion bombardment results in the same etch rate independently of crystallographic orientation; conversely, at high O<sub>2</sub> flow rates, chemical etching may predominate than physical etching, resulting in etch rates that vary per orientation of the crystal planes in the single-crystal TiO<sub>2</sub> substrates.<sup>17</sup> Moreover, the above reasoning is supported by the fact that TiO<sub>2</sub> etch rates (**Fig. S3g**†) increase significantly (~3-fold) while Cr etch rates (**Fig. S3h**†) remain nearly the constant (~3 nm/min), as we increase O<sub>2</sub> flow rate. The removal of Cr mask layer is possible only by the physical etching but not by the chemical etching based on fluorine chemistry while both etching modes induce TiO<sub>2</sub> etching. We attribute this to a decreased thickness of the CHF<sub>3</sub> plasma-generated fluorocarbon passivation layer on TiO<sub>2</sub> surfaces in the presence of O<sub>2</sub> plasma.<sup>18</sup> As such a layer protects TiO<sub>2</sub> etch rates whilst those of Cr remain nearly unaffected, enhancing etch selectivity (**Fig. S3i**†).

#### The reproducibility of different sidewall profiles in CHF<sub>3</sub>-etched single-crystal TiO<sub>2</sub> nanocylinders

A repetition of this experiment in the second nominally identical RIE system yields the similar trends but at slightly altered  $O_2$  flow rates (using the F2 etching system; etching conditions in **Table S2**<sup>†</sup>). For example, etching nanocylinders with vertical sidewall angles requires an  $O_2$  flow rate of 4–8 sccm (compared to ~0.5 sccm in the first RIE system (**Fig. S3b**<sup>†</sup>)). Similarly, etching nanocylinders into hourglass-shapes requires an  $O_2$  flow rate of ~16 sccm (compared to 5–10 sccm in the first RIE system (**Fig. S3d,e**<sup>†</sup>)). We attribute this discrepancies in parameters to the differences in instrument calibration, e.g. of the mass flow controllers for the control and measurement of  $O_2$  flow rates.



Fig. S4. Dimensions of fabricated single-crystal TiO<sub>2</sub> nanocylinders. (a-c) SEM images of etched single-crystal TiO<sub>2</sub> nanocylinders (light gray). Scale bars denote 1  $\mu$ m. (a) Top-view of a single-crystal TiO<sub>2</sub> substrate with partially cleaved nanocylinders. An array of rigidly fixed nanocylinders is visible in the top left corner, and the cleaved substrate surface is bottom right corner. The released nanocylinders are positioned at the interface of these regions. (b) Top-view of a substrate with etched nanocylinders. The inset shows a zoom-in as an example for image analysis. The red (blue) contour, dotted line, and dot represent the boundary, equivalent radius, and center of nanocylinder top (bottom) surface, respectively. (c) Tilted-view (60°) of a substrate with etched nanocylinders. The green dotted horizontal lines are crossing the centers of top and bottom surfaces, and the green vertical line indicates the height that will be converted to the actual height considering the tilting angle. (d-i) Nanocylinder dimensions extracted from the SEM images. The graphs display the (d) top diameter, (e) bottom diameter, (f) height, (g) top roundness, (h) bottom roundness, and (i) volume as a function of the radial distance from the substrate center. The roundness (defined as  $4\pi A/P^2$ , A: area, P: perimeter) measures how closely the shape of a nanocylinder's cross section resembles that of a circle, where 1 corresponds to a perfect circle and smaller values imply deviations from circular. Measurement points are spaced by 0.5 mm from the center of the substrate to its edge. At each point, the diameters (heights) are calculated from n = 12 (n = 10) different nanocylinders. The square markers and the error bars in the graphs represent the mean and the standard deviation of the local uniformity, respectively. The horizontal dotted black lines and the top and bottom sides of the gray shaded boxes in the graphs represent the mean and the standard deviation of the global uniformity, respectively.

#### Distributions of fabricated single-crystal TiO<sub>2</sub> nanocylinder dimensions

The comparison between single-crystal  $TiO_2$  nanocylinder dimensions presented in **Fig. S4**<sup>†</sup> and those presented earlier (**Fig. 3**) provides more information for the analysis of the nanocylinder dimensions. We etch the different batches of nanocylinders under the same conditions, except for the etch duration (**Table S2**<sup>†</sup>). The etch time of the nanocylinders analyzed in **Fig. S4**<sup>†</sup> (8 min) is approximately twice less than that of the

nanocylinders shown in Fig. 3 (15 min). Both etch times demonstrate a similar trend regarding the nanocylinder dimensions (analysis results are summarized in Table S3<sup>+</sup>). The observed irregular Cr mask erosion effect, which depends on the roughness of the mask surface, causes stronger deformation of the top surface geometry than the bottom of the nanocylinders; we expect that the bottom surface geometry is mostly determined by the initial round shape of etch mask while the top surface geometry is the same as the eroded etch mask until the end of the etching process. This geometry deformation is also observable in the roundness analysis, in which the top roundness values are smaller than the bottom roundness values for both batches of nanocylinders. For this reason, the top diameters show less local uniformity than the bottom diameters. As this effect is more profound for extended etch times, etch time of 15 min results in less local uniformity than etch time of 8 min for both top and bottom diameters. For each measurement point, the top and bottom diameters are directly correlated as observable in Fig. S4<sup>†</sup> and Fig. 3. However, variations in the top and bottom diameters across a substrate are random, possibly due to the instability of e-beam size or current during ebeam patterning process. Regardless of these variations, the top and bottom diameters exhibit still high global uniformity for both batches of nanocylinders (RSD ≤5%), which lie in the same order of magnitude as their local uniformity. Further, we observe high global uniformity in nanocylinder heights across the substrates (RSD  $\leq$  3%) regardless of the loading effect. These excellent global uniformity in both diameters and heights leads to nanocylinder volumes with high global uniformity (RSD ≤5%) across a substrate which is desirable for the application of torque in an OTW.<sup>1</sup>



**Fig. S5.** Quantitative comparison of surface functionalization efficiencies on single-crystal TiO<sub>2</sub> for different linker molecules. **(a)** Schematic of the different functionalization strategies. At left, the TiO<sub>2</sub> (or other oxide materials, e.g. SiO<sub>2</sub>) surface provides hydroxyl groups (-OH) to which the linkers bind covalently. In the center, the used linker molecules (ETA, GPDMES, APDMES, and BADMSCP) are shown with their molecular structures, including the PEGylation of ETA-coated surface. At right, target molecules are NHS-ester or amine modified ATTO 647N fluorophores (F), binding to the different functional group of each linker (**ESI Methods**†). The fluorophores allow quantitative measurements via fluorescence microscopy. However, in the same manner, other organic molecules, such as biomolecules or polymers, can be bound to the surface linkers. **(b)** Quantitative comparison of differently functionalized single-crystal rutile TiO<sub>2</sub> (red bars) and quartz SiO<sub>2</sub> (blue squares) substrates. The measured fluorescence intensity represents the surface coating density while the error bar (standard deviation) reflects the homogeneity of the coatings (**ESI Methods**†).

#### Comparison of TiO<sub>2</sub> and SiO<sub>2</sub> in surface functionalization efficiency

It is known that TiO<sub>2</sub> has lower functionalization efficiency compared to other widely used oxide materials, e.g. SiO<sub>2</sub> and Al<sub>2</sub>O<sub>3</sub>.<sup>19,20</sup> To quantify this difference in functionalization efficiency, we compare the surface coating efficiency of TiO<sub>2</sub> (rutile) with that of SiO<sub>2</sub> (quartz). We select two linkers for this comparison: ETA, based on its highest coating efficiency on TiO<sub>2</sub> surfaces (**Fig. S5b**†), and GPDMES that we used for TiO<sub>2</sub> nanostructure functionalization (**Fig. 4c**) and OTW measurements (**Fig. 5**). For SiO<sub>2</sub> substrates, which are functionalized under the same conditions as TiO<sub>2</sub>, both ETA and GPDMES coatings show ~30% higher functionalization density than on TiO<sub>2</sub>. However, the coating efficiencies of TiO<sub>2</sub> are sufficient to perform single molecule OTW experiments (**Fig. 5b,c**). If higher coating density is required, TiO<sub>2</sub> substrates can be treated with extended O<sub>2</sub> plasmatreatment time to increase the density of surface hydroxyl groups.<sup>20</sup>



**Fig. S6.** DLS measurements of single-crystal TiO<sub>2</sub> nanocylinder aggregation in relation to surface coatings and buffer conditions. In the DLS graphs, each curve is an average of 10 measurements with a duration of 10 s each, with 2 min between successive curves (see legend at bottom of figure). Each green shaded box within the panels displays the range of nanocylinder sizes measured previously in SEM (left edge: diameter, right edge: height). Top-left corner denotes the individual test conditions, which are surface coating (1<sup>st</sup> row) and buffer solution (2<sup>nd</sup> row). (a-d) Results for non-coated nanocylinders dispersed in (a) DI water, (b) DI water with 2% (m/v) BSA (New England Biolabs, UK), (c) PBS buffer (pH 7.4), and (d) PBS buffer (pH 7.4) with 2% BSA. (e-g) DLS data for PEGylated nanocylinders in (e) DI water, and in PBS buffer (pH 7.4) after bioconjugation with (f) biotin and (g) DNA. (h) Result for GPDMES-coated nanocylinders with bound streptavidin

in PBS buffer (pH 7.4): this condition is used for the presented single-molecule OTW experiments (**Fig. 5**). The SEMmeasured nanocylinder dimensions are as follows where D (H) denotes diameters (heights): (**a,b,d**) D: ~110 nm, H: ~430 nm; (**c,e**) D: ~195 nm, H: ~585 nm; (**f**) D: ~255 nm, H: ~625 nm; (**g**) D: ~290 nm, H: ~460 nm; (**h**) D: ~105 nm, H: ~505 nm.

#### Measurements and analysis of DLS data

We probe the aggregation of cleaved, isolated single-crystal TiO<sub>2</sub> nanocylinders with different surface coatings and buffer solutions via DLS (Zetasizer Nano ZS, Malvern, UK). We compare the degree of nanocylinder aggregations by characterizing the nanocylinder size distributions with 173° backscattering angle at 25 °C. To achieve monodispersed nanocylinders in aqueous solution, we vortex the nanocylinder solutions for 1 min before each measurement. However, for the nanocylinders in **Fig. S6a**† and **Fig. S6b**†, vortexing is insufficient to obtain monodispersity. For these samples, we sonicate the solutions for 10 min before measurements. We attribute the mismatch between nanocylinder dimensions (green boxes) based on SEM image analysis and size measurements via DLS to the highly scattering nature of TiO<sub>2</sub> and non-spherical shape of the nanocylinders. Besides the aggregated nanocylinder solutions (**Fig. S6a,b,d**†) which are apparent from the severe broadening of the size distributions, the monodispersed nanocylinder solutions (**Fig. S6c,e-h**†) exhibit only sedimentation of nanocylinders over time, observable by intensity decrease.



**Fig. S7.** Optical trap calibration of single-crystal TiO<sub>2</sub> nanocylinders. The power spectral density (gray curve) of a trapped TiO<sub>2</sub> nanocylinder with a Lorentzian fit (red dotted line) provides corner frequency ( $f_c$ ) and baseline amplitude ( $A_b$ ). A piezo stage drives the flow cell sinusoidally in time (with amplitude of 1 µm and frequency of 25 Hz) along the z-axis (inset) to induce a drag force to the trapped nanocylinder. The driving frequency ( $f_d$ ) of this modulation appears as a spike in the power spectrum (blue arrow), with amplitude  $A_d$ . Analysis of the three measured values ( $A_b$ ,  $A_d$ , and  $f_c$ ) yields the three necessary parameters for the force calibration in an OTW: nanocylinder drag coefficient, PSD sensitivity, and trap stiffness.<sup>21</sup>

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