Supporting Information for

“Bubble Free Propulsion of Ultrasmall Tubular Nanojets Powered by Biocatalytic Reactions”

Xing Ma, Ana C. Hortelao, Albert Miguel-López, and Samuel Sánchez*

*e-mail: sanchez@is.mpg.de; ssanchez@ibecbarcelona.eu

Experimental Section

Materials and Instruments

Tethaethylorthosilicate (TEOS, 99%), ammonia (25%), cetyltrimethylammonium bromide (CTAB, 99%), 3-aminopropyltriethoxysilane (APTES), ethanol (EtOH, > 99%), Jack Bean urease from Canavalia ensiformis (Sigma-Aldrich, Type IX, powder, 50,000-100,000 units/g solid), hydrochloride (HCl, assay 37%), nitric acid (HNO₃, assay 65%), isopropanol (IPA, 99.5%), phosphate buffered saline (PBS, pH = 6.5) glutaraldehyde solution (GA, 25% in H₂O), fluorescamine (≥98%), urea (assay 99-100.5%), ethanol (EtOH, analytical grade), and silver nanowires (D×L – 115 nm×20-50 µm, 0.5 % in IPA) were purchased commercially. Scanning electron microscopy (SEM) images were captured by a Zeiss ULTRA™ 55 SEM at 5 kV. Optical videos and fluorescence images were captured by a Leica inverted optical microscope with 63×air objective. UV-vis absorption spectra were acquired by a Specord 50 Plus UV-vis spectrometer.

Synthesis of Silica Nanotubes (SNT) and amine group grafting (SNT-NH₂)

For a total volume of 12 mL, 0.5 mL of AgNW were dispersed in a solution of IPA and de-ionized (DI) water in a ratio of 10:2, then 24 µL of ammonia were added to the solution under stirring. Then, 80 µL of TEOS was added. The mixture solution was kept stirring for 45 minutes at room temperature. The silica coated silver nanowires (AgNW@SiO₂) were then collected and washed with an IPA: DI H₂O mixture (10:2) three times. Following this, the silica coated silver nanowires were sonicated for 3 hours in order to break the nano-wires into shorter length. Afterwards, the AgNW template was removed by etching in aqua regia (mixture of HCl(37%) and HNO₃(65%) in a 3:1 ratio) etching overnight. The silica nanotubes (SNT) were then collected by centrifugation and washed again with a mixture of IPA: DI water (10:2)
three times. The SNT were suspended in EtOH (1 mL) solution containing APTES (20 µL), and kept under stirring for 48 h. The amine grafted SNT, SNT-NH₂ were collected by centrifugation and washed with EtOH for three times.

Selective functionalization of amine (-NH₂) Inside (I), Outside (O) and All over (A):

The silica nanotubes with different distributions of amine groups were synthesized using co-condensation methods.

**SNT-NH₂-A**: To obtain silica nanotubes with amino groups distributed all over, a mixture solution of silver nanowires (0.5 mL), IPA (9.5 mL), de-ionized water (2 mL) and aqueous ammonia (0.24 mL) was gently sonicated and stirred at room temperature. Then, 80 µL of a mixture of TEOS and APTES (3:1) were added and the solution was kept stirring at room temperature for 45 minutes. After, the silica coated silver nanowires were collected by centrifugation and washed three times with a EtOH:H₂O mixture (1:1).

**SNT-NH₂-I**: To obtain silica nanotubes with amine groups distributed only on the inside surface of the nanotube, a second step was added to the reaction that yields nanotubes with amino groups all over by using 40 µL of a mixture of TEOS and APTES (3:1). After washing the silica coated nanowires, the latter were re-suspended in a mixture solution containing IPA (9.5 mL), de-ionized water (2 mL) and aqueous ammonia (0.24 mL), 40 µL of TEOS were added to the solution and it was kept stirring at room temperature for 45 minutes. Ultimately, the silica coated nanowires were collected by centrifugation and again washed three times with a mixture solution of EtOH:H₂O (1:1).

**SNT-NH₂-O**: To obtain silica nanotubes with amine groups only on its outer surface a two-step co-condensation reaction was employed. Similarly to the previous syntheses, a mixture solution of silver nanowires (0.5 mL), IPA (9.5 mL), de-ionized water (2 mL) and aqueous ammonia (0.24 mL) was gently sonicated and stirred at room temperature. Then, 40 µL of TEOS were added to the mixture and it was kept stirring at room temperature for 30 minutes. After, 40 µL of a mixture solution of TEOS and APTES (3:1) were added and the reaction was kept stirring for 30 minutes. The silica coated nanowires were collected by centrifugation and washed with a mixture solution of EtOH:H₂O (1:1).

The silver nanowire template was removed by *aqua regia* etching overnight. During the AgNW removal process by strong acid (regia water) etching, the organic functional groups (-NH₂) on the external surface were removed due to strong oxidation. In the co-condensation process, both TEOS (silica precursor) and APTES (-NH₂ groups source) were added together, the amine (-NH₂) groups were also imbedded inside the whole tube, so by further controlled etching with ammonia which reacted with external layer of silica, the amine (-NH₂) groups inside the silica could be further exposed. Typically, the amine groups were
exposed by suspending the silica nanotubes in a solution of aqueous ammonia (pH = 11.6) for 3 hours with gently shaking to gently etch out the surface silica. Then, the silica nanotubes were collected by centrifugation and washed with EtOH.

**Functionalization of SNT-NH$_2$ with urease**

The amine modified silica nanotubes were washed with PBS (pH = 6.5) once and suspended in the buffer. Then, 100 μL of glutaraldehyde solution were added drop wise to the suspension and it was kept shaking for 3 hours. Then, the silica nanotubes containing the linker molecule were washed with PBS three times and suspended in a PBS solution containing urease (3 mg mL$^{-1}$) and kept shaking overnight. The urease-functionalized silica nanotubes (SNT-Urease) were then collected by centrifugation and washed with DI H$_2$O three times.

**Silica tracer nanoparticles (680 nm) preparation**

The solid silica nanoparticles were prepared by modified Stöber method.$^1$ Typically, a mixture solution containing EtOH (70 mL), DI H$_2$O (10 mL) and NH$_3$·H$_2$O (25%, 5 mL) was stirred for 5 minutes. Then, silica precursor TEOS (6 mL) was added. The solution was kept under stirring for 24 h before collection of the nanoparticles by centrifugation.

**Optical video recording**

The motion of the urease conjugated tubular nanojets was assessed using a Leica optical inverted microscope equipped with a 63× air objective. The nanojets were placed on petri dish filled with DI water containing 100 mM of urea (or with tracer nanoparticles as well), and the well was covered by a glass slide in order to minimize drifting effect. Videos with duration of 30 seconds were recorded with a CCD camera at a frame rate of about 20 fps under phase contrast. Video recording was performed at the initial 3 minutes of the experiment, ensuring constant fuel concentration, enzymatic reaction rate, and avoiding fuel depletion effect.

**Video analysis**

*Velocity and MSD:* Accurate tracking of the tubular nanojets was performed on the recorded videos to compute velocity and the mean square displacement (MSD). In order to achieve decent statistical data, a script in Python using the OpenCV library was developed. The tubular nanojets were clearly differentiated from the background, so their edges are detected by taking the gradient of the image. Since numerical derivatives are strongly affected by noise, different types of blurring are applied on the image before proceeding with the gradient. The contours of the nanojets are then obtained by thresholding the
absolute of this gradient. Bayesian decision making (the closest shape to a previous position is always assumed to be the correct one) is enough to accurately follow the trajectory of the detected motors along each frame. After obtaining the tracking trajectory of each nanojet, the length of tracking trajectory (ΔL) of a known time period (Δt) were directly measured for a tubular nanojet to calculate the velocity $V = \frac{ΔL}{Δt}$. The MSD is calculated using the following formula:

$$MSD(Δt) = \langle (\bar{r}(t + Δt) - \bar{r}(t))^2 \rangle_t$$

Where $\bar{r}$ is a 2-dimensional position vector. The translational diffusion coefficient ($D_t$) is obtained by fitting the MSD data according to:

$$MSD(Δt) = 4D_t Δt$$

which is valid at low time intervals and for small particles/tubes with low rotational diffusion.$^{[2]}$

Angular information and mean square angular displacement (MSAD): In the case of tubular nanojets, the orientation can be computed from their elongated shape $S$ using image moments.$^{[4]}$ We can define the major and minor axis of a non-symmetric shape as the axis along which the pixel variance is maximum and minimum, respectively. The nanotube orientation is taken as the major axis, with an angle $θ$ given by:

$$\tan θ = \frac{2σ_{11}}{σ_{20} - σ_{02}}$$

The central image moments ($σ_{nm}$) are computed on a binary image which includes the shape of the tube, with their general expression being:

$$σ_{nm} = \sum_{x,y \in S} (x-m_{10})^n(y-m_{01})^m \text{ Area of } S; \quad \text{and} \quad m_{ij} = \sum_{x,y \in S} x^i y^j \text{ Area of } S$$

Once the angle information is obtained, the rotational diffusional constant ($D_r$) is computed from the mean square angular displacement (MSAD), calculated as:

$$MSAD(Δt) = \langle (θ(t + Δt) - θ(t))^2 \rangle_t$$

Which is then fitted to

$$MSAD(Δt) = 2D_r Δt$$

The angular auto-correlation of the nanomotor is calculated by the equation $\cos(Δθ)$, where angular change $Δθ = θ(t + Δt) - θ(t)$, (t=0).
For the theoretical calculation of the translational diffusion coefficient for rod-like (tube here) structure with length $L$ and width $w$, the translational diffusion coefficient is given by: $^{[3]}$

$$D_t = \frac{k_B T}{3\pi\eta L} (\ln p + v)$$

Where $k_B$ is the Boltzmann constant, $T$ the temperature, $\eta$ the viscosity of the solvent and $p = \frac{L}{w}$ the aspect ratio of the tube. $v$ represents the so-called end-effect corrections, which can be expressed as a function of its lengthwise $v_\perp$ and sidewise $v_\parallel$ components, fulfilling the following equations:

$$v = \frac{v_\parallel + v_\perp}{2}$$

$$v_\perp = 0.866 - \frac{0.15}{\ln 2p} - \frac{8.1}{(\ln 2p)^2} + \frac{18}{(\ln 2p)^3} - \frac{9}{(\ln 2p)^4}$$

$$v_\parallel = -0.114 - \frac{0.15}{\ln 2p} - \frac{13.5}{(\ln 2p)^2} + \frac{37}{(\ln 2p)^3} - \frac{22}{(\ln 2p)^4}$$

The rotational diffusion coefficient is similarly given by:

$$D_r = \frac{3k_B T}{\pi\eta L^3} (\ln p + \delta_\perp)$$

The end-effect correction parameter $\delta_\perp$ in this case follows:

$$\delta_\perp = -0.446 - \frac{0.2}{\ln 2p} - \frac{16}{(\ln 2p)^2} + \frac{63}{(\ln 2p)^3} - \frac{62}{(\ln 2p)^4}$$

These expressions are assumed to be valid for $p > 4.6$. In our experiments the length ranges from about $2 \ \mu m$ to $30 \ \mu m$, so for a constant width of $200 nm$ $p$ varies between the values of 10 and 150, making this a good theoretical fit.

**Figure S1.** SiO$_2$ coated AgNW and SNT after removal of AgNW. (Average Diameter±SD=220±24 nm, N=30)
Figure S2. Amine groups grafting onto SNT. a) Detection of amine groups by fluorescamine, indicated by the fluorescence emission at 477 nm; b) Zeta-Potential of the nanotubes before and after grafting. (Before: -39±11 mV; after 44±11 mV)

Figure S3. a) and b) are bright field and fluorescence field images of SNT-Urease (red fluorescence color indicates the presence of urease on tube surface); The urease was conjugated onto both internal and external surface of the SNT, as the average internal diameter of the SNT is 115 nm, which is sufficient for the free diffusion of urease molecules whose hydrodynamic size is about 7 nm by DLS measurement.\[^{5}\]

Figure S4. Tracking trajectories of tubular motor without fuel, exhibiting Brownian motion only.
Figure S5. a) Video snapshots of a typical tracking of a moving tubular motor (9.28 µm); b) tracking trajectory of the directional movement of the tubular nanojet and corresponding parabolic MSD plot; c) angular tracking result and MSAD plot; the rotational diffusion $D_r$ was calculated by the equation $\text{MSAD}=2D_r\Delta t$.

Figure S6. Two step synthesis of the selectively functionalized tubes with amine groups inside only. SEM images of SiO$_2$ coated AgNW after a) 1$^{\text{st}}$ step growth of silica with amine groups by co-condensation method; and b) after 2$^{\text{nd}}$ step growth of silica only.
**Figure S7.** Zeta-Potential of AgNW@SiO$_2$ with selectively grafting amine groups, SNT-NH$_2$ All, SNT-NH$_2$ Inside and SNT-NH$_2$ Outside. (Inside: -27±5mV; Outside: 21±5mV; All: 20±6mV). The positive charges of SNT-NH$_2$ All, and SNT-NH$_2$ Outside indicates the presence of amine groups on nano-tube’s surface. While negative value for SNT-NH$_2$ Inside indicates the external surface of the nanotube is fully covered by second layer of bare silica.

**Figure S8.** Exposure of amine groups by controlled etching of silica with ammonia (pH=11.6 for 3h); TEM of SNT a) before and b) after etching.
Figure S9. Exposure of amine group by ammonia (pH=11.6 for 3h) etching. Fluorescamine reacted with exposed amine groups and exhibited emission at 477 nm. The amine groups were removed during the AgNW removal process by *aqua regia* etching. By further controlled etching with ammonia which reacted with external layer of silica, the amine groups pre-embedded during co-condensation growth were exposed again and detected by fluorescamine, indicated by the peak at 477 nm (Figure S9).

**Figure S10.** a) Protein concentration measurement and b) enzyme activity assay represented by the time dependent ammonia production (1 µg protein (urease)) of the three samples, SNT-Urease-A, SNT-Urease-I, and SNT-Urease-O, respectively. Before the evaluation of the enzymatic activity, the protein concentration of the three samples was quantified by using a Coomassie Brilliant Blue (CBB) based protein quantification kit according to the instructions (Figure S10 (a)). The protein concentrations were found to be 13.7 µg/mL, 8.6 µg/mL, and 6.7 µg/mL for the three samples, respectively. Then, we diluted 73 µL (×13.7 µg/mL), 116 µL (×8.6 µg/mL), 149 µL (×6.7 µg/mL) of each sample (1 µg protein) into the solution of 1 mL (in total) for enzymatic activity assay according to previous reported method,[6] in which way we ensured the same concentration of protein for the enzymatic assay. The continuous generation of ammonia up to 35 minutes indicates that the conjugated enzymes on the three samples were all active.
Figure S11. a) Video snapshots and b) schematic illustration of a self-propelled tubular motor with tracer nanoparticles (680 nm); blue tracking trajectory indicate nanoparticle trapped on the tube surface and red tracking trajectory of nanoparticle under Brownian motion; c) Average Mean-square-displacement (MSD) plot of the two kinds of nanoparticles (N=10). The scale bar in the inset figure is 1 µm.

Short Discussion: The velocity of SNT-Urease-A is higher than that of SNT-Urease-I only, suggesting that the enzymes attached on the external surface might help to enhance the velocity given the presence of enzymes inside the tube pushing the directional movement. We tracked the tracer nanoparticles next to the active tubular nanojets and found that the tracer nanoparticles were “attracted” towards the tubular nanomotor’s surface, implying a flow field on the surface of the nanojet. As shown in Figure S11 (a) in the SI, the tracking trajectory of a tracer nanoparticle next to the surface of the tubular nanojet was “elongated” (blue track), compared to a free Brownian motion nanoparticles in the same video (red track). We plotted the mean-square-displacement (MSD) curve of the affected tracer nanoparticles next to the tube surface and found a parabolic shape due to elongation effect along the tubular surface in one-dimension, however the MSD plot of free Brownian motion nanoparticles showed typical linear curve (red). The presence of fluidic flow field on the external surface proved the activity of enzymes outside the nano-tube, leading to trapping effect on the tracer nanoparticles. It is anticipated that the enzymatic reaction on the external surface will create a chemical gradient of both products (NH$_3$(aq) and CO$_2$(aq)) and reactants (urea) along the transversal direction on the nanojet surface, leading to density-driven
convective flows due to catalytic reactions, e.g. fuel of urea moving towards the tube surface and products moving away from the tube surface, as previously reported by Sen and co-authors. This flow field affected the Brownian motion of the tracer nanoparticles as shown in Figure S11, resulting in a “trapping” effect of these nanoparticles along the tube surface. Such external flow field might contribute to the velocity enhancement for the SNT-Urease-All. However, further in-depth investigation should be carried out before reaching a conclusive answer to this phenomenon.

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