Supporting information file for:

Fluorescence Anisotropy Using Highly Polarized Emitting Dyes Confined inside BNNTs

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1- Materials

Boron nitride nanotubes (BNNT) were provided by BNNT LLC. Only reagent grade solvents were used. 3-aminopropyltriethoxysilane (APTES) (99%), α -sexithiophene (6T) and poly(methyl methacrylate) (PMMA, Mw=120000 g.mol⁻¹) were purchased from Sigma-Aldrich and used as received.

2- Sample preparation

Preparation of surfaces with localisation markers

Si/SiO₂ surfaces (100 nm oxide) were dehydrated in a YES oven. OIR 674 resist was spin-coated on the substrate at 4000 rpm for 30s and then baked for 60s at 90°C. Photolithography was done with an exposition dose of 36.5 mJ/cm² using a Karl Suss MA6 mask aligner. After development, 5 nm of titanium and 20 nm of gold were deposited on the substrate using e-beam deposition. Lift-off was done in warm acetone.

Preparation of surfaces with BNNTs

BNNTs were provided from BNNT LLC and purified as previously described.^[1] Briefly, BNNTs were treated in nitric acid and/or at high temperature in air, and then centrifuged at 12 350g. The purified BNNTs were dispersed in DMF at a concentration of 50 µg/mL.

Si/SiO₂ surfaces with localisation markers were sonicated for 10 min each in acetone and isopropanol and immersed in a piranha solution (3:1) for 5 min. Substrates where rinsed copiously with miliQ water and dried with N₂. Then, an APTES treatment was applied to the surfaces using a vapor-phase method. The substrates were placed on glass slides suspended above a crystallization dish with 1 mL of APTES, and the dessicator was vacuum pumped for one minute and sealed for 30s. The APTES layer was annealed in air for 30-45 minutes at about 100 °C in a conventional oven. The BNNT solution was then spin-coated onto the surface at 3000 rpm for 30s.

Encapsulation of 6T on surfaces

The Si/SiO₂ surface with BNNTs was annealed in a vacuum oven at 800°C for 1h and immediately used for encapsulation. The encapsulation was done in a flask equipped with a condenser, under reflux. The concentration of the 6T solution was fixed at $5x10^{-6}$ M and the encapsulation was carried out for 24h at 115°C. After refluxing, the surface was rinsed with fresh solvent and IPA and dried with N₂. Figure S1 shows an AFM image of the prepared surface after encapsulation.



Figure S1 - AFM image of 6T@BNNTs on a substrate with metallic localisation marks

Encapsulation of 6T in BNNT for TEM grid (Mo/SiO₂)

About 20μ L of BNNT solution in DMF was drop-casted on a Molybdenum grid with SiO₂ membrane decorated with holes. Before encapsulation, the TEM grid was annealed under vacuum at 800°C for two hours. The grid was inserted in the 6T encapsulation solution at 115°C for 6 hours. Following the encapsulation, the grid was rinsed for a few seconds in DMF, cleaned using an oxygen plasma (100W, 10 minutes) and a piranha treatment (2 minutes) to completely remove the excess of non-encapsulated dyes.

(10) (9) (8) (7) (4) (5) BS 90/10 (6) (6) (1) (2) (3) (6) sample

3- Optical set up for polarized fluorescence experiments

Figure S2. List of the elements used for polarized micro-fluorescence experiment.

(1) Oxxius Laser: 532 nm, 100mW, polarization extinction ratio (1:1000)

- (2) Glan prism
- (3) $\lambda/2$ or $\lambda/4$ wave plates

(4) Focalisation lens (300 mm)

- (5) Non polarized prism cube beam-splitter (Tr90:R10)
- (6) Objective 60X, NA 0.9
- (7) Band-Pass filter 660 nm \pm 5 nm
- (8) Polarized prism cube as analyzer
- (9) Focalisation lens (75mm)
- (10) Nitrogen cooled Pylon camera (Princeton) (1340x400)

4- Fit of deviation angle distribution

The angle distribution was adjusted using a non-linear least-square minimization and curve-fitting package,^[2] using the built-in Lorentzian model. The center of the Lorentzian was fixed to an angle of 0 degree, while all the other fitting parameters were free. The Half-Width Half-Maximum (HWHM) was directly extracted from the fitting parameters.

5- Ac-HRTEM experiments

The high-resolution images presented in figure 3 were performed at 80 kV on a JEOL ARM microscope, equipped with an aberration corrector.

6- Estimation of the maximum 6T rows number inside BNNTs.

We estimated the minimum inner diameter (d_{inner}) of BNNTs allowing the encapsulation of one, two and three rows of 6T molecules by considering the vdW distances for a BN wall and 6T molecules.

- The effective vdW radius of a BN tube wall is 173.5 pm, corresponding the average of the value of the vdW radii of B and N atoms, 192 pm and 155pm, respectively.
- The effective vdW radius of 6T molecules is the sum of their covalent distance (d_{cov}) between H and S atoms and the vdW radius of the top and bottom atom: H and S atoms, 120 pm and 180pm, respectively.

Based on these values we have calculated from references in the literature the complimentary data for the models A and B.

-Model A: From d_{vdW} we calculate the d_{cov} (H-S) in 6T molecules by subtracting the sum of vdW radius of B and N atoms (347pm).

-Model B: From d_{cov} we calculate the d_{vdW} of 6T molecules by adding vdW radius of H and S atoms (300pm).

 $\label{eq:solution} \begin{array}{l} \textbf{Table S1}: Comparison of different models for the calculation of the covalent and vdW \\ distances (d_{cov} \mbox{ and } d_{vdW}) \mbox{ of } 6T \mbox{ molecules}. \end{array}$

	d _{cov} (H-S in 6T)	Half of d_{cov}	d _{vdW}
Estimation model	(in m)	(in m)	(in m)
А	3.70E-10	1.85E-10	6.70E-10 ref ^[3]
В	4.65E-10 ref ^[4]	2.32E-10	7.65E-10

Average 4.18E-10	2.09E-10	7.18E-10
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Conditions required for the first 6Ts row:

The boundary condition for the entry of a first 6T molecule row is imposed by the section H/S-C-C-S/H. By following the average d_{vdW} value (718pm) found in literature on 6T (see Table S1) and the sum of vdW radius of B and N (347pm) we obtain a minimum d_{inner} of 1.07 nm for allowing the formation of one 6T row inside the BNNT.

Condition for reaching two and three 6Ts rows

The spacing conditions for two and more 6Ts row is no longer the boundary condition of the H/S-C-C-S/H section but more the vdW distances between the BNNT walls and the two or more 6T rows. In this case, the vdW radius of the S (180pm) was used for the 6T molecules. We applied the Pythagorean theorem to trace back the d_{inner} of the BNNT at the S/H atoms height (half of the d_{cov} of 6T molecules) in the circle. Hence, we obtained a favourable BNNT inner diameter for the entry of two 6T rows of 1.15 nm. In the same way, we calculated a minimum d_{inner} of 1.49 nm for the stacking of three adjacent rows of 6T molecules inside a BNNT.

7- Fabrication and stretching of the PMMA/6T@BNNT films.

An amount of 0.33g of PMMA (Mw=120000 g.mol-1) was dissolved in 10 mL of DMF. 2.4 μ L of a centrifugated 6T@BNNT solution in DMF were added and the solution were stirred during 1h. The solution was then poured into a 6 cm diameter glass petri dish. The petri dish was placed on a hot plate at 40°C to accelerate the evaporation of DMF for 4 hours.

Samples of dimensions 5x15mm were cut with scissors in the films. They were then installed in the traction system presented in figure S3. The starting distance between the two jaws is 5 mm. The heating plate is heated to a temperature of 125°C. The system is heated for 5 min. The



stretching is then carried out by stretching the film using the translation stage at a speed of 1mm/min roughly. The distance between the two edges is then measured to quantify the stretching.

Figure S3. Custom-built stretching machine, based on optical translation stages, installed on a hot plate. The PMM/6T@BNNT film are placed at a distance of 10 mm of the hot plate.

8- Confocal fluorescence Imaging of PMMA/6T@BNNT and analysis

- Preparation of samples for confocal microscopy :

To ensure appropriate immobilization and optimized optical collection under the microscope, the films were covered by refractive index liquid and sealed between a microscope slide and a glass cover slide. See Figure S4.



Figure S4 – Immobilization of the films prior confocal imaging.

- Confocal imaging parameters:

The confocal fluorescence images data presented in Figure 5 and 5e (main text) were acquired with a Leica microscope SP8 WLL2 on an inverted stand DMI6000 (Leica Microsystems, Mannheim, Germany) using a HCX Plan Apo CS2 63X oil NA 1.40 objective. The spectral interval of the excitation and collection channels are 510-520 nm and 600-780 nm, respectively. A z-step of 600 nm were used for the z-stack. The scan area is 50x50µm²

- Image processing:

Background Subtraction: The image stacks were processed using the FIJI software. In a first processing step a rolling ball with a radius of 2.0 pixels was applied. Then Gaussian blur of radius 2 was then applied on the stacks. The Extented Maxima 3d function (MorpholibJ plugin) was then used with a connectivity of 26 and a dynamic of 1000. ^[5,6]

3D segmentation: The 3D Manager function (3Dsuite plugin) allows to segment the different nanotubes on the whole height of the image stack. 1138 objects were identified in the reference sample stack and 1191 objects in the stretched sample.

BNNT orientation analysis: For each object, The 3D Region Analysis function (MorpholibJ plugin) was applied to extract the function returns the centroid (center of gravity) as well as the size and the orientation of the inertia ellipse or ellipsoid. The objects with a sphericity index lower than 0.6 were not kept into account to be in agreement with the tubular geometry of the nanotubes. Finally, a list of 387 objects for the reference sample and 437 objects for the stretched sample were selected for the statical analysis presented in Figure 5e.

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