Supporting Information

A three-branched DNA template for Carbon Nanotubes self-assembly into nanodevice configuration

Sébastien Lyonnais[1], Chia-Ling Chung[1], Laurence Goux-Capes[1], Christophe Escudé[2], Olivier Pietrement[3,4], Sonia Baconnais[3,4], Eric Le Cam[3,4], Jean-Philippe Bourgoin[1], Arianna Filoramo[1,*]

[1]*Laboratoire d'Electronique Moléculaire CEA Saclay, DSM/IRAMIS/SPEC, F-91191 Gif/Yvette, France*

[2]*Régulation et Dynamique des Génomes, USM 0503 Muséum d'Histoire Naturelle, CNRS UMR 5153, INSERM U565, Case Postale 26, 43 rue Cuvier, F-75231 Paris Cedex 05, France*

[3]*CNRS, Laboratoire de Microscopie Moléculaire et Cellulaire, UMR 8126 Interactions Moléculaire et Cancer, Institut de cancérologie Gustave Roussy, Villejuif, F-9485, France*

[4] *Univ. Paris Sud, Villejuif, F-94805, France.*

** Corresponding author : arianna.filoramo@cea.fr*

Experimental Procedures

Compounds: Streptavidin, T4-DNA Ligase and BsaI were purchased from New England Biolabs. Streptavidin was stored at +4°C into 20µl aliquots at a concentration of 0.5mg/ml in 10mM sodium phosphate, 150mM NaCl pH 7.2. Poly-L-lysine was purchased from Sigma-Aldrich. DNA adapter oligodeoxynucleotides (**Table S1**) and PCR primers (**Table S2**) were synthesized by Eurogentec (Belgium). Nucleic acids concentrations were measured using a Genequant-pro microspectrophotometer (GE Healthcare).

Electrophoresis: Polyacrylamide gel electrophoresis (19:1 acrylamide:bisacrylamide) was performed on 18x16cm gels (SE600 Amersham) at 10V/cm in 1 X TBE. The gels were stained with a SybrGreen I & II (Molecular Probes) mix diluted at 1/10000. Agarose gel electrophoresis was performed at 50V using Ethidium Bromide (0,5mg/ml) pre-stained gels in 0.5 x TBE. Image acquisition was performed with a CCD camera (Biorad) under UV illumination at 254nm.

Carbon Nanotubes: pristine laser ablation SWNT[1] were prepared as already described.[2] 25 mg of raw material were sonicated 30' (120 W in a 40 kHz Fisherbrand US bath 2.8 L) in 80ml nitric acid (35 vol. %) and refluxed at 100°C for 3 to 5 h. The suspension was then cooled and vacuum filtered through a PTFE membrane (Sartorius, 0.45 µm pores). The thick SWNT buckypaper formed on the filtration membrane was washed by 80 ml of deionised water, followed by 15 ml of CH_2Cl_2 and 15 ml of acetone. The buckypaper was then sonicated 30' in 80 ml of hydrogen peroxide (30 %) and refluxed at 100°C for 1 hour. The suspension was filtered through a PTFE membrane. The new buckypaper was washed by 80 ml of deionised water, then sonicated 30' in nitric acid (35 vol. %) and refluxed at 100°C for 2-3 hours. The third buckypaper was washed by 80 ml of deionised water, and finally dried at 60°C. Samples purity and characteristics were analyzed by Raman spectroscopy on a T64000 triple micro-Raman spectrophotometer. The spectra were collected under visible (λ=514nm) Ar laser excitation at room temperature. The Raman spectrum of such bucky paper is reported in **figure S1.**

Carbon Nanotube Solutions: SWNT were dispersed in water (final concentration estimated at 0.1mg/ml) by overnight sonication at minimum power (about 12 hours at 24 W in a 40 kHz Fisherbrand US bath 2.8 L). Centrifugation (64000g) was subsequently carried out during 3h to remove insoluble particles. The typical absorption spectrum is reported in **figure S2**. Note that to obtain the whole spectrum including first energy transitions for semiconducting nanotubes heavy water has to be used. [3]

In our experiment normal water is always used. This solution is subsequently diluted (as described below) for the realization of SWNT-DNA conjugates.

Adapter: T1, T2 and T3 oligonucleotides (**Table S2**) were annealed in 20mM Tris HCl (pH7.5) and 2mM $MgCl_2$ at a 1:1:1 molar ratio by incubation at 95°C for 3 min followed by slow cooling to room temperature. The product was purified by anion exchange chromatography on a miniQ 4.6/50 PE using an Aktäpurifier system (GeHealthcare) with a linear NaCl salt gradient (0 mM to 2 M).

DNA Scaffold: The 1.2kbp, 2.3kbp (long arm) and 468bp fragments were synthesized by PCR, using pBluescript SK^+ as a template. The sequences of the primers were designed with non-hybridizing 5'-ends in order to introduce cleavage sites for the restriction enzyme BsaI. This class IIS restriction enzyme cleaves outside its recognition site and therefore can generate any 4 nucleotides overhang, the sequence of which being chosen by designing the primers. The 468bp fragment was biotinylated by incorporation of Biotin-14-dUTP (Roche) during the PCR. The PCR reactions were carried out according to previously described processes. [4] For both fragments, primers and unincorporated dNTP were removed using a Qiagen PCR purification kit. Fragments were next digested overnight at 50°C with BsaI according to manufacturer's instructions and the cleaved extremities were removed by ultrafiltration using a microcon YM50 column (Millipore) which was centrifugated at 5000rpm for 5min. at room temperature. The short arms (1.2kbp + 468bp) were assembled by incubating a 3:1 ratio of fragments 1.2kbp and 468bp (about 0.3µM for the shortest fragment) and 100U of T4 DNA ligase (New England Biolabs) in 50µl of the recommended buffer overnight at 16°C. The fragments were then purified by gel extraction using a commercial kit (Qiagen). The final structure was assembled by incubating a 5:5:1 ratio of respectively short and long arms, the adapter (0.1µM) and 300U of T4 DNA ligase in 50µl in the recommended buffer. The sample was first incubated at 37°C for 10min. and cooled to 16°C by steps of 1°C/min in a PCR apparatus (Mastercycler Eppendorf), and left at 16°C overnight. The ligation product (see **Figure S1b**) was purified by cutting the appropriate band gel from a 0.6% agarose gel stained with Ethidium bromide, extracted from the gel using a commercial kit (Qiagen) and stored in 10mM Tris HCl (pH7.5) containing 1mM EDTA. A control DNA template was assembled using the same procedure, except that the 468bp fragments were synthesized by PCR without biotin-dUTP.

Gold nanoparticles on the DNA Scaffold:. Conjugation with streptavidin-gold particles was performed using 5nm STV-colloidal gold (Sigma): 50µl of the nanoparticles (~1unit/ml) were purified twice by gel filtration on a Smart system (Amersham) with a Superose 6 column (GEHealthcare) using a 10mM

Tris HCl (pH7.5) buffer containing 50mM NaCl. The particles where then diluted 10000 times in the same buffer and incubated for 10min. at room temperature in a 20µL solution containing 0.5ng of the DNA scaffold. A 5µL-drop was then deposited for TEM imaging. A control experiment was performed using a DNA template assembled using the same procedure, except that the 468bp fragments were synthesized by PCR without biotin-dUTP. No gold nanoparticles were found in this case.

Transmission Electron Microscopy : TEM sample preparation and observations by positive staining were performed as previously described.[5] Briefly, 5 µl of a solution containing 0.2-0.5 µg/ml of DNA were deposited onto a 600 mesh copper grid covered with a thin carbon film activated by a glow discharge in the presence of pentylamine according to Dubochet.[6] Grids were washed with aqueous 0.2% uranyl acetate, dried and the samples were observed with a Zeiss/LEO CEM-912 electron microscope in tilted dark-field mode, filtering out inelastic electron with the spectrometer in order to improve image quality. Using this spreading procedure DNA molecules were rapidly adsorbed onto the carbon film with no major loss in the tridimensional information.[7] Electron micrographs were obtained using a Proscan digital camera. DNA length was measured using the SIS Soft Imaging Software. For Figure 2, the DNA scaffold was diluted to 1/50 in 10mM Hepes (pH 7.0), 50mM NaCl, and 2.5mM $MgCl_2$.

Atomic Force Microscopy sample preparation and imaging: For Figure 2a, the DNA template was diluted (1/50) in a buffer solution containing 20 mM Tris (pH 7.5), 50 mM NaCl and 1.5mM $MgCl_2$. A 5 µl droplet of this solution was incubated for 2 min onto the surface of freshly cleaved muscovite mica pre-treated with 2.5mM $NiCl_2$ for 1 min.[8] The surface was rinsed with 0.02% uranyl acetate and dried. AFM imaging was performed in Tapping ModeTM with a MultimodeTM system (Veeco) operating with a Nanoscope IIIaTM controller (Veeco). AFM probes were NSC35/A1BS (µmasch) cantilevers (resonant frequency of 150 to 300 kHz, force constant of 5 to 15 N/m). The scan frequency was typically 1Hz per line and the modulation amplitude was a few nanometers. A first or second order polynomial function was used to remove the background slope. Mica activation with poly-L-lysine was performed as follows: a droplet of 0.1% of poly-L-Lysine freshly diluted in milliQ water is deposited on freshly cleaved mica for 5min. The mica surface is then extensively rinsed with water and dried with a soft nitrogen flow.

Streptavidin coated SWNT : streptavidin coated SWNT were obtained by mixing 45µl of the SWNT dispersion (final concentration of about 0.01mg/ml in 20mm Hepes pH 7.0) and 5µl of streptavidin (50pg/µl) for 1hour at 20°C in Hepes buffer (10mm, pH 7.0) containing NaCl (50mm). Excess of

unbound streptavidin was removed by dialysis for 6h at 4°C (SpectraPor Float A Lyzer) in the same buffer. AFM image of such this solution deposited on a silicon substrate is presented in **figure S3.**

DNA-SWNT conjugate: The DNA-SWNT conjugate presented in Figure 4c was obtained by gentle mixing of a 0.01mg/ml streptavidin -coated SWNT solution with the DNA template at a final concentration of 5ng/µl for 30 min. at 30°C. The sample was then diluted 2x to 5x-fold in water and a 5µl-droplet was deposited on the carbon grid for TEM imaging, after uranyle acetate staining. For Figure 4(b,d-e), a 5µl droplet of solution containing the DNA template (1ng/µl) diluted in 20mM Hepes pH 7.0, 50mM NaCl was deposited on the surface of poly-L-lysine coated mica for 2min. Then, the surface was rinsed and dried with a soft nitrogen flow and a 10µl droplet of a streptavidin coated nanotubes solution was deposited as shown in figure S4 right panel. The deposition yield of the streptavidin-coated nanotubes depends on the concentration of the solution and incubation time. One nanotube for DNA template is obtained with the aforementioned parameters. The mica was then placed in a humid chamber at 30°C for 30min., rinsed with a droplet of 0.02% uranyl acetate, 200µl of milliQ water and air dried. Note that if this protocol is not followed and solutions of DNA and streptavidin coated nanotube are mixed before fixing the DNA on the surface (as schematized in **figure S4** left panel) some network and enrolled DNA structure around nanotube can be found in the images. This is partially shown in figure 4c (since for TEM imaging we realized the complexes before depositing on the surface) and shown in a more evident manner in the images reported in **figure S5.**

Supporting Tables:

Table S2. *DNA adapter sequences*

Size	*Oligo*	*Sequence (5' – 3')*
54nt	*T1*	*CGGTGCGATAGTCTCTAGACAGCATGTCCGGACATCTTTGCCCACGTTGACCCG*
54nt	*T2*	*CGGTCGGGTCAACGTGGGCAAAGATGTCCTAGCAATGTAATCGTCTATGACGTC*
54nt	*T3*	*GAGCGACGTCATAGACGATTACATTGCTAGGACATGCTGTCTAGAGACTATCGC*

Table S1. *PCR primers*

Fragment Size	*Oligo*	*Sequence (5' – 3')*
468bp	*468rv*	*ATGCTGGTCTCTACCGGCGATAAGTCGTGTCTTAC*
	468fw	*CGCTTGGTCTCTGCTCGGTATCAGCTCACTCAAAG*
1.2kbp	*1230rv*	*ATGCTGGTCTCTGAGCATTGGTAACTGTCAGACC*
	1230fw	*TGGAGCTCCAGCTTTTGTTC*
2.3kbp	*2300rv*	*GCATTGGTAACTGTCAGACC*
	2300fw	*CGCTTGGTCTCTGCTCTCTCAAGGATCTTACCGCT*

Supporting figure:

Figure S1

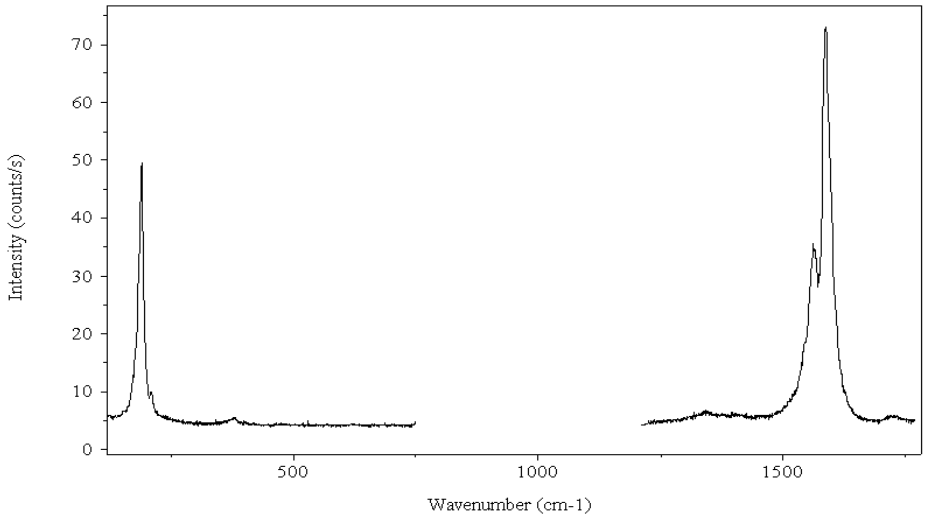


Figure S1 Typical Raman spectra of purified single wall carbon nanotubes (bucky paper). Note the Radial Breathing Mode (RBM) Raman line that corresponds to the atomic vibration of the C atoms in the radial direction. This line is typical of single wall carbon nanotube and it is often used to determine the nanotube diameter. The features in the Raman spectra in the range 1540-1600 cm-1 can be understood by the application of zone folding of 2D graphite phonon dispersion. In single wall carbon nanotubes due to their low dimensionality this tangential stretch G band mode gives rise to a multi-peak feature, also named the G band. Finally, the Raman spectra of all sp2 carbon materials present a feature called D band around 1250 and 1450 cm-1. This band is usually associated with the presence of in-plane substitutional hetero-atoms, vacancies, grain boundaries or other defects and by finite size effects, all of which lower the crystalline symmetry of the quasi-infinite lattice. In our case the nanotubes present little D band feature.

Figure S2

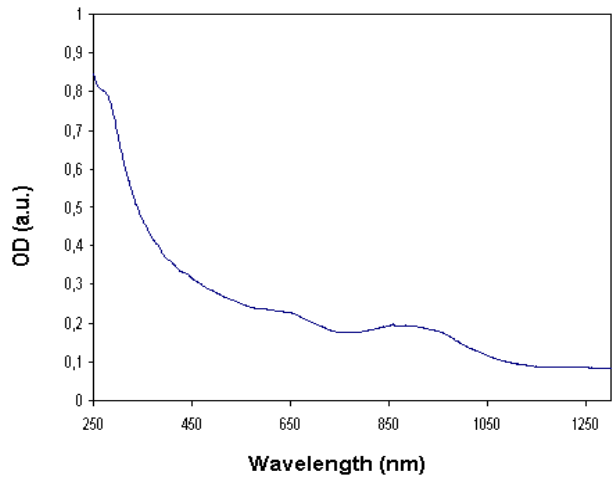
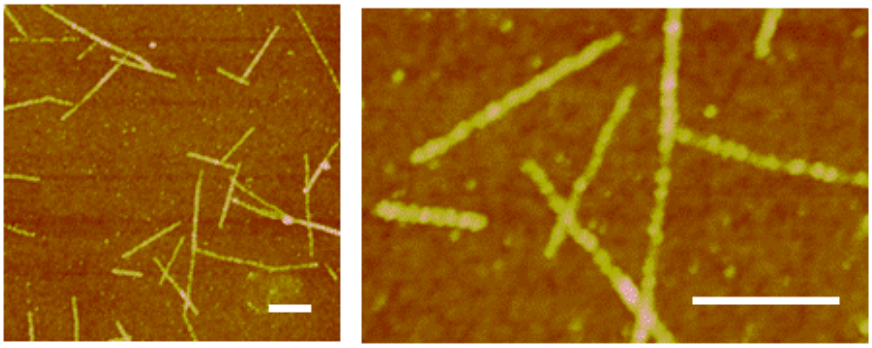


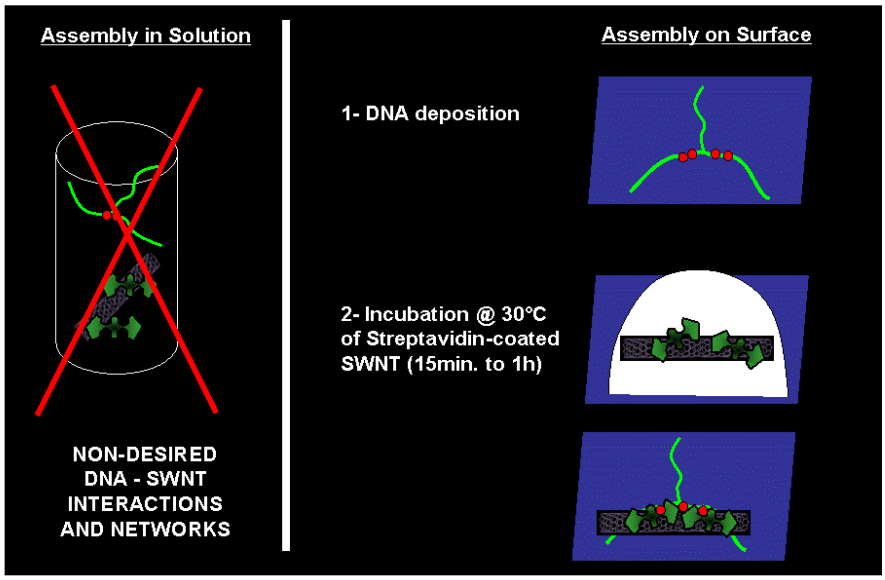
Figure S2 Typical absorption spectra of single wall carbon nanotubes solution.

Figure S3



FigureS3. AFM images of SWNT completely coated by the streptavidin deposited on a silicon substrate. Scale bars: 250nm

Figure S4



FigureS4. Schema of the protocols used to realize the conjugates. Left side: Assembly of DNA with streptavidin coated nanotube performed by mixing DNA solution with streptavidin-nanotube solution. Right side: Assembly of the conjugates by first depositing DNA on a surface, rinsing and subsequently incubating this surface with the streptavidin -nanotube solution.

Figure S5

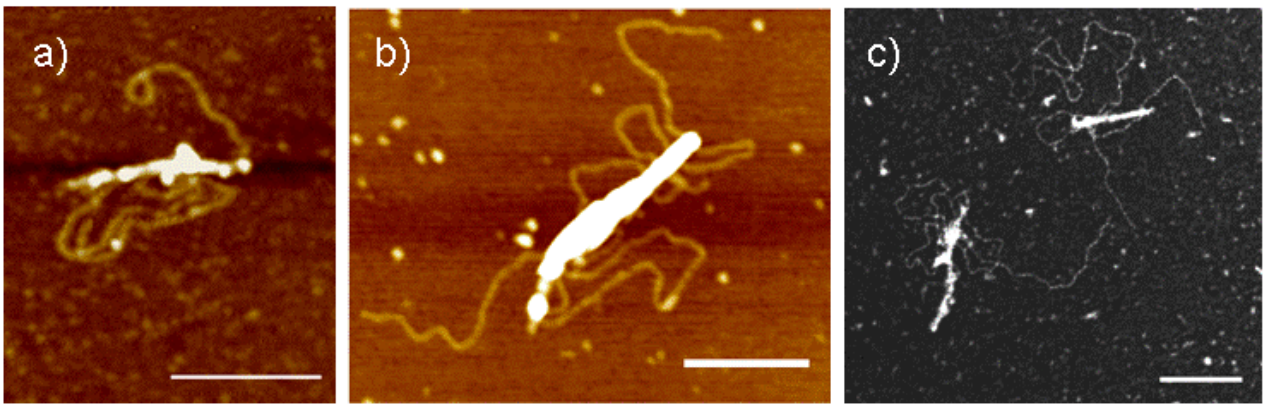


Figure S5: AFM (a-b) and TEM (c) images showing "bad examples" of the realized conjugates when the whole assembly process is performed in solution. We note that: (i) generally the problems related to DNA scaffold flexibility are increased (ii) sometimes more than one scaffold can be linked to a nanotube and finally (iii) more interrelated and not controlled geometries can be found. Scale bars: 250nm

References

[1] A. A. Gorbunov, R. Friedlein, O. Jost, M. S. Golden, J. Fink, W. Pompe, *Appl. Physic. A*, 1999**, 69**, 593-596.

[2] L. Capes, E. Valentin, S. Esnouf, A. Ribayrol, O. Jost, A. Filoramo, J. N. Patillon. *in Proceedings of the 2nd IEEE Conference on Nanotechnology*, 2002. Washington, D.C., USA.

[3] M. J. O'Connell, S. M. Bachilo, C. B. Huffman, V. C. Moore, M. S. Strano, E. H. Haroz, K.L. Rialon, P. J. Boul, W. H. Noon, C. Kittrell, J. Ma, R. H. Hauge, R. B. Weisman, R. E. Smalley, *Science* 2002, **297**, 593-596.

[4] A. Crut, B. Géron-Landre, I. Bonnet, S. Bonneau, P. Desbiolles, C. Escudé, *Nucl. Acids Res.* 2005**, 33**, e98-.

[5] E. Le Cam, *in Vizualisation of Nucleic Acids* (Eds.: G. Morel), CRC Press, Boca Raton, Fl: 1995.pp 331-356.

[6] J. Dubochet, M. Ducommun, M. Zollinger, K. E., *J. Ultrastruct. Res*. 1971**, 35**, 147-167.

[7] M. Joanicot, B. Revet, *Biopolymers* 1987**, 26**, 315-326.

[8] O. Pietrement, D. Pastre, S. Fusil, J. Jeusset, M. O. David, F. Landousy, L. Hamon, A. Zozime, E. Le Cam, *Langmuir* 2003, **19**, 2536-2539.