Grafting Single-Walled Carbon Nanotubes with Highly Hybridizable DNA Sequences: Potential Building Blocks for DNA-Programmed Material Assembly

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Experimental section:

DNA-SWNT conjugates: 100 μL of aqueous solution containing about 0.1 mg HiPco single walled carbon nanotubes (Carbon Nanotechnologies, Inc.), 0.025 mg DNA strand 1 or 2 and 0.1 M NaCl was sonicated for 30 min at a power of about 4 W using a UH-100A probe-type sonicator (Tianjin AutoScience Instrument Co., Ltd, China). The sample was kept in an ice-water bath during sonication. The resulting solution containing carbon nanotubes wrapped by 1 or 2 (denoted as SWNT-1 or SWNT-2) was centrifuged at 16000 g for 1 hour and the resulting black precipitate was discarded. To the supernatant containing SWNT-1 after centrifugation, oligonucleotide strand 3, 4, or 5 (custom-synthesized by Shanghai Sangon Biological Engineering Technology and Services Co., Ltd., China) was added in three times excess followed by an overnight incubation to get the corresponding SWNT-[1,3], SWNT-[1,4], or SWNT-[1,5] conjugate for the assembly experiments.

Free DNA removal: This step must be done before any assembly experiments. Here we developed a simply but efficient protocol to remove free DNA from a carbon nanotube solution. Up to 30 mM MgCl₂ was added to the DNA and SWNT mixture to precipitate the DNA-SWNT conjugate from the solution assisted with a centrifugation at 2000 g for 2 min. The supernatant solution was carefully removed using a pipette tip. The resulted DNA-SWNT conjugate was redispersed in 0.5xTBE buffer (Tris, 44.5 mM; EDTA, 1mM; and boric acid, 44.5 mM, pH 8.0) containing 30 mM NaCl plus 10 mM extra EDTA to complex with residual Mg²⁺ in the precipitate, and SWNT concentration could be easily readjusted at this step. Multiple precipitation-dispersion steps can be done if desired. It is interesting that we now find Mg²⁺ seems to work better than Na⁺, the latter sometimes leads to incomplete precipitation as well as some permanent aggregates in the redispersed SWNT sample.

Hybridization driven SWNT aggregation: Two parts of DNA-SWNT conjugates bearing complementary DNA sequences were mixed together in 0.5xTBE buffer containing 30 mM NaCl and 10 mM extra EDTA disodium salt. The resulting mixture was incubated at 25 °C for different time periods, and assembled products were then checked by gel electrophoresis or AFM.

Agarose gel electrophoresis: DNA-SWNT conjugates were loaded into 0.5% agarose gel and run in 0.5xTBE at 10 V/cm. The SWNT aggregates, after being formed, could not run into 0.5% agarose gel and appeared as black deposits in the gel-loading wells.
Assembly of gold nanoparticles onto DNA wrapped SWNT: Please see Figures S6 and S7 for details.

Atomic force microscope imaging: A typical sample for AFM imaging was prepared by first treating the freshly cleaved mica surface with 1 M MgCl₂ for 1 minute, and then spotting 5 μL of sample solution onto the mica surface. The liquid drop was blown away in one minute by compressed air followed by washing the surface twice with doubly distilled water (ddH₂O). The sample was then imaged with a Nanofirst-3000 (Shanghai Haizisi Optical-Electronics Co., Ltd., China) atomic force microscope (AFM) operated in tapping mode using a MikroMasch NSC11 AFM tip. All AFM images used in this paper were processed by a freely available software: WSxM 4.0 (www.nanotec.es). S1

**Figure S1.** Cartoon showing the self-assembly of DNA grafted carbon nanotubes into 3D aggregates upon hybridizations, and the disassembly of the as formed aggregates by DNA strand-displacement. (a) Tail strategy: hybridization happens between SWNT-[1,3] and SWNT-[1,4] with grafted DNA tails colored in blue and green, respectively; (b) Tail-free strategy: hybridization happens between SWNT-1 and SWNT-2, with the two complementary sequences embedded (marked in black and red, respectively) in the disperser strands 1 and 2; (c) Reversing the SWNT aggregation by using a DNA strand-displacement strategy: In this case, strand 3 is replaced by 5, and the basepairing between 5 and 4 responsible for the formation of SWNT aggregates can then be disrupted after adding strand 6 as a stripper strand. All DNA strands are color-coded in this figure, and the same colors within the drawings correspond to the same base sequences.
Figure S2. A centrifugation-assisted procedure showing the re-dispersion of the aggregates formed in a solution containing a mixture between SWNT-[1,4] and SWNT-[1,5] by adding strand 6 as a stripper strand. Tubes 1 and 2 correspond to the SWNT-[1,5] and SWNT-[1,4] solutions, respectively. Tube 3 corresponds to the mixture between SWNT-[1,4] and SWNT-[1,5]. Tube 4 corresponds to the re-dispersion of the precipitate as in tube 3 after adding a stripper strand 6 (Note that strand 6 was added after the precipitate similar to that in tube 3 had already formed and been verified after a quick centrifugation at 2000 g). The picture after a centrifugation at 2000 g for 30 seconds clearly shows the aggregation and re-dispersion of the DNA-SWNT conjugates switched by DNA hybridizations. Tube 3 in the second row of the images shows the precipitates of carbon nanotubes after hybridization, and tube 4 in the same row shows that a stripper strand 6 can disperse the precipitates again into a stable solution that cannot be precipitated out after a centrifugation at 2000 g.
**Figure S3.** AFM images of the DNA conjugated SWNT-[1,3] before (left picture) and after (right picture) hybridization with SWNT-[1,4]. Samples were purposely deposited with a lower density, which show a cleaner background for the aggregated sample (right), as well as less overlapped carbon nanotubes for the dispersed sample (left) compared to Figure 3.

**Figure S4.** More AFM pictures showing the DNA-SWNT aggregates induced by DNA hybridization.
Figure S5. AFM pictures corresponding to Figure 4 (gel electrophoresis) showing the carbon nanotube aggregates (middle), as well as the carbon nanotubes before aggregation (left) and after the aggregates being disrupted using a stripper strand (right). Note that for the aggregated samples the AFM image has a clean background, implying most of the carbon nanotubes in this sample exist as big aggregates as shown in the middle image.
**Figure S6.** Schematic representation of a linear AuNP-SWNT conjugate formed through DNA hybridizations. In order to form this structure, SWNTs were first dispersed with the black strand. After that, the green strand was grafted to the carbon nanotubes following the procedures as described in the experimental section. On the other hand, the blue strand was hybridized with the thiolated red strand and then the resulting hybrid was conjugated to 6 nm gold nanoparticles at a molar ratio of 0.5:1 between DNA and gold nanoparticles. Since the ratio between DNA and gold nanoparticles was very low, we then avoided the step to remove free DNA from the gold nanoparticle solution, which might be challenging for the 6 nm gold nanoparticles since the DNA conjugated nanoparticle with such a small diameter are hard to get completely precipitated. The buffer used for hybridization is 0.5xTBE containing 0.05 M NaCl.
**Figure S7.** An agarose gel (1.5%) recovery strategy used to isolate the gold nanoparticle-SWNT conjugates after hybridizing DNA functionalized gold nanoparticles with DNA grafted carbon nanotubes. In the left gel in (a), the first gel loading well has a clear layer of red precipitates (corresponding to gold-SWNT conjugates). However, in the control experiment (no hybridizations happened) as shown in the right gel in (a), no any conjugation products can be observed from the gel. It can also be seen from the picture in (b) that the product (left tube) washed out from the first gel loading well of the left gel in (a) has an reddish color coming from the assembled gold nanoparticles on the carbon nanotubes; while the buffer recovered from the first gel loading gel in the right gel in (a) (right tube) does not have any discernible red color, indicating no actual conjugation happens between the gold nanoparticles and the single walled carbon nanotubes grafted with non-complimentary sequences. Note that the gels were not stained. For the control experiment (the right gel in (a)) the corresponding blue strand as shown in Figure S6 was changed to another strand with a sequence of: GGACGGAATCTCTACCTATACGGGCTCGGCAAAAAAAAAAAA (5’ to 3’), which could not hybridize with the green tails on carbon nanotubes (see Figure S6).
Figure S8. More AFM images corresponding to SWNT-AuNP assemblies. Some crosslinkings are observed, which might be caused mainly due to the following reason: some gold particles bear multiple DNA strands and thus can bridge two or more carbon nanotubes together. Using mono-functionalized gold nanoparticles and further optimizing the hybridization conditions are expected to reduce the chance of forming crosslinked structures. It can also be observed that some discrete gold nanoparticles scatter around on the background of these images. We assume most of these background particles were detached gold nanoparticles from the assemblies during the repetitive sample washing process, because the fluidic force could partially destroy and denature the samples especially when deionized water was used in order to get a clean sample to facilitate AFM imaging.
Black strand 1 (lower SWNT): 5’ GTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGAGGCTATTCT 3’
Green strand: 5’ GGACGGAATCTCTACCAGAATAGCCTCGCATC 3’
Blue strand (linker or target strand): 5’ GGTAGAGATTCGTCATGACTGCTCAGATCG 3’
Black strand 2 (upper SWNT): 5’ GATGCGAGGCTATTCTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTG
Figure S10. A gel electrophoretic assay demonstrating the possibility for the detection of a linker DNA (or target DNA) using our carbon nanotube aggregation strategy. Each target DNA added to the solution can bridge two different tails grafted on two carbon nanotubes accordingly, and therefore leads to the formation of 3D aggregates (please refer to Figure S9 for details). After addition of the target DNA to a mixture of two different SWNT solutions, aggregations happened. 0.8% agarose gel electrophoresis was used to check the aggregated samples. For the lower gel, carbon nanotubes had a concentration about 1/4 as that in the upper gel, while the DNA concentrations were kept identical in the two gels. Stars indicate the gel lanes where the aggregation happened most efficiently; arrows point to the gel lanes corresponding to the limit where a difference can still be distinguished with unaided eyes as compared to the gel lanes with lower DNA concentrations, i.e., the detection limit by this gel electrophoretic detection assay. It can be seen that the detection limit shifted from 70.4 nM to 17.6 nM when we decreased the concentration of the carbon nanotubes 4 times, which implies if a more sensitive method can be used for monitoring the aggregation of carbon nanotubes, a much lower concentration of carbon nanotubes can then be used, and the detection limit of the resulting analytical assay for DNA can possibly be further pushed down to a much lower level than the current case. We also expect that tuning the density of the grafted tails on the carbon nanotubes should also improve the detection limit of this assay, and this can be very easily realized experimentally. Note that, the gels were not stained, and the black colored bands and deposits in the gel loading wells correspond to dispersed and aggregated carbon nanotubes, respectively. It can also be seen from this gel that neither very low nor very high concentration of the target DNA will favor an efficient aggregation of carbon nanotubes, and this agrees well with our expectations based on the structural model in Figure S9, which should be also true for other aggregation-based sensing systems such as gold nanoparticles.