nature nanotechnology

ARTICLES

PUBLISHED ONLINE: 8 NOVEMBER 2009 | DOI: 10.1038/NNANO.2009.311

Self-assembly of carbon nanotubes into two-dimensional geometries using DNA origami templates

Hareem T. Maune†, Si-ping Han*†, Robert D. Barish†, Marc Bockrath, William A. Goddard III, Paul W. K. Rothemund and Erik Winfree

A central challenge in nanotechnology is the parallel fabrication of complex geometries for nanodevices. Here we report a general method for arranging single-walled carbon nanotubes in two dimensions using DNA origami—a technique in which a long single strand of DNA is folded into a predetermined shape. We synthesize rectangular origami templates (∼75 nm × 95 nm) that display two lines of single-stranded DNA 'hooks' in a cross pattern with ∼6 nm resolution. The perpendicular lines of hooks serve as sequence-specific binding sites for two types of nanotubes, each functionalized non-covalently with a distinct DNA linker molecule. The hook-binding domain of each linker is protected to ensure efficient hybridization. When origami templates and DNA-functionalized nanotubes are mixed, strand displacement-mediated deprotection and binding aligns the nanotubes into cross-junctions. Of several cross-junctions synthesized by this method, one demonstrated stable field-effect transistor-like behaviour. In such organizations of electronic components, DNA origami serves as a programmable nanobreadboard; thus, DNA origami may allow the rapid prototyping of complex nanotube-based structures.

Single-walled carbon nanotubes (SWNTs) have exceptional electronic properties that suggest their use in nanoscale information-processing devices. Towards this goal, there have been advances in SWNT synthesis[1], dispersion[2], sorting by electronic property[3] or length[4], and modification[5]. Methods for the parallel alignment of SWNTs have allowed the creation of lithographically defined high-performance electronic devices[6]. However, the arrangement of individual SWNTs into complex nanoscale geometries is an open challenge. Lithographic methods that produce the smallest arbitrarily complex patterns, such as dip-pen[7] and electron-beam[8], are serial processes; nanoimprint lithography can replicate such patterns[9], but methods for solving challenges such as alignment are still being developed[10]. Thus, although the organization of SWNTs by lithographically patterned affinity templates[11] or electrodes[12] could allow the creation of complex circuits, scaling up production remains difficult. Approaches based on protein and/or DNA self-assembly potentially provide parallelism. Many such methods have only created one-dimensional SWNT structures[13,14] and devices[15,16] in which a single SWNT positioned between a pair of electrodes is switched by the substrate back-gate. One method has created structures in which DNA linkers define the connectivity between three carbon nanotubes[17]; however, the angles between the nanotubes are uncontrolled. Two-dimensional control over SWNT organization is necessary to deterministically and reproducibly create circuits of many devices in which SWNTs gate other SWNTs directly.

DNA nanotechnology[18,19] provides, simultaneously, parallel and geometrically complex nanofabrication by making use of the binding specificity and structural predictability of nucleic acids. Over two decades ago, it was proposed[20] that DNA nanostructures could be used to template a three-dimensional memory. So far, DNA has been used to organize gold nanoparticles[21] into arrays and self-assemble one-dimensional SWNT electronic devices[15]. Scaffolded DNA origami[22] allows construction of arbitrary, ∼100 nm, two-dimensional shapes that can display desired patterns of 200 chemical modifications with ∼6 nm resolution. Trillions of origami can be self-assembled in millilitre reaction volumes in a single step. These properties suggest that DNA origami could be used to organize SWNTs into desirable device architectures[23–25]. Interfacing such circuits with the macroscale may require some top-down lithography, but the goal of using DNA templates is to shift more of the burden of creating complex geometries from lithography to self-assembly.

Cross-junction assembly scheme

Our approach is to align nucleic acid-labelled SWNTs (NL-SWNTs) along lines of complementary single-stranded DNAs (ssDNA) 'hooks'[26] on DNA origami. In principle, multiple populations of NL-SWNTs with different properties (for example, semiconducting or metallic) could be labelled with different sequences, and self-assemble simultaneously into a complex geometry defined by the layout of lines on an origami. Fortuitously, when ssDNAs are sonicated with SWNTs, they attach by means of physisorption of DNA bases to SWNT sidewalls[3] and cause the SWNTs to disperse[2] in aqueous solution. This non-specific interaction allows non-covalent attachment of DNA labels to SWNTs without disrupting their electronic properties[27] and provides a simple route to NL-SWNTs.

It is difficult, however, to design a DNA molecule that both disperses SWNTs and serves as an efficient label, because any ssDNA label it carries can also bind the SWNTs and either crosslink the SWNTs or become unavailable for binding hooks. Such SWNT-bound labels are capable of partial desorption and hybridization to free DNA hooks, but they do so prohibitively slowly[28]. In many applications such as those in which SWNTs are purposefully aggregated by DNA labels[29], it is only necessary that a fraction of DNA labels bind cognate hooks. However, to bind and align a SWNT

California Institute of Technology, Pasadena, California 91125, USA; †These authors contributed equally to this work. *e-mail: si-ping@wag.caltech.edu

with high fidelity to a row of relatively few DNA hooks on an origami it seems important that a high fraction of the SWNT labels bind. This suggests any DNA label intended to attach to the hooks must be protected from sticking to the SWNT, for example by making it double-stranded DNA (dsDNA). However, this presents the secondary challenge of removing the complementary 'protection strand' at the right time so that the DNA label can attach to hooks while remaining attached to the SWNT. Previous methods using protecting strands[30] or other secondary strands[31] do not protect ssDNA labels during critical assembly steps; thus these schemes appear to lack the level of control required for two-dimensional organization.

Here we prepare NL-SWNTs using a protection scheme borrowed from the construction of DNA nanomachines[32] and self-assemble them on DNA origami templates to create two-dimensional cross-junctions. In this scheme, protection strands are removed by the process of labels hybridizing to the origami hooks. Thus throughout our method, ssDNA labels remain almost completely protected until they bind the DNA origami; only short 'toehold' sequences are ever exposed as ssDNA. We created two types of NL-SWNTs (labelled 'blue' and 'red' for convenience) by using two different linkers to disperse separate aliquots of high-pressure CO conversion (HiPco) SWNTs (Fig. 1a). Each aliquot comprised a mixed population of semiconducting and metal SWNTs. In principle, pure populations of semiconducting and metallic SWNTs could be used to specify exclusive assembly of semiconductor–metal cross-junctions, the arrangement most likely to act as a field-effect transistor (FET.) Each linker is a two-stranded, partially duplex complex that adsorbs onto a SWNT via a 40-base poly-thymine (poly-T) dispersal domain. Its 20 nucleotide labelling domain (design methods in Supplementary Information, Text S1 and ref. 33) has a sequence specific to its colour and is complementary to similarly coloured hooks on a DNA origami template (Fig. 1b). A 15-base protection strand leaves 5 bases of the labelling domain unprotected. These 5 bases comprise the toehold, which is composed of locked nucleic acid (LNA). During dispersal, we expect the poly-T dispersal domain to adsorb on the SWNT while the protection strand prevents adsorption of the labelling domain. The relative instability of SWNTs dispersed by short ssDNA (4 or 6 nt)[34] suggests that the interaction of the short toeholds with the SWNT sidewalls is dynamic, making them available for binding hooks. (Short toeholds also seem important, because the use of 7 or 10-nt ssDNA toeholds resulted in crosslinked SWNTs during dispersal.) At the same time, the toehold is long enough that initiation of deprotection is still fast (toeholds should be $\geq$4 bases to maximize the reaction rate[35]). During assembly (Fig. 1c), a DNA hook complementary to all 20 labelling domain bases binds first to the 5-LNA-base toehold and initiates branch migration (Fig. 1d); this allows the hook to displace the protection strand and bind to the entire labelling domain[32,36]. We chose LNA for toeholds because branch migration efficiency increases with toehold binding stability[37], and LNA–DNA duplexes are more stable than their DNA counterparts.

Our template design (Fig. 1b, Supplementary Figs S1–S3 and Text S2) is based on the 'tall rectangle' origami[22], formed by ~200 DNA staples that fold a long scaffold strand into the desired sheet of B-form helices. The sequence of each staple (typically 32 bases) determines its unique position in the sheet. Hence, a DNA hook can be placed at any position by extending the 3′ end of the appropriate staple. DNA helical twist (10.5 bases per turn) determines the angle of the backbone relative to the plane of the origami; this allows hooks to be added to either face. We added a row of 11 red hooks to the bottom, and a column of 16 blue hooks to the top. In the original design, all staple ends fall on the bottom; thus, to project red hooks down, we concatenated the red hook sequence onto 3′ ends of staples in the desired row. For each staple in the blue column, we shifted the staple's 3′ end by half a turn (5 nucleotides) to position it on top and concatenated the blue hook sequence onto the end. Between each hook and staple sequence, we inserted a four-thymine spacer.

Origami aggregate by means of stacking interactions between helix ends along their vertical edges. Thus we omitted the leftmost column of staples from the original design[22]. This resulted in a column of single-stranded loops that inhibited stacking (Fig. 1b). Also, we replaced the rightmost column of staples with DNA strands that nucleated growth of a ~100-nm-wide, typically >500-nm-long, DNA ribbon (Fig. 1b) through algorithmic self-assembly of DNA tiles[38,39]. Addition of ribbons made image interpretation easier and appeared to increase the deposition rate of SWNT/DNA constructs.

Fidelity of alignment

To measure the efficiency, specificity and orientation of attachment for red and blue NL-SWNTs (independently) we imaged more than 200 SWNT/DNA constructs assembled using only red or blue SWNTs. Constructs were assembled by separately mixing either blue or red NL-SWNTs with templates displaying the cross-pattern of red and blue hooks (Fig. 1b). In each case, SWNTs had an opportunity to bind to either red or blue hooks. The desired outcome for each construct was a single SWNT aligned over the complementary hook array. Non-specific attachment would result in incorrect alignment or binding of more than one tube. Constructs were deposited on mica and scanned under buffer; 86% of templates mixed with red SWNTs had at least one SWNT attached, as did 80% of templates mixed with blue SWNTs. Of templates with attached SWNTs, ~25% were distorted or aggregated. Overall, ~50% of all templates were intact and had a single SWNT attached as desired. Figure 2 shows the distribution of alignments between templates and attached SWNTs. The angle of the ribbon with respect to the origami (Supplementary Information, Fig. S4a) allowed us to distinguish between red and blue faces and to define SWNT alignment angles. Figure 2 shows that the angular distribution for blue SWNTs peaks at ~0° (as expected) with 56% oriented within ±15° of the peak. The distribution of red SWNTs peaks at ~90° (as expected) with 50% within ±15° of the peak. These data suggest that NL-SWNTs strongly prefer their complementary hook array and align parallel to it. The importance of the protection strands for binding efficiency was verified in a control experiment: when blue SWNTs were prepared without protection strands <10% of DNA templates had SWNTs attached.

Cross-junctions

We assembled cross-junctions (Supplementary Text S3) by mixing templates with both red and blue NL-SWNTs simultaneously, and visualized them by atomic force microscopy (AFM) (Fig. 1e,f and Supplementary Fig. S5). Cross-junctions, like these examples, are frequently asymmetric because NL-SWNTs often bind near their ends (for unknown reasons), even appearing to align so that their ends are flush with the edge of the origami template. In the final constructs, red and blue NL-SWNTs are separated by a layer of DNA composed of their respective linkers (at least 1 nm where linkers attach due to the thickness of the poly-T dispersal domains, potentially up to a few nanometres depending on the detailed configuration of linkers) and the DNA origami (2 nm thick) that lies between them. AFM height measurements of the cross-junctions (~4 nm) provide a weak upper bound for the thickness of the layer given that we cannot measure the thickness of naked SWNTs for the exact structures in question. In principle, the intervening DNA layer is thicker, with the SWNT on opposite sides of the origami, and we chose this geometry over binding both SWNTs to the same side. We hypothesized that, if retained, a thicker intervening DNA layer might function as a better insulator